Expression of Parkinson's disease and associated neurophenotypes in 22q11.2 deletion syndrome

by

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A thesis submitted in conformity with the requirements
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Institute of Medical Science
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Abstract

The etiology of Parkinson's disease (PD) remains largely unknown with the exception of a few genetic mutations that affect a small proportion of patients. Case reports suggest that individuals with 22q11.2 deletion syndrome (22q11.2DS), a multisystem genomic disorder associated with hemizygous 22q11.2 deletions, may be at increased risk of early-onset PD. The aim of this thesis was to investigate 22q11.2 deletions as a risk factor for early-onset PD.

The prevalence of PD was assessed in a well-characterized cohort of adults with 22q11.2DS. Neuropathological studies were performed in cases with available post-mortem tissue. Whole-genome sequencing was used to investigate the possible contribution of genome-wide rare coding mutations to disease penetrance in neuropathologically confirmed PD cases. Assessment of putative pre-diagnostic clinical and neuroimaging markers of PD in a subset of the older adults (30 to 54 years) provided evidence of an elevated prevalence of pre-morbid motor and olfactory deficits, and of nigrostriatal dopaminergic dysfunction assessed using ^{11}C-dihydrotetrabenazine and positron emission tomography.

The clinical context of early-onset PD in this adult cohort of individuals with 22q11.2DS was also investigated. Treatment response was excellent to the atypical antipsychotic, clozapine, a

candidate for use in psychotic patients at risk of, or with, PD. Cognitive level and severe psychiatric disorders were mediators of baseline functional capacity in 22q11.2DS adults. Practical guidelines were developed from a review of the 22q11.2DS literature to help inform the management of 22q11.2DS-associated conditions, including PD.

The results of these studies provided evidence that early-onset PD is associated with the 22q11.2 deletion with important implications for PD pathogenesis and for the clinical management of 22q11.2DS.

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I would first and foremost like to thank my supervisor, mentor, and friend, Dr. Anne Bassett, for her unwavering encouragement, support, and intellectual guidance over the course of my doctoral studies. It has been my honour and privilege to train with her.

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Contributions

Chapter 1: Introduction & Chapter 2: Research aims and hypotheses

Contribution statement and acknowledgements: The content of these chapters was conceived, drafted, and revised by me, with the following qualifiers. Anne Bassett provided helpful scientific comments and revisions. Table 1-1 presents the United Kingdom Parkinson's Disease Society Brain Bank Clinical diagnostic criteria.[1] Table 1-3 describes the Diagnostic and Statistical Manual of Mental Disorders diagnostic criteria for schizophrenia.[2] Figure 1-1 was reproduced with permission from Fusar-Poli et al. (2012).[3] Figure 1-2 was reproduced with permission from Berg et al. (2011).[4] Figures 1-3[5] and Figures 1-4[6] were prepared under our direction by Sean Bekeschus.

Chapter 3: Association between early-onset Parkinson's disease and hemizygous 22q11.2 deletions

Citation: Butcher NJ*, Kiehl T-R*, Hazrati L-N, Chow EWC, Rogaeva E, Lang AE, Bassett AS. 2013. Individuals with 22q11.2 deletion syndrome are at increased risk of early-onset Parkinson disease: Identification of a novel genetic form of Parkinson disease and its clinical implications. *JAMA Neurology*. 70:1359-1366. *Co-first authors

Contribution statement and acknowledgements: My roles in this study included the following: 1) conceiving and designing the study, 2) Identifying and characterizing cases of PD in the existing 22q11.2DS cohort, 3) Checking and correcting demographic data in existing 22q11.2DS database, 4) Statistical analyses, 5) Coordinating and participating in one of the three autopsies of a 22q11.2DS patient with PD, 6) Providing input on immunohistochemistry methods, interpretation, and presentation of results, 7) Coordinating and interpreting genetic test results, 8) Writing the manuscript, 9) Submitting and revising the manuscript for publication, and 10) Communicating results through conference presentations and press interviews.

I thus played a primary role in every aspect of this study with the exception of performing wet lab work (immunohistochemistry, genetic testing, and autopsies completed prior to the beginning of my doctoral studies).

The roles of the co-first author, Rasmus Kiehl, were as follows: 1) Initiating neuropathological studies of adults with 22q11.2DS, 2) Coordinating and/or performing autopsies, 3) Immunohistochemistry experiments and interpretation, and 4) Critically revising the manuscript.

Anne Bassett supervised all aspects of my involvement in this study, and made critical contributions to the study design, data interpretation, and writing the manuscript. Adults with 22q11.2DS were recruited and assessed over a period of many years as part of a longitudinal study of 22q11.2DS, directed by Anne Bassett and Eva Chow, which made this study possible. Lili-Naz Hazrati conducted one of the three autopsies, and performed immunohistochemistry experiments including interpretation and quantification (Table 3-3). Eva Chow contributed to statistical analyses. Anthony Lang provided critical input on data interpretation and contributed the early-onset PD cohort for testing for the 22q11.2 deletion, together with Ekaterina Rogaeva, who directed the genetic testing of known Parkinson's disease genes in our four 22q11.2DS-PD cases. All authors provided critical revision of the manuscript for important intellectual content.

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Chapter 4: Genetic markers of Parkinson's disease in 22q11.2 deletion syndrome

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I thus played a primary role in every aspect of this study, with the exception of genome sequencing and core bioinformatic processing, performed by Complete Genomics and The Centre for Applied Genomics.

Anne Bassett supervised all aspects of my involvement in this study, and made critical contributions to the study conception and design, data interpretation, and revising the manuscript. Daniele Merico and Mehdi Zarrei performed bioinformatic processing of the whole-genome sequencing data. Lucas Ogura assisted with data analyses and quality checking. Thomas Nalpathamkalam and Bhooma Thiruvahindrapuram performed variant annotation. Christian Marshall and Stephen Scherer contributed to study conception and design. Eva Chow, Daniele Andrade, and Anthony Lang contributed clinical data. All authors provided critical revision of the manuscript for important intellectual content.

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Chapter 5: Prodromal markers of Parkinson's disease in 22q11.2 deletion syndrome

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Butcher NJ*, Marras C, Pondal M, Rusjan P, Christopher L, Strafella A, Lang AE, Bassett AS. 2015. Investigating prodromal markers of Parkinson's disease in adults with hemizygous 22q11.2 deletions. *Movement Disorders* (S1): S1035 (*abstract*).

Contribution statement and acknowledgements: My roles in the study were as follows: 1) Conceiving and designing the study, 2) Protocol and consent form writing, 3) Obtaining Research Ethics Board and Health Canada approval, 4) Recruiting subjects, 5) Performing study procedures, 6) All data coding and entry, 7) Analyzing all of the data including neuroimaging and statistical analyses, 8) Interpreting all of the data, 9) Ensuring study procedures complied with Health Canada regulatory guidelines, 10) Performing regular study reviews with study monitor, 11) Completing a study audit, 12) Communicating findings to clinicians, scientists, and patients at international meetings.

I thus played the primary role in every aspect of this study.

Anne Bassett supervised all components of this study, and contributed to study conception and design and patient recruitment. Connie Marras and Margarita Pondal performed the transcranial sonography and motor assessments (UPDRS, Tremor Rating Scale). Pablo Rusjan contributed to PET methodology and analyses. Leigh Christopher and Antonio Strafella provided critical input and expertise on the PET data. Anthony Lang contributed input to study design. All authors provided important input on data interpretation. We thank core facility staff at the Centre for Addiction and Mental Health Research Imaging Centre. We also thank the staff of Clinical Genetics Research Program and the Dalglish Family Hearts and Minds Clinic for Adults with 22q11.2 Deletion Syndrome for study support, especially Fiona Fu for assistance with creation of a database for data management and helping with neurocognitive testing of the 22q11.2DS patients, Lucas Ogura for assistance with 22q11.2DS patient clinical data, and Drs. Eva Chow and Erik Boot for assisting with patient recruitment for the study.

Chapter 6: Safety and efficacy of antipsychotic treatment in 22q11.2 deletion syndrome

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with Anne Bassett, provided the (blinded) clinical ratings for patients treated with clozapine. Laura Fitzpatrick conducted data collection and telephone follow-up for the comparison idiopathic-schizophrenia group under my supervision. Alina Guna assisted with retrospective data collection for the 22q11.2DS group under my supervision. Danielle Andrade, Anthony Lang, and Eva Chow made contributions to 22q11.2DS patient data collection and interpretation. All authors critically revised the manuscript. Recruitment and longitudinal phenotypic characterization of the patient groups occurred over many years (directed by Anne Bassett for both cohorts, together with Eva Chow for the 22q11.2DS cohort).

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Chapter 7: Neurophenotypic mediators of baseline adaptive functioning in 22q11.2 deletion syndrome

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Anne Bassett closely supervised all components of this study, and made critical contributions to conception and design of the study. Eva Chow and Gregory Costain made contributions to 22q11.2DS patient data collection and interpretation. Dominique Karas and Andrew Ho assisted with data collection and data coding. All authors critically revised the manuscript. Recruitment and phenotypic characterization of the 22q11.2DS patients that made this study possible was directed by Anne Bassett and Eva Chow and occurred over many years.

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Chapter 8: Management of neurological and co-morbid multisystem conditions in adults with 22q11.2 deletion syndrome

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revising the manuscript. The remaining co-authors assisted in the drafting of manuscript text in their areas of expertise and/or provided critical review of the manuscript draft. The authors wish to acknowledge the support and endorsement provided by The 22q11.2 Society (www.22qsociety.org) with special thanks to its President, Dr. Peter Scambler, for his helpful comments on the manuscript.

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Chapter 9: General discussion and future directions

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List of Abbreviations

22q11.2DS	22q11.2 deletion syndrome
22q11.2DS-PD	22q11.2 deletion syndrome-Parkinson's disease
2D	two dimensional
3D	three dimensional
^{11}C-DTBZ	^{11}C-dihydrotetrabenazine
µm	micrometre
AAO	age at onset
ABC	adaptive behavior composite
ANC	absolute neutrophil count
BID	bid in die (two times a day)
bp	base pairs
BP_{ND}	non-displaceable binding potential
CGI	Clinical Global Impression scale
CHD	congenital heart disease
CI	confidence interval
CNV	copy number variation
COMT	catechol-*O*-methyltransferase
CT	computerized tomography
DNA	deoxyribonucleic acid

DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders (Fourth edition, text revision)
EEG	Electroencephalography
F-dopa	fluorodopa
FISH	fluorescence *in situ* hybridization
g	gram
GWAS	genome-wide association study
H&Y	Hoehn and Yahr staging
HRRT	high-resolution research tomograph
ID	intellectual disability
In/del	insertion/deletion
IQ	intelligence quotient
kb	kilo base pairs
L	litre
LB	Lewy body
LCR	low copy repeats
L-dopa	levodopa
MAF	minor allele frequency
Mb	mega base pairs
MCI	mild cognitive impairment
MDS	Movement Disorders Society

mg	milligram
mm	millimeters
MoCA	Montreal Cognitive Assessment
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	magnetic resonance imaging
nt	nucleotides
OMIM	Online Mendelian Inheritance in Man
OR	odds ratio
PCR	polymerase chain reaction
PD	Parkinson’s disease
PET	positron emission tomography
prn	pro re nata (when necessary)
RBD	rapid eye movement behaviour disorder
REM	rapid eye movement
SCA	spinocerebellar ataxia
SD	standard deviation
SMR	standardized morbidity ratio
SMST	sensorimotor striatum
SNP	single nucleotide polymorphism
SNV	single nucleotide variant

SPECT	single-photon emission computerized tomography
TCS	transcranial sonography
TH	tyrosine hydroxylase
TID	ter in die (three times a day)
UPDRS	Unified Parkinson’s Disease Rating Scale
UPSIT	University of Pennsylvania Smell Identification Test
VABS	Vineland Adaptive Behavior Scales
VMAT2	vesicular monoamine transporter type 2
WAIS-III	Wechsler Adult Intelligence Scale III
WAIS-R	Wechsler Adult Intelligence Scale-Revised
WBC	white blood cell
WGS	whole-genome sequencing

Chapter 1 Introduction

1 Overview

1.1 Clinical and epidemiologic features of Parkinson's disease

Parkinson's disease (PD; OMIM #168600) is a chronic neurodegenerative disorder associated with motor, cognitive, and autonomic dysfunction. First described by Dr. James Parkinson in 1817 as the "shaking palsy," PD is now recognized as one of the most common neurological disorders world-wide, affecting approximately 1% of individuals over the age of 60 years.[9,10] PD is clinically diagnosed based on the presentation of symptoms that include slowness of movement (bradykinesia), resting tremor, rigidity, and postural instability (Table 1-1).[1] Non-motor symptoms, including cognitive deficits and sensory, sleep, and psychiatric disturbances are now also recognized as important clinical features of PD.[11,12] The underlying neuropathology of the disease is predominantly characterized by the progressive death of dopaminergic neurons in the substantia nigra, a region of the midbrain, and the accumulation of Lewy bodies, abnormal aggregates of protein, within neurons.[13] The expression and severity of clinical features correlates with the extent of the neuropathological progression.[13-15]

PD occurs as a sporadic disorder with no clear cause in the majority of cases. Approximately 10-15% of patients have a positive family history of the disease. This common, complex disease is generally considered a multifactorial condition arising from a combination of genetic and environmental factors. Causative monogenic mutations have been identified in a small proportion of patients to date.[16-18] PD risk is most strongly associated with age, with a nearly exponential increase in incidence between the ages of 60 and 80 years. Juvenile or early-onset (e.g., <50 years) of the disease occurs infrequently, and is more likely to be related to genetic factors.[17,19,20] Epidemiological studies consistently show that men are approximately 1.5 times more likely to develop PD than women, possibly related to a protective effect of estrogens or increased rates of head trauma and toxin exposures in men.[10,21] Many environmental risk factors that may contribute to disease pathogenesis have been extensively examined, but either have a small impact (e.g., odds ratios$\leq$2) or exposures are rare.[10,19] Factors such as pesticide and heavy

Table 1-1 United Kingdom Parkinson's Disease Society Brain Bank Clinical diagnostic criteria

Step 1. Diagnosis of parkinsonian syndrome

Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions) and at least one of the following:

- Muscular rigidity
- 4-6 Hz rest tremor
- Postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction

Step 2. Exclusion criteria for Parkinson's disease

- History of repeated strokes with stepwise progression of parkinsonian features
- History of repeated head injury
- History of definite encephalitis
- Oculogyric crises
- Neuroleptic treatment at onset of symptoms
- More than one affected relative[a]
- Sustained remission
- Strictly unilateral features after three years
- Supranuclear gaze palsy
- Cerebellar signs
- Early severe autonomic involvement
- Early severe dementia with disturbances of memory, language, and praxis
- Babinski sign
- Presence of cerebral tumor or communication hydrocephalus on imaging study
- Negative response to large doses of levodopa in absence of malabsorption
- MPTP exposure

Step 3. Supportive prospective positive criteria for Parkinson's disease

Three or more required for diagnosis of definite Parkinson's disease in combination with step 1:

- Unilateral onset
- Rest tremor present
- Progressive disorder
- Persistent asymmetry affecting side of onset most
- Excellent response (70-100%) to levodopa
- Severe levodopa-induced chorea
- Levodopa response for five years or more
- Clinical course of ten years or more

[a]This criterion is no longer used[22,23]

MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry.* 1992; 55:181-184

metal exposures, for example, are associated with slightly increased PD risk. Smoking and coffee drinking may be protective.[10,19]

1.2 Natural history of Parkinson's disease

1.2.1 Pre-motor Parkinson's disease

As a progressive neurodegenerative disorder, PD has a long pre-diagnostic, or prodromal period, that precedes its formal clinical diagnosis. Prodromal PD is characterized by olfactory deficits, autonomic dysfunction, and neuropsychiatric and sleep problems.[11,12] Pathological studies indicate that PD begins in structures outside the substantia nigra, such as the olfactory bulb and brainstem nuclei, which correspond to manifestation of these early and detectable pre-motor symptoms of the disease.[11,13] The possibility of identifying patients in this early pre-symptomatic stage of the disease, before the onset of motor signs, is an area of considerable interest given the implications for the development of early interventions to slow or halt disease progression. Striatal dopamine denervation is estimated to begin 5 to 15 years before motor symptoms manifest. An estimated 50% of nigral dopaminergic neurons and 80% of striatal dopamine content is lost by the time a patient is clinically diagnosed,[24,25] suggesting a large window of opportunity that remains largely unexploited by current therapeutic strategies.

The earliest known pre-motor manifestations of PD are recognizable clinical features that include olfactory loss, rapid eye movement behaviour disorder (RBD), excessive daytime sleepiness, constipation, and depression.[12] One or more of these symptoms are present in nearly all PD patients prior to diagnosis. Disruptions to non-dopaminergic neurotransmitter systems, including glutamatergic, noradrenergic, serotonergic, and cholinergic pathways, are increasingly appreciated to be involved in the expression of these features.[26,27] With the exception of RBD, these symptoms are also experienced by a significant proportion of the general population, most of whom do not go on to develop PD, limiting their utility in early diagnostic strategies for PD.[12,28,29] RBD is characterized by complex vigorous dream-enacting behavior due to loss of REM-related muscle atonia. RBD affects up to one half of patients with PD,[30] and up to ~65% of patients with RBD develop a neurodegenerative disease within ten years.[28,31-33] Roughly half of these cases develop PD, and most of the remainder develop the closely associated disease of Lewy body dementia. The high conversion rate suggests patients with RBD constitute an important population in which other predictive markers should be studied and in which the

efficacy of neuroprotective agents could be examined.[11,28] Periodic follow-up neurological examinations are suggested for patients with RBD in order to detect treatable symptoms early.[11]

Although not highly specific to PD, hyposmia is an early and sensitive symptom that may be useful in early diagnosis or prodromal detection of PD.[28,34] Lewy body pathology affecting olfactory structures, such as the olfactory bulbs and the anterior olfactory nucleus, is among the earliest of neuropathogical changes observed in PD.[13,34] Accordingly, approximately 90% of individuals diagnosed with PD have olfactory deficits. These appear to be unrelated to disease stage and duration, medication status, and severity of cognitive symptoms.[34,35] Olfactory loss may be useful for distinguishing PD from other neurological diseases in which olfactory deficits are absent or less severe, such as multiple system atrophy, progressive supranuclear palsy, corticobasal degeneration, and essential tremor.[34] The strongest evidence for olfaction as a predictive PD marker comes from a large prospective study of asymptomatic elderly Asian men, where olfactory deficits at baseline were associated with an odds ratio of 5.2 for developing PD within four years.[36] Additional longitudinal studies are needed but cohorts enriched for olfactory dysfunction may be helpful in stratifying for PD risk, particularly when combined with other signs of PD.[34,37]

1.2.2 Emergence of clinically manifest Parkinson's disease

Parkinson's disease is diagnosed based on standard clinical criteria, marked by the manifestation of bradykinesia together with muscular rigidity, rest tremor, and/or postural instability (Table 1-1). As the underlying neurodegenerative pathophysiology of PD progresses to later prodromal stages of the disease, patients begin to exhibit mild signs of parkinsonism that are insufficient to fulfill the criteria for a clinical diagnosis of PD, analogous to the mild cognitive impairment that precedes a formal diagnosis of Alzheimer's disease.[12] In the early stages of motor symptom development, making a definitive diagnosis of PD may be challenging.[1,38] PD can be confused with numerous other conditions with overlapping parkinsonian features (e.g., medication side effects, metabolic disorders, structural brain lesions, other neurodegenerative conditions). Thorough and systematic clinical assessment should guide the diagnosis. Bradykinesia and a typical resting pill-rolling tremor with good response to L-dopa is highly suggestive of true PD. It is important to rule out other secondary causes of parkinsonism and consider alternative diagnoses in the presence of atypical clinical signs (Table 1-1).[39,40] A recent study showed that

clinical diagnostic accuracy of PD in untreated or not clearly medication-responsive patients was only 26% based on follow-up neuropathological validation.[38] Diagnostic accuracy improves substantially, to 80-90%, in the later stages of the disease.[1,38] Common alternative diagnoses include drug-induced or vascular parkinsonism, essential tremor, multiple system atrophy, and progressive supranuclear palsy. Neuropathological confirmation of a diagnosis of PD thus remains the gold-standard.[13] Neuropathological assessment may not be routinely available, however, and is not informative for clinical management given that these are post-mortem investigations.

Neuroimaging may be a useful tool in patients with clinically uncertain parkinsonism to help confirm or refute a suspected diagnosis of PD.[39,40] Dopaminergic cell loss can be visualized using single-photon emission computed tomography (SPECT) and positron emission tomography (PET) using radiotracers that localize to various components of the presynaptic dopaminergic system (Figure 1-1). Evidence of nigrostriatal degeneration can help distinguish PD from non-degenerative conditions such as drug-induced parkinsonism and essential tremor.[41,42] Presynaptic dopaminergic neuroimaging is restricted to use in research settings in Canada and most other countries. SPECT imaging using DaTscan™ (^{123}I-ioflupane injection; ^{123}I-FP-CIT) is approved in the USA and Europe to visualize the striatal dopamine transporter (DAT) to assist in the evaluation of cases with a clinically uncertain parkinsonian syndrome.[42] DAT is a presynaptic membrane protein that transports dopamine from the synapse back to the cellular cytosol. The use of DaTscan™ in patients with clinically uncertain parkinsonism (typically those in early disease stages) may help improve clinical diagnostic accuracy in some cases.[42] The diagnostic accuracy of DaTscan™ and clinical assessment are equivalent once a clinical diagnosis has been reached, however, rendering its use largely redundant for most cases.[43,44] Additional clinical information must be considered to aid in the differentiation of PD from other conditions associated with dopaminergic neurodegeneration apparent on DaTscan™, such as multiple system atrophy, progressive supranuclear palsy, and corticobasal degeneration.[40,45,46] Patients with vascular parkinsonism cases may also exhibit dopaminergic abnormalities when the nigrostriatal system is involved.[45]

Well-characterized markers of presynaptic dopaminergic integrity commonly used in PD research studies (Figure 1-1) include ^{18}F-fluoro-L-dopa (^{18}F-FDOPA), ^{11}C-methylphenidate (^{11}C-MP), and ^{11}C-dihydrotetrabenazine (^{11}C-DTBZ). ^{18}F-FDOPA is stored in synaptic vesicles in the

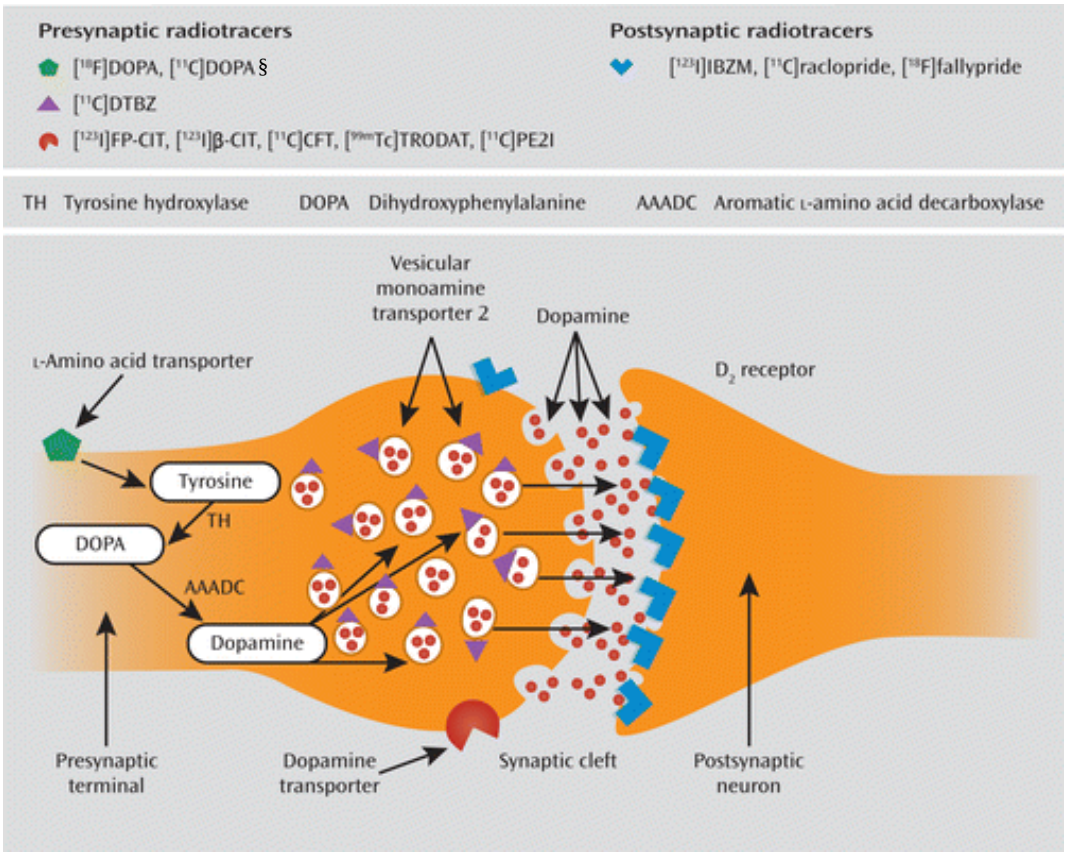

Figure 1-1 Dopaminergic radiotracers useful in the study of Parkinson's disease

A simplified diagram of a striatal dopaminergic synapse is shown. Presynaptic dopaminergic radiotracers show progressive decline in Parkinson's disease. [^{18}F]-DOPA and [^{11}C]-DOPA provide a measure of dopamine synthesis capacity using positron emission tomography (PET). In the case of [^{18}F]-DOPA, the radiotracer is taken up by the L-Amino acid transporter (§), decarboxylated to ^{18}F-dopamine (^{18}F-DA) by aromatic L-amino acid decarboxylase (AAADC), and stored temporarily in presynaptic vesicles by the vesicular monoamine transporter type 2 (VMAT2). ^{11}C-dihydrotetrabenazine [^{11}C]-DTBZ labels VMAT2 in PET neuroimaging, which provides an index of dopamine presynaptic terminal density in the striatum. The dopamine transporter (DAT) can be labelled with several suitable agents in single-photon emission computed tomography (SPECT) and PET studies. DAT neuroimaging provides another measure of dopaminergic presynaptic terminals. Post-synaptic D2/D3 dopamine receptor availability can be assayed using ^{11}C-raclopride (sensitive to synaptic dopamine levels), or numerous other radiotracers including those not sensitive to endogenous dopamine (depending on affinity) in PET and SPECT neuroimaging. A D2 receptor is illustrated for simplicity.

Fusar-Poli P, Rubia K, Rossi G, Sartori G, Balottin U. Striatal dopamine transporter alterations in ADHD: pathophysiology or adaptation to psychostimulants? A meta-analysis. Am J Psychiatry. 2012; 169:264-272

presynaptic dopaminergic terminal following decarboxylation to ^{18}F-dopamine (^{18}F-DA). DAT is labeled by ^{11}C-MP, or one of several other available radioligands. ^{11}C-DTBZ labels the vesicular monoamine transporter type 2 (VMAT2), responsible for pumping dopamine from the cytosol into presynaptic vesicles in dopaminergic cells. Each of these radiotracers is sufficient for detecting dopaminergic cell loss in PD although the rates of decline in PD show subtle differences between them.[39,47] VMAT2 shows the earliest decline relative to the other dopamine markers[47] and is considered the most stable marker of PD progression because of its relative resistance to disease- and drug-compensatory regulation.[48-50] Efforts are underway to develop an α-synuclein PET radiotracer to assess *in vivo* Lewy body pathology, which promises to further advance neuroimaging in PD patients.[51]

Transcranial sonography (TCS) imaging of the substantia nigra offers promise as a rapid, low-cost, and non-invasive alternative imaging tool in the differential diagnosis and in early diagnosis of PD.[4,52-54] Approximately 70-90% of patients with PD show an increased area of hyperechogenicity at the anatomical site of the substantia nigra[4,54] (Figure 1-2). Hyperechogenicity is typically defined as an echogenic area exceeding a cut-off point set by the 90th percentile of a healthy population with no clinical signs of PD (often ~ 0.20 cm^2). Hyperechogenicity appears to be a stable marker of PD, with most studies reporting no substantial association with disease severity (although usually larger contralateral to the most clinically affected side) or duration - at least up to a time interval of six years.[4,53]

There is increasing evidence that substantia nigra hyperechogenicity is present even prior to the onset of clinically manifest PD. Hyperechogenicity, by definition, is observed in ~10% of healthy subjects. It is posited that at in at least some of these cases, the increased echo signal marks a vulnerability to PD.[4,52,54] Unaffected individuals with a hyperechogenic signal have an increased prevalence of other non-motor and motor symptoms associated with PD,[4] including olfactory dysfunction,[37,55] depression,[56] RBD,[57] mild extrapyramidal signs,[55] and evidence of nigrostriatal abnormalities using presynaptic neuroimaging techniques.[58] Moreover, a prospective, longitudinal study recently demonstrated that healthy individuals over the age of 50 years with hyperechogenicity but no clinical signs of PD had more than a 20 fold increased risk of converting to PD within five years than those without this feature.[54] The pathophysiological mechanism underlying nigral hyperechogencity remains unclear, but pathological studies

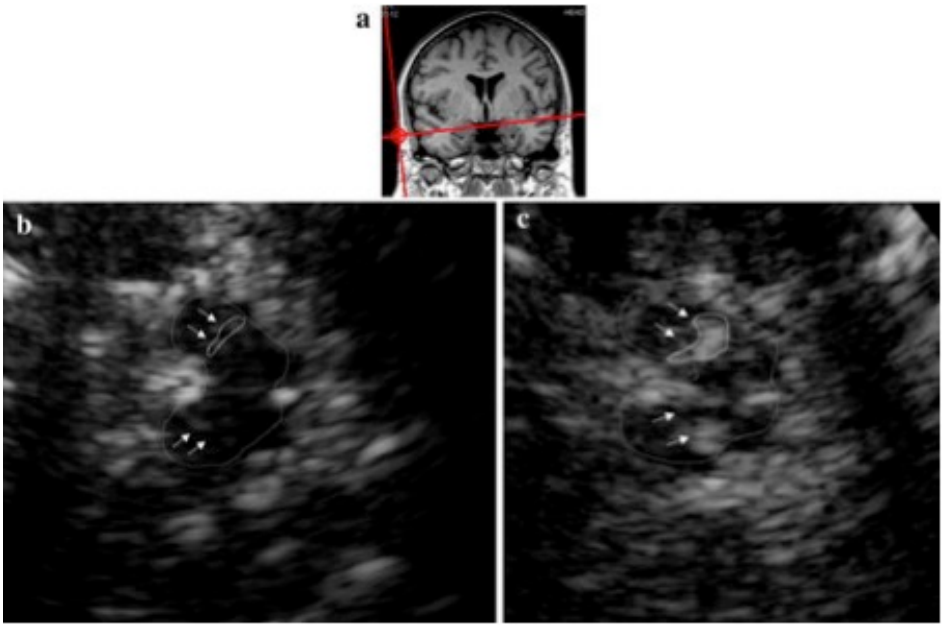

Figure 1-2 Visualizing the substantia nigra using transcranial sonography

The midbrain is examined parallel to the orbitomeatal positioning line (a). The echogenic substantia nigra is measured ipsilaterally (encircled and marked with arrows) within the butterfly-shaped midbrain in a healthy control (b) and a patient with Parkinson's disease (c). The contralateral substantia nigra is marked with arrows only. Note the hyperechogenicity at the anatomical location of the substantia nigra in the Parkinson's disease case compared with the healthy control.

Berg D. Hyperechogenicity of the substantia nigra: pitfalls in assessment and specificity for Parkinson's disease. *J Neural Transm.* 2011; 118:453-461

indicate a correlation with local iron accumulation (non-ferritin bound) and microglial activation.[58,59]

1.2.3 Parkinson's disease course and progression

In the early stages of clinically manifest PD, the emerging motor symptoms are generally mild and do not have a significant impact on daily life and functioning. As the severity of the motor symptoms increases, antiparkinsonian agents become necessary to manage the disease. Most medications used in routine practice act directly on the dopaminergic system. These include levodopa, the gold-standard treatment, as well as dopamine agonists and both catechol-O-methyl transferase and monoamine oxidase B inhibitors.[60,61] The disease course is variable between patients, but severity of the motor symptoms progressively worsens and tends to become more difficult to manage. Greater baseline severity of motor symptoms and early cognitive impairment appear to be predictive of more rapid motor decline and disability.[62] Older age at onset and lack of rest tremor at onset may also be important factors in predicting disability.[62,63] Response duration to each dose of levodopa decreases as PD advances (the so-called 'wearing off-effect'), and unpredictable motor fluctuations including freezing and involuntary movements may develop.[64] Patients in advanced stages of the disease may be considered for high-frequency deep brain stimulation to manage worsening motor disability.[65] Deep brain stimulation requires the surgical implantation of stimulation electrodes into the subthalamic nucleus or the globus pallida interna, which deliver high-frequency pulses of electrical stimulation.[66] Deep brain stimulation is thought to help alleviate motor symptoms in PD by suppressing abnormal signaling in its neural targets, but its therapeutic mechanism of action remains unclear and an active area of investigation.[67]

It is now well-recognized that the non-motor symptoms of PD are also important clinical features of the disease that can have a significant impact on progression of disability and quality of life.[60,68,69] This diverse group of symptoms may include sensory abnormalities (e.g., hyposmia, visual discrimination defects, pain), autonomic dysfunction (e.g., orthostatic hypotension, constipation, incontinence), neuropsychiatric abnormalities (e.g., depression and anxiety), sleep disturbances, and cognitive impairments.[26,28,70] These largely non-dopaminergic features respond poorly, if at all, to levodopa treatment and often remain unrecognized and untreated in patients with PD.[71,72] Select motor disturbances, such as dysphagia, drooling, gait dysfunction, freezing,

and postural instability, are also considered non-dopaminergic features of PD.[70] Neuronal loss and/or Lewy pathology in regions other than the nigrostriatal dopamine system are likely to be involved in the various manifestations of non-dopaminergic PD symptoms.[27,70] These regions may include the peripheral autonomic nervous system, the spinal cord, the olfactory structures, the dorsal motor nucleus of the vagus nerve in the medulla oblongata, the noradrenergic neurons of the locus coeruleus, the serotonergic neurons of the raphe nuclei, the cholinergic neurons of the nucleus basalis of Meynert, and the cerebral cortex.[27,70]

Existing non-motor symptoms may continue to worsen, and others may emerge, as the disease progresses. The development of serious cognitive and psychiatric disorders as PD progresses is of particular concern. The spectrum of cognitive dysfunction in PD ranges from mild cognitive impairment (PD-MCI) to dementia.[73-76] PD-MCI is broadly considered as cognitive decline that is not normal for age but with essentially normal functional activities (e.g., symptoms are sufficiently mild that they do not substantially impede daily functioning at home or at work).[73] The risk of PD-MCI and progression to dementia increases with age, disease duration, and disease severity. Up to 80% of patients eventually develop dementia.[73,77] Approximately 30% of PD patients are diagnosed with psychosis.[78] The strongest risk factor in PD for developing psychotic symptoms is the presence of severe cognitive impairment or dementia.[79] Patients are most commonly affected by visual hallucinations.[79,80] Managing psychosis in patients with PD presents a significant therapeutic challenge given the propensity of antipsychotic medications to worsen parkinsonian symptoms due to their dopamine-blocking properties.[69,78] The propensity of antiparkinsonian medications to promote or worsen psychosis is a further complicating clinical issue.[78,79] By the end stages of the disease, patients are completely disabled from the progression of motor and non-motor symptoms and are dependent on others for basic self-care.[81] The associated burden on caregivers of patients with PD includes substantial physical, psychological, and socioeconomic strains.[82]

A clear and comprehensive delineation of the natural progression of PD from disease onset to its end stages remains elusive. There is considerable variability in age at onset, number and severity of symptoms, and disease course between patients. It is unclear whether and how disease emergence, symptoms, and course may relate to individual causes and pathogenetic mechanisms. Exciting genetic advances suggest that there are numerous molecular pathways to PD. There are mutations in genes that disrupt key cellular pathways, including lysosomal autophagy,

mitochondrial metabolism, endosomal trafficking, and synaptic neurotransmission.[83,84] Identifying the genetic causes of PD provides not only new information about such pathogenetic mechanisms but also presents an opportunity to identify individuals in the presymptomatic stages of the disease. This in turn could advance early detection and neuroprotective strategies.

1.3 Genetics of Parkinson's disease

PD has emerged from being a disease with little to no known genetic predisposition to a disease with a genetic basis for an increasing proportion of patients over approximately the last two decades. Prior to the first molecular genetic cause of PD being identified in 1997 in *SNCA,*[85] the role of heritability in PD was contentious.[86] PD was largely considered a non-genetic disease, caused by environmental agents such as viruses or neurotoxins. In part, this was related to the pandemic of post-encephalitic parkinsonism after World War I,[87] and to the discovery of MPTP-related parkinsonism.[88] In fact, familial clustering of PD cases had been observed for more than a century. Gowers (1902)[89] noted that about 15% of his patients reported a positive family history of the disease ('familial PD').[90] There was a sentiment however that familial forms of the disease did not constitute 'real' PD,[86] reflected by the diagnostic exclusion criterion of having more than one affected relative in the widely used 1992 United Kingdom Parkinson's Disease Society Brain Bank Clinical diagnostic criteria (Table 1-1).[1] The attribution of PD to non-genetic causes was further strengthened by results for several small cross-sectional monozygotic twin studies in the 1980s. These failed to demonstrate higher concordance rates for PD in monozygotic compared with dizygotic twin pairs.[86,91-93] In contrast, the 2011 longitudinal population-based Swedish Twin Registry revealed that concordance rates for PD were 11% for monozygotic twin pairs and 4% for same-sexed dizygotic twin pairs, and that the overall heritability estimate was 34%.[10]

Studies of pedigrees where PD was segregating led to the eventual identification of rare highly-penetrant mutations that can cause PD (Table 1-2). These are considered monogenic forms of the disease in which mutation of a single gene is apparently sufficient for disease expression (though disease penetrance is often reduced and age-related.[17]) Genes reported to have causative PD mutations have been assigned the term 'PARK' loci. Follow-up genetic studies have shown inconsistent results for some of these loci. For others, conclusive data remains pending (Table 1-2). However a number of genes mapped to PARK loci have been established as well-validated causative genes for PD with Mendelian inheritance patterns (Table 1-2). These monogenic forms

Table 1-2 Causative and risk genes reported in Parkinson's disease

Gene symbol	PARK locus	Chromosome	OMIM (PD[a])	Inheritance	Clinical phenotype	Pathological features	Initial ref.	Mutations[b]	Status and remarks
PARK-designated genes **(Bold indicated confirmed locus)**									
SNCA	PARK1/4	4q21	163890	AD	Variable onset age, aggressive course	LBs	[85]	Missense (n=5), whole gene duplications and triplications	Confirmed
PARK2	PARK2	6q25.2-q27	602544	AR	Early-onset, slow course	No LBs in most	[94]	>100 mutations, including missense, nonsense, in/dels and splice-site mutations, exonic deletions and duplications	Confirmed
UCHL1	PARK5	4p14	191342	AD	Late-onset, typical	Unknown	[95]	Missense mutation	Unconfirmed (single family)
PINK1	PARK6	1p36	608309	AR	Early-onset, slow course	LBs (n=1)	[96]	>60 mutations, including missense, nonsense, in/dels, whole gene and exonic deletions	Confirmed
PARK7 (DJ-1)	PARK7	1p36.23	602533	AR	Early-onset, slow course	Unknown	[97]	>10 mutations, including missense, exonic deletions and duplications	Confirmed
LRRK2	PARK8	12q12	609007	AD	Late-onset, typical	LBs in most	[98,99]	>50 mutations, including missense and nonsense mutations	Confirmed
ATP13A2	PARK9	1p36	610513	AR	Juvenile onset, atypical (KRS)	Unknown	[100]	Missense, exonic deletions	Confirmed
GIGYF2	PARK11	2q37.1	612003	AD	Late-onset	Unknown	[101]	Missense mutations	Unconfirmed
HTRA2	PARK13	2p12	606441	AD	Late-onset	Unknown	[102]	Missense mutations	Unconfirmed
PLA2G6	PARK14	22q13.1	603604	AR	Juvenile onset dystonia-parkinsonism	Typical LBs	[103]	Missense mutations	Confirmed; other mutations cause INAD and NBIA
FBXO7	PARK15	22q12.3	605648	AR	Juvenile onset, atypical	Unknown	[104]	Missense and splice-site mutations	Confirmed
VPS35	PARK17	16q12	601501	AD	Late-onset, typical	Unknown	[105,106]	Missense mutations	Confirmed
EIF4G1	PARK18	3q27.1	600495	AD	Late-onset, typical	Typical LBs	[107]	Missense mutations	Unconfirmed
DNAJC6	PARK19	1p31.3	615528	AR	Juvenile onset, atypical	Unknown	[108,109]	Nonsense and splice-site mutations	Recently identified
SYNJ1	PARK20	21q22.2	604297	AR	Early-onset, atypical	Unknown	[110,111]	Missense mutation	Recently identified
Other possible rare causes/risk factors for Parkinson's disease									
ATXN2[c]	N/A	12q24.1	601517	AD	Late-onset, typical	LBs (n=1)	[112]	Trinucleotide expansions	Larger expansions cause SCA2
ATXN3[c]	N/A	14q21	607047	AD	Late-onset, typical	Unknown	[113,114]	Trinucleotide expansions	Usually cause SCA3
TBP[c]	N/A	6q27	600075	AD	Late-onset, typical	Unknown	[115]	Trinucleotide expansions	Usually cause SCA17
TH	N/A	11p15.5	191290	AD	Late-onset, typical	Unknown	[116]	Whole gene deletion	Unconfirmed (single patient); AR mutations cause DRD
Moderate/high genetic risk factors for Parkinson's disease									
GBA	N/A	1q21	606463	Risk factor	Late-onset, typical	Typical LBs	[117]	OR>5 (het.,or homo. many mutations)	Confirmed; AR mutations cause Gaucher's disease
GCH1	N/A	14q22.1-q22.2	600225	Risk factor	Variable onset age, typical	Unknown	[118]	OR>7 (het., nonsense and missense mutations)	Recently identified; AD mutations cause DRD, including overlapping variants
SMPD1	N/A	11p15.4-p15.1	607608	Risk factor	Late-onset, typical	Unknown	[119]	OR>9 (het., missense mutation)	Recently identified; AR mutations cause Niemann-Pick disease

Genes include those identified as relevant to PD through curation of OMIM entries for Parkinson's disease (#168600) and manually inspecting the literature for causative and high risk PD genes. Four PARK loci without an identified gene (PARK3, PARK10, PARK12, PARK16) are not included.

AR, autosomal recessive; AD, autosomal dominant; DRD, dopa-responsive dystonia; het., heterozygous; homo., homozygous; INAD, infantile neuroaxonal dystrophy; LBs, Lewy bodies; NBIA, neurodegeneration with brain iron accumulation; OR, odds ratio; PD, Parkinson's disease; ref., reference; SCA, spinocerebellar ataxia

[a] indicates annotation for Parkinson's disease entry in OMIM (#168600); all except *TH*, *GCH1*, and *SMPD1*

[b] Note that not all mutation classes have been investigated in all genes and not all mutations may be pathogenic.

[c] Parkinson's disease or Parkinson's disease-like phenotypes reported in multiple patients; additional studies including pathology necessary to better delineate the clinical heterogeneity of SCA mutations

of PD collectively account for up to 30% of familial forms of the disease and 3-5% of "sporadic" PD cases, where there is no family history of the disease.[17] Some of these monogenic forms of PD, such as those related to autosomal dominant mutations in *LRRK2*, *SNCA*, and *VPS35*, show phenotypes similar to clinically typical, late-onset sporadic, or idiopathic, PD (Table 1-2).[17,120] Others exhibit a younger onset of symptoms, such as those caused by mutations in *PARK2*, *PINK1*, and *PARK7* (more familiarly known as *DJ-1*). Monogenic juvenile forms of PD such as those involving recessive *ATP13A2*, *PLA2G6*, and *FBXO7* mutations (Table 1-2) may present at a very young age (<20 years) and often with additional atypical clinical symptoms (e.g., dystonic, oculomotor, pyramidal, and cognitive features).[17,120]

Mutation types associated with PD pathogenicity are diverse. These include rare nonsynonymous sequence variants (point mutations including nonsense and missense mutations) and less frequently, copy number variants (Table 1-2). Copy number variants, a form of genomic structural variation, involve the gain (e.g., insertions or duplications) or loss (deletion) of a stretch of DNA sequence. Pathogenic copy number variants reported in PD to date are relatively small variants that encompass either a single gene or its exons (Table 1-2). This is in notable contrast to other brain-based diseases such as autism and schizophrenia where large (e.g., >500 kb) are prevalent.[121,122] Trinucleotide repeat expansions in genes more typically associated with the spinocerebellar ataxias may also be rare causes of a typical late-onset PD phenotype (Table 1-2).[112,123] The contributions of each mutation type to the overall genetic architecture of PD remain unclear as few studies have employed both exon sequencing and assessment of structural variants.[18]

1.3.1 Autosomal dominant forms of Parkinson's disease

There are three genes confirmed with autosomal dominant expression of PD (Table 1-2): *LRRK2* (leucine-rich repeat kinase 2), *SNCA* (alpha-synuclein), and *VPS35* (vacuolar protein sorting 35 homolog). The role of a fourth possible autosomal dominant PD gene, *EIF4G* (eukaryotic translation initiation factor 4-gamma 1) remains to be established.

Mutations in *LRRK2* are the most frequent genetic cause of familial and sporadic PD. *LRRK2* mutations account for 5-10% of all familial cases and 1-2% of sporadic cases in most populations.[18,124] Patients with *LRRK2* mutations typically display symptoms of PD that are

indistinguishable from typical late-onset PD.[124-126] Of the more than 50 *LRRK2* mutations reported to date, seven nonsynonymous variants are considered of proven pathogenicity. The recurrent Gly2019Ser mutation (glycine to serine exchange in position 2019 of the peptide sequence) is especially prevalent in specific ethnic populations, accounting for up to 40% of PD cases in Ashkenazi Jews and North African Arabs.[124,127] Penetrance of PD associated with *LRRK2* mutations is incomplete and age-dependent, rising to about 75% by age 80 years.[124,127]

The second most common cause of dominant PD is mutations in the *SNCA* gene, which encodes α-synuclein, a key component of Lewy bodies. These mutations are most often genomic duplications or triplications of *SNCA*, but can also rarely include pathogenic missense mutations (Table 1-2).[128-130] The phenotype of PD patients with *SNCA* variants may be related to the type of mutation and include atypical features such as psychosis, depression, dysautonomia, and dementia.[131]

LRRK2 and *SNCA* were both identified by genome-wide linkage mapping, a method that has been highly successful in identifying highly-penetrant PD genes through the meticulous mapping of large families with PD segregation patterns. The increasing availability and reduced cost of next generation sequencing is expected to increase the yield of PD genes. In 2011, the first PD gene, *VPS35* was identified through a direct next generation sequencing method. Two independent whole exome sequencing (assesses protein-coding regions of the genome) studies on Swiss[107] and Austrian[106] families identified the same nonsynonymous missense mutation in *VPS35*. Patients with a *VPS35* mutation appear to present with classical features of late-onset PD, albeit with a slightly earlier age at onset (~early 50s). Mutations in *VPS35* appear to be a rare cause of PD in Caucasian populations, estimated to account for ~0.1% cases.[132] A nonsynonymous missense mutation identified in the *EIF4G* gene, recently identified by traditional linkage methods in a large French family, has also been suggested be another rare cause of autosomal dominant PD.[107] A role for *EIF4G1* in PD remains to be conclusively demonstrated, however, as the reported mutations were not found in other PD studies, and have now been reported in healthy controls.[16]

1.3.2 Autosomal recessive, early-onset typical Parkinson's disease

Homozygous or compound heterozygous mutations in *PARK2* (parkin RBR E3 ubiquitin protein ligase), *PINK1* (PTEN induced putative kinase 1), and *PARK7* (*DJ-1;* parkinson protein 7) can

cause early-onset autosomal recessive PD (Table 1-2). Clinically, patients with these mutations tend to be indistinguishable from one another or from individuals with other forms of early-onset PD. Autosomal recessive *PARK2* mutations account for up to half of familial early-onset PD cases and about 15% of sporadic early-onset cases. Mutations in *PINK1* and *DJ-1* are less common (~1-8%, and 1-2% of sporadic early-onset cases, respectively).[133] Heterozygous *PARK2, PINK1,* and *DJ-1* variants in PD cases have been reported, but are difficult to assess for pathogenicity since these have also been detected in unaffected individuals.[18] Mutation types implicated in early-onset recessive PD include point mutations and copy number variants, necessitating gene dosage assays in addition to sequencing, for sensitive mutation screening.[18,129] Copy number variations, including single or multiple exon deletions and duplications, are particularly common in *PARK2* but are also occasionally reported in *DJ-1*. *PINK1* copy number variations are rare but include single or multiple exon deletions and whole gene deletions.[18]

1.3.3 Genetic risk factors for Parkinson's disease

Genome-wide association studies (GWAS) have been widely used in the study of PD to identify loci that may mediate risk of developing PD. Recent meta-analyses of GWAS provide evidence for more than 20 susceptibility PD risk loci,[133-137] which collectively appear to explain a small fraction (3-7%) of PD heritability.[135,138] Notably, these include common variants in *SNCA* and *LRRK2*, in which rare pathogenic variants cause autosomal dominant PD. The true significance of many of these loci in the underlying etiology of PD remains to be elucidated, however. The risk of PD associated with each of these polymorphisms is typically low (e.g., OR <2), with correspondingly limited utility with respect to diagnostics or for disease prediction.[133,137]

In contrast, mutations in the glucocerebrosidase (*GBA)* and the sphingomyelin phosphodiesterase (*SMPD1*) genes have been reported as high risk susceptibility alleles for PD (Table 1-2). Autosomal recessive mutations in these genes cause the lysosomal storage disorders Gaucher's disease and Niemann-Pick disease type A, respectively. Astute clinical observations that patients with Gaucher's disease[117] and their relatives[139] developed PD more often than would be expected eventually led to the confirmation of *GBA* mutations as important worldwide risk factors for PD.[140] Approximately 5%-10% of PD patients have GBA mutations,[140] making these numerically one of the most important genetic risk factors involved in PD. The discovery of the role of *GBA* in PD prompted recent study of the impact of the heterozygous state of other

recessive causes of lysosomal storage disorders on PD, revealing a rare heterozygous missense mutation in *SMPD1* that dramatically increases risk of PD (OR=9.4), at least in the genetically homogenous Ashkenazi Jewish population.[119,141]

It is suspected that at least some of the "missing heritability" of PD left unexplained by the rare mutations of large effect and the common variants of small effect may be explained by these types of moderate PD risk variants, that are not penetrant enough to be found by traditional family linkage studies but are not common enough to be detected by GWAS.[16] Others may be *de novo* mutations not amenable to linkage mapping. Indeed, new moderate risk candidates continue to emerge. For example, nonsynonymous mutations in *GCH1,* a gene associated with childhood dopa-responsive dystonia, have now also been identified in typical PD cases and associated with a significant risk of PD (Table 1-2).[118] Hemizygous 22q11.2 deletions, associated with 22q11.2 deletion syndrome (22q11.2DS), have also been suggested as a possible PD risk locus but systematic studies are lacking.[142-144]

1.4 Hemizygous 22q11.2 deletions and the associated 22q11.2 deletion syndrome

1.4.1 Overview of 22q11.2 deletion syndrome

22q11.2DS (OMIM #192430, #188400) is the most common microdeletion syndrome in humans, with an estimated incidence of at least 1 in 4000 live births.[145,146] 22q11.2DS is a multi-system condition associated with congenital cardiac and palatal anomalies, developmental delay, hypoparathyroidism and hypocalcemia, and subtle facial dysmorphism.[146-148] Later onset disorders affecting the nervous system, including seizure and anxiety disorders, and schizophrenia, are relatively common.[5,146,149,150] Penetrance is high for any of the associated features (Figure 1-3), but expression is variable and as yet unpredictable. The variable manifestations of the syndrome have contributed to 22q11.2DS being known historically by several names (e.g., DiGeorge, velocardiofacial, conotruncal anomaly face, Opitz G/BBB, and Cayler cardiofacial syndromes).[146,151] These names were applied before the common deletion was identified, and have contributed to persistent under-diagnosis of patients.[152-154]

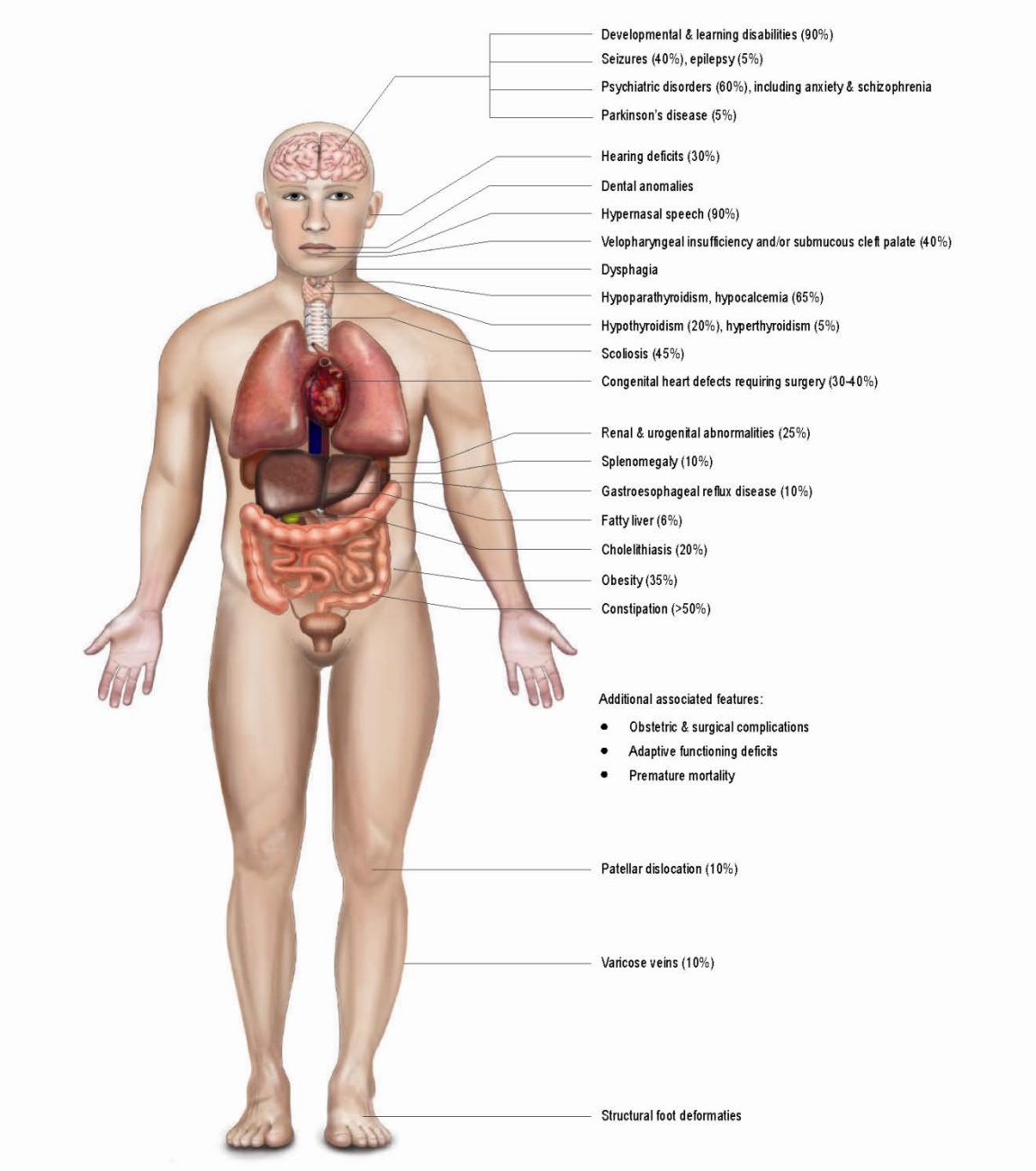

Figure 1-3 Common features of 22q11.2DS with clinical management implications in adulthood

Percentages are approximate estimates of lifetime prevalence among adults with 22q11.2DS.[146,147,155] Further data may alter these estimates. There is a wide range of number and severity of features in any individual with 22q11.2DS.

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Fung WL*, **Butcher NJ***, Costain G*, et al. 2015. Practical guidelines for managing adults with 22q11.2 deletion syndrome. *Genet Med.* 17:599-609.

The associated recurrent 22q11.2 deletion usually occurs as a spontaneous *de novo* mutation.[156] These mutations are mediated by flanking segmental duplications, or low copy repeats (LCRs), that confer susceptibility of the region to copy number variation through non-allelic homologous recombination.[157,158] About 90% of individuals with 22q11.2DS have a large (~2.6 Mb) deletion that spans about 90 non-coding and coding genes (Figure 1-4).[6,156,159] The smaller proximal deletion that occurs in about 10% of cases is approximately 1.5 Mb long.[160,161] There appears to be no relationship between either deletion length or parental origin of the deletion occurrence with expression of the associated clinical features.[162-165] Proposed mechanisms for the variable expression include gene dosage sensitivity of one or more 22q11.2 regions genes,[166,167] mutations on the intact chromosome, and genome-wide variants outside the 22q11.2 region, involving protein-coding genes and regulatory pathways.[167-171]

1.4.2 Movement abnormalities and associated neurophenotypes in 22q11.2DS

Marked neuromotor delays and motor abnormalities are commonly reported in children and adolescents with 22q11.2DS. These include delayed gross motor milestones, low muscle tone (hypotonia), and fine and gross motor deficits.[172-178] Other reported motor disturbances include muscle weakness,[175] asymmetric crying facies,[179] and specific deficits in axial stability[180] and psychomotor speed.[181] Many have poor balance and coordination difficulties relative to their peers,[175,181] even after accounting for level of intellect.[177,182] Congenital heart defects and corrective surgery do not appear to impact neuromotor development or abilities in children with 22q11.2DS.[172,175,178]

Other conditions associated with 22q11.2DS could contribute to disturbed motor functioning (Figure 1-3). For example, hypocalcemia (either overt or latent/subclinical) can arise at any age and affects the majority (>60%) of 22q11.2DS patients.[146,147,183-187] Hypocalcemia can cause tremor, muscle cramps and pains, stiffness, numbness, tetany, and seizures.[146,188-190] Hypocalcemia associated with 22q11.2DS is typically attributed to hypoparathyroidism that may be related to developmental defects of the parathyroid glands.[184,187,191] The condition is usually managed with calcium and Vitamin D supplementation.[146] Thyroid dysfunction, affecting approximately 25% of patients with 22q11.2DS, can also be associated with motor abnormalities including muscle stiffness, spasms, and cramps.[190]

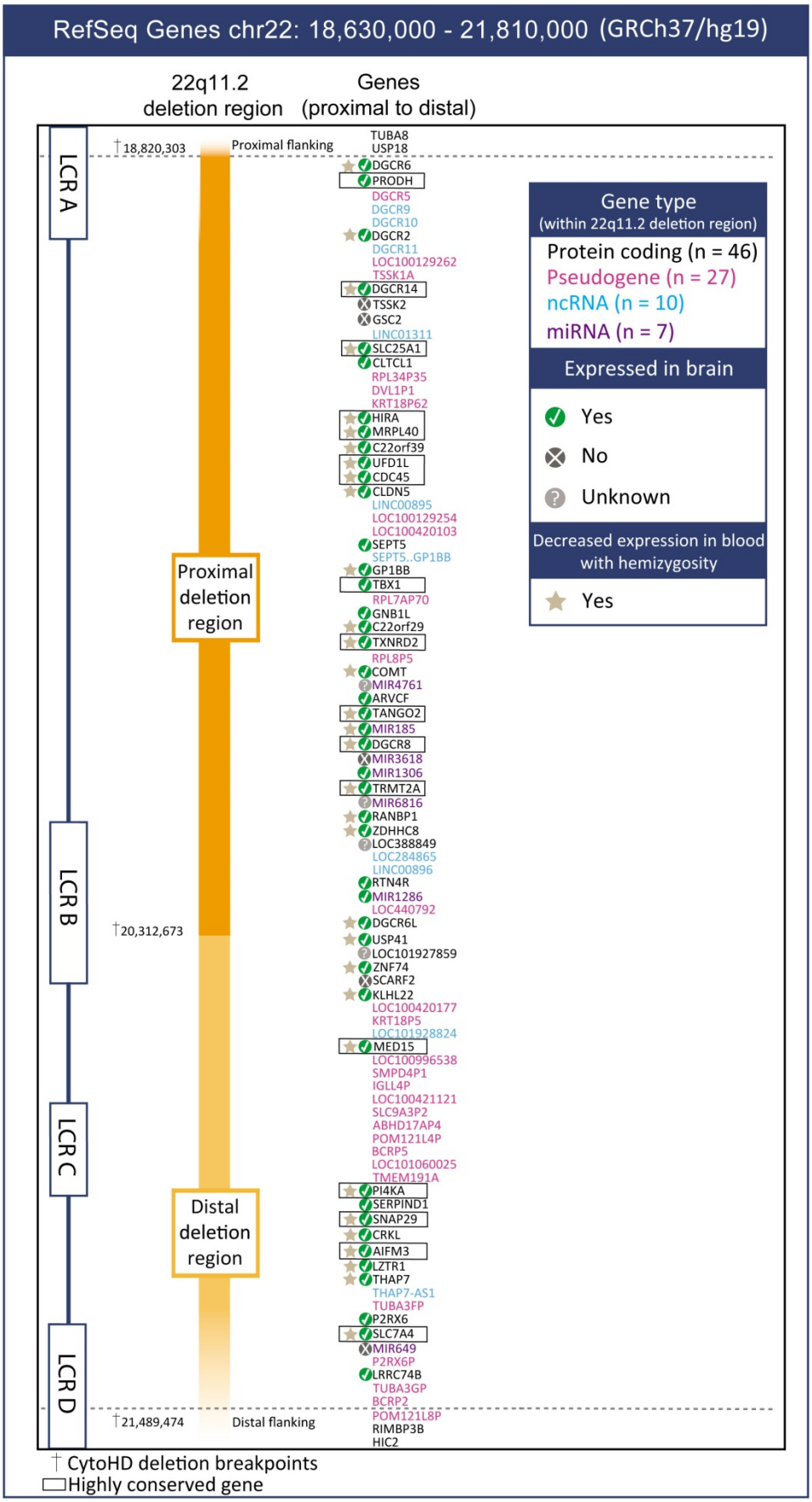
RefSeq Genes chr22: 18,630,000 - 21,810,000 (GRCh37/hg19)
22q11.2 deletion region
Genes (proximal to distal)
LCR A
LCR B
LCR C
LCR D
†18,820,303
Proximal flanking
Proximal deletion region
†20,312,673
Distal deletion region
†21,489,474
Distal flanking
TUBA8
USP18
DGCR6
PRODH
DGCR5
DGCR9
DGCR10
DGCR2
DGCR11
LOC100129262
TSSK1A
DGCR14
TSSK2
GSC2
LINC01311
SLC25A1
CLTCL1
RPL34P35
DVL1P1
KRT18P62
HIRA
MRPL40
C22orf39
UFD1L
CDC45
CLDN5
LINC00895
LOC100129254
LOC100420103
SEPT5
SEPT5..GP1BB
GP1BB
TBX1
RPL7AP70
GNB1L
C22orf29
TXNRD2
RPL8P5
COMT
MIR4761
ARVCF
TANGO2
MIR185
DGCR8
MIR3618
MIR1306
TRMT2A
MIR6816
RANBP1
ZDHHC8
LOC388849
LOC284865
LINC00896
RTN4R
MIR1286
LOC440792
DGCR6L
USP41
LOC101927859
ZNF74
SCARF2
KLHL22
LOC100420177
KRT18P5
LOC101928824
MED15
LOC100996538
SMPD4P1
IGLL4P
LOC100421121
SLC9A3P2
ABHD17AP4
POM121L4P
BCRP5
LOC101060025
TMEM191A
PI4KA
SERPIND1
SNAP29
CRKL
AIFM3
LZTR1
THAP7
THAP7-AS1
TUBA3FP
P2RX6
SLC7A4
MIR649
P2RX6P
LRRC74B
TUBA3GP
BCRP2
POM121L8P
RIMBP3B
HIC2
Gene type
(within 22q11.2 deletion region)
Protein coding (n = 46)
Pseudogene (n = 27)
ncRNA (n = 10)
miRNA (n = 7)
Expressed in brain
Yes
No
Unknown
Decreased expression in blood with hemizygosity
Yes
† CytoHD deletion breakpoints
Highly conserved gene

Figure 1-4 22q11.2DS is caused by recurrent hemizygous deletions at chromosome 22q11.2

LCR A-D indicate the approximate locations of the four chromosome specific low-copy repeats that mediate the 22q11.2 region breakpoints. Green circled check marks denote gene expression as established using The Human Brain Transcriptome. Data for decreased expression with hemizygosity was collated from experimentally demonstrated reductions in gene expression in blood cells from patients with 22q11.2DS.[192-198] Gene names within a rectangle (n=17) are those conserved across the mouse (*M. musculus*), zebrafish (*D. rerio*), fruit fly (*D. melanogaster*), and worm (*C. elegans*).

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Movement disturbances in patients with 22q11.2DS can also be related to seizure activity. Seizures may involve abnormal movements such as twitching, twisting, and abnormal postures.[199] Single and recurrent seizures are frequent across the lifespan in patients with 22q11.2DS, affecting more than 40% of individuals in total. Approximately 5% are diagnosed with epilepsy.[147,189] The associated seizures can be of generalized or focal onset. Myoclonic epilepsy and myoclonus has been reported in a few cases.[200-202] Seizures in patients with 22q11.DS may be unprovoked, or related to identifiable factors such as fever, hypoxia, ischemia, or surgery.[146,189] Hypocalcemic seizures are particularly common[186,203,204] and generally resolve with appropriate supplementation and monitoring of ionized calcium levels.[5,146,186,203,205] Medications could also play a role in seizure risk.[206] There are case reports of 22q11.2DS patients developing seizures following antipsychotic treatment.[7,142,207-209] In some cases, seizures may be related to neurodevelopmental cortical malformations such as polymicrogyria, cortical dysplasia, or periventricular nodular heterotopia.[186,210,211] Other rare neurological disorders may be possible. Three children with spastic hemiplegia have been reported,[175,212] and in one case this was associated with polymicrogyria.[212]

Other associated conditions, and their treatments, may also have an impact on motor functioning in patients with 22q11.2DS. Approximately one in four develop schizophrenia (Table 1-3) in adolescence or early adulthood.[5,150,156,213-217] Schizophrenia associated with 22q11.2DS (22q11.2DS-Schizophrenia) is indistinguishable from other forms of schizophrenia with respect to prodrome, age at onset, core signs and symptoms, and cognitive profile.[149,150,165,215,217-222] There are as yet no formal comprehensive studies of motor dysfunction in adults with 22q11.2DS and schizophrenia, either related to the schizophrenia itself or related to side effects from the associated treatments. Disturbances in motor functioning have long been associated with idiopathic forms of schizophrenia.[223,224] Often attributed to the effects of antipsychotic medications used to manage the associated psychotic symptoms, motor symptoms can also represent features of schizophrenia itself. Extrapyramidal disturbances, including spontaneous parkinsonism and dyskinesias (repetitive involuntary movements usually involving the tongue, mouth, or limbs), are reported in first-episode, medication-naïve, patients with idiopathic schizophrenia.[223,225,226] Estimates vary widely but spontaneous parkinsonism and dyskinesias may affect approximately 15-20% and 10% of untreated idiopathic schizophrenia patients, respectively.[227] Muscle rigidity and bradykinesia are the most common parkinsonian symptoms

Table 1-3 Diagnostic and Statistical Manual of Mental Disorders (Fourth edition, text revision) diagnostic criteria for schizophrenia

A. *Characteristic symptoms:* Two (or more) of the following, each present for a significant portion of time during a one month period (or less if successfully treated): • Delusions • Hallucinations • Disorganized speech (e.g., frequent derailment or incoherence) • Grossly disorganized or catatonic behaviour • Negative symptoms (i.e., affective flattening or avolition) *Note: At least one of the symptoms must be delusions, hallucinations, or disorganized speech.*
B. *Social/occupational dysfunction:* For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care are markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).
C. *Duration:* Continuous signs of the disturbance persist for at least six months. This six month period must include at least one month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).
D. *Schizoaffective and mood disorder exclusions:* Schizoaffective disorder and depressive or bipolar disorder with psychotic features have been ruled out because either 1) no major depressive, manic, or mixed episodes have occurred concurrently with the active-phase symptoms, or 2) if mood episodes have occurred during active-phase symptoms, they have been present for a minority of the total duration of the active and residual periods of the illness.
E. *Substance and general medical condition exclusions:* The disturbance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.
F. *Relationship to a pervasive developmental disorder:* If there is a history of autistic disorder or a childhood onset communication disorder, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least one month (or less if successfully treated).

reported.

Treatment with any antipsychotic medication may induce, exacerbate or diminish pre-existing motor abnormalities.[227-230] The possible exception is the atypical antipsychotic clozapine, which is not thought to typically cause or aggravate motor disturbances.[228,230,231] Drug-induced parkinsonism, tardive dyskinesia, akathisia (motor restlessness), and dystonia (involuntary muscle contractions causing twisting and repetitive movements or abnormal postures) are among the possible motor side effects of antipsychotic medications. Case reports of 22q11.2DS patients treated for psychosis are suggestive of an increased vulnerability to antipsychotic-related movement disorders that may include drug-induced parkinsonism, myoclonus, and oculogyric crises.[7,142,209]

Drug-induced parkinsonism is one of the most common extrapyramidal side effects of antipsychotic medications, affecting 15-30% of patients with long-term antipsychotic use. Drug-induced parkinsonism is indistinguishable from PD in some patients, particularly in the early stages of PD.[232,233] Antipsychotics induce a functional dopamine deficiency by blocking post-synaptic dopamine receptors.[234,235] This leads to a similar clinical picture to PD. Compared with PD, drug-induced parkinsonism is most often characterized by bradykinesia and rigidity, but tremor and postural instability are also seen.[236,237] Onset of symptoms (e.g., bilateral, acute/subacute onset compared with the typically unilateral, slow onset of PD) can help guide the differential diagnosis.[236,237] Symptoms of drug-induced parkinsonism emerge within the first several months of treatment in the majority of cases.[237] Notably, recent functional neuroimaging evidence suggests that approximately 50% of patients with drug-induced parkinsonism have associated signs of nigrostriatal degeneration.[232,238] Manifestation of parkinsonism in patients treated for schizophrenia can thus create a diagnostic challenge. Differential diagnosis included consideration of medication choice and dose, the timing of symptom onset and progression of symptoms, and the possible use of neuroimaging.[45,233,237]

1.5 22q11.2 deletions as a risk factor for Parkinson's disease

Adults with 22q11.2DS represent an increasing population as a consequence of improved childhood survival following advances in pediatric care and improved availability of genetic testing.[148,239,240] Knowledge about movement abnormalities beyond childhood is important to inform best-practices for diagnosis and treatment, but available data are limited. Motor

impairments in adults with 22q11.2DS are typically attributed to extrapyramidal symptoms related to antipsychotic use or other 22q11.2DS-related conditions (e.g., hypocalcemia).[7,146] The onset of motor and other neurological abnormalities in adulthood could be indicative of the development of age-related disorders, such as PD, in patients with 22q11.2DS.

1.5.1 Case reports of early-onset parkinsonism in 22q11.2DS

Several case reports of patients with 22q11.2DS suggest that neurodegenerative conditions, including PD, may manifest in adulthood with early-onset (Table 1-4).[142-144,241] The first report of a neurodegenerative disorder in a patient with 22q11.2DS was described in 1995.[241] A 28 year old man developed progressive neurological symptoms, including gait difficulty, muscle stiffness, and speech problems (dysarthria). There was no history of exposure to antipsychotic medications. Neurological evaluation at age 31 years revealed bradykinesia and features of cerebellar ataxia. This patient was not diagnosed with PD, presumably because a structural MRI showed significant cerebellar atrophy. There were minimal changes (calcification) observed in the basal ganglia. No functional neuroimaging was performed.

The first patient with a clearly parkinsonian phenotype was described three years later (Table 1-4).[142] A 30 year old man with 22q11.2DS and treatment-resistant schizophrenia (onset at age 15 years) presented with progressive resting tremor, rigidity, and generalized bradykinesia that had developed sometime during late adolescence and young adulthood. Attempts to manage these symptoms with the antiparkinsonian medications amantadine, diphenhydramine, and benztropine were unsuccessful. Motor symptoms persisted even after switching his antipsychotic medication from fluphenazine to clozapine. Psychiatric functioning improved with clozapine, but he was unable to tolerate doses higher than 125 mg, which induced seizures, considered unusual for such a low dose. A wheelchair became necessary due to worsened muscle rigidity. He was treated with L-dopa for his extrapyramidal symptoms. Differential diagnoses included drug-induced Parkinson's disease and idiopathic juvenile-onset parkinsonism. This initial report suggested a novel association between parkinsonism and 22q11.2DS, and highlighted the clinical challenges that arise when the co-management of psychosis and parkinsonism becomes necessary.

Reports of three other adults with 22q11.2DS and parkinsonism (Table 1-4) followed more than a decade later. These three unrelated cases were clinically diagnosed with early-onset (<50 years) Parkinson's disease,[143,144] raising the possibility that patients with 22q11.2DS may be at

Table 1-4 Reported clinical features of 22q11.2DS patients with parkinsonian symptoms suggestive of early-onset Parkinson's disease

	Krahn 1998[142]	Zaleski 2009[143]		Booij 2010[144]
		Patient 1	Patient 2	
Demographic features and parkinsonian phenotype				
Sex	Male	Male	Male	Male
Ethnicity	N/A	Caucasian	Caucasian	N/A
Age				
Motor symptom onset (y)	Adolescence/ early adulthood	~42	43	~42
Diagnosis (y)	Parkinsonism (<27)	PD (42)	PD (44)	PD (52)
Motor symptoms				
Tremor	Yes[a]	No	Yes[a]	N/A
Bradykinesia	Yes	Yes[a]	Yes[a]	N/A
Postural instability	No	Yes	Yes[a]	N/A
Rigidity	Yes[a]	Yes[a]	Yes	N/A
Laterality of onset	N/A	Yes	No	N/A
Dysarthria	Yes	-	Yes	N/A
Nonmotor symptoms				
Drooling	-	-	Yes	N/A
Mood disturbances	-	Yes	Yes	N/A
Cognitive decline	-	No	Yes	N/A
Functional decline	Yes	Yes	Yes	N/A
L-dopa responsive	Uncertain[c]	Yes	Yes	N/A
DAT scan[b]	N/A	N/A	N/A	Positive
Family history of PD	No	No	No	N/A
Reported 22q11.2DS features				
Age at diagnosis (y)	30	42	44	52 (?)
Congenital features				
Congenital heart defect	Yes[d]	No	-	N/A
Cleft palate and/or VPI	No	-	-	N/A
Hypernasal speech	No	Yes	Yes	N/A
Mild facial dysmorphism	Yes	Yes	Yes	N/A
Scoliosis	-	-	Yes	N/A
Neuropsychiatric				
Intellect	Mild ID	Borderline ID	None	Learning disabilities
Schizophrenia (onset, y)	Yes (15)	No	No	Psychotic disorder
Antipsychotic	Clozapine (125 mg)	No	No	N/A
Seizures	Recurrent (clozapine-induced)	-	-	N/A
Other				
Hypoparathyroidism	-	Yes	-	N/A
Hypothyroidism	No	-	-	N/A

ID, intellectual disability; N/A, not reported: limited phenotypic information available; VPI, velopharyngeal insufficiency; y, years

[a]Initial symptom(s) presenting on history

[b]DAT scan, dopamine transporter imaging with [^{123}I]FP-CIT SPECT (single-photon emission computerized tomography)

[c]Patient reported as taking L-dopa but clinical response was not described, was unresponsive to amantadine, benztropine, diphenhydramine

[d]Krahn 1999: Repaired ventricular septal defect and ductus arteriosus

[e] ”-“ indicates not included in description of patient’s clinical features but not explicitly reported as a negative finding

increased risk of developing an early-onset form of the disease. Importantly, two of these cases had no history of psychotic symptoms and were free of antipsychotic medications. The third had a treated psychotic disorder that complicated diagnosis for ten years, given the possibility of drug-induced parkinsonism, until presynaptic dopamine imaging (dopamine transporter imaging with [^{123}I]FP-CIT SPECT; Figure 1-1) indicated degeneration of the nigrostriatal dopamine system.[144]

It remains possible that the co-occurrence of early-onset PD and 22q11.2DS was a chance or spurious association in one or more of the four reported 22q11.2DS cases (Table 1-4). None was tested for disease-causing mutations in established PD genes (Table 1-2), which collectively account for up to 10% of all cases.[242] Two were treated with antipsychotic medications, although presynaptic dopamine imaging in one showed striatal denervation consistent with PD.[142,144] PD is a relatively common disease, with a lifetime risk of approximately 1-2%. Patients with early-onset forms of the disease, however, account for only a very small minority of PD cases. The relative rarity of both conditions suggests that the 22q11.2 deletion may indeed be associated with increased risk of early-onset PD. Systematic investigation and gold-standard neuropathological confirmation of PD in 22q11.2DS is warranted.

1.5.2 Implications of an association between Parkinson's disease and 22q11.2DS

Characterizing a novel link between 22q11.2DS and PD could provide important new insights into the causes and progression of PD. The 22q11.2 deletion would be the first example that a large rare copy number variant may play a role in PD pathogenesis, further expanding the genetic architecture of the disease. Moreover, the region contains several possible candidate genes that could mediate susceptibility to PD (Figure 1-4). Notable candidates include *COMT,* encoding catechol-*O*-methyltransferase, an important regulator of dopamine levels and an established therapeutic target in Parkinson's disease,[243] and *SEPT5,* encoding septin-5, which functionally interacts with the protein product of *PARK2.*[244] The 22q11.2 mitochondrial genes (*MRPL40, PRODH, SLC25A1, TANGO2, TXNRD2,* and *ZDDHC8*)[245] could contribute to mitochondrial dysfunction that has been implicated in Parkinson's disease pathogenesis.[246] Novel genome-wide risk modifying mechanisms, and interaction with non-genetic factors, could also mediate PD risk in 22q11.2DS.

On a clinical research level, patients with 22q11.2DS could provide a unique opportunity to investigate PD biomarkers and disease states. The identification of predictive biomarkers and the development of preventative and disease-modifying therapies for PD have historically been hampered by the challenges of identifying patients in early stages of the disease. Unlike other genetic forms of PD, patients with 22q11.2DS are clinically identifiable and molecular confirmatory testing is available world-wide in clinical genetic laboratories. Investigation of early pre-diagnostic signs of PD in patients with 22q11.2DS such as subtle movement problems, associated non-motor symptoms (e.g., cognitive and sensory deficits), and neuroimaging abnormalities could help improve early diagnostic and screening strategies. Identification of known non-motor and neuroimaging markers of PD in patients with 22q11.2DS would provide additional support for the involvement of the deletion in PD. Although other conditions associated with 22q11.2DS (e.g., antipsychotic-induced movement disorders)[7] may perturb motor functioning in these patients, careful clinical assessment and the use of presynaptic neuroimaging tools promises to aid in the differentiation of the onset of PD from other causes of parkinsonism.

An association between 22q11.2DS and early-onset PD would have major implications for clinical monitoring, treatment, and genetic counselling for patients with 22q11.2DS.[5] Patients may require periodic assessments for changes in motor functioning, particularly those treated with antipsychotic medications. Patients and families may require support for planning for the future following a diagnosis of PD, given the progressive and significant declines known to occur in patients with PD.[247] The motor, cognitive, and psychiatric manifestations of PD all significantly contribute to functional decline and disability. The high baseline prevalence of psychiatric disorders and cognitive dysfunction in patients with 22q11.2DS suggest that broad functional impairments may be common in adulthood, even in the absence of PD. Baseline measures of functional abilities of adults with 22q11.2DS are needed to establish the typical functional capacity of adults with 22q11.2DS and how these may be affected by later-onset of PD. Otherwise, the functional impact of PD could be overestimated in adults with 22q11.2DS. In addition, it would be important to discern between lifetime 22q11.2DS-related neurophenotypes and PD-associated changes when considering diagnoses (e.g., PD, PD with dementia, dementia with Lewy bodies, psychosis in PD).

An increased risk of PD would likely have a considerable impact of the clinical care of patients with 22q11.2DS, particularly those taking antipsychotic medication. The choice and dose of antipsychotic medication requires careful consideration in patients with a vulnerable nigrostriatal system. There are no studies to date however that assess side effect risks or treatment response to antipsychotic medications in patients with 22q11.2DS-Schizophrenia. Standard treatments and clinical practice guidelines are recommended.[146] The atypical antipsychotic clozapine would be expected to have good tolerance and efficacy based on studies of the management of psychosis in PD.[248] Several case reports suggest that low doses of clozapine may be necessary in patients with 22q11.2DS (median therapeutic dose, 200 mg, range 75 to 350 mg).[142,207-209,249,250] Interestingly, seizures were reported in four patients on clozapine,[142,207-209] and a dangerously low white blood cell count (agranulocytosis) in another.[251] Further studies are needed to establish the safety and efficacy of clozapine in patients with 22q11.2DS.

1.6 Summary

PD is a progressive neurological disorder characterized by motor and non-motor symptoms including cognitive, autonomic, and neuropsychiatric abnormalities. Genetic mutations are associated with an increasing proportion of patients. Additional causative variants are likely to be discovered. Case reports of four individuals with 22q11.2DS and parkinsonism suggest that the hemizygous 22q11.2 deletion confers an increased risk to early-onset PD. In the absence of prevalence studies of PD in populations with 22q11.2DS and gold-standard confirmatory neuropathology, it remains to be determined with certainty if or how strongly PD is associated with 22q11.2DS, however. 22q11.2DS is associated with a multitude of other conditions that may make diagnosis difficult on the basis of a clinical examination alone, including schizophrenia treated with antipsychotic medications. Neuroimaging may aid in distinguishing drug-induced parkinsonism from onset of PD. Genetic analyses are necessary to confirm that PD in diagnosed 22q11.2DS cases is not related to mutations in known PD genes and to probe the genetic mechanism underlying the manifestation of PD in these patients. Identifying the 22q11.2 deletion as a novel genetic risk factor for PD could provide a clinically identifiable population amenable to the study of PD in its earliest stages, which could lead to improved screening, detection, and treatment. Neurobehavioural and neuroimaging markers may identify those at highest risk, and provide further evidence for a role of the 22q11.2 deletion in PD. An

association between early-onset PD and 22q11.2DS would have important clinical and genetic counselling implications for patients with 22q11.2DS, including careful screening and management of PD together with the multitude of other neuropsychiatric and congenital abnormalities associated with 22q11.2DS, particularly schizophrenia.

Chapter 2 Research aims and hypotheses

2 Summary of objectives

The overarching goal of this thesis was to investigate 22q11.2 deletions as a novel risk factor for early-onset Parkinson's disease (PD) and begin to evaluate the corresponding pathogenesis and implications for individuals with the associated 22q11.2 deletion syndrome (22q11.2DS). The first three data chapters (Chapters 3-5)[252-254] examine the expression of PD in adults with 22q11.2DS and its clinical, genetic, and neuroimaging correlates. The possible impact of earlier-onset 22q11.2DS neurophenotypes on the clinical study and management of PD were assessed in the second half of this thesis (Chapters 6-8).[5,255,256] The implications of these findings for the increasing adult 22q11.2DS population, and other forms of PD, are discussed.

2.1 Specific aims

The specific aims of this thesis were as follows:

Aim 1: To test for an association between 22q11.2DS and Parkinson's disease (Chapter 3[252])

An observational study of PD in the world's single largest cohort of well-characterized adults with 22q11.2DS was performed to provide the first prevalence estimate and neuropathological confirmation of PD in 22q11.2DS. The study involved (i) identifying and characterizing 22q11.2DS patients with PD, (ii) determining if PD occurred significantly more often in adults with 22q11.2DS relative to standard Canadian population estimates, (iii) assessing the neuropathology of affected cases with available brain tissue compared with those with 22q11.2DS and no history of parkinsonism and matched controls with no history of either condition, and (iv) performing targeted genetic testing in the 22q11.2DS patients with PD for known causative PD mutations. We hypothesized that 22q11.2DS patients would be significantly enriched for early-onset PD with characteristic neuropathology and in the absence of other known causative mutations. We proposed that this could provide substantive evidence for the 22q11.2 deletion as a novel genetic risk factor for early-onset PD. We also predicted that

22q11.2 deletions would account for a small proportion of all early-onset PD cases, and (v) assessed a clinical series of individuals with early-onset PD for 22q11.2 deletions.

Aim 2: To identify genetic (Chapter 4[253]) and clinical (Chapter 5[254]) predictors of Parkinson's disease in adults with 22q11.2DS

A whole-genome sequencing study was undertaken to identify possible genetic modifiers of PD penetrance in patients with 22q11.2DS that may be useful for identifying those at highest risk of developing the disease. Susceptibility to PD could be mediated by changes on the intact chromosomal 22q11.2 region and/or mutations elsewhere in the genome. In a pilot study we investigated genome-wide protein-coding variants in 22q11.2DS patients with and without early-onset PD to begin to delineate the genetic etiology of PD in the context of the 22q11.2 deletion. We hypothesized that the expression of PD in 22q11.2DS may be associated with rare mutations in (i) a novel recessive locus on the intact 22q11.2 chromosome or in (ii) PD-relevant genes outside of the 22q11.2 deletion region.

Unaffected adults with 22q11.2DS at increased age-related risk of PD (≥30 years) were prospectively recruited from our larger cohort and investigated for motor and non-motor symptoms and neuroimaging markers consistent with prodromal stages of PD. Age and sex-matched healthy controls, and our only available living patient with a diagnosis of L-dopa responsive 22q11.2DS-PD, were assessed for comparison purposes. Imaging included transcranial sonography to investigate substantia nigra echogenicity and positron emission tomography (PET) using ^{11}C-dihydrotetrabenazine, a vesicular monoamine transporter (VMAT2) radioligand, to assess striatal dopamine neuron integrity. We hypothesized that patients with 22q11.2DS would exhibit a higher prevalence of (i) motor dysfunction including mild signs of parkinsonism, (ii) non-motor symptoms such as olfactory, visual, and cognitive deficits, and (iii) PD-associated neuroimaging abnormalities including enlarged substantia nigra hyperechogenicity and reduced striatal VMAT2 binding.

Aim 3: To assess the clinical context and implications of early-onset PD in 22q11.2DS by:

i) investigating the safety and efficacy of the use of antipsychotic treatments in patients with 22q11.2DS (Chapter 6[256])

Side effects and treatment response to the atypical antipsychotic medication clozapine, an excellent candidate for use in patients at high risk of, or with, PD, was investigated in patients with 22q11.2DS-associated schizophrenia, including one also treated with L-dopa for PD. Results were compared with schizophrenia patients treated with clozapine who were molecularly confirmed to have no pathogenic copy number variant. Long term safety and efficacy of clozapine was evaluated through review of lifetime medical records and follow-up semi-structured interviews regarding the patient's experiences with clozapine. An independent blinded review of comprehensive lifetime clinical summaries compiled for each patient was used to retrospectively evaluate mental illness severity and improvement following treatment with clozapine. The hypothesis was that (i) 22q11.2DS patients would show good clinical response to clozapine and (ii) low rates of extrapyramidal and other neurological side effects, comparable to that of those with idiopathic forms of schizophrenia. These results would have important implications for the co-management of parkinsonism and psychosis.

ii) identifying baseline mediators of functional impairment (Chapter 7[255])

A cross-sectional study of 22q11.2DS adult functioning was performed to assess the typical functional capacity and independence levels of adults with 22q11.2DS that may later be further impacted by the onset of PD. We hypothesized that (i) adults with 22q11.2DS would demonstrate widespread deficits in adaptive functioning skills that would have an impact on all major aspects of adult life, as measured using standard assessment tools and demographic proxies, and ii) that these functional deficits would be mediated by intellect level and the presence or absence of neuropsychiatric conditions associated with 22q11.2DS including schizophrenia and non-psychotic mood and anxiety disorders. These findings would have implications for informing family and clinician expectations for adulthood and highlight the importance of accounting for pre-existing cognitive and functional deficits in patients presenting with PD.

iii) reviewing the evidence to inform management of 22q11.2DS neurophenotypes and other associated adult comorbidities (Chapter 8[5])

We identified a need for a comprehensive resource for families and clinicians to guide management and expectations for 22q11.2DS based on these and other important advances in our knowledge of issues affecting patients with 22q11.2DS in adulthood. A review of the existing 22q11.2DS adult literature was performed to develop the first practical guidelines for the clinical management of 22q11.2DS neurophenotypes together with other associated comorbidities in adulthood. Systematic literature searches were performed to identify articles with a substantive focus on adult issues in 22q11.2DS. These were reviewed to create guidelines focused on managing the neuropsychiatric, neurodegenerative, endocrine, cardiovascular, reproductive, psychosocial, genetic counselling, and other issues that are the focus of attention in adults with 22q11.2DS. The draft was then circulated amongst an international panel of multidisciplinary experts in 22q11.2DS for expert opinion and feedback. These guidelines would provide an important novel resource detailing strategies for the recognition, evaluation, surveillance, and collective management of 22q11.2DS-associated morbidities.

Chapter 3 Association between early-onset Parkinson's disease and hemizygous 22q11.2 deletions

3 Overview

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Butcher NJ*, Kiehl T-R***,** Hazrati L-N, Chow EWC, Rogaeva E, Lang AE, Bassett AS. 2013. Individuals with 22q11.2 deletion syndrome are at increased risk of early-onset Parkinson disease: Identification of a novel genetic form of Parkinson disease and its clinical implications. *JAMA Neurology*. 70:1359-1366. ***Co-first authors**

ABSTRACT

Background: Clinical case reports of parkinsonism co-occuring with hemizygous 22q11.2 deletions and the associated multisystem syndrome, 22q11.2 deletion syndrome (22q11.2DS) suggest that 22q11.2 deletions may lead to increased risk of early-onset Parkinson's disease (PD). The frequency of PD and its neuropathological presentation remain unknown in this common genetic condition. **Methods:** An observational study of the occurrence of PD in the world's largest cohort of well-characterized adults with 22q11.2DS (n=159; ages 18.1 to 68.6 years; n=6 with post-mortem tissue) was conducted in Toronto, Ontario, Canada. Rare post-mortem brain tissue from individuals with 22q11.2DS and a clinical history of PD was investigated for neurodegenerative changes and compared with those with no history of a movement disorder. **Results**: We found that adults with 22q11.2DS had a significantly elevated occurrence of PD compared with standard population estimates (SMR=69.7; 95% CI, 19.0-178.5). All four cases showed early-onset and typical PD symptom pattern, treatment response, and course. All were negative for family history of PD and known pathogenic PD-related mutations. The common use of antipsychotics in 22q11.2DS to manage associated psychiatric symptoms delayed diagnosis of PD by up to 10 years. Post-mortem brain tissue revealed classic

loss of midbrain dopaminergic neurons in all three post-mortem 22q11.2DS-PD cases. Typical α-synuclein positive Lewy bodies were present in the expected distribution in two cases but absent in another. **Conclusions:** These findings suggest that 22q11.2 deletions represent a novel genetic risk factor for early-onset PD with variable neuropathological presentation reminiscent of *LRRK2* associated PD neuropathology. Individuals with early-onset PD and classic features of 22q11.2DS should be considered for genetic testing, and those with a known 22q11.2 deletion monitored for the development of parkinsonian symptoms. Molecular studies of the implicated genes, including *DGCR8*, may help shed light on the underlying pathophysiology of PD in 22q11.2DS and idiopathic PD.

3.1 Introduction

Parkinson disease (PD) is a progressive neurodegenerative disorder associated with motor, cognitive, and autonomic dysfunction. It is one of the most common neurological disorders world-wide, affecting approximately 1% of individuals over the age of 65 years.[9,10] Early-onset of the disease (< 50 years) is far less common,[9,10] and is associated with mutations in genes including *LRRK2*, *PARK2*, *SNCA*, *PARK7* (*DJ-1*), and *PINK1*.[257] Known genetic mutations account for 4 to 16% of early-onset PD cases.[258,259]

Previous clinical case reports of four individuals with parkinsonism and a hemizygous deletion of chromosome 22q11.2 have suggested that this genetic anomaly may also confer an increased risk of early-onset PD.[142-144] Three of these cases were reported to be treated with dopamine replacement therapy (L-dopa) and presynaptic dopamine imaging in the other indicated degeneration of the nigrostriatal dopamine system.[142-144] The 22q11.2 deletion affects at least 1 in 4000 live births[145,146] and occurs as a spontaneous mutation in approximately 90% of identified cases.[156] The associated 22q11.2 deletion syndrome (22q11.2DS) (OMIM #192430/188400), shows variable expression including congenital, neurodevelopmental, and later onset features e.g. schizophrenia.[146] Classic manifestations of 22q11.2DS include learning difficulties, palatal anomalies such as velopharyngeal insufficiency causing hypernasal speech, congenital heart defects, hypocalcemia, and subtle facial dysmorphic features.[146-148] The effect of the 22q11.2 deletion on aging and neurodegenerative processes is less clear. Antipsychotic medication used to manage psychosis may obfuscate diagnosis of PD in 22q11.2DS patients.[144] Neuropathological confirmation of PD, including midbrain dopaminergic cell loss and the presence of Lewy bodies,[13,260] remains essential in establishing a 22q11.2 deletion as a risk factor for PD.

To investigate the proposed association between 22q11.2 deletions and PD, we assessed the occurrence of a clinical diagnosis of PD in a well-characterized cohort of adults with 22q11.2DS. We examined available brain tissue from three individuals with 22q11.2DS and an antemortem diagnosis of PD and compared results to those with a 22q11.2 deletion and no history of parkinsonism as well as with age and sex-matched controls with no history of either condition. We also assessed a clinical series of individuals with early-onset PD for 22q11.2 deletions. The results of this study provide the first prevalence estimate and neuropathological confirmation of

PD in 22q11.2DS, supporting the 22q11.2 deletion as a novel genetic risk factor for developing early-onset PD.

3.2 Methods

Subjects

We conducted an observational study in Toronto, Ontario, Canada of a large, well-characterized cohort of 159 adults (≥18 years) clinically diagnosed with 22q11.2DS and molecularly confirmed to have a chromosome 22q11.2 deletion using standard methods.[147,156] Most subjects were ascertained through adult congenital cardiac, psychiatric, and genetic services using active screening and/or clinical referrals[148,261] with no known bias to ascertaining individuals with a neurodegenerative condition. All but five of these Canadian subjects were from Eastern Canada, mostly from Ontario. Comprehensive neuropsychiatric phenotypic data and other clinical and demographic information were available from direct assessments and lifetime medical records.[147,262] We recorded all clinical diagnoses of PD made by a neurologist as of March 2013 that included documentation of standard treatment(s) for PD, and family history of neurodegenerative disease in first and second degree relatives. The diagnosis of PD was made using the United Kingdom Parkinson's Disease Society Brain Bank criteria (Table 1-1).[1] We did not include cases of "suspected PD" lacking strong confirmatory evidence. Eighteen of the 159 patients had died (median age=44.7 years, range 18.1 to 68.6 years). Written informed consent was obtained for all subjects, and from next of kin as necessary for autopsy including use of tissue for research purposes. The study was approved by the Research Ethics Boards of the University of Toronto, Centre for Addiction and Mental Health, and University Health Network.

Statistical analyses

The observed number of PD cases in our 22q11.2DS cohort was compared with the expected number of PD cases using an age- and sex-adjusted standardized morbidity ratio (SMR). The expected number was calculated using Canadian population norms[9]. Given the small numbers, we also calculated the SMR for the main age group examined (35 to 64 years). Analyses were performed with SAS version 9.3 software (SAS Institute, Cary, NC), with statistical significance defined as $p < 0.05$.

Neuropathological investigation of PD

Immunohistochemistry: Brain tissue from three individuals with 22q11.2 deletions and a clinical diagnosis of PD was fixed in 10% buffered formalin for two weeks. Standard blocks were taken from the left hemisphere for light microscopy (5 μm, hematoxylin-eosin and hematoxylin-eosin-Luxol fast blue staining). Immunohistochemistry using an avidin–biotin complex, peroxidase-based method was performed for tyrosine hydroxylase (TH, 1:1000, Sigma, Oakville, Canada) and for proteins commonly found to aggregate in PD, including α-synuclein (1:400, Zymed, South San Francisco, USA; 1:3000, Santa Cruz Biotechnology, Santa Cruz, USA) and ubiquitin (1:400, Dako, Glostrup, Denmark). Markers of other neurodegenerative disorders were also used (beta-amyloid, Aβ, 1:10, Dako, Glostrup, Denmark; paired helical filament-tau, 1:400, Innogenetics, Ghent, Belgium, and TAR DNA-binding protein 43, TDP-43, 1:6000, Cosmo Bio Co., Tokyo, Japan). To investigate the presence of gliosis and microglial activation, tissue was double labelled with glial fibrillary acidic protein (GFAP, 1:1000, Millipore, Canada) and Iba-1 (1:200, Millipore, Canada) in a sequential manner. The chromogens used were diaminobenzidine and Fast Red, respectively.

The severity of overall neuronal loss in subcortical and cortical areas of interest was classified semi-quantitatively as: 0=none (no apparent neuronal loss), 1=mild (mild gliosis with some free pigments), or 2=extensive (neuronal loss involving more than 70% of the structure) by examining the hematoxylin-eosin stained sections. Dopaminergic cell loss in the substantia nigra *pars compacta* was further examined using TH-immunoreactivity using the same classification system. Sections immunostained against α-synuclein were used to qualitatively evaluate the visible presence or absence of Lewy bodies and Lewy neurites.

Comparable brain tissue obtained from three individuals with 22q11.2 deletions but no history of parkinsonism[210] and ten age and sex-matched subjects with unremarkable brain autopsies with a negative clinical history for diagnoses or features of 22q11.2DS or PD was prepared and examined using the methods described above. Additional tissue control experiments included known positive material from patients with PD, diffuse Lewy body disease, and Alzheimer's disease. No reactions were observed following the omission of primary antibodies.

Genetic investigations: We used genomic DNA available to conduct genetic analysis of the *LRRK2*, *PARK2*, *PARK7* (*DJ-1*), *PINK1*, and *SNCA* genes[97,263-266] in individuals with a 22q11.2 deletion and a history of PD (Table 3-1). In addition, we performed real-time PCR to investigate the presence of copy number variations for each exon of *PARK2* and *SNCA* using SYBR Green reagent (TaKaRaMirus Bio, Madison, USA) on an ABI7500 system (Applied Biosystems, Foster City, USA).

To ascertain the occurrence of 22q11.2 deletions in early-onset PD, we performed a quantitative PCR study using DNA samples from a clinical series of patients ascertained from the Movement Disorders Centre, Toronto Western Hospital (n=225, age of onset ≤ 50 y). Nearly half (n=106) of these unrelated individuals had a positive family history of PD; most were Caucasian. Copy number variations in two 22q11.2 genes (TBX1 and SNAP29) were examined on a ViiA(tm) 7 Real-Time PCR System (Life Technologies), performed by The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada. This study was approved by University Health Network and written informed consent was obtained from all individuals in this study.

3.3 Results

Occurrence of PD and genetic findings

The occurrence of PD in the entire cohort of adults with a hemizygous 22q11.2 deletion was significantly elevated compared with Canadian population norms[9] (SMR=69.7; 95% CI, 19.0-178.5). Post-hoc subgroup analyses showed no subjects aged 18 to 34 years (n=90) diagnosed with PD. Four (5.9%) of 68 subjects aged 35 to 64 years with 22q11.2 deletions had been diagnosed with PD (SMR=91.7; 95% CI, 25.0-234.8). The one subject aged >65 years had suffered a stroke and showed no signs of PD.

Age of onset of motor symptoms ranged from 39 to 48 years (Table 3-2; see Case Histories below). The use of antipsychotic (and other) medications often delayed or complicated the diagnosis of PD. However, the early-onset of typical motor symptoms and progressive course, despite reduction or discontinuation of antipsychotics and/or the use of atypical agents (e.g. clozapine), together with a characteristic response to L-dopa supported the consideration of PD in all cases. All had typical 22q11.2 deletions, three with the most common ~3 Mb deletion and

Table 3-1 Post-mortem and demographic features of four adults with 22q11.2 deletions and early-onset Parkinson's disease identified in a cohort of Canadian adults (n=159) with 22q11.2DS

	Case 1	**Case 2**	**Case 3**	**Case 4**
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian
Age at death (y)	56	58	61	Living
Sex	Female	Male	Male	Male
Post-mortem interval (h)	11	60	6	N/A[a]
Brain weight (g)	1260	1130	1350	N/A[a]
Features of 22q11.2DS				
Intellect[b]	Borderline to mild ID	Average	Average	Borderline to mild ID
Schizophrenia Age at onset	Yes 17 y	Yes 22 y	No	Yes 22 y
Seizures	Single (age 52 y)	No	No	Multiple (age at onset 28 y)
Hypocalcemia	Yes	Yes	Yes	Yes
Hypothyroidism[c]	No	Yes	No	Yes
Congenital heart defects	No	Tetralogy of Fallot	No	No

[a]Not applicable, living patient
[b]ID, intellectual disability
[c]Treated with thyroxine

Table 3-2 Clinical features of Parkinson's disease associated with 22q11.2DS

	Case 1	**Case 2**	**Case 3**	**Case 4**	**Zaleski et al.**[143a]	**Booij et al.**[144]	**Krahn et al.**[142]
Sex	Female	Male	Male	Male	Male	Male	Male
22q11.2 deletion size (Mb)	1.5	3	3	3	N/A[b]	N/A[b]	N/A[b]
Motor symptom onset (y)	45	48	43	36	~42	~42	<27 y
Age at PD diagnosis (y)	55	54	44	48	42	52	Parkinsonism (<27 y)
Age at death (y)	56	58	61	N/A[c]	N/A[c]	N/A[c]	N/A[c]
Motor symptoms							
Tremor	Yes[d]	Yes	Yes[d]	Yes	No	N/A[e]	Yes[d]
Bradykinesia	Yes	Yes	Yes[d]	Yes[d]	Yes[d]	N/A[e]	Yes
Postural instability	Yes	Yes[d]	Yes[d]	Yes	Yes	N/A[e]	No
Rigidity	Yes[d]	Yes	Yes	Yes[d]	Yes[d]	N/A[e]	Yes[d]
Laterality of onset	Yes	Yes	No	Yes	Yes	N/A[e]	N/A[d]
Nonmotor symptoms							
Incontinence	Yes	Yes[f]	Yes	Yes	No	N/A[e]	N/A[e]
Mood disturbances	No	No	Yes	Yes	Yes	N/A[e]	N/A[e]
Cognitive decline	No	Yes	Yes	Yes	No	N/A[e]	N/A[e]
L-dopa responsive	Yes	Yes	Yes	Yes	Yes	N/A[e]	Uncertain[g]
History of antipsychotics (before PD onset)	Yes	Yes	No	Yes	No	Yes	Yes
Family history of PD	No	No	No	No	No	N/A[e]	No

[a]Reported American patient, not overlapping with our Case 3 who was reported in the same publication at the time as a living patient
[b]Not reported; FISH used to identify 22q11.2 deletion
[c]Not available; living patient
[d]Initial symptom(s) presenting on history
[e]Not reported
[f]Concomittent chronic renal insufficiency
[g]Patient reported as taking L-dopa but their clinical response was not specified

one with the proximal nested 1.5 Mb deletion (Table 3-2).[156] All were negative for the tested PD risk mutations and had no family history of neurodegenerative disease or parental consanguinity.

In contrast to the general population estimate of 1 in 4000 (0.025%),[145,146] we found one (0.4%) of 225 individuals in the early-onset PD series to have a 22q11.2 deletion. Closer examination of records revealed that this individual was our Case 3. Long before this study of PD in 22q11.2DS began, he was recruited into the early-onset PD cohort following a clinical referral to the Movement Disorders Clinic by one of the study authors (EWCC). His 22q11.2 deletion was known at the time but not considered to have contributed to his PD when he entered the PD cohort.

Clinical case reports of adults with 22q11.2 deletions and early-onset PD

***Case 1*:** This 56 year old Caucasian woman had onset of psychosis at age 17 years, at which time she was hospitalized with symptoms meeting DSM-IV criteria for schizophrenia. The illness had a chronic course. Despite good compliance to antipsychotic treatments and the addition of paroxetine and low dose lorazepam, auditory hallucinations and multiple delusions persisted. Second generation antipsychotics (e.g., olanzapine) were tried but weight gain prompted a return to first generation antipsychotics (haloperidol, up to 6 mg) and risperidone (up to 4 mg) with anticholinergic medications (procyclidine or benztropine) for the extrapyramidal side effects. Tremors and muscle stiffness were initially attributed to side effects of antipsychotic medications. Stooped posture was present from at least age 40 years and gradually worsening bradykinesia began at age 45 years. A psychiatrist noted bilateral tremor of the hands and forearms as well as pronounced jaw tremor of unknown duration at age 52 years. Despite reducing antipsychotic medications, discontinuing paroxetine, and reducing caffeine intake, bilateral (left greater than right) pill-rolling tremors, bradykinesia, slurred speech, cogwheel rigidity, stooped posture, shuffling gait and problems with balance worsened. Occasional incontinence was also noted. There was rapid deterioration over several weeks at age 55, with severe tremors and akinesia that prompted hospitalization. Low dose risperidone and benztropine had been discontinued just prior to admission. A consultant neurologist diagnosed PD and started levodopa-carbidopa with rapid symptomatic response. At 100/25 mg TID levodopa-carbidopa, motor symptoms were significantly improved and mobility restored. Risperidone (0.5 mg) was subsequently restarted and levodopa-carbidopa reduced to BID because of auditory

hallucinations. Some worsening of tremor and bradykinesia prompted the addition of benztropine (1 mg BID). The patient was discharged after several weeks and her last year was noted as the best in over a decade in terms of functioning and quality of life, despite some residual tremor, balance and speech problems, and mild psychotic symptoms.

Other history of note included a single generalized tonic-clonic seizure at age 52 years attributed to a respiratory tract infection and/or antipsychotic medications, although was also documented as hypocalcemic. CT scans and MRI scan revealed no intracranial abnormalities. An EEG showed epileptiform discharges intermittently from the left temporal region; a jaw tremor was also noted. A previous EEG at age 17 years had shown paroxysmal dysrhythmia of the fronto-central region.

***Case 2*:** This 58 year old Caucasian man met DSM-IV criteria for schizophrenia with an onset of psychosis at age 22 years. The illness had a chronic course but was managed fairly well with multiple first and second generation antipsychotics over his lifetime. He began feeling off-balance and stumbling when walking at approximately age 48 and presented with proximal muscle weakness at age 53 years. A consultant neurologist (AEL) diagnosed PD at age 54 years and began carbidopa-levidopa treatment (100/25 mg). A brain MRI at age 56 showed a few nonspecific foci of increased T2 signal intensity in hemispheric white matter compatible with microangiopathic change, as well as generalized enlargement of the cortical sulci. At age 57 years, bilateral (left greater than right) hand tremor was mild to moderate and intermittent. He sometimes had leg tremor. Schizophrenia was well-controlled with quetiapine (350 mg, increased to 375 mg following worsened psychotic symptoms). An EEG was normal. At age 58 years, proximal muscle weakness and akinesia had worsened. His speech became more slurred and there was significant drooling. He began to show severe and constant left hand and jaw tremors. Beginning about age 57 years, there was some gradual decline of memory and increasing emotional lability. Quetiapine had been increased to 400 mg following a relapse of some psychotic and depressive symptoms of schizophrenia, which may have contributed to the increased severity of his motor symptoms. As a result, his antipsychotic medication was going to be switched to clozapine. The patient received only two test doses of clozapine, however, over a two day period (total 18.75 mg) two days before death. Cause of death was secondary to cardiovascular failure and unrelated to clozapine initiation.

***Case 3*:** This Caucasian man developed bradykinesia, bilateral tremors, mild difficulties with balance, and incoordination in his hands at age 43 years. He also had occasional stuttering and slurring of speech, and a lack of facial expression. He was diagnosed with PD at age 44 years by a neurologist and responded well to levodopa-carbidopa for several years. He had no history of psychiatric illness. Within a year of diagnosis with PD, he began to exhibit symptoms of depression and anxiety including feelings of worthlessness, social withdrawal, and altered eating and sleeping habits. He noted episodes of uncontrollable episodes of laughing and crying. His motor symptoms stabilized for several years then progressively worsened despite increases in his medications. He experienced akinestic rigid episodes and developed more severe gait difficulties, losing his balance at times. An MRI at age 53 years showed non-specific bright foci in hemispheric white matter and cavum velum interpositum. He began gambling at age 54 y, which was controlled with counselling. At this time, his rigidity, tremors, and slurred speech were under good control with levodopa-carbidopa (100/25 mg, 8 tablets for 6 equal doses; also ropinirole, 5 mg four times a day; entacapone 200 mg tid; and domperidone 10 mg, 7 tablets for 5 equal doses) although would reoccur as medication wore off before next dose. He continued to deteriorate and decline in response to antiparkinsonian medications, moving to a nursing home at age 58. Symptoms of depression and anxiety worsened with poor response to antidepressants. Urinary retention and incontinence developed. He was treated for delusions and agitation with olanzapine (5 mg). He remained in the nursing home for almost 3 years, with gradual wasting because of feeding difficulties, and died of pneumonia at age 61. Additional details have been previously reported on this case.[1]

***Case 4*:** This 48 year old Caucasian man began showing signs of schizophrenia at age 17, including delusions and disorientation, and was diagnosed with schizophrenia at age 22. The illness had an intermittent but severe course. He had numerous generalized seizures associated with antipsychotic medication (ages 28, 33, 37, 42, 43, 46, and 47 years) and was treated with various anticonvulsant medications. After responding poorly to a series of first and second generation antipsychotics, the patient began on clozapine at age 33 years. He began exhibiting slurred speech and stuttering at the age of 34. At age 36, the mother reported right-sided leg and arm weakness and rigidity and dragging of the right leg while walking. The right-sided weakness became so severe by the following year that he lost right-handed dominance for eating. At age 40, clozapine was reduced from 250 mg to 125 mg in an attempt to reduce excessive drooling.

His stiffness worsened and was felt to be parkinsonian; he responded well to benztropine (2 mg bid). Choreoathetoid movements of his head and tongue were noted. His affect was downcast and he was treated with electroconvulsive therapy. Clozapine was reduced to 75 mg and benztropine was apparently not maintained. Later in the year, he demonstrated poor gag reflex and difficult swallowing. His speech became unintelligible and he had a lack of facial expression. He deteriorated to a catatonic state and exhibited urinary incontinence. Clozapine and then risperidone (2 mg) were completely discontinued at age 41 because of his movement problems. He began to require the use of a geri-chair due to balance problems. Following worsening of his stiffness, benztropine (2 mg tid) and levodopa-carbidopa (100/25 mg 2 tid) were started. He responded well to the antiparkinsonian treatments and clozapine was restarted. Bowel incontinence began at age 42. Clozapine was again discontinued, following a seizure. At age 44, his freezing episodes showed worsening. At attempt to taper the patient off of benztropine, levodopa-carbidopa, and lorazepam worsened his symptoms, leading to right-sided rigidity and affective changes. He showed a positive response to the addition of pramipexole (0.75 mg tid). He was diagnosed with depression and anxiety. A neurologist noted his parkinsonian symptoms but felt that the patient did not have typical PD due to a lack of “rigidity and hypokinesia”. At age 45, he was spending hours frozen in one position, was unable to keep himself from falling, and had difficulty controlling his body. He showed some deterioration of intellectual capacity. Stiff Man Syndrome was suspected by a neurologist and a trial of diazepam conducted without benefit. Entacapone was added to help control motor symptoms (200 mg). At age 46, a psychiatrist suggested that his symptoms and history were consistent with severe PD, including tremors, affective flattening, flexed posture, difficulty initiating gait, a tendency to fall, and hypersalivation. Freezing episodes became more frequent and prolonged and prevented voluntary movement. He was unable to swallow oral medications and regurgitated his food. At age 48, his antiparkinonsian medications included levodopa-carbidopa (100/25 mg, 3/4 tablets, 5 times a day) with 200 mg entacapone with each dose in addition to pramipexole 1.5 mg tid. He showed dramatic levodopa-carpodopa dose-related motor fluctuations, ranging from being completely immobilized in the Off state and able to walk, speak, and feed himself during the On state. He was not on any antipsychotic medication. The diagnosis of PD was confirmed at age 48, by one of the study authors (AEL), a movement disorders neurologist.

Other history of note for this patient includes a breech forceps delivery. An EEG at 46 years was normal. Brain MRI was normal at age 41 and showed nonspecific white matter lesions at age 47. At age 38, an EMG (electromyography) and CT scan of the craniocervical junction was normal. A head CT scan showed cavum septum pellucidum but was otherwise normal. A cervical and lumbar spine x-ray revealed the absence of a portion of the odontoid process and bifid spinous process at C1 (cervical vertebra 1).

Neuropathology

Neuropathological examination showed similar neurodegenerative pathology in Cases 1 and 2. Classical PD synucleinopathy, including the subcortical and cortical presence of α-synuclein positive Lewy bodies and Lewy neurites, corresponding to Braak stages V and VI,[13] respectively, was observed (Table 3-3). There was extensive degeneration of TH-positive cells in the substantia nigra and depletion of TH in the striatum (Figure 3-1). Neuronal loss was also visible in the locus coeruleus and the dorsal motor nucleus of the tenth cranial nerve (Table 3-3).

Case 3 showed no Lewy body or Lewy neurite pathology, but like the first two cases, showed extensive nigral degeneration and loss of TH immunoreactivity in the striatum. Investigations for abnormal neuronal inclusions or aggregates, including immunohistochemisty for α-synuclein, tau, TDP-43, and ubiquitin, were all negative. Neuronal loss was observed in the expected pattern, with prominent cell loss in the ventrolateral aspect of the substantia nigra pars compacta with extensive gliosis and free pigments (Figure 3-2 A-D). There was neuronal loss in the locus coeruleus and dorsal motor nucleus of the 10th cranial nerve, and some gliosis reminiscent of neuronal loss in the following structures: pedunculopontine nucleus, amygdala, and thalamus, all areas usually described to be affected in PD (Figure 3-2 E,F; Table 3-2). Like Cases 1 and 2, TH staining revealed extensive degeneration of TH-positive cells in the substantia nigra *pars compacta* and depletion of TH in the motor territory of the striatum (dorsolateral putamen) (Figure 3-2 G-I).

There were no neuropathological features of PD, including midbrain dopaminergic cell loss or Lewy body pathology, in individuals with 22q11.2DS who had no history of parkinsonism (Figure 3-1 D-F), or in the matched controls (e.g. Figure 3-2 A,C). Alzheimer-type pathology was limited to Aβ-positive diffuse plaques that were rare and restricted to the cortex in Case 2

but more widespread and with moderate neuritic plaques and cortical neurofibrillary tangles in Case 1 (Figure 3-3). There was no evidence of Alzheimer-type pathology in any other 22q11.2DS case or in any of the matched controls (data not shown). All cases and controls were immuno-negative for TAR DNA-binding protein 43 aggregates.

Table 3-3 Staging of pathological features in three autopsy cases with Parkinson's disease and 22q11.2DS

	Severity of pathological features[a]								
	Case 1			**Case 2**			**Case 3**		
Brain region	**Lewy bodies**	**Lewy neurites**	**Neuronal loss**	**Lewy bodies**	**Lewy neurites**	**Neuronal loss**	**Lewy bodies**	**Lewy neurites**	**Neuronal loss**
Olfactory bulbs and tracts	1	1	N/A	1	1	N/A	0	0	N/A
Dorsal motor nucleus X[b]	1	1	1	1	1	1	0	0	2
Locus coeruleus	1	1	1	1	1	1	0	0	1
Substantia nigra	1	1	2	1	1	2	0	0	2
Thalamus	1	1	1	1	1	1	0	0	1
Nucleus basalis of Meynert	1	1	1	1	1	1	0	0	1
Anterior cingulate cortex	1	1	0-1	1	1	0-1	0	0	0-1
Temporal, insular cortex	1	1	0-1	1	1	0-1	0	0	0-1
Allocortex	1	1	0-1	1	1	0-1	0	0	0-1

[a]Lewy bodies and neurites: 0=absent; 1=present; Neuronal cell loss: 0=none;1=mild; 2=extensive; N/A= Not assessable

[b]Dorsal motor nucleus of the 10th cranial nerve (vagus)

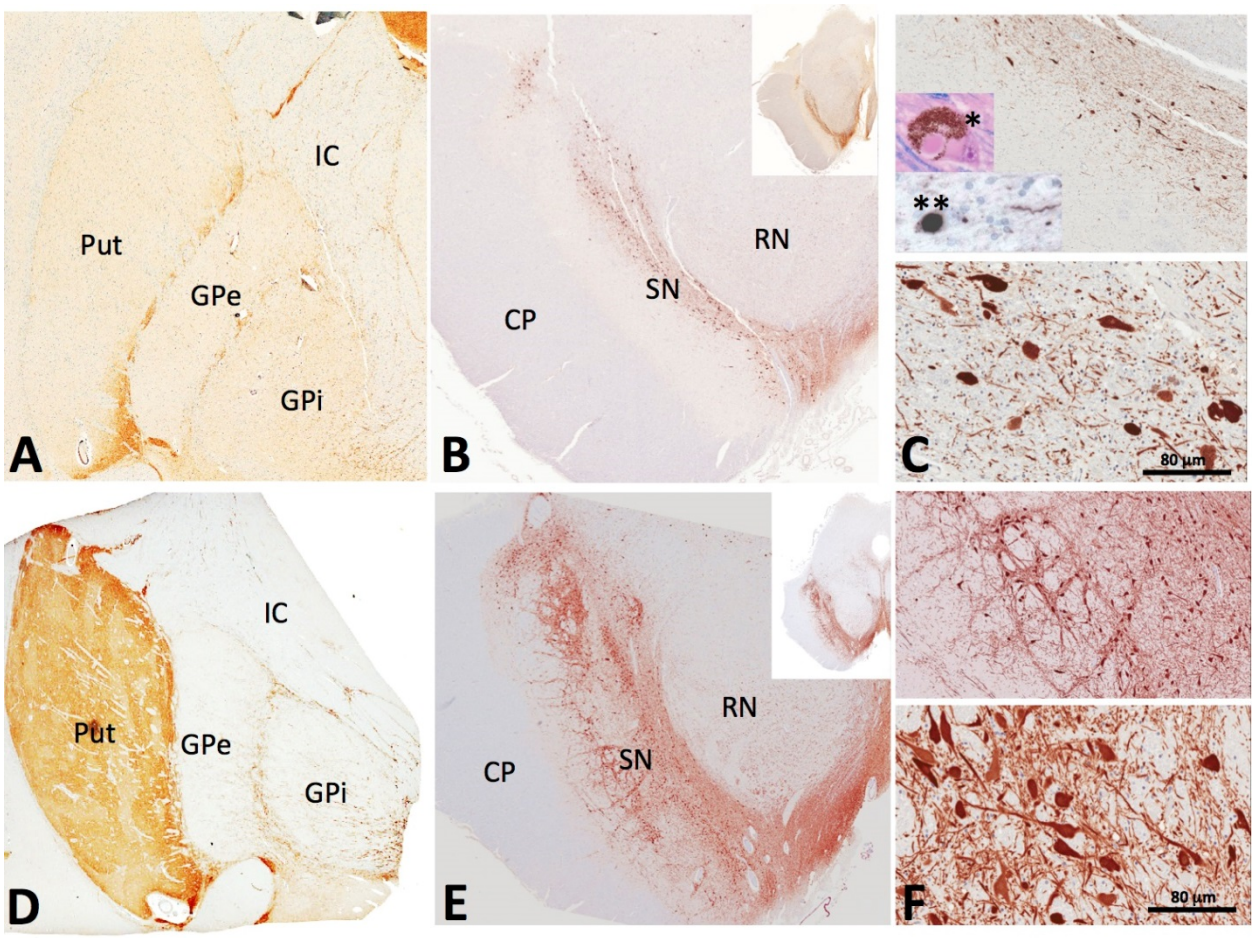

Figure 3-1 Cases 1 and 2 with hemizygous 22q11.2 deletions and diagnosed Parkinson's disease show loss of dopamine cells and α-synuclein pathology

Results of immunohistochemical studies for tyrosine hydroxylase (TH) in the striatum (A,D) and substantia nigra (B,C; E,F) are shown from a representative 22q11.2DS case with Parkinson's disease (Case 1; A-C) and a case of 22q11.2 deletion syndrome without Parkinson's disease (D-F). C and F depict the density of TH-positive neurons in the substantia nigra at low (upper panels) and high power (lower panels). Insets in C shows an example of a Lewy body visualized with hematoxylin & eosin staining (*) and an α-synuclein positive Lewy body and neurites (**) in the substantia nigra *pars compacta*. Put: putamen; GPe: external segment of globus pallidus; GPi: internal segment of globus pallidus; IC: internal capsule; CP: cerebral peduncle; SN: substantia nigra; RN: red nucleus. (original magnification ×1 in A, B, D, E, and insets in B and E; ×10 in upper panels of C and F; ×25 in lower panels of C and F; and ×40 in insets in C).

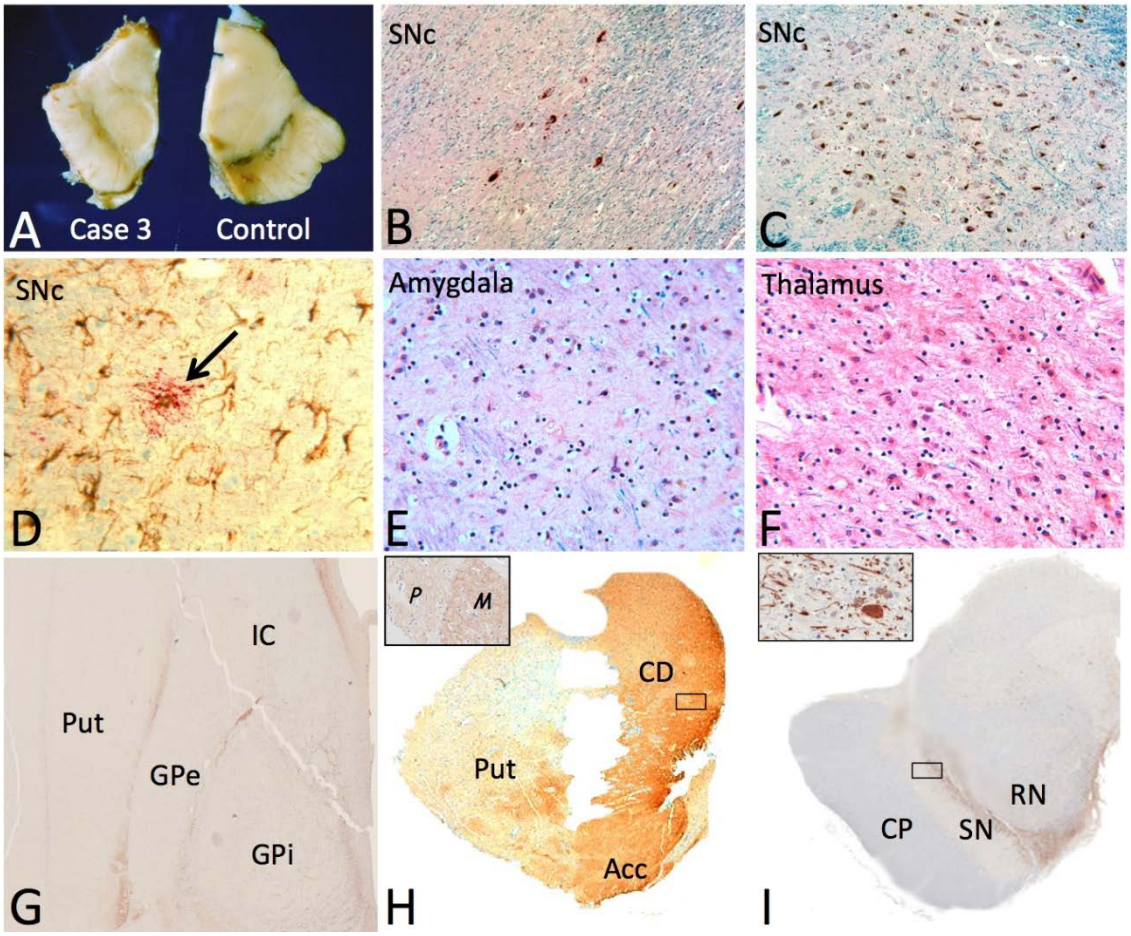

Figure 3-2 Details of Parkinson's disease-associated neuropathological changes in Case 3 with 22q11.2DS and Parkinson's disease

A) Case 3 showed a smaller midbrain with gross depigmentation of the substantia nigra (left) compared with a control case without either 22q11.2DS or parkinsonism (right). B) Hematoxylin-eosin-Luxol fast blue staining of the substantia nigra *pars compacta* revealed decreased density of pigmented cells compared with a control (C). D shows extensive gliosis and microglial activation in the substantia nigra as visualized with glial fibrillary acidic protein (brown chromogen) and Iba-1 (red chromogen, arrow), respectively. The amygdala (E) and thalamus (F) also showed gliosis and neuronal loss (hematoxylin-eosin-Luxol fast blue). G,H) Severe depletion of tyrosine hydroxylase (TH) positive axons was visible in the dorsal putamen (G) and dorsolateral aspect of rostral putamen (H). TH immunoreactivity was preserved in the caudate and the accumbens (H) and was more abundant in the matrix compared with the patches (inset in H). I) TH-positive neurons were severely depleted in the ventrolateral aspect of substantia nigra (inset). SNc: substantia nigra pars compacta; Put: putamen; GPe: external segment of globus pallidus; GPi: internal segment of globus pallidus; IC: internal capsule; Acc: accumbens; CD: caudate; M: matrix; P: patches; CP: cerebral peduncle; SN: substantia nigra; RN: red nucleus. (original magnification ×25 in B-F; ×1 in G-I; and ×25 in insets in H and I).

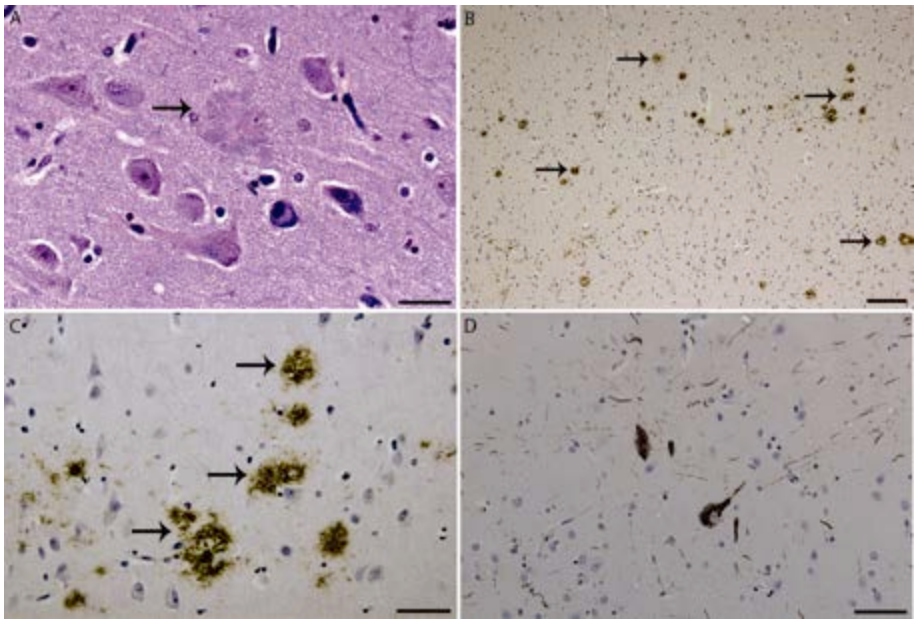

Figure 3-3 Other neurodegenerative pathology in Case 1 with 22q11.2DS and Parkinson's disease

A) The amygdala contained frequent amyloid plaques visible with hematoxylin & eosin staining staining (arrow). B, C) The parahippocampal gyrus showed moderate numbers of diffuse and neuritic plaques immunopositive for β-amyloid (arrows). D) The superior frontal gyrus contained tau-positive neurofibrillary tangles (brown). Scale bars = 50 μm in A, D; 300 μm in B; 100 μm in C.

3.4 Discussion

The results of this study provide evidence of a significantly increased occurrence of PD with early-onset and typical symptom pattern, treatment response, and disease course in adults with 22q11.2 deletions. Neuropathological data confirmed the antemortem clinical diagnoses, providing for the first time details of the neurodegenerative pathology of PD associated with this genetic anomaly. Together with previous reports of unrelated patients[142-144] (Table 3-2), the findings suggest the 22q11.2 deletion as a mutation increasing risk for early-onset PD. The development of early-onset PD in this population is likely related to the effects of the hemizygous 22q11.2 deletion and involve one or more dosage sensitive genes in the 22q11.2 region.

Two of our three pathologically confirmed 22q11.2DS cases had typical neuropathological features of PD with prominent Lewy body and Lewy neurite formation in a classical distribution. Our third case had extensive neuronal loss in the substantia nigra pars compacta along with neuronal loss in the locus coeruleus and the dorsal motor nucleus of the 10th cranial nerve. Gliosis was also observed in the midline nuclei and the anterior and posterior group of the intralaminar nuclei but in the absence of α-synuclein pathology. This variable presence of α-synuclein aggregation is identical to the situation found in PD associated with *LRRK2* mutations, the most common known genetic cause of PD. Lewy body pathology is most frequent in *LRRK2,* however some cases, even in the same family as Lewy body cases, have a "bland" nigral degeneration as seen in our Case 3, while others have tau-related pathology.[99,257] *PARK2*-associated PD typically lacks α-synuclein pathology but some cases with Lewy bodies have been reported (although these may represent examples of incidental Lewy body disease coincident with *PARK2* mutations).[257,267]

Candidate genetic pathways in 22q11.2DS-associated PD

The proximal 22q11.2 deletion region shared by the individuals affected with PD involves approximately 30 genes, none of which overlap any known PD loci (OMIM #168600). However, this region does contain plausible candidate genes implicated in PD-related pathways. These include a microRNA, miR-185, predicted to target *LRRK2,*[268] and *DGCR8,* a key gene in the biogenesis of brain microRNA.[269] Disruption of microRNA mediated post-transcriptional

regulation of gene expression in 22q11.2DS could directly or indirectly impact the expression of PD risk genes elsewhere in the genome (without the necessity of associated mutations).[171] Other possible candidate genes in this 22q11.2 deletion region include *SEPT5*, encoding a protein that functionally interacts with the product of *PARK2*,[244] *COMT*, essential to dopamine level regulation, and six mitochondrial genes.[245] Of these genes, all have shown brain expression and all examined have shown gene dosage effects in mouse models of the 22q11.2 deletion.[170,195,245,270] However, there are as yet no studies of these models at older ages that could help inform potential changes relevant to PD.

Advantages and limitations

Our ascertainment strategies ensured that sample selection was unbiased with respect to recruiting adults with 22q11.2 deletions who had PD, minimizing the risk of artificially inflating the observed frequency of their co-occurrence. Importantly, we had access to exceedingly rare post-mortem tissue from adults with 22q11.2 deletions who had been extensively genetically and phenotypically characterized. The pathological signs of PD found in the 22q11.2DS cases with and without schizophrenia strongly suggest that the parkinsonian clinical features were not (entirely) due to side effects from antipsychotics used to manage schizophrenia, though these may have contributed to the motor phenotype. A recent epidemiological study has proposed that antipsychotic use may be associated with risk of developing PD.[271] However, neither the extensive nigrostriatal dopaminergic cell loss nor Lewy body pathology typical of PD has been observed in neuropathogical studies of antipsychotic medication effects[272] or of individuals with schizophrenia treated with antipsychotics.[273] A recent meta-analysis of neuroimaging studies examining striatal presynaptic dopamine in patients with schizophrenia found no evidence of altered density of dopamine terminals and no significant effect of antipsychotic medication.[274] The absence of dopaminergic cell loss in our three cases with 22q11.2DS and treated schizophrenia without PD corresponds with these findings. Further studies on the possible effect of antipsychotic drugs on dopamine cell degeneration however are needed.

There were some unavoidable limitations of our study. The occurrence of PD in the overall cohort with 22q11.2DS is likely a minimum figure. Most individuals in our cohort were younger than 50 years of age and may yet go on to develop PD at an age that would still be considered early-onset. There may be as yet undiagnosed adults with 22q11.2DS and PD, including those in

the early stages of the disease. For example, we know of two other individuals (ages 36 and 54 years) who were noted as exhibiting parkinsonian symptoms possibly indicative of early-onset PD but who had not received a definitive PD diagnosis. Additional studies including functional imaging of the nigrostriatal dopamine system may help to further clarify this. Small numbers, likely related to the early mortality associated with the 22q11.2 deletion,[262] prevented assessment of the occurrence of PD over age 65 years in patients with 22q11.2DS. Our 22q11.2DS sample was over-represented by individuals with schizophrenia and congenital heart defects, given our ascertainment strategies. This could affect the generalizability of the findings to adults with milder manifestations of 22q11.2DS, many of whom remain unrecognized.[146]

Replication in an independent sample of adults with 22q11.2 deletions would be desirable. However, to our knowledge, our cohort remains the largest available in this age range with extensive phenotypic data of all participants.[146] Although we ruled out known PD mutations elsewhere in the genome and family history of PD, other very rare or as yet unknown PD risk variants are possible, as for any individual with PD. Our relatively small early-onset PD clinical series precluded a formal analysis of the occurrence of 22q11.2 deletions. The series was also biased toward individuals with a positive family history. We note however that if the sample was restricted to cases with no family history of PD that the occurrence of 22q11.2DS would have been 1 in 119. Evaluation of larger cohorts of individuals with early-onset PD will be needed to determine the relative contribution of 22q11.2 deletions to this disease.[258,259] The few published studies of genome-wide copy number variation in PD have not reported any 22q11.2 deletions.[116,275-277] However, these have used ascertainment strategies that would bias against inclusion of individuals with 22q11.2 deletions, e.g., restricting to patients with a positive family history of PD,[277] older age and/or later onset of PD,[276] and/or excluding individuals with history of antipsychotic exposure.[116,275] In contrast, most 22q11.2 deletions are new mutations, therefore family history of 22q11.2DS, let alone 22q11.2DS with PD is unlikely, there is premature mortality in 22q11.2DS, and one in four individuals with a 22q11.2 deletion develops schizophrenia requiring antipsychotic treatment.[146]

Clinical implications

The results of this study may help inform best practices for individuals with early-onset PD and for individuals with 22q11.2DS. Patients with PD who have features associated with a 22q11.2

deletion, such as learning difficulties, congenital palatal, cardiac or other birth defects, and/or schizophrenia,[146] should be considered for clinical genetic referral and/or testing. Management changes for PD with a diagnosis of 22q11.2DS, such as anticipatory care recommendations and attention to associated multisystem conditions would be essential.[146] For example, hypocalcemia, a common treatable feature of 22q11.2DS that can induce or aggravate tremors, should be monitored carefully especially when considering dosage changes in dopaminergic replacement therapy.[146] Neurological assessment for signs of parkinsonism in younger adults with 22q11.2DS should become part of standard practice and when these occur in patients taking antipsychotic medications for schizophrenia, consideration should be given to functional imaging (SPECT or PET) of the presynaptic nigrostriatal dopamine system,[45,144] where available.

Importantly, the results of our study indicate that although individuals with PD and the 22q11.2 deletion associated with 22q11.2DS present with classical motor symptoms, diagnosis of PD may be delayed or obscured by antipsychotic treatment for schizophrenia. Individuals with 22q11.2DS reported without psychosis were diagnosed within a year of motor symptoms onset, compared with six to ten years in individuals with schizophrenia (Table 3-2). Identifying PD in adults with 22q11.2 deletions and schizophrenia presents a unique challenge in clinical practice. As indicated, future studies will need to include careful evaluation and monitoring of signs of PD, perhaps aided by functional neuroimaging.[45,144] Management in these patients will entail careful balancing of anti-parkinsonian medication with antipsychotic treatments. Based on studies of the management of psychosis in PD, one would expect good tolerance to quetiapine and clozapine but better efficacy with clozapine.[248] Other "atypical" antipsychotics are generally much less tolerated by patients with PD, and the development of parkinsonism in individuals with schizophrenia and a 22q11.2 deletion on previously well tolerated doses of these medications might be an important indicator of underlying progressive PD. We can not rule out the possibility that antipsychotic use may have prompted earlier detection of PD in our three cases with a long-term history of antipsychotic use. However, we note the similar age of onset of parkinsonian motor symptoms with individuals with 22q11.2DS who had no prior history of antipsychotic use. The possible effects of antipsychotic medications on either accelerating or unmasking clinical PD merit further study.

Conclusions

Hemizygous 22q11.2 deletions may constitute a new genetic risk factor for early-onset PD with important clinical implications. Additional studies are needed to replicate these results and further establish the prevalence of 22q11.2 deletions in adults with early-onset PD, especially for those with no family history of PD. Further postmortem studies in similar patients are critical to advancing our knowledge of this disorder. Our initial findings suggest that, as with *LRRK2* mutations, the pathogenesis of PD associated with 22q11.2 deletions may or may not be accompanied by α-synuclein aggregates in Lewy bodies and Lewy neurites. Understanding how these genetic disorders are capable of causing nigral degeneration both with and without synuclein aggregation will be critical to advances in understanding of sporadic PD and developing effective disease modifying therapies. Although 22q11.2DS is under-recognized in adults and there is a degree of premature mortality associated with the condition,[262] these patients could represent an identifiable, high risk group for future studies of PD. Studies of 22q11.2 deletions and other genetic forms of PD promise to add to the global understanding of the genetic architecture of this common neurodegenerative disease.

Chapter 4 Genetic markers of Parkinson's disease in 22q11.2 deletion syndrome

4 Overview

ABSTRACT

Background: The hemizygous 22q11.2 deletion associated with 22q11.2 deletion syndrome (22q11.2DS) has been identified as a novel genetic risk factor for early-onset Parkinson's disease (PD). The mechanism underlying this increased risk remains unclear. **Methods:** We performed a pilot whole-genome sequencing study to investigate rare variants in three individuals with 22q11.2DS with neuropathologically confirmed PD, compared with six individuals with 22q11.2DS without PD, that may contribute to the expression of PD in 22q11.2DS. We used standard methods to form gene-sets relevant to PD. **Results:** No mutations common to the cases with PD were found in the intact 22q11.2 region. All were negative for coding sequence mutations in the candidate PD gene-set. There was a significantly (nominal P=0.029) higher burden of putatively damaging missense variants in PD cases in genes part of a genome-wide PD-relevant gene-set. **Conclusions:** PD in 22q11.2DS does not appear to be caused by the unmasking of a recessive locus in the 22q11.2 region or related to rare mutations in known PD genes. The results suggest that the cumulative burden of mutations perturbing a functional PD-relevant network may potentially contribute to expression of PD in the presence of the threshold-lowering effect of a 22q11.2 deletion. Multiple within-individual rare variants may contribute to the penetrance of other genetic forms of PD as well as to the expression of idiopathic PD.

4.1 Introduction

Since the discovery of *SNCA* mutations in 1997[85] as the first genetic cause of Parkinson's disease (PD), PD has emerged from being considered a disease with little to no genetic predisposition to a disease with a Mendelian basis for an ever increasing proportion of patients. In addition to causative pathogenic point mutations in well-established disease genes, it is now clear that copy number variants, deletions and multiplications that respectively decrease or increase gene dosage, also play a critical role in PD pathogenesis given findings, for example, in *SNCA* and *PARK2*.[17,18] We recently identified typical (1.5-2.5 Mb) hemizygous 22q11.2 microdeletions as a novel risk factor for early-onset (<50 years) PD.[252] This deletion affects at least 1 in 4000 live births,[145,146] usually occurring as a spontaneous mutation[156] and manifesting multiple congenital and later onset features (OMIM #192430, #188400).[5,146,147]

The 22q11.2 deletion region contains several potential candidate genes for mediating susceptibility to PD.[252] These include: *COMT*, a regulator of dopamine levels, *SEPT5*, a substrate of parkin, and six mitochondrial genes (*MRPL40*, *PRODH*, *SLC25A1*, *TANGO2*, *TXNRD2*, and *ZDDHC8*). Reduced dosage of one or more 22q11.2 region genes could permit expression of PD, or the deletion could unmask a mutation in a novel recessive locus on the intact 22q11.2 chromosome. Alternatively, expression of PD in 22q11.2DS could be mediated by mutations in PD-associated genes outside of the 22q11.2 deletion region. We investigated rare genome-wide protein-coding variants in 22q11.2DS patients with and without early-onset PD in a pilot study, to begin to delineate the genetic etiology of PD in the context of the 22q11.2 deletion.

4.2 Methods

Participants

We performed whole-genome sequencing using DNA from nine unrelated Canadian adults of European descent with chromosome 22q11.2 deletions (Table 4-1).[147,156] Direct clinical assessments and review of comprehensive lifetime medical records and clinical histories provided deep phenotyping. The three subjects (PD1-PD3) with early-onset PD met United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria and had confirmed

Table 4-1 Clinical and 22q11.2 deletion-related characteristics of individuals with 22q11.2DS of European ancestry with whole-genome sequencing results

	22q11.2DS-Parkinson's disease			22q11.2DS-No Parkinson's disease					
	PD1	**PD2**	**PD3**	**NP1**	**NP2**	**NP3**	**NP4**	**NP5**	**NP6**
Demographic features and PD phenotype									
Sex	Female	Male	Male	Female	Male	Female	Male	Male	Female
Age (y)									
At last follow-up or at death	56	58	61	21	38	48	44	53	52
PD motor symptom onset	45	48	43	-	-	-	-	-	-
PD diagnosis	55	54	44	-	-	-	-	-	-
Neuropathology[a]	Nigral cell death, LBs	Nigral cell death, LBs	Nigral cell death	Living	Living	Living	No PD pathology	Living	Living
Family history of PD	No	No	No	No	No	No	No	Father, late-onset	No
Other major 22q11.2DS features									
Congenital heart defect	No	Yes[b]	No	Yes[b]	No	No	Yes[b]	No	Yes[b]
Intellectual disability	Borderline	None	None	Mild	Mild	Borderline	Borderline	Borderline	Borderline
Schizophrenia (age at onset, y)	Yes (17)	Yes (22)	No	Yes (12)	Yes (15)	Yes (18)	Yes (21)	No	No
Other neuropsychiatric disorders[c]	No	No	No	OCD	No	No	No	GAD	GAD
Seizures[c]	Single	No	No	Recurrent	Recurrent	Recurrent	Single	No	No
Hypocalcemia[c]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cleft palate and/or VPI	No	No	Yes	Yes	No	Yes	No	No	Yes
22q11.2 deletion region									
Deletion type (age at diagnosis, y)	Nested – proximal (53)	Typical (52)	Typical (44)	Typical (4)	Typical (21)	Typical (27)	Typical (42)	Typical (41)	Typical (39)
De novo 22q11.2 deletion[156]	Probable	Probable	Probable	Probable	Yes	Yes	Probable	Yes	Probable

22q11.2DS, 22q11.2 deletion syndrome; GAD, generalized anxiety disorder; LBs, Lewy bodies; NP, No Parkinson's disease; OCD, obsessive compulsive disorder; VPI, velopharyngeal insufficiency

[a]Detailed phenotypic reports published previously for PD1-PD3[252] and NP4[34]

[b]Repaired Tetralogy of Fallot (PD2); Repaired ventricular septal defect (VSD) and atrial septal defect (NP4, NP6); Perimembranous VSD (NP1)

[c]Lifetime history

PD neuropathology.[252] We had previously established these subjects had no known pathogenic point mutations in *LRRK2, PARK2, PARK7* (*DJ-1*), *PINK1,* or *SNCA*, or copy number variants in *PARK2* or *SNCA*.[252] No other major feature of 22q11.2DS was unique to the PD subjects (Table 4-1). Informed consent was obtained in writing, and the study was approved by local research ethics boards.

Pipeline overview

An overview of our analysis pipeline is summarized in Figure 4-1. The overall WGS approach is based on methods used in recent studies of autism[278] and schizophrenia.[279] In brief, genomic DNA was extracted from whole blood and sequenced using the Complete Genomics platform.[280,281] Single-nucleotide variants (SNVs) and small insertions and deletions (in/dels), copy number variants (deletions and multiplications >2kb), and other larger structural variants (insertions and deletions, translocations, and inversions) were called using the Complete Genomics analysis pipeline (version 2.2) and annotated using a custom pipeline based on ANNOVAR.[282] We examined high-quality SNVs and in/dels affecting coding genes, prioritized based on rarity (minor allele frequency [MAF]≤0.01, a standard rarity threshold for very rare variants, in each of three standard[283], and one in-house, databases) and predicted protein damaging effects.[278] Deleterious non-synonymous variants included loss-of-function (nonsense, frameshift in/dels, and essential splice site alterations) and missense mutations.[278,279] High-quality copy number and other structural variants were prioritized by rarity.[278]

The analysis was restricted to a candidate gene approach of rare variants involving coding regions of the genome. Rare coding sequence variants are enriched for those that are deleterious and have a moderate to large effect on disease risk.[284,285] In contrast, the well-studied single nucleotide polymorphisms (SNPs) reported to contribute to the expression of Parkinson's disease necessitate large samples because the common variants individually have a very small impact on disease risk.[133,137] We examined all rare variants on the intact 22q11.2 chromosome and performed targeted PD-gene relevant analyses for the rest of the genome. We investigated rare variants in a gene-set comprising disease-causing and PD risk genes (n=24; Table 4-2). This gene-set was generated through curated OMIM entries and manually inspecting the literature for genes involved in PD. We also assessed protein-coding genes associated with the "top hits" of common susceptibility loci in PD (n=22; Table 4-3), extracted from the PDgene database

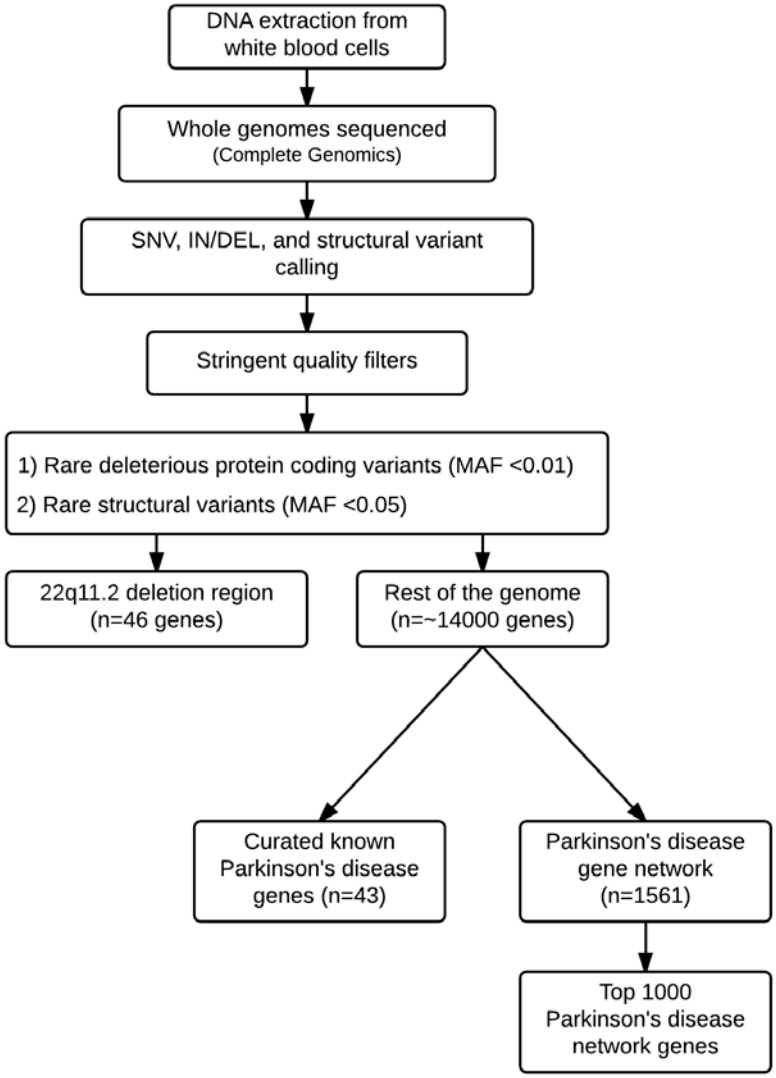

Figure 4-1 Overview of whole-genome sequencing methods

See text for details.

Table 4-2 List of causative and risk genes implicated in Parkinson's disease

Gene	PARK locus	Chromosome	Inheritance	Status & Remarks
PARK-designated genes (n=15)				
SNCA	PARK1/4	4q21	Dominant	Confirmed
LRRK2	PARK8	12q12	Dominant	Confirmed
VPS35	PARK17	16q12	Dominant	Confirmed
PARK2	PARK2	6q25.2-q27	Recessive	Confirmed
PINK1	PARK6	1p36	Recessive	Confirmed
PARK7 (DJ-1)	PARK7	1p36.23	Recessive	Confirmed
ATP13A2	PARK9	1p36	Recessive	Confirmed
PLA2G6	PARK14	22q13.1	Recessive	Confirmed
FBXO7	PARK15	22q12.3	Recessive	Confirmed
UCHL1	PARK5	4p14	Dominant	Unconfirmed (single family)
GIGYF2	PARK11	2q37.1	Dominant	Unconfirmed
HTRA2	PARK13	2p12	Dominant	Unconfirmed
EIF4G1	PARK18	3q27.1	Dominant	Unconfirmed
DNAJC6	PARK19	1p31.3	Recessive	Recently identified (2012)[108,109]
SYNJ1	PARK20	21q22.2	Recessive	Recently identified (2013)[110,111]
Moderate/high risk genetic risk factors for Parkinson's disease, no PARK locus (n=5)				
GBA	N/A	1q21	Risk factor	Confirmed
ADH1C	N/A	4q23	Risk factor	Unconfirmed
MC1R	N/A	16q24.3	Risk factor	Unconfirmed
SMPD1	N/A	11p15.4-p15.1	Risk factor	Recently identified (2013)[119]
GCH1	N/A	14q22.1-q22.2	Risk factor	Recently identified (2014)[118]
Other putative rare causes/risk factors for Parkinson's disease (n=4)				
ATXN2	N/A	12q24.1	Dominant	Usually cause SCA2
ATXN3	N/A	14q21	Dominant	Usually cause SCA3
TBP	N/A	6q27	Dominant	Usually cause SCA17
TH	N/A	11p15.5	Dominant	Unconfirmed (single patient)

Genes include those identified as relevant to PD through curation of OMIM (Online Mendelian Inheritance in Man) entries for Parkinson's disease (#168600) and by manually inspecting the literature. We inclusively assessed all genes (e.g., confirmed and unconfirmed) reported to be involved in PD to limit the possibility of a false negative in reported PD genes. Trinucleotide repeat expansions in *ATXN2*, *ATXN3*, and *TBP* that are more typically associated with the spinocerebellar ataxias (SCA2, SCA3, SCA17, respectively) may also be rare causes of a late-onset PD phenotype.[112,123]

Table 4-3 Top genome wide-association study "hits" implicated in Parkinson's disease in Caucasian populations

Gene implicated (n=22)[a]	Location (hg19)	Meta OR (95% CI)	Meta P-value
SNCA[b] [-19139 bp]	chr4:90626111	1.34 (1.30-1.38)	1.85E-82
LRRK2[b]	chr12:40614434	1.16 (1.11-1.20)	4.86E-14
GCH1[b]	chr14:55347827	1.12 (1.08-1.15)	1.25E-10
MAPT	chr17:43994648	0.77 (0.75-0.80)	6.11E-49
TMEM175	chr4:951947	1.26 (1.22-1.31)	6.00E-41
ASH1L	chr1:155359992	0.52 (0.46-0.58)	6.86E-28
MCCC1	chr3:182762437	0.84 (0.81-0.87)	5.38E-22
STK39 [+24494 bp]	chr2:169129145	1.21 (1.16-1.26)	1.67E-20
BST1	chr4:15737101	0.89 (0.87-0.91)	4.26E-17
NUCKS1 [+4168 bp]	chr1:205723572	0.89 (0.87-0.92)	1.96E-16
TMEM229B	chr14:67984370	1.15 (1.11-1.19)	5.70E-16
GPNMB	chr7:23293746	0.90 (0.87-0.92)	5.62E-14
HLA-DQB1[+30500 bp]	chr6:32666660	0.80 (0.75-0.85)	5.81E-13
BCKDK	chr16:31121793	1.10 (1.07-1.14)	3.63E-12
INPP5F	chr10:121536327	1.77 (1.50-2.08)	1.21E-11
FAM47E	chr4:77198986	0.91 (0.88-0.93)	1.85E-11
RIT2	chr18:40673380	1.10 (1.07-1.14)	2.15E-11
CCDC62	chr12:123303586	0.91 (0.88-0.93)	3.08E-11
SIPA1L2	chr1:232664611	1.13 (1.09-1.18)	1.76E-10
UBOX5 [+12661 bp]	chr20:3153503	0.91 (0.88-0.94)	3.30E-10
TMPRSS9 [-26450 bp]	chr19:2363319	1.14 (1.09-1.19)	2.52E-09
DLG2	chr11:83544472	0.91 (0.88-0.94)	2.59E-08

Abbreviations: bp, base pairs; OR, odds ratio

[a]The top hits thought to be associated with protein-coding genes (accessed October 2014 from www.pdgene.org), generated from up to 15 independent genome-wide association study datasets of European descent (up to 13,708 PD cases and 95,282 controls).[137,286] The top hits list included genes that contained at least one variant that showed genome-wide ($p<1x10^{-5}$) association with PD in the GWAS meta-analysis and, if attempted, showed significant ($p<0.05$) association replication on the NeuroX chip.[137,286] SNPs were automatically annotated to the nearest gene within an interval of +/- 50kb based on Ensembl; the distance is indicated in brackets following the gene name.[137,286] Details on the included datasets as well as all genotyping procedures and statistical analyses can be found in Nalls et al. 2014[137] and online at www.pdgene.org.

[b]Overlaps with genes with rare variants associated with Parkinson's disease (Table 4-2).

(www.pdgene.org; accessed October 2014), for rare variants.[137,286] These were included as rare and common variants can involve the same PD risk gene (Table 4-3).[137,286] Together, these two lists yielded a total of 43 unique PD-associated genes (*SNCA*, *LRRK2*, and *GCH1* were in both lists). Using these as input training genes, we generated a global PD network (n=1609 genes) using the genome-wide candidate gene prioritization tool, Endeavour,[287] with data imported from standard annotation databases (Gene Ontology, Kegg, SwissProt), protein-protein interaction databases (BIND, BioGRID, Hprd, InNetDb, Intact, Mint), and a human gene expression database.[288] Of these, 1561 were mapped to current unique HGNC gene symbols, excluding the 22q11.2 region and the known PD-associated genes that were examined separately. To minimize the risk of false positives, we restricted our PD gene network burden analyses to the top 1000 genes ranked genome-wide. We used one-sided independent *t*-tests in these exploratory analyses to assess variant burden between groups, performed with SAS version 9.2 software. Statistical significance was defined as $P<0.05$.

Sequencing and variant discovery methods

Genomic DNA was extracted from white blood cells blood and sequenced using the Complete Genomics platform (pipeline and assembly version 2.2; GRCh37).[280,281] DNA concentration was measured by picogreen in triplicates and quality checked on a 2% agarose gel. Approximately 11 μg of DNA was submitted for whole-genome sequencing to Complete Genomics. Complete Genomics uses high-density DNA nanoarrays that are concatamers of mate pair reads each ~500 bp long. Base identification was performed using a proprietary non-sequential, unchained read technology known as combinatorial probe-anchor ligation (cPAL). Each mate pair included 35 nt of genomic DNA sequence and adaptor sequences required for cPAL sequencing. The average mate gap length is 300 bp. On average, 99.0% of the genome was covered with at least 5x sequence depth; 97.7% of the genome was covered with at least 10x depth.

Single-nucleotide variants and in/dels

Local realignment and quality recalibration were done with the Genome Analysis Toolkit (GATK-1.4-30).[289] Sequence Alignment/Map tools (SAMtools)[290] was used for removal of duplicated reads. The effects (e.g., nonsense, missense, or frameshift mutations) and classifications (e.g., in exonic, intronic, or intergenic regions) of variants across the genome were

annotated by ANNOVAR.[282] Rarity was annotated using the three major publicly-available reference data-sets based on WGS and whole exome sequencing, i.e., 1000 Genomes project,[291] NHLBI-ESP exomes,[292] and Broad Institute's Exome Aggregation Consortium (ExAC); in addition, we also used platform-matched whole-genome reference data-sets including more than 1,000 subjects. Only variants with CGI high quality variant call scores (''VQHIGH'' tag in the data files) were included. Additional quality parameters[278] applied including the following: sequencing depth >= 5 AND allele quality VQHIGH for both alleles AND alternate allele fraction >= 80% for haploid and homozygous variants, alternate allele fraction >= 35% for heterozygous variants AND equal allele fraction (EAF) score >= 40 for heterozygous variants, EAF allele score >= 20 for homozygous variants, AND called ploidy = 1 for haploid variants, called ploidy = 2 for other variants.

Missense mutations categorized as deleterious[278] were required to show at least moderate nucleotide level conservation (UCSC PhyloP placental mammal or vertebrate)[293] and meet at least two of the six following predictive criteria: high conservation by PhyloP placental mammal (≥2.34), high conservation by PhyloP 100vertebrate (≥4), predicted damaging by SIFT (≤0.05),[294] Polyphen2 (≥0.90),[295] Mutation Assessor (≥1.9),[296] or CADD (Phred score ≥15).[297] The few nonframeshift substitutions, in/dels, and stoploss with nucleotide level conservation (PhyloP placental mammal ≥1.5 or vertebrate ≥2.5) and no overlap with exact match or overlap with common polymorphisms were considered as "other" damaging non-synonymous variants and not considered further. To help manage the risk of false positives, we restricted the analyses to diploid regions of the genome, with the exception of the 22q11.2 deletion. Brain expression was assessed using the BrainSpan spatiotemporal brain expression database (brain expression defined as log2 [rpkm] threshold of 3.21 in at least five data points; www.brainspan.org).

Copy number and other structural variants

Copy number variant (CNV) calls made using custom scripts were based on normalized sequencing coverage. Structural variants (SV) calls were based on abnormal junction and discordant mate pair clusters.[278] We considered only high-quality rare CNVs (not found in the 69 Complete Genomics public genomes[280] and MAF ≤0.05 in the Caucasian parents of 85 autism spectrum disorder multiplex families[278]) that overlapped a coding gene exon and where less than 80% of the CNV was overlapped by a region of segmental duplication. We restricted to high-

quality SVs that overlapped a coding gene exon not found in the 69 Complete Genomics public genomes dataset with mate pair counts ≥20. We annotated data for rarity using the Database of Genomic Variants (http://dgv.tcag.ca).

4.3 Results

Results are summarized in Table 4-4. Targeted analysis of the intact 22q11.2 deletion region did not reveal mutations in any putative 22q11.2 region PD candidate genes.[252] There was a single rare missense variant in each of three subjects (PD1, PD3, NP6) involving three different brain-expressed genes located in the proximal 22q11.2 deletion region (Table 4-4). None was implicated in PD-related pathways. A novel variant in case PD1 was identified in *TRMT2A*, a cell cycle regulated protein of largely unknown function.[298] Case PD3 had a novel variant in *DGCR2*, which encodes a putative transmembrane adhesion receptor protein of unknown function. [299] NP6 had a rare variant in *GNB1L*, a putative schizophrenia risk gene,[300] who had no history of psychosis.

We then investigated known PD causative/risk and susceptibility genes for rare mutations. No deleterious nonsynonymous, or copy number or other structural, variants involving any of these 43 known PD-associated genes was found (Table 4-4).

Extending our analyses to all genome-wide non-synonymous variants outside the 22q11.2 region showed non-significantly more rare deleterious missense (P=0.07) and fewer loss-of-function (P=0.06) mutations in the PD group compared with the patients without PD (Table 4-4). In contrast, functional restriction to the top 1000 ranked PD-relevant gene-set revealed the PD cases to have a significantly higher (nominal P=0.029; Table 4-4) burden of rare deleterious missense mutations (Table 4-5). The result remained significant (P=0.044) after correcting for the total number of rare deleterious missense variants per subject. Restricting to the top 500 ranked candidates in this PD network gene-set showed nearly three-fold enrichment in the PD group (P=0.034; Table 4-4). Moreover, the genes with these variants in the PD patients ranked higher (mean rank, 196) in the PD-relevant gene-set than those in the cases without PD (mean rank, 316; P=0.009). Loss-of-function variants were similarly few in both groups (Table 4-4). There were no genome-wide PD-relevant copy number or structural variants identified (data not shown).

Table 4-4 Summary of nonsynonymous mutations in 22q11.2 deletion-associated early-onset Parkinson's disease patients compared with 22q11.2 deletion patients without Parkinson's disease

	Rare deleterious coding variant counts[a]											
	22q11.2DS-Parkinson's disease			22q11.2DS-No Parkinson's disease						Analyses		
	PD1	PD2	PD3	NP1	NP2	NP3	NP4	NP5	NP6	PD Mean (SD)	NP Mean (SD)	*P*[b]
22q11.2 deletion region genes[c] (n=46)	1	0	1	0	0	0	0	0	1	0.7 (0.6)	0.2 (0.4)	0.14
Known PD causative or risk genes (n=43)	0	0	0[d]	0	0	0	0	0	0	-	-	-
Genome-wide total												
Loss-of-function	9	7	14	22	17	22	13	9	11	10.0 (3.6)	15.7 (5.6)	0.06
Missense	97	98	117	102	79	95	90	82	89	104.0 (11.3)	89.5 (8.4)	0.07
Functional gene-sets												
PD-relevant network genes (top 1000)												
Loss-of-function	1	1	0	1	0	1	1	3	0	0.7 (0.6)	1.7 (1.1)	0.29
Missense	12	7	11	3	4	4	5	8	5	10.0 (2.6)	4.8 (1.7)	**0.029**
PD-relevant network genes (top 500)												
Loss-of-function	1	0	0	0	0	1	0	1	0	0.3 (0.6)	0.3 (0.5)	0.50
Missense	8	4	5	1	0	2	4	4	1	5.7 (2.1)	2.0 (1.7)	**0.034**

[a]Rare (MAF<0.01) autosomal heterozygous deleterious variant counts. Only one homozygous variant was identified (missense variant in *ZNF418*, no apparent functional relevance to PD, in PD3). The PD-relevant gene network was generated using the genome-wide candidate gene prioritization tool, Endeavour.[19] See text for details.

[b]Nominal p value for one-sided independent *t*-test. Non-parametric Wilcoxon testing yielded the same pattern of results.

[c]*TRMT2A*, tRNA methyltransferase 2 homolog A [*S. cerevisiae*] (PD1); *DGCR2*, DiGeorge syndrome critical region 2 (PD3); *GNB1L*, guanine nucleotide binding protein [G protein], beta-polypeptide 1-like (NP6); all three are missense variants in brain-expressed genes in the proximal typical deletion region and none are 22q11.2 PD candidate genes.[252]

[d]A false positive missense variant was initially identified in *PARK2*; confirmatory Sanger sequencing showed no mutation.[252]

Table 4-5 Nonsynonymous variants in genes in a genome-wide Parkinson's disease-relevant gene-set in unrelated patients with 22q11.2DS-associated Parkinson's disease

Case	Gene symbol	Gene Name	Mutation	Rank	Coordinates [GRCh37]			Ref. allele	Alt. allele	Entrez ID	OMIM ID	dbSNP	Rarity	PhyloP Mam.	PhyloP Vert.	SIFT score	Polyphen 2 score	Mutation Assessor Score	CADD Phred score
					Chr.	Start	End												
PD1	TTN[a]	titin	Missense	57	2	179560789	179560789	T	C	7273	188840	NA	0	2.20	3.94	0.00	1.00	1.94	15.13
	MAP2	microtubule-associated protein 2	Missense	147	2	210543361	210543361	C	A	4133	157130	rs138065981	0.005	2.32	6.55	0.86	0.99	0.70	29.40
	HAL	histidine ammonia-lyase	Missense	180	12	96371731	96371731	C	T	3034	609457	rs61937878	0.005	0.79	4.69	0.00	1.00	3.05	15.34
	GPATCH8	G patch domain containing 8	Missense	184	17	42476786	42476786	G	A	23131	614396	NA	0.005	2.72	4.54	0.03	0.64	0.90	10.77
	DOCK4	dedicator of cytokinesis 4	Missense	213	7	111368481	111368481	G	A	9732	607679	rs199706346	0.01	2.64	7.22	0.01	1.00	1.79	35.00
	MYH9	myosin, heavy chain 9, non-muscle	Missense	229	22	36681790	36681790	G	A	4627	160775	rs201021615	0.005	2.30	6.36	0.00	0.95	2.82	27.60
	KHK	ketohexokinase (fructokinase)	Missense	256	2	27320515	27320515	G	A	3795	614058	rs41288797	0.005	1.35	3.57	0.01	0.82	3.19	23.10
	HSD17B4	hydroxysteroid (17-beta) dehydrogenase 4	Missense	265	5	118844919	118844919	C	T	3295	601860	rs201455193	0.005	2.46	2.79	0.00	0.98	3.43	15.29
	EYA1	EYA transcriptional coactivator and phosphatase 1	Missense	648	8	72246370	72246370	G	A	2138	601653	rs201434219	0.005	2.82	8.28	0.01	0.66	1.25	22.80
	ATXN7	ataxin 7	Missense	735	3	63968025	63968025	A	T	6314	607640	rs140270787	0.005	1.08	5.03	0.01	0.60	2.09	25.80
	RNF123	ring finger protein 123	Missense	804	3	49737107	49737107	C	T	63891	614472	rs372770015	0.005	2.55	3.82	0.01	0.64	0.35	16.05
	G3BP1	GTPase activating protein (SH3 domain) binding protein 1	Missense	997	5	151176801	151176801	G	C	10146	608431	rs201691899	0.005	2.74	5.41	0.12	0.40	2.08	18.27
	PDE1A	Phosphodiesterase 1A, calmodulin-dependent	LOF (del)	177	2	183106620	183106623	GTTT		5136	171890	NA	0.01	.	.	.	.	.	11.41
PD2	KLF11	Kruppel-like factor 11	Missense	75	2	10188597	10188597	C	T	8462	188840	rs188185141	0.005	1.08	1.88	0.00	0.04	0.07	14.68
	GCA	grancalcin, EF-hand calcium binding protein	Missense	86	2	163208877	163208877	G	T	25801	176878	rs61748154	0.005	0.88	6.76	0.77	0.01	-1.42	.
	PTPRG	protein tyrosine phosphatase, receptor type, G	Missense	165	3	62189076	62189076	C	T	5793	172250	NA	0.005	2.12	9.02	0.20	0.06	1.92	18.39
	ADCY6	adenylate cyclase 6	Missense	408	12	49165650	49165650	C	T	112	603301	rs35476458	0.005	2.59	6.06	.	0.87	2.65	19.66
	NFATC1	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	Missense	887	18	77171480	77171480	T	C	4772	600489	rs200663312	0.01	1.36	5.19	0.03	0.92	1.55	.
	LARS2[a]	leucyl-tRNA synthetase 2, mitochondrial	Missense	921	3	45537795	45537795	G	A	23395	604544	rs116826217	0.01	2.58	9.41	0.00	1.00	1.95	34.00
	TYR	tyrosinase	Missense	970	11	89017973	89017973	C	T	7299	606933	rs104894313	0.01	2.17	7.38	.	1.00	2.51	24.00
	C19orf80	chromosome 19 open reading frame 80	LOF (nonsense)	936	19	11350874	11350874	C	T	55908	.	rs145464906	0.005	0.94	0.78	0.14	.	.	21.20
PD3	ANKHD1	ankyrin repeat and KH domain containing 1	Missense	22	5	139815809	139815809	C	G	54882	610500	rs61758138	0.01	2.53	7.78	0.06	0.93	1.04	19.68
	ARG1	arginase 1	Missense	29	6	131904553	131904553	C	T	383	608313	NA	0.005	2.87	4.52	0.00	0.99	3.45	33.00
	TTN[a]	titin	Missense	57	2	179430433	179430433	C	T	7273	188840	NA	0.005	2.70	7.82	0.00	1.00	3.12	14.54
	MTMR14	myotubularin related protein 14	Missense	469	3	9714418	9714418	A	G	64419	611089	rs201904466	0.005	2.12	8.41	0.02	0.99	1.67	26.50
	MC5R	melanocortin 5 receptor	Missense	489	18	13826678	13826678	C	T	4161	600042	rs143262370	0.01	2.25	3.04	0.00	0.78	2.13	15.59
	DHTKD1	dehydrogenase E1 and transketolase domain containing 1	Missense	502	10	12129639	12129639	G	T	55526	614984	rs146741810	0.005	2.63	5.02	0.01	0.10	0.73	11.18
	ALDH4A1	aldehyde dehydrogenase 4 family, member A1	Missense	512	1	19209862	19209862	A	G	8659	606811	NA	0	2.01	8.49	0.05	0.06	2.33	18.50
	SMG6	SMG6 nonsense mediated mRNA decay factor	Missense	585	17	2202573	2202573	G	A	23293	610963	rs376396697	0.005	2.52	4.19	0.02	0.85	0.81	15.53
	HARS	histidyl-tRNA synthetase	Missense	815	5	140070517	140070517	C	T	3035	142810	rs144588417	0.005	2.94	4.50	0.98	1.00	2.74	35.00
	LARS2[a]	leucyl-tRNA synthetase 2, mitochondrial	Missense	921	3	45537795	45537795	G	A	23395	604544	rs116826217	0.01	2.58	9.41	0.00	1.00	1.95	34.00
	PGLYRP4	peptidoglycan recognition protein 4	Missense	972	1	153303392	153303392	C	T	57115	608198	rs146118434	0.01	1.83	1.36	0.09	0.96	2.25	18.14

Abbreviations: Alt. allele, alternate allele; Chr., chromosome; del, frameshift deletion; LOF, loss-of-function; NA, not applicable; PhyloP Mam., UCSC PhyloP Placental mammal; PhyloP Vert., UCSC PhyloP 100vertebrate; Rank, generated using the genome-wide candidate gene prioritization tool, Endeavour[287]; Rarity, minor allele frequency compiled from major publicly-available reference data-sets; Ref. allele, reference allele.

[a]Variants involving the same gene in two patients with 22q11.2DS-associated Parkinson's disease.

There were two genes in the PD-relevant gene-set with rare variants that affected more than one case with Parkinson's disease (Table 4-5). One variant in *LARS2* (rs116826217) with rarity ≤0.01 in subjects PD2 and PD3 implicates a mitochondrial aminoacyl-tRNA synthetase that has been reported to be significantly down-regulated (~1.45 fold) in substantia nigra dopamine neurons of patients with idiopathic PD.[301] Two different rare variants in *TTN*, one of the largest genes in the genome, were identified in PD1 and PD3. The large size and highly polymorphic nature of this gene warrants increased caution in the interpretation of potential pathogenicity of variants.[302]

4.4 Discussion

The results of this pilot study provide preliminary evidence that increased burden of genome-wide mutations in genes that are part of a PD-relevant gene network may collectively act as modifiers of PD penetrance in patients with the 22q11.2 deletion. The genes with variants in the affected subjects included, for example, *KLF11*, a regulator of monoamine oxidase B expression,[303] and *MAP2*, a neuronal cytoskeletal protein found in Lewy bodies in PD patients.[304,305] These novel findings provide initial proof-of-principle of the utility of next generation sequencing for studying the etiology of PD, and the advantage of using a more genetically homogenous population to identify putative mechanisms.

We did not find evidence that the association between early-onset PD and 22q11.2DS is mediated by the unmasking of a recessive allele on the intact 22q11.2 chromosome,[252,306] or was impacted by pathogenic mutations in known causative or high risk PD genes. A lowered threshold for expression of PD (in this case, conferred by the 22q11.2 deletion), together with each individual's cumulative genome-wide burden of deleterious variants in PD-relevant pathways, could represent a common multi-hit mechanism generalizable to other forms of PD.[307] Interestingly, loss-of-function mutations affecting genes in the PD-relevant network did not appear to contribute to this result. Additional clinical, animal, and molecular studies are needed to determine the identity of the mechanism of this threshold effect in 22q11.2DS. Of the 46 protein-coding genes within the typical 22q11.2 deletion region, *SEPT5* and *MED15* were among the top 500 genes in our PD-relevant gene-set. Other factors may be involved. For example, Vitamin D deficiency, a common manifestation of 22q11.2DS,[5,146,147] could increase PD risk.[308] Common variants, i.e., SNPs, implicated as possible PD susceptibility loci in the general population may play a minor contributory role but were not studied here. Future studies using

larger samples could help elucidate the likely multifactorial mechanisms underlying the expression of PD in 22q11.2DS.[118,122]

Additional and larger scale studies are needed to replicate findings from this preliminary study. We relied on currently known PD-associated genes to seed a network to prioritize rare variants across the genome; more are likely to be discovered. We were underpowered in this pilot study to include clinical covariates in our analyses. However, there were no significant differences in the PD network variant burden using other major neurological phenotypes (e.g., schizophrenia)[279] and our findings were specific to PD-relevant genes. Whole-genome sequencing methods permit detection of all classes and sizes of variants, which may be missed by technologies such as exome sequencing and microarrays.[278,309,310] Our stringent quality control measures[278] are associated with an experimental validation rate >95% for SNVs.

Advances in whole-genome sequencing bioinformatics methods will permit reliable analyses of non-coding regions in future studies.[311] Increasing evidence suggests that misregulation of splicing contributes to PD.[312,313] Splicing regulatory variants located in intronic regions further away from essential splice sites that negatively affect exon inclusion may be involved in PD pathogenesis in 22q11.2DS. Variants affecting long-intergenic non-coding RNA (lincRNA) should also be considered.[314] Notably, the 22q11.2 region also includes *DGCR8*, a key gene in the biogenesis of brain microRNAs, in addition to seven microRNAs.[8] The true penetrance of PD in adults with 22q11.2DS remains to be reliably estimated, and there are as yet no predictive markers. Longitudinal studies, possible as more patients with 22q11.2DS-PD are identified, will help resolve such questions. All but one of our cases without PD had reached or was beyond the age-at-onset range for 22q11.2DS-associated PD.[143,144,252,315] The absence of PD pathology was confirmed in the case with available brain tissue,[210] and there was no evidence of striatal dopamine denervation in two others (NP2, NP6) from positron emission tomography using ^{11}C-dihydrotetrabenazine (^{11}C-DTBZ),[316] a vesicular monoamine transporter radioligand and index of striatal dopamine neuron density.[317-320] It remains possible that one or more of the cases without PD may go on to develop PD at a later age. Nonetheless, the results of this study provide an important first step in elucidating the genetic aetiology of early-onset PD in 22q11.2DS. These findings have implications for idiopathic PD and could eventually help inform early identification and intervention strategies for individuals at risk.

Chapter 5 Prodromal markers of Parkinson's disease in 22q11.2 deletion syndrome

5 Overview

Published in abstract form in *Movement Disorders*

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Butcher NJ*, Marras C, Pondal M, Rusjan P, Christopher L, Strafella A, Lang AE, Bassett AS. 2015. Investigating prodromal markers of Parkinson's disease in adults with hemizygous 22q11.2 deletions. *Movement Disorders* (S1): S1035.

ABSTRACT

Background: The hemizygous 22q11.2 deletion and the associated multisystem syndrome, 22q11.2 deletion syndrome (22q11.2DS), has recently been identified as a novel genetic risk factor for early-onset Parkinson's disease (PD). Adults with 22q11.2DS may exhibit early pre-diagnostic markers of PD that could help identify those at highest risk. **Methods:** We investigated clinical and neuroimaging features relevant to PD in 13 adults with 22q11.2DS at risk of PD (mean age 41.5, range 30-54 years), 10 healthy age and sex-matched controls, and a case of confirmed L-dopa responsive early-onset 22q11.2DS-PD. **Results:** Using standard motor assessments (MDS-UPDRS, Purdue Pegboard test), the 22q11.2DS group showed significant motor deficits compared with controls. All exhibited at least one parkinsonian symptom, most commonly bradykinesia. The majority (n=10) had olfactory deficits. Transcranial sonography showed that substantia nigra echogenicity was similar to controls. On positron emission tomography using ^{11}C-dihydrotetrabenazine (^{11}C-DTBZ), a vesicular monoamine transporter radioligand and index of striatal dopamine neuron density, the positive control with 22q11.2DS-PD showed the expected pattern of severely reduced striatal ^{11}C-DTBZ binding. Results for the 22q11.2DS group unexpectedly revealed significantly higher binding of ^{11}C-DTBZ in the striatum relative to controls (P<0.01). Consistent with a pattern predicted from idiopathic PD

however, lower striatal ^{11}C-DTBZ binding correlated with increased severity of bradykinesia (r=-0.75, *P*=0.002) in 22q11.2DS. **Conclusions:** These novel findings collectively support patients with 22q11.2 deletions as a readily identifiable population in which to investigate the PD prodrome. The presynaptic dopaminergic abnormality identified using PET imaging suggests a novel pathway to parkinsonism and/or PD in 22q11.2DS. Longitudinal studies are needed to evaluate the observed motor and olfactory deficits, and abnormal pattern of ^{11}C-DTBZ binding, as potential predictive markers of PD in patients with 22q11.2DS and/or as part of the variable clinical presentation of 22q11.2DS in adulthood.

5.1 Introduction

Parkinson's disease (PD) is a common progressive neurodegenerative disorder, affecting approximately 1% of individuals over the age of 60 years.[9,10] Early-onset of the disease that begins before the age of 50 occurs infrequently (~5 to 10% of cases). An increasing number of genetic mutations have been identified for early-onset PD.[120] We recently identified the hemizygous 22q11.2 deletion as a novel genetic risk factor for early-onset PD.[252] This deletion affects at least 1 in 4000 live births,[145,146] usually occurring as a spontaneous mutation.[156] Earlier manifestations of the associated multisystem syndrome, 22q11.2 deletion syndrome (22q11.2DS; OMIM #192430, #188400) include congenital heart and palatal defects, learning disabilities, hypocalcemia, hypoparathyroidism, hypothyroidism, and neuropsychiatric disorders including schizophrenia.[5,146,147] The phenotype of PD associated with 22q11.2DS (22q11.2DS-PD) includes classic loss of midbrain dopaminergic neurons and variable Lewy body neuropathology.[143,144,252]

Patients at high genetic risk of developing PD may exhibit early markers of PD that could help inform early detection and treatment strategies.[11,12] The typical motor symptoms of PD emerge after approximately 50% of nigral dopaminergic neurons and 80% of striatal dopamine are lost during an extensive pre-diagnostic, or prodromal, period estimated to be 5-15 years in duration.[24,25] Early non-motor symptoms that may antedate the classic motor features of PD include olfactory deficits, autonomic dysfunction, and neuropsychiatric and sleep problems.[11,12] Single-photon emission computed tomography (SPECT) and positron emission tomography (PET) with presynaptic dopaminergic markers can reveal early signs of striatal dopamine nerve terminal degeneration suggestive of subclinical PD.[39] Hyperechogenicity of the substantia nigra, visualized using transcranial sonography (TCS), may also indicate a prodromal stage of PD in asymptomatic individuals.[4,54]

In this study, we investigated adults with 22q11.2DS at increased age-related (≥30 years) risk of 22q11.2DS-PD for markers associated with prodromal stages of PD using standard assessments of non-motor and motor functioning. Neuroimaging included TCS, and PET using ^{11}C-dihydrotetrabenazine (^{11}C-DTBZ), a vesicular monoamine transporter (VMAT2) radioligand, to assess striatal dopamine neuron density (Figure 1-1).[317-320] Of the different presynaptic dopaminergic ligands studied, VMAT2 shows the earliest decline in PD[47,321] and is considered

the “gold standard” presynaptic dopamine marker because of its relative resistance to both disease and drug compensatory regulation mechanisms.[48-50,322] Healthy controls, and a patient with a diagnosis of L-dopa responsive 22q11.2DS-PD (positive control), were assessed for comparison purposes. We hypothesized that adults with 22q11.2DS, but without a PD diagnosis, would exhibit (i) a higher prevalence of motor and non-motor symptoms associated with pre-diagnostic stages of PD and (ii) enlarged substantia nigra hyperechogenicity and reduced striatal ^{11}C-DTBZ binding, relative to healthy controls. We expected that the 22q11.2DS-PD patient would show a similar but more severe profile of symptoms and neuroimaging abnormalities.

5.2 Methods

Participants

A total of 24 Canadian adults participated in this study. The main comparison groups comprised 13 individuals with 22q11.2DS (‘22q11.2DS group’) at increased age-related risk of PD (≥30 years) and ten healthy age and sex-matched controls (‘HC group’; Table 5-1). We also assessed one previously unreported patient with 22q11.2DS and clinically diagnosed, L-dopa responsive early-onset PD (50 year old woman of East Indian/Portuguese descent; symptom onset ~45 years, diagnosis ~48 years) for comparison purposes. None of the 24 participants had a first-degree family history of PD.

Chromosome 22q11.2 deletions were molecularly confirmed in all 22q11.2DS patients (diagnosed age 18-48 years) with fluorescence *in situ* hybridization (FISH) using standard methods and probes.[147,156] All were *de novo* or probable *de novo* mutations except in one case where the deletion was maternally inherited.[147,156] The 22q11.2DS-PD case additionally had clinical microarray testing that showed a maternally inherited ~500 kb 3q29 duplication (variant of unknown significance) in addition to the *de novo* 22q11.2 deletion. Research microarray results (Affymetrix® genome-wide human SNP 6.0 and/or 250k microarrays[121,156]) available for 12 of the 13 subjects in the 22q11.2DS group showed the typical ~2.5 Mb 22q11.2 deletion (LCR A-D).[6,147] The subject without microarray results had a confirmed 22q11.2 deletion with FISH with standard probes for the typical 22q11.2 deletion region. Informed consent was obtained in writing and the study was approved by the research ethics boards at the Centre for Addiction and Mental Health and University Health Network.

All patients with 22q11.2DS were recruited from a larger cohort of adults with 22q11.2DS (n=182 ≥17.5 years) primarily ascertained through adult congenital cardiac, psychiatric, and genetic services using active screening and/or clinical referrals, with no known bias to ascertaining patients with movement disorders.[148,252,261] Healthy controls free of major neurological, psychiatric, or any other major illness were recruited using advertisements. A comprehensive clinical history was performed for all participants. The absence of motor, neuropsychiatric, and cognitive impairments were confirmed in the control group using the Movement Disorders Society-Unified Parkinson's Disease Rating Scale part III (MDS-UPDRS III), the Mini Neuropsychiatric Exam, and the Mini Mental Status Examination. Psychiatric diagnoses in patients with 22q11.2DS were made using DSM-IV criteria.[220,255,323] Seven patients in the 22q11.2DS group were treated with an antipsychotic for psychosis (Table 5-2), including five with schizophrenia or schizoaffective disorder and two with a psychotic mood disorder. One was currently treated with the anticholinergic medication procyclidine for associated motor side effects. One non-psychotic patient was treated with propranolol for symptoms of essential tremor (Table 5-2).[7] Other current psychotropic medications in the 22q11.2DS group included antidepressants (n=9), benzodiazepines (n=5), and anticonvulsants (n=1). Using our established protocol to assess intellect,[220,255,323] including standard neurocognitive testing, nine (64.3%) individuals in the 22q11.2DS group had borderline to normal intellect and the remainder (n=4, 30.8%) met criteria for mild intellectual disability (Table 5-3). The mean full-scale IQ assessed using the Wechsler Adult Intelligence Scale-Revised[324] or the Wechsler Adult Intelligence Scale III[325] was 73 (range 62-91) for the 12 subjects with available IQ results (IQ testing performed mean ~9 years previously, range 1 month to 17 years).[220,255,323] The patient with 22q11.2DS-PD had a lifetime history of moderate intellectual disability (IQ=49 at age 44 years) and was treated for anxiety and depression with an antidepressant. There was no history of psychosis or treatment with an antipsychotic medication. L-dopa treatment for this participant was withdrawn for 12 hours prior to performing PET scanning and motor assessments. Healthy controls had no lifetime history of treatment with psychotropic medications.

Assessments

Participants were tested using a battery of motor and non-motor assessments (Table 5-1). Severity of parkinsonian symptoms were assessed using the MDS-UPDRS (22q11.2DS, parts I-III; controls, part III), performed by a movement disorders specialist (C.M. and/or M.P.), and

classified according to modified Hoehn & Yahr staging. Motor subscores were calculated from the MDS-UPDRS for all subjects as follows: rigidity (item 3.3), bradykinesia (items 3.4+3.5+3.6+3.7+3.8+3.14; finger tapping, hand movements, pronation-supination movement of hands, toe tapping, leg agility, and body bradykinesia, respectively), axial impairment (items 3.9+3.10+3.12+3.13; arising from a chair, gait, postural instability, posture, respectively), and tremor (items 3.15+3.16+3.17; postural hand tremor, kinetic hand tremor, and rest tremor amplitude, respectively). Tremor was further assessed in 22q11.2DS patients using the Modified Fahn-Tolosa-Marin Tremor Rating Scale. All participants were also assessed with the following: the Purdue pegboard test for manual dexterity and coordination, the University of Pennsylvania Smell Identification Test (UPSIT) for olfactory function, the Farnsworth-Munsell 100-Hue test for color discrimination, and the Montreal Cognitive Assessment (MoCA) as a measure of global cognition. Screening for symptoms of rapid eye movement (REM) sleep behaviour disorder (RBD) was performed through self-report of dream-enacting behavior and self-injury or harm to others while asleep based on questions derived from the International Classification of Sleep Disorders criteria for diagnosis of RBD.[326]

Transcranial sonography

Transcranial sonography of the substantia nigra was independently performed in sequence by two experienced sonographers (M.P. and C.M.) using a Siemens Acuson X300 PE using a 2.0-3.5 MHz transducer with a penetration depth of 14-16 cm and a dynamic range of 40-45 dB. Off-line measurements were performed by the other sonographer in the few cases where both sonographers were unavailable at the time of sonography. We selected the larger area of hyperechogenicity in the substantia nigra of each participant or, in cases of an insufficient temporal bone window on one side, the area from the analyzable side.

MRI and PET scanning

MRI was performed to obtain high-resolution proton density weighted structural MR images (GE 3 Tesla, oblique axial scan with 1 mm slice thickness) for co-registration with the PET images. Each subject was scanned using ^{11}C-DTBZ, a VMAT2 PET radioligand. VMAT2 is a vesicular membrane protein that transports cytosolic dopamine (and other monoamines) into presynaptic vesicles.[327] The majority (>95%) of VMAT2-related activity in the striatum derives from dopaminergic presynaptic terminals.[322,328] VMAT2 binding is a well-established index of

presynaptic dopaminergic terminal density in other populations.[317,318,322,329,330]

For PET scanning, ^{11}C-DTBZ was injected as a bolus into an antecubital vein through an intravenous line. Acquisition of emission data occurred over a period of 60 minutes while participants were at rest. PET scans were performed using a 3D high-resolution research tomograph (HRRT) brain tomograph (Siemens), which measures radioactivity in 207 brain sections (section thickness=1.22 mm). The HRRT detectors were LSO/LYSO phoswich detectors (crystal elements=2 x 2 x 10 mm^3). To correct for attenuation, a ten minute transmission scan measured using a single-photon point source (^{137}Caesium; $t_{1/2}$=30.2 years, Eγ=662 kiloelectron volts) was acquired immediately prior to the emission scan. Custom-made thermoplastic facemasks were used to minimize subject head movement together with a head-fixation system (Tru-Scan Imaging). Following acquisition completion, the emission list mode data were rebinned into a series of 3D sinograms. The data were normalized with attenuation and scatter correction before applying Fourier rebinning to convert the 3D sinograms into 2D sonograms,[331] which were then reconstructed into image space using a 2D filtered back projection algorithm, with a ramp filter at Nyquist cut-off frequency.

A region of interest analysis was performed for ^{11}C-DTBZ,[320,332] delineated as described in Rusjan et al. (2006)[333] using ROMI software based on previously described atlases.[334,335] Our primary region of interest (ROI) was the sensorimotor striatum (posterior putamen), which shows the earliest signs of dopamine depletion in PD.[336] We also examined the associative (anterior putamen and caudate nucleus) and limbic (ventral striatum) striatal subdivisions.[333,337] In brief, a standard brain template (International Consortium for Brain Mapping/Montreal Neurological Institute 152 MRI) containing predefined cortical and subcortical regions of interest[334,335] was non-linearly transformed using Statistical Parametric Mapping software (SPM8; Wellcome Department of Imaging Neuroscience, London, UK) to fit the individual high-resolution MRI. Regions of interest were aligned and resliced to match the dimension of the PET images using a normalized mutual information algorithm. The region of reference (i.e., devoid of significant levels of VMAT2) used was the occipital cortex.[338,339] ^{11}C-DTBZ binding to VMAT2 was estimated in each region of interest using the simplified reference tissue model[340] and the occipital cortex time activity curve as an input function[339,341] using Receptor Parametric Mapping software. The simplified reference tissue model has been established as an appropriate model to quantify DTBZ without arterial input function.[339,341] The outcome measure derived from this

analysis is BP_{ND}, the specific to non-specific partition coefficient, commonly termed as the non-displaceable binding potential. BP_{ND} is equal to $Bmax/K_D$ where Bmax is unoccupied VMAT2 density, and $1/K_D$ is the in vivo affinity of ^{11}C-DTBZ.

Statistical analyses

To compare demographic and clinical variables between the 22q11.2DS group and healthy controls, we used Fisher's exact tests for independent categorical variables and *t*-tests for independent continuous variables. ^{11}C-DTBZ binding in the regions of interest was contrasted between groups with analysis of covariance and corrected for multiple testing using a Bonferroni correction. Age was used as a covariate due to age-related decreases in VMAT2 binding.[318] An absolute lateralization index[342] was calculated to investigate possible hemispheric asymmetry of ^{11}C-DTBZ binding (|right-left|/[right+left]/2) for each region of interest and compared between groups using the same analysis of covariance approach. Pearson product moment correlation coefficients were used to examine the putative relationships between regional ^{11}C-DTBZ binding and clinical symptoms. All statistical analyses were two-tailed and performed with SAS version 9.4 software (SAS Institute, Cary, NC), with statistical significance defined as $P<0.05$.

5.3 Results

Demographic and clinical features of the 22q11.2DS group (n=13) at genetic risk of early-onset PD and age and sex-matched healthy controls (n=10) are presented in Table 5-1. There were no significant between-group differences in age, sex, or ethnicity. Patients with 22q11.2DS had a lower level of education and performed significantly worse on the MoCA than controls, consistent with the level of intellect expected in 22q11.2DS.[146,220,255] A fourteenth 22q11.2DS patient who was diagnosed with early-onset PD was considered separately as a positive control for PD.

Motor symptoms

Consistent with our hypothesis, the 22q11.2DS group at elevated risk of PD showed several motor features compared with age and sex-matched healthy controls (Table 5-1). The control subjects had normal motor functioning (zero scores on the MDS-UPDRS Motor Exam in all but three individuals who had clinically insignificant scores of 1 to 3). This was expected given their

Table 5-1 Demographic and clinical variables of 22q11.2DS subjects (≥30 years) at genetic risk of early-onset Parkinson's disease and age and sex-matched healthy controls

	Healthy controls (n=10)	22q11.2DS (n=13)	Analyses (*P*)[a]
Demographic features			
Age (years)	42.8±9.4 (31-56)	41.5±7.3 (30-54)	0.72
Sex (male)	6 (60%)	8 (61.5%)	1.0
Ethnicity (Caucasian)	9 (90%)	14 (100%)	0.44
Education (years)[b]	15.1±1.8	12.8±1.8	**0.007**
MoCA (score 0-30)	27.0±2.7	22.5±3.5	**0.003**
Psychotic disorder[c]	-	7 (53%)	-
Age at onset (years)	-	25.3±7.2	-
Motor and neurobehavioural assessments			
MDS-Unified Parkinson's Disease Rating Scale (UPDRS)[d]			
I: Non-motor EDL (score 0-52)	-	11.2±7.6 (1-25)	-
II: Motor EDL (score 0-52)	-	5.6±6.5 (0-25)	-
III: Motor exam (score 0-132)	0.6±1.1 (0-3)[e]	11.5±9.0 (2-33)	**0.0006**
Bradykinesia subscore (score 0-44)	0.3±0.7 (0-2)	5.8±6.1 (0-20)	**0.007**
Tremor subscore (score 0-36)	0.3±0.7 (0-2)	1.3±1.6 (0-4)	**0.043**
Rigidity subscore (score 0-20)	0±0	0.6±1.0 (0-3)	0.055
Axial impairment subscore (score 0-20)	0±0	1.6±1.4 (0-4)	**0.001**
Tremor Rating Scale (score 0-136)	-	9.6±8.9 (0-29)	-
Purdue pegboard (age and sex-adjusted z-score)			
Dominant hand	-0.7±1.1	-2.8±1.1	**0.0002**
Non-dominant hand	-1.1±1.4	-2.9±1.1	**0.0035**
Hyposmia[f] (UPSIT)	4 (40%)	10 (76.9%)	0.10
100-Hue test error score	131.6±87.0	227.1±77.4	0.39[g]
REM sleep behaviour disorder symptoms	1 (10%)	1 (7.1%)	1.0

Data are mean (SD) or n (%). Range is indicated in brackets for selected variables.

Abbreviations: 100-Hue Test, Farnsworth-Munsell 100-Hue test; EDL, Experiences of Daily Living; max.; maximum score possible; MDS, Movement Disorders Society; MoCA, Montreal Cognitive Assessment; UPSIT, University of Pennsylvania Smell Identification Test; Tremor Rating Scale, Modified Fahn-Tolosa-Marin Tremor Rating Scale.

[a]*P*-values from Fisher's exact tests for categorical variables and independent *t*-tests for continuous variables unless otherwise noted. A similar pattern of results was obtained using the non-parametric Mann-Whitney *U* test for continuous variables. [b]Includes grades achieved with special education, where applicable, in 22q11.2DS group only [c]Schizophrenia, n=3, schizoaffective disorder, n=2, psychotic mood disorder, n=2 in the 22q11.2DS group; controls selected for absence of major psychiatric illness. [d]See text for subscore calculation details [e]Three healthy controls had slight clinically insignificant motor features. [f]Hyposmia classified using age- and sex-adjusted normative scores (http://sensonics.com/). Two of the four (50%) healthy controls and one of the ten (10%) 22q11.2DS patients with hyposmia were smokers. [g]Adjusted for cognitive level (MoCA) due to test sensitivity to mild cognitive deficits.[343] Significant group differences ($p<0.001$) did not survive this correction. N=12 in 22q11.2DS group; one 22q11.2DS patient was unable to complete 100-Hue Test due to comprehension difficulties.

relatively young age and our inclusion criteria. Relative to controls, the 22q11.2DS group had a significantly higher mean score on the MDS-UPDRS Motor Exam subscale (22q11.2DS mean=11.5, SD=9.0 vs. control mean=0.6, SD=1.1; P=0.0006). This higher mean score is comparable to scores expected from patients with early stage PD.[344] The mean 22q11.2DS scores on the Non-Motor (mean=11.2, SD=7.6) and Motor (mean=5.6, SD=6.5) Experiences of Daily Living (EDL) subscales were similarly in line with early stage PD scores.[344] The MDS-UPDRS scores for the single 22q11.2DS-PD (H&Y stage 3) case were as follows: Non-motor EDL=3, Motor EDL=3, and Motor Exam=64 (following 12-hour withdrawal from L-dopa). Subscore analyses of the Motor Exam indicated significantly higher bradykinesia (P=0.007), tremor (P=0.043), and axial impairment (P=0.001) subscores in the 22q11.2DS group relative to healthy controls. Rigidity subscores were non-significantly higher in the 22q11.2DS group (P=0.055). Subjects also performed significantly worse on the Purdue Pegboard test compared with controls (dominant hand, P=0.0002; non-dominant hand, P=0.0035; Table 5-1).

To better delineate the variable motor profile of patients with the 22q11.2 deletion, we examined the pattern of motor abnormalities in 22q11.2DS (Table 5-2). Each subject in the 22q11.2DS group at risk of PD exhibited at least one parkinsonian symptom on the MDS-UPDRS motor exam (Table 5-2). Bradykinesia was the most commonly observed, affecting 11 (84.6%) of the 13 patients, including all of those (n=6) not treated with any antipsychotic medication. Four subjects had rigidity (n=1 with no antipsychotic treatment) and four had postural instability (n=3 with no antipsychotic treatment). There were eight with action tremor (kinetic, with or without postural tremor), including two that were clinically significant (Case 1, treated with 60 mg propranolol,[7] and Case 12, untreated and with no response to a prior trial of propranolol). Half (n=4) of those with action tremor had no history of antipsychotic treatment. As expected, the 22q11.2DS-PD patient showed typical signs of PD, including bradykinesia, rest tremor, rigidity, and postural instability (Table 5-2).

Table 5-2 Movement disorders and abnormalities in 13 adults with 22q11.2DS at genetic risk of early-onset Parkinson's disease and one with 22q11.2DS and Parkinson's disease

Case	Age (y), Sex	H&Y	Bradykinesia[a] (subscore)	Tremor[a]	Rigidity[a]	Post. Inst.[a]	Clinical classification	Additional remarks	Hyposmia (UPSIT score)	DTBZ BP_{ND} (SMST)	TCS (cm^2)	Antipsychotic treatment[g] (daily, mg)
1[b]	30, F	0	Yes (6)	Postural + Kinetic (R)	-	-	Postural/kinetic tremor[c]	Treated with propranolol (60 mg)	None (37)	3.65	0.14	-
2	34, M	0	Yes[d] (3)	Kinetic (L)	Yes[d] (LLE)	Slight	-	-	None (40)	2.96	0.13	-
3	36, M	2	Yes (9)	Kinetic (L)	-	-	Drug-induced parkinsonism	Decreased armswing and facial expression, speech monotonous	Moderate (26)	3.71	0.24	Risperidone 3 [254.7]
4	37, F	0	- (0)	-	Yes (UEs)	-	-	-	Mild (31)	3.53	0.15	[f]Fluphenazine 25 depot q2 weeks [407.3]
5	38, M	2	Yes (17)	-	-	-	Parkinsonism	Stooped posture, masked face	Mild (33)	2.78	0.09	Quetiapine 700 [682.5] Risperidone 6 [513.4]
6	39, F	1	- (1)[e]	Kinetic (R)	-	-	Parkinsonism	Speech monotonous, masked face	Severe (24)	4.10	0.09	Quetiapine 700 [682.5]
7	39, M	1	Yes (2)	-	Yes (neck)	-	Parkinsonism	Decreased R armswing	Moderate (29)	4.71	0.22	Quetiapine 100 [16.2]
8	42, F	2.5	Yes (5)	-	-	Slight	Parkinsonism	-	None (37)	3.73	-	-
9	42, M	2.5	Yes (5)	Postural + Kinetic (B)	-	Slight	Drug-induced parkinsonism	Treated with procyclidine (5 mg)	Severe (23)	2.62	0.14	Zuclopenthixol 30, oral [120]
10	46, M	0	Yes (3)	Kinetic (R)	-	-	-	-	Mild (27)	2.95	0.10	-
11	51, F	2.5	Yes (3)	Postural + Kinetic (B)	-	Slight	Parkinsonism + postural/kinetic tremor[c]	-	Severe (21)	5.74	0.07	-
12	52, M	0	Yes (2)	-	-	-	-	-	Mild (30)	2.82	0.10	-
13[b]	54, M	2	Yes (20)	Postural + Kinetic (B)	Yes (neck)	-	Parkinsonism + myoclonus	Myoclonus treated with clonazepam	Severe (23)	2.21	0.11	Clozapine 400 [478.4] Risperidone 1 [82.4]
PD1	50, F	3	Yes (35)	Rest (RLE, LLE)	Yes (neck, UEs, LEs)	Slight	PD (symptom onset, 45 years)	Good L-dopa response; dyskinesia	Anosmia (14)	0.37	0.13	-

Abbreviations: B, bilateral hand tremor; DTBZ BP_{ND} (SMST), ^{11}C-DTBZ non-displaceable binding potential in the sensorimotor striatum; F, female; H&Y, Modified Hoehn and Yahr Staging; L, left hand tremor; LLE, lower left extremity; M, male; R, right hand tremor; UPSIT, University of Pennsylvania Smell Identification Test (max. score 40); LE, lower extremeties; TCS, transcranial sonography area measurement of the substantia nigra, UEs, upper extremities.

[a]Evaluated using the Movement Disorders Society-Unified Parkinson's Disease Rating Scale and defined as follows[38]: bradykinesia, score of ≥1 in 2 motor tests on the same side of the body or a score of ≥2 in one motor test (items 3.4-3.8); tremor, score of ≥1 (postural, item 3.15; kinetic, 3.16; rest, item 3.17); rigidity, score of ≥1 (item 3.3), postural instability, score of ≥1 (item 3.12). See text for details on bradykinesia subscore calculation.

[b]Detailed phenotypic information published previously.[7]

[c]Phenomenology consistent with essential tremor, including bilateral tremor of arms and legs (Case 1) and bilateral hand tremor and lip tremor (Case 11).

[d]Possibly related to plates in left ankle and left knee injury.

[e]Slightly impaired on finger tapping test (item 3.4).

[f]Typical prescription is 10 mg depot injection q 4 weeks but had been temporarily increased due to missed doses. Last injection (25 mg) was eight weeks prior to study participation.

[g]Chlorpromazine equivalent (mg) denoted in square brackets. Equivalent doses were calculated using linear regression conversion formulas medication reported by Andreasen and colleagues (2010)[345] and the Maudsley Prescribing Guidelines[346] (zuclopenthixol only).

Hypocalcemia, a common neuroendocrine manifestation of 22q11.2DS, can cause tremor, stiffness, and other involuntary movements,[146,188-190] but we did not find higher rates of hypocalcemia (Table 5-3) in the 22q11.2DS patients with action tremor (n=7, 87.5% vs. n=4, 80.0% without action tremor and with hypocalcemia) or rigidity (n=3, 75.0% vs. n=8, 88.9% without rigidity and with hypocalcemia). 22q11.2DS-associated hypothyroidism can also be associated with muscle stiffness,[190] but none of the patients with rigidity had hypothyroidism. Notably, neuroendocrine disorders were managed in all subjects in this study using standard treatments (Table 5-3). Three (7.7%) patients in the 22q11.2DS group had a history of seizures (Table 5-3).

The assessing neurologist clinically classified each patient's symptoms following the neurological exam (Table 5-2), blind to antipsychotic medication status to avoid over-attribution of symptoms to drug-induced parkinsonism. Suspected cases of drug-induced parkinsonism based on symptomatology were confirmed following the motor exam. Using these methods, six patients were classified as having parkinsonism. These included four treated with atypical antipsychotic medications and two with no antipsychotic treatment (including Case 12 who additionally exhibited action tremor; Table 5-2). Two others were classified with drug-induced parkinsonism; they were treated with the atypical antipsychotic risperidone (3 mg; Case 3), and the typical antipsychotic zuclopenthixol (30 mg; Case 9), respectively. There was one subject (Case 1) with a postural/kinetic tremor where the phenomenology was consistent with essential tremor, and no history of antipsychotic treatment (Case 12).[7]

Non-motor symptoms

Also consistent with our hypothesis, the majority (n=10, 76.9%) of patients with 22q11.2DS at risk of PD had olfactory deficits based on age- and sex-adjusted normative scores (Tables 5-1, 5-2). One was a smoker. UPSIT raw scores did not significantly correlate with full-scale IQ (r=0.04, *P*=0.90) or MoCA (r=0.45, *P*=0.12) scores in the 22q11.2DS group, as expected; the UPSIT is a relatively simple test that can be completed by children as young as five years old.[347] The prevalence of hyposmia in 22q11.2DS did not differ significantly from the control group, however, related to the small numbers and the high proportion of smokers in the control group (n=5, 50%; *P*=0.10). Hyposmia was absent (n=2) or mild (n=3) in the 22q11.2DS patients without parkinsonism. In contrast, six of the eight patients with parkinsonism or drug-induced

Table 5-3 Psychiatric, neurological, and neuroendocrine clinical characteristics of 13 adults with 22q11.2DS at genetic risk of early-onset Parkinson's disease and one with 22q11.2DS and Parkinson's disease

Case	Age (y), Sex	H&Y	Psychiatric diagnoses[a]	Antidepressant medications (mg)	Neurocognitive			Seizures[a] (treatment, mg)	Neuroendocrine disorders[a]	
					Intellectual disability	IQ (age, y)	MoCA score		Hypocalcemia[b]	Hypothyroidism[c]
1[b]	30, F	0	MDD	Citalopram (40)	Borderline	75 (21)	24	None	Yes	Yes
2	34, M	0	GAD	None[d]	None	N/A	24	None	No	No
3	36, M	2	SA	Fluoxetine (20)	Mild	67 (33)	20	Recurrent (clonazepam, 0.5)	Yes	Yes
4	37, F	0	PM, GAD	Citalopram (30)	Mild	62 (29)	24	None	Yes	No
5	38, M	2	SZ	None	Mild	65 (34)	22	Recurrent (clonazepam, 0.5)	Yes	No
6	39, F	1	SZ, MDD	Citalopram (40)	Borderline	81 (25)	21	None	Yes	Yes
7	39, M	1	PM, MDD	Citalopram (50)	Borderline	75 (35)	22	None	Yes	No
8	42, F	2.5	MDD, GAD	Citalopram (25)	Borderline	72(34)	26	None	No	Yes
9	42, M	2.5	SA, OCD	Fluvoxamine maleate (200)[e]	Borderline	65 (32)	17	None	Yes	No
10	46, M	0	MDD	Citalopram (30)	Borderline	78 (32)	24	None	Yes	No
11	51, F	2.5	GAD	Sertraline (100)	Borderline	77 (39)	27	None	Yes	No
12	52, M	0	None	None	None	91 (49)	26	None	Yes	No
13[b]	54, M	2	SZ	None	Mild	69 (43)	15	Recurrent (clonazepam, 2; divalproex, 750; gabapentin, 3200)	Yes	No
PD1	50, F	3	MDD, GAD	Venlafaxine (150)	Moderate	49 (44)	9	None	Yes	No

Abbreviations: GAD, generalized anxiety disorder; MDD, major depressive disorder; MoCA, Montreal Cognitive Assessment; N/A, not applicable; PM, psychotic mood disorder; SA, schizoaffective disorder; SZ, schizophrenia; y, years

[a]Lifetime history

[b]All treated with calcium and/or Vitamin D supplementation, except Cases 3 and 6 who were treated with calcitriol. Cases 4 and 12 reported non-compliance.

[c]Treated with levothyroxine.

[d]Not treated with medication at time of study participation; symptoms had remitted following improved psychosocial situation.

[e]Also treated with the anxiolytic busprione (10 mg).

parkinsonism had moderate to severe hyposmia (Table 5-2). The patient with 22q11.2DS-PD met criteria for anosmia.

Although there were higher error scores (worse performance) on the 100-Hue test of colour discrimination in the 22q11.2DS group (Table 5-1), there was no significant between group difference after adjusting for cognitive level (MoCA scores), consistent with previously reported effects of cognitive impairments on colour discrimination defects.[343] One participant in each group self-reported symptoms of REM sleep behaviour disorder (Table 5-1).

Neuroimaging

Transcranial sonography results for area of substantia nigra echogenicity were non-significantly larger in patients with 22q11.2DS at risk of PD (mean area=0.16 cm^2, SD=0.05) and healthy controls (mean area=0.12 cm^2, SD=0.08; *P*=0.11; Figure 5-1). The patient with 22q11.2DS-PD did not show a hyperechogenic signal (area=0.08 cm^2). Independent analyses by a second sonographer showed no significant difference between groups (*P*=0.68). Substantia nigra echogenicity did not correlate with any motor symptoms or with ^{11}C-DTBZ binding results (data not shown).

Contrary to our main hypothesis, the 22q11.2DS group at risk of PD had significantly higher mean striatal ^{11}C-DTBZ binding in all regions of the striatum compared with healthy controls (age-adjusted $P \leq 0.01$; Figure 5-2). Specifically, there was a 27.1% increase of ^{11}C-DTBZ binding in the sensorimotor striatum (mean BP_{ND}=3.50, SD=0.96) relative to controls (mean BP_{ND}=2.55, SD=0.39; *P*=0.01). ^{11}C-DTBZ binding was similarly elevated, by 26.1%, in the associative striatum (22q11.2DS mean BP_{ND}=3.40, SD=0.70; HC mean BP_{ND}=2.51, SD=0.39; *P*=0.002), and by 23.9% in the limbic striatum (22q11.2DS mean BP_{ND}=2.71, SD=0.42; HC mean BP_{ND}=2.06, SD=0.28; *P*=0.0005). Indeed, the majority of the 22q11.2DS subjects had ^{11}C-DTBZ binding results that did not overlap the control range in the striatal subdivisions (n=7, 53.8% in sensorimotor striatum; n=9, 69.2% in each of the associative and limbic striatum regions, respectively; Figure 5-2). Similar results were obtained after implementing a partial volume effects correction on time activity curve data using the Rousset algorithm[348] (e.g., ~20% higher ^{11}C-DTBZ binding in each striatal subdivision in the 22q11.2DS group relative to controls).

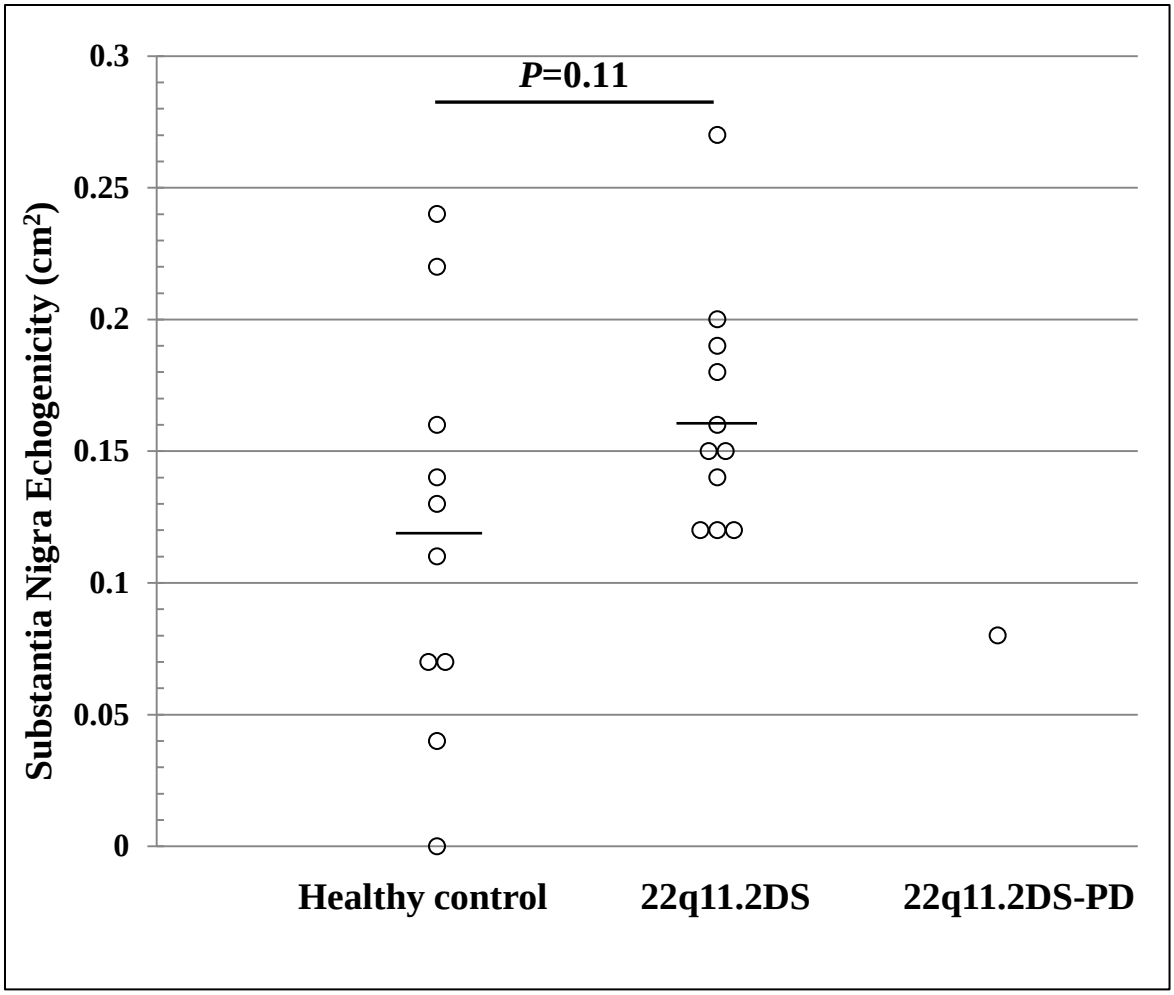

Figure 5-1 Transcranial sonography findings in 13 adults with 22q11.2DS at genetic risk of early-onset Parkinson's disease and one with 22q11.2DS and Parkinson's disease

The mean echogenic area corresponding to the anatomical location of the substantia nigra was non-significantly larger in adults with 22q11.2 deletion syndrome (22q11.2DS; n=13) compared with age and sex-matched controls (n=10). Horizontal lines indicate group means. Independent analyses by a second sonographer showed no significant difference between groups (*P*=0.68). The 22q11.2DS patient with Parkinson's disease (22q11.2DS-PD) did not display a hyperechogenic signal related to either group. The largest bilateral measurement is shown for all cases. Zero measurements indicate instances where the mesencephalic brainstem was clearly visualized but there was no echogenic signal in the location of the substantia nigra. The bilateral temporal bone window was insufficient in one 22q11.2DS patient.

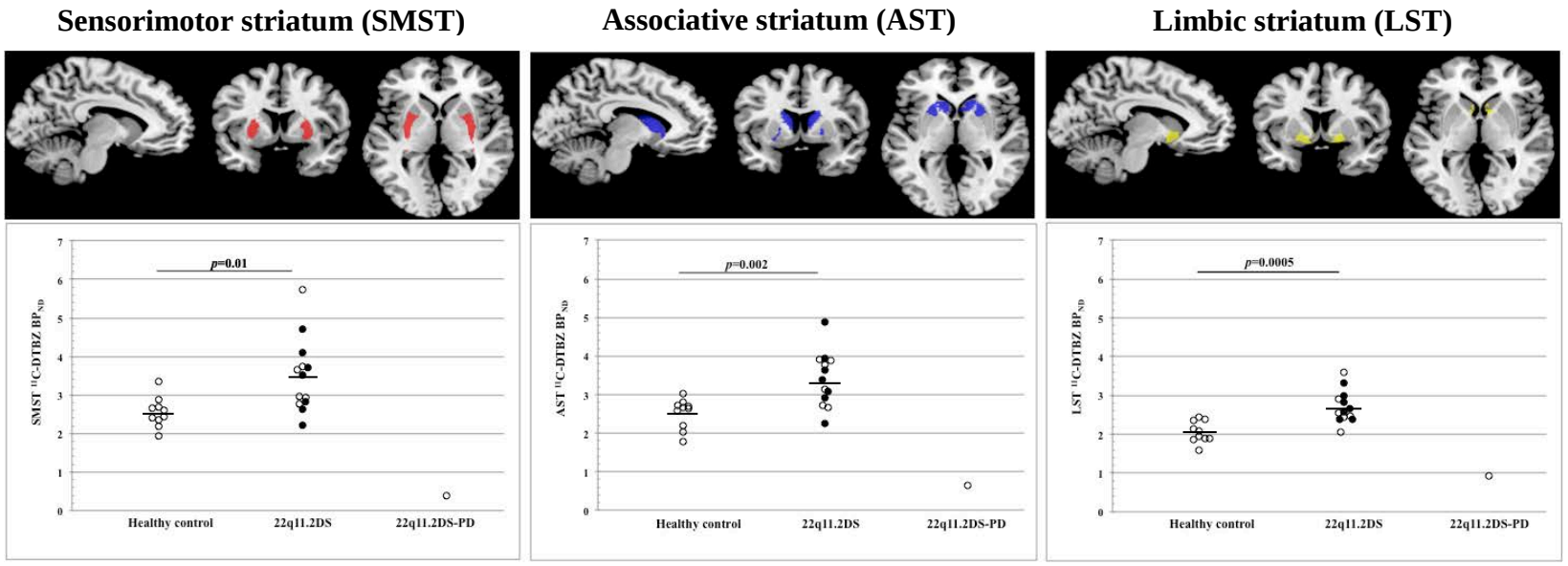

Figure 5-2 ^{11}C-DTBZ binding in 13 adults with 22q11.2DS at genetic risk of early-onset Parkinson's disease and one with 22q11.2DS and Parkinson's disease

Adults with 22q11.2 deletion syndrome (22q11.2DS without diagnosed PD; n=13) show elevated ^{11}C-DTBZ binding potential (BP_{ND}), compared with healthy control subjects (n=10) using PET. ^{11}C-DTBZ is a vesicular monoamine transporter 2 radioligand. The single 22q11.2DS patient with Parkinson's disease (22q11.2DS-PD) shows the expected pattern of grossly reduced ^{11}C-DTBZ binding. Black circles denote 22q11.2DS patients with psychosis. Anatomical images provided by Dr. Antonio Strafella.

Time-activity curves calculated using decay-corrected standard uptake values for the occipital reference region showed no significant difference between patients and controls. There was no significant difference (P>0.3) in the laterality index calculated for hemispheric ^{11}C-DTBZ binding between groups in any of these three striatal regions. Within the 22q11.2DS group, striatal binding potentials were similar for patients with and without parkinsonism (P>0.1), with and without postural and/or kinetic tremor (P>0.4), and with and without psychosis (P>0.6), in all three striatal subdivisions. In contrast, consistent with expectations, the 22q11.2DS patient with PD showed the expected pattern of severely reduced striatal ^{11}C-DTBZ binding (Figure 5-2).

Despite these unexpected findings, we investigated the relationship between striatal ^{11}C-DTBZ binding and motor functioning in patients with 22q11.2DS at risk of PD. We identified a correlation trend between higher (worse) MDS-UPDRS bradykinesia subscores and lower ^{11}C-DTBZ binding in the sensorimotor striatum (r=-0.48, P=0.10; Figure 5-3), but not in the associative (r=-0.25, P=0.39) or limbic (r=-0.01, P=0.98) striatum. No relationships were found between ^{11}C-DTBZ binding in any of the three striatal regions and other motor measures (MDS-UPDRS tremor, rigidity, and axial instability subscores, Tremor Rating Scale and Purdue Pegboard scores). We further examined the relationship between bradykinesia severity and reduced ^{11}C-DTBZ binding in the sensorimotor striatum by including the patient with 22q11.2DS-PD as a measure of an advanced motor disease state. This revealed a strong linear relationship between bradykinesia and ^{11}C-DTBZ binding (r=-0.75, P=0.002) in adults with 22q11.2DS, which remained significant after adjusting for age (P=0.005).

As a secondary analyses, we explored putative correlations between striatal ^{11}C-DTBZ binding and cognitive measures in the 22q11.2DS group, to examine if abnormal ^{11}C-DTBZ binding may be related to the neurodevelopmental cognitive deficits commonly associated with the syndrome.[5,146,255] There was a trend for lower ^{11}C-DTBZ binding in the sensorimotor striatum and lower (worse) total MoCA score (r=0.49, P=0.086), but not in the associative (r=-0.21, P=0.49) or limbic (r=0.07, P=0.82) striatum. We found no correlation between ^{11}C-DTBZ binding results and full-scale IQ scores in any of the striatal subdivisions (P>0.4).

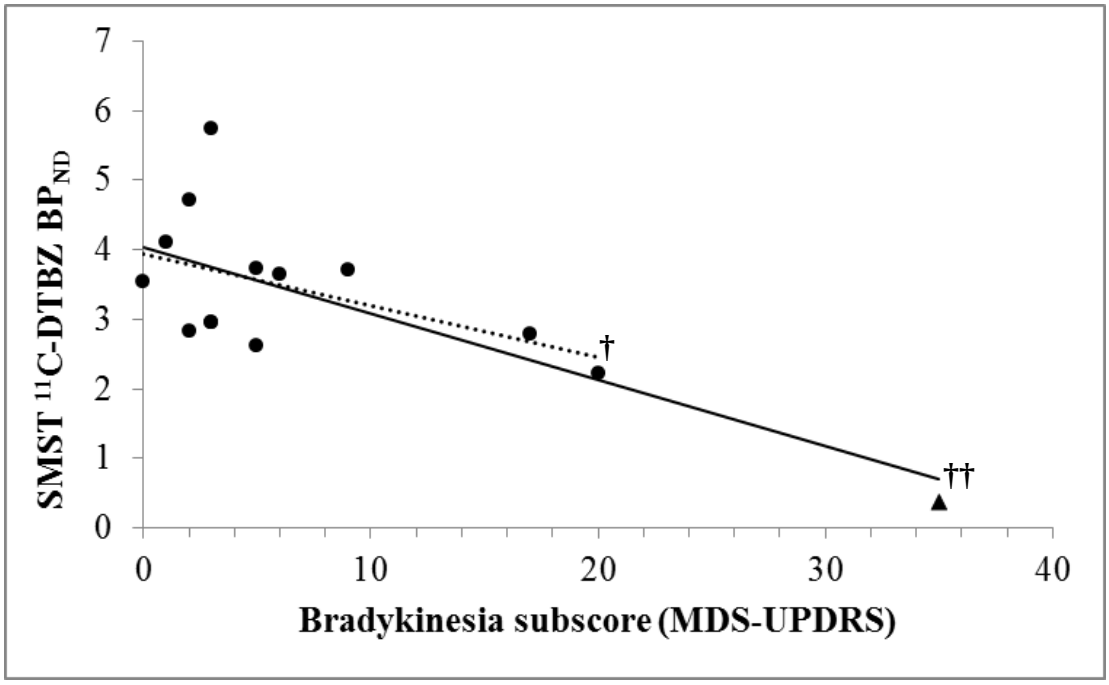

Figure 5-3 Bradykinesia severity correlates with levels of ^{11}C-DTBZ binding in the sensorimotor striatum of adults with 22q11.2DS

Circles indicate patients with 22q11.2 deletion syndrome without diagnosed PD (22q11.2DS; n=13) with varying severity of bradykinesia, as measured using the Movement Disorders-Unified Parkinson's Disease Rating Scale (MDS-UPDRS). The triangle indicates a 22q11.2DS patient with clinically diagnosed Parkinson's disease (22q11.2DS-PD). SMST, sensorimotor striatum.

†The correlation trend (r=-0.48, *P*=0.10) among 22q11.2DS patients not diagnosed with PD.

††Correlation result (r=-0.75, *P*=0.002) when including the 22q11.DS-PD patient who represents an advanced motor disease state (black line). Note the comparable trajectory.

5.4 Discussion

As a progressive neurodegenerative disorder, PD has a long prodromal period that precedes its clinical diagnosis, characterized by the gradual loss of dopaminergic nigrostriatal neurons and the emergence of non-motor and subclinical motor symptoms.[11,12] Here we report the results of the first investigation of prodromal markers of PD in individuals with hemizygous 22q11.2 deletions, a newly identified genetic risk factor for early-onset PD.[252]As hypothesized, we found that parkinsonian features and olfactory deficits associated with prodromal PD are common in adults (aged 30-54 years) with 22q11.2DS. The results of the first ever assessment of presynaptic dopaminergic functioning in adults with 22q11.2DS without PD using PET neuroimaging with ^{11}C-DTBZ, a radioligand for the presynaptic vesicular monoamine transporter (VMAT2) and an established index of striatal dopamine neuron density in other populations,[317-320] unexpectedly revealed elevated ^{11}C-DTBZ binding levels in each of the anatomo-functional subdivisions of the striatum (sensorimotor, limbic, and associative striatal regions) relative to healthy age and sex-matched controls. This provides initial evidence of a higher pre-morbid availability of presynaptic dopamine storage vesicles in 22q11.2DS. Consistent with a pattern predicted from idiopathic PD, lower striatal ^{11}C-DTBZ binding correlated with increased severity of bradykinesia in patients with 22q11.2DS in the sensorimotor striatum. These findings collectively indicate a novel pathway to parkinsonism and/or PD in this genetic population. Longitudinal studies are needed to evaluate the observed motor symptoms, olfactory deficits, and abnormal ^{11}C-DTBZ binding levels as potential predictive markers of early-onset PD in 22q11.2DS and/or as part of the variable clinical presentation of 22q11.2DS in adulthood.

Possible clinical predictors of PD in 22q11.2DS

The results of this study suggest that patients with 22q11.2 deletions provide a unique opportunity to study prodromal stages of PD. These clinically and genetically identifiable patients commonly exhibited motor and olfactory deficits that are well-established signs of prodromal PD. Patients performed significantly worse on tests of motor functioning (i.e., MDS-UPDRS Motor Exam, Purdue Pegboard test) relative to age and sex-matched healthy controls. All 13 patients with 22q11.2DS exhibited at least one parkinsonian motor feature, most commonly mild bradykinesia, with eight of these subjects deemed to have “parkinsonism” clinically. Most (n=10) exhibited olfactory deficits, independent of intellect, consistent with the high prevalence of olfactory dysfunction reported in PD populations.[34,35] In contrast, we found

no evidence of increased rates of symptoms of REM sleep behaviour disorder in patients with 22q11.2DS. Symptoms of REM sleep behaviour disorder, present in up to one half of patients with PD,[30] may not be a major early prodromal feature of 22q11.2DS-PD. Our finding that poor performance in the 22q11.2DS group on the Farnsworth-Munsell Hue test, a test of colour discrimination abilities used in PD studies, was mediated by the lower overall level of intellect adds to increasing evidence that this may not be a useful test in patients with compromised cognitive functioning.[343]

The observed motor and olfactory impairments may prove to be useful measures in stratifying patients with 22q11.2DS for PD risk. Longitudinal follow-up of these patients for clinical onset of PD will be necessary to elucidate the possible clinical utility of these initial findings. Motor and olfactory deficits are considered sensitive, but non-specific, prodromal markers of PD. The penetrance of PD in 22q11.2DS requires further study, but is certainly incomplete (~6% in adults older than 35 years in our recent cohort study.[252]) We therefore expect that at most, a subset of the patients identified here with motor and olfactory impairments may be progressing towards PD. In the others, the observed olfactory and motor deficits may represent clinical manifestations of 22q11.2DS and/or its associated comorbidities and treatments. Based on the PET neuroimaging results, bradykinesia severity may be a potential clinical indicator of progression towards PD in 22q11.2DS (discussed below).

This study is among the few but increasing number of studies investigating the clinical features of patients genetically predisposed to PD, but who have not yet developed the disease. These studies promise to provide new insights into the PD prodrome. One of the most notable of these involved 60 individuals (aged 50 to 89 years) with *GBA* mutations, risk factors for typical late-onset PD, which revealed significant motor, olfactory, and cognitive deficits relative to controls.[349] The severity of these impairments appeared to be milder, on average, in these older patients with *GBA* mutations than those identified here in 30 to 54 year old patients with the 22q11.2 deletion. Importantly, two-year clinical follow-up revealed significant declines in these clinical measures in those with *GBA* mutations, but not in controls,[350] suggestive of an evolving PD prodrome that is detectable using selected clinical markers. There was a subset (five of the 60 subjects) with parkinsonian symptoms insufficient for a clinical diagnosis of PD, three of whom showed substantial worsening of symptoms at follow-up.[349,350] This suggests that the subset of

eight subjects with 22q11.2DS in the current study classified as having parkinsonism may contain those who may be at highest risk for progression of symptoms.

Several cross-sectional studies of older (e.g., ≥50 years) individuals with *LRRK2* mutations at high genetic risk of typical late-onset PD have reported subtle motor deficits compared with controls, including slightly increased UPDRS scores (e.g., mean ≤6)[126,351-353]and minor gait abnormalities.[352] Olfactory deficits do not appear to be a common manifestation of the *LRRK2* prodrome, consistent with evidence that hyposmia is a less frequent feature of *LRRK2*-PD compared with idiopathic forms of the disease.[126,351,354] Interestingly, the only difference in motor functioning reported in one study of *LRRK2* mutation carriers compared with relatives with no *LRRK2* mutation was an increased frequency of postural or kinetic tremor (38% vs. 10%).[126] In the current study, we also identified a high prevalence of postural and kinetic tremor in patients with 22q11.2DS (n=8, 61.5%; not found in controls). Longitudinal evaluation of patients with the 22q11.2 deletion and other genetic risk mutations will be critical for identifying which pre-diagnostic motor or other phenotypes may best inform early predictive or diagnostic methods for PD.

Substantia nigra hyperechogenicity

An increased area of hyperechogenicity at the anatomical site of the substantia nigra is reported in 70-90% of patients with PD.[4,54] Elevated rates of hyperechogenicity have also been reported in patients at clinical (e.g., 36% of REM sleep behaviour disorder patients[355]) and genetic (e.g., 85% of *LRRK2* G2019S mutation carriers[354]) high risk of the disease. Contrary to our expectation, given these previous findings, we did not find evidence that echogenicity of the substantia nigra was significantly increased in patients with 22q11.2DS relative to controls. There was also no enlargement in the 22q11.2DS-PD patient. Although the presence of substantia nigra hyperechogenicity appears to have good positive predictive value in patients with already well-characterized PD,[4,54] some studies (including from our own group)[356-358] have reported relatively poor diagnostic sensitivity. Poor diagnostic sensitivity appears to be a particular issue in patients in the early stages of the disease.[357,359] Methodological differences between transcranial sonography studies, such as the use of various different transducers and scoring methods, may also play a role.[358]

We found no relationship between echogenicity and ^{11}C-DTBZ binding, consistent with previous reports that failed to show a correlation between echogenicity and dopamine transporter levels,

another presynaptic dopaminergic marker, in studies of patients with PD[360,361] or at clinical high risk of PD.[355] Consistent with studies of idiopathic PD,[4,53] there was also no relationship of substantia nigra echnogenicity with any clinical symptoms of PD. It remains possible that the subjects with larger substantia nigra echogenic signals relative to others in this sample may be at increased risk of "converting" to PD.[54] Larger and longitudinal studies are needed to better evaluate substantia nigra hyperechogenicity as a possible prodromal/predictive marker of PD in patients with 22q11.2DS.

Nigrostriatal dysfunction in 22q11.2DS

We investigated nigrostriatal functioning for the first time in patients with 22q11.2DS using ^{11}C-DTBZ PET neuroimaging. ^{11}C-DTBZ binds to VMAT2 sites on presynaptic dopamine storage vesicles (1 to 3 sites per synaptic vesicle[362,363]). VMAT2 binding is an established index of striatal dopamine neuron density in other populations,[317-320] based on evidence that striatal VMAT2 levels are a linear function of the number of dopaminergic neurons in the substantia nigra *pars compacta*,[322,329,330] its strong correlation with striatal levels of the dopamine transporter, another presynaptic dopamine terminal marker, in healthy subjects,[330] and its robustness to both disease and drug compensatory regulation mechanisms.[48-50,322] Patients diagnosed with PD reliably show severely reduced striatal ^{11}C-DTBZ binding, reflecting the neurodegenerative loss of dopamine synaptic terminals in the striatum in PD.[317-320] Indeed, the single 22q11.2DS patient diagnosed with early-onset PD in this study showed this expected pattern of severely reduced striatal ^{11}C-DTBZ binding. This was consistent with the severe striatal dopamine denervation described in other reports of patients with early-onset 22q11.2DS-PD, including our own neuropathological study of three patients[252] and a report of a single 22q11.2DS-PD patient using dopamine transporter imaging with single-photon emission computed tomography (SPECT).[144]

Contrary to our hypothesis, patients with 22q11.2DS without PD showed substantially elevated striatal ^{11}C-DTBZ binding relative to healthy age and sex-matched controls. This result is consistent with an increased availability of presynaptic dopamine storage vesicles and is suggestive of some form of dysfunction of dopaminergic neurons in 22q11.2DS that is pre-morbid to PD. Notably, all three anatomo-functional subdivisions of the striatum were similarly affected, with ^{11}C-DTBZ binding increased relative to controls by ~27% in the sensorimotor striatum (posterior putamen), ~26% in the associative striatum (anterior putamen and caudate

nucleus), and ~24% in the limbic (ventral) striatum. These novel findings suggest that baseline dopaminergic neurotransmission in the basal ganglia circuitry is likely to be disturbed in 22q11.2DS. These results were unexpected because motor signs of PD, common in this group of 22q11.2DS patients, typically become apparent only after striatal dopamine loss has occurred in idiopathic PD.[17,18] Moreover, asymptomatic *LRRK2* mutation (associated with late-onset PD) carriers[364,365] and clinically at risk individuals with REM sleep behaviour disorder[366] have shown reduced striatal ^{11}C-DTBZ binding consistent with prodromal loss of striatal presynaptic dopaminergic terminals.

Exploration of the effects of abnormal ^{11}C-DTBZ binding on motor functioning in patients with 22q11.2DS revealed that lower striatal ^{11}C-DTBZ binding was linearly correlated with increased severity of bradykinesia, but not with other clinical features. This relationship is consistent with results of a similar PET study using the ^{11}C-DTBZ radioligand in early stage untreated idiopathic PD,[319] as well as studies using other presynaptic dopaminergic markers in PD.[319,367-369] Together, these studies support the notion that the severity of bradykinesia, a dopa-responsive symptom of PD, is directly related to the (underlying) dopaminergic deficits typical of PD.[367-369] Notably, we found that the correlation between reduced ^{11}C-DTBZ binding and increased bradykinesia severity in 22q11.2DS was specific to the sensorimotor striatum (posterior putamen), the region of the striatum first affected by dopaminergic terminal loss in idiopathic PD.[336] This suggests that a typical progressive loss of striatal dopamine neurons may occur in 22q11.2DS prior to the onset of clinically manifest PD, even though baseline ^{11}C-DTBZ binding appears to be elevated. Severity of bradykinesia may therefore represent a possible clinical marker of early striatal neurodegeneration and increased PD risk in patients with 22q11.2DS.

Bradykinesia generally does not manifest in idiopathic PD until significant nigrostriatal degeneration has occurred.[17,18] The results of our initial cross-sectional study could suggest that the early stages of dopaminergic degeneration in 22q11.2DS may be masked by higher pre-morbid presynaptic dopamine levels. In this scenario the relative loss of dopamine from a higher baseline level would lead to the manifestation of bradykinesia in 22q11.2DS. Interestingly, the 22q11.2DS patient (Case 14, age 54 years with severe hyposmia) with the highest bradykinesia subscore on the MDS-UPDRS Motor Exam and the lowest ^{11}C-DTBZ binding of the entire non-PD but at risk 22q11.2DS group, has shown progressive parkinsonism (onset age 48 years; H&Y stage 2) with functional and cognitive decline. We have reported a detailed clinical history of this

case elsewhere (Boot, Butcher et al., 2015).[7] The patient with the second highest bradykinesia score (Case 5, age 38 years with mild hyposmia), and within the lower bound for ^{11}C-DTBZ binding in the 22q11.2DS group (fourth lowest, and the youngest amongst these four), also showed parkinsonism (H&Y stage 2). Longitudinal studies are necessary to evaluate the putative relationship between ^{11}C-DTBZ binding levels and bradykinesia severity in 22q11.2DS to determine if bradykinesia severity itself, or perhaps more likely bradykinesia progression/worsening, may be a useful clinical predictor of PD in 22q11.2DS. Other non-neurodegenerative factors are also likely to be involved in the manifestation of bradykinesia and other motor symptoms in 22q11.2DS (discussed below).

Possible mechanisms for increased ^{11}C-DTBZ binding in 22q11.2DS

We identified an abnormal pattern of ^{11}C-DTBZ binding in patients with 22q11.2DS relative to healthy controls, indicative of increased availability of presynaptic dopamine storage vesicles. This finding could potentially be the consequence of excess striatal dopaminergic innervation in this genetic syndrome.[322,329,330] The linear correlation between decreased levels of ^{11}C-DTBZ binding and increased bradykinesia severity in the sensorimotor striatum in patients with 22q11.2DS, as reported in patients with idiopathic PD,[319] and the expected gross reduction of ^{11}C-DTBZ in the patient with diagnosed 22q11.2DS-PD, provide some support for ^{11}C-DTBZ as an index of dopaminergic terminal density in patients with 22q11.2DS. Confirmation of this finding awaits the use of other additional markers of dopaminergic functioning in 22q11.2DS neuroimaging studies, such as the dopamine transporter. Neuropathological studies to measure dopamine neuron numbers in patients with 22q11.2DS who have not developed PD would be helpful. Nevertheless, there are several other possible factors that might influence the high levels of ^{11}C-DTBZ binding in 22q11.2DS that warrant consideration.

First, elevated levels of ^{11}C-DTBZ in 22q11.2DS could reflect an increase in the number of dopamine storage vesicles per synaptic terminal (i.e., via more densely packed vesicles or larger synaptic terminals), rather than increased density of synaptic terminals in 22q11.2DS. We cannot exclude this possibility in the absence of neuropathological study of patients with 22q11.2DS without a diagnosis of PD. Under normal conditions, synaptic vesicle function is tightly controlled by the movement of a relatively stable number of vesicles between the active and reserve synaptic vesicle pool rather than the addition or removal of synaptic vesicles.[370] Notably,

presynaptic vesicle counts in hippocampal neurons in a mouse model of 22q11.2DS were typical.[371]

Second, elevated ^{11}C-DTBZ binding could be due to upregulation of VMAT2 expression. VMAT2 expression does not appear to be easily modified, however. Unlike other presynaptic dopaminergic markers (i.e., dopamine transporter binding and fluorodopa uptake), VMAT2 is not thought to be subject to regulatory or compensatory changes in expression.[372] The number of VMAT2 sites per synaptic vesicle appears to be relatively stable.[362,363] Animal studies have shown no effects of standard antiparkinsonian, antipsychotic, and serotonin reuptake inhibitor medications on VMAT2 expression.[48,373-375]

Third, multiple previous studies have shown that ^{11}C-DTBZ binding is sensitive to altered intravesicular dopamine levels (but not dopaminergic alterations elsewhere in the synapse, e.g., dopamine D2 receptor blockade with antipsychotic medications),[48,322,376] thought to be due to competition with intravesicular dopamine for the same binding site on VMAT2.[332,377-380] Elevated ^{11}C-DTBZ binding has been reported previously in two populations known to have a low intravesicular dopamine concentration. A small study of five adults with dopa-responsive dystonia showed a ~17-21% increase in striatal ^{11}C-DTBZ binding relative to healthy controls.[377] Also, a sample of recently abstinent methamphetamine users (n=16), a group investigated for possible dopamine neuron loss from methamphetamine use, demonstrated an ~11-22% increase in striatal ^{11}C-DTBZ binding. The 24-27% mean increase in binding over control values identified here in 22q11.2DS is comparable to or even higher than these examples. None of the patients with 22q11.2DS participating in this study had a history of using methamphetamines, or any other amphetamine derivatives, and none had a family history of, or signs of, dopa-responsive (or other) dystonia. Whole-genome sequencing results (Chapter 4) available for two participants, including the one with the highest ^{11}C-DTBZ binding in the 22q11.2DS group, revealed no rare sequencing or structural variants involving the genes for dopa-responsive dystonia (*GCH1*, *TH*) or involving the VMAT2 gene itself (*SLC18A2*).[253]

As far as we are aware, there is no evidence to indicate that hemizygosity of the 22q11.2 deletion region confers a reduction in presynaptic vesicular dopamine concentration. Post-synaptic striatal dopamine D_2 receptor binding was not found to be abnormal in a resting state study of 12 non-psychotic adults with 22q11.2DS (without PD; aged 18-39 years) relative to matched controls as assessed with single-photon emission computed tomography using [^{123}I]IBZM.[381] This result

suggests normal basal dopaminergic neurotransmission in 22q11.2DS. Furthermore, one would expect poor tolerability to antipsychotic treatments with a high affinity to, and slow dissociation from, the dopamine D_2 receptor if patients with 22q11.2DS had depleted vesicular dopamine levels. While there is some evidence for overall sensitivity to neurological side effects to antipsychotic medications in 22q11.2DS, demonstrated for seizures and myoclonus related to the atypical antipsychotic clozapine,[7,256] this does not appear to be the case for most patients with 22q11.2DS.[5] Also, fluphenazine, zuclopenthixol, and risperidone were all used to successfully treat patients participating in this study.

Fourth, increased ^{11}C-DTBZ binding might reflect structural brain differences in 22q11.2DS patients, which could influence parameter measurements (BP_{ND}). Variable and often subtle structural brain abnormalities have been reported in patients with 22q11.2DS.[210,382,383] The volume of the caudate may be slightly enlarged in children with 22q11.2DS[384-387] though this was not found in an adult population,[388] nor was caudate volume significant in a meta-analysis of 22q11.2DS MRI studies.[382] No volumetric differences have been reported in the putamen,[384,388] though smaller occipital volumes have been reported.[382] We do not expect that any minor structural brain differences in patients with 22q11.2DS relative to controls would account for the large differences in ^{11}C-DTBZ binding potential observed in this study. Moreover, partial volume effects correction on time activity curve data using the Rousset algorithm[348] similarly resulted in greater striatal VMAT2 binding in the 22q11.2DS patients relative to controls. We found no difference in non-specific binding levels in the reference occipital region between patients and controls that might have affected our results. Notably, there were no gross structural abnormalities in the striatum or the occipital lobe in patients in this study.

Motor dysfunction in the context of 22q11.2DS

We provide here a comprehensive description of motor functioning in 22q11.2DS prior to the onset of PD that may help inform clinical care of the growing adult 22q11.2DS population and lead to the identification of predictive markers of PD for patients with the 22q11.2 deletion. We identified a high prevalence of motor features in adults with 22q11.2DS, most notably bradykinesia and postural and/or kinetic tremor. At most, a small subset of these individuals would be expected to progress to PD given the strongly reduced penetrance of PD in 22q11.2DS.[252] These findings indicate that a spectrum of motor dysfunction may be part of the

variable clinical presentation of 22q11.2DS in adulthood, in keeping with the variable expressivity of other 22q11.2DS phenotypes.[5]

Additionally, 22q11.2DS-associated conditions and their associated treatments, most notably schizophrenia and neuroendocrine disorders, may contribute to a spectrum of motor impairment in adulthood. These will require careful study to evaluate their relative contribution to the neurological presentation of adults with 22q11.2DS.[5,7,256] A general neurological exam may be useful to assess the potential impact of effects of other possible neurological abnormalities. We note that all six of the non-psychotic patients in this study showed some degree of bradykinesia including three assessed with a movement disorder diagnosis (a 30 year old with postural/kinetic tremor,[7] a 42 year old with parkinsonism, and a 51 year old with both of these diagnoses). Treated hypocalcemia and hypothyroidism did not appear to have any major effect on the motor symptoms observed in patients in this study. Other medications used to treat patients with 22q11.2DS (e.g., valproic acid and phenytoin for seizure management, lithium for mood stabilizing treatment, and serotonin reuptake inhibitors for mood disorders) may also infrequently cause parkinsonism or other motor abnormalities.[389] Additional studies will be necessary to delineate the spectrum and aetiology of motor dysfunction in adults with 22q11.2DS.

Possible implications of olfactory deficits in 22q11.2DS

The results of this study add to the limited data on olfactory functioning in adulthood in 22q11.2DS. We previously reported low scores on the UPSIT in 18 adults with 22q11.2DS as part of a larger neurocognitive study.[390] To our knowledge, olfaction has otherwise remained unexamined in adults with 22q11.2DS. Interestingly, we found absent or mild hyposmia in the 22q11.2DS patients without parkinsonism, moderate to severe hyposmia in six of the eight patients with parkinsonism, and that the patient with 22q11.2DS-PD was anosmic. These results suggest that olfactory dysfunction could be a progressive marker of PD risk in 22q11.2DS. Olfactory deficits appear to be frequent in children and adolescents with 22q11.2DS.[391,392] This raises the question as to whether olfactory dysfunction is a feature of the syndrome itself and/or could be a predictive marker of early-onset PD. A study of olfaction in 39 children with 22q11.2DS (aged 5 to 15 years)[391] with the UPSIT revealed olfactory deficits in a similar (68%) proportion of subjects than that reported here in our adult sample (77%). The only other study of

olfaction in 22q11.2DS children (n=27, aged 7 to 16 years) used a different olfactory assessment tool, “Sniffin Sticks”, and reported global olfactory dysfunction in 22q11.2DS children relative to matched controls.[392] Olfactory impairment was not associated with any of 22q11.2DS comorbidities examined (lower intellect, psychiatric disease, velopharyngeal insufficiency, or otorhinolyrngologic problems) suggesting an independent pathophysiological mechanism underlying olfactory deficits in 22q11.2DS.[392] Additional studies are needed to define the relationship between parkinsonism and olfactory dysfunction in 22q11.2DS.

Advantages and limitations

The results of this study provide the first assessment of prodromal markers of PD in patients at risk of 22q11.2DS-PD. The study of patients at increased risk of developing PD, such as those with the 22q11.2 deletion, can provide a unique opportunity to investigate early biomarkers of the disease and gain insights into the natural history of the disease. Longitudinal assessment of our study participants will help inform the interpretation of the results of this initial cross-sectional study.

Neurological assessments were not able to be completed blind to 22q11.2 deletion status as 22q11.2DS patients are clinically identifiable (e.g., due to hypernasal speech and mild dysmorphic features). However, all assessments were performed blind to PET imaging results. The correlation between the severity of ^{11}C-DTBZ binding and bradykinesia on PET imaging suggests that the lack of blindness to assess 22q11.2DS status did not influence motor ratings in a systematic way. It is possible that patients with 22q11.2DS with some motor problems were more likely to volunteer for participation in this study. This may have influenced the high proportion of patients with motor dysfunction observed in this study.

Our study was limited by our sample size. Even in this relatively small sample, however, pronounced differences in motor functioning and ^{11}C-DTBZ binding were detectable relative to controls. Larger samples of adults with 22q11.2DS may reveal differences of smaller effect size. The number of participants was typical for a PET neuroimaging study. We included the patient with diagnosed PD as a measure of advanced disease state in our correlation analyses to probe the initial relationship between bradykinesia severity and ^{11}C-DTBZ binding in the 22q11.2DS group without PD. Notably, we observed a similar linear trajectory upon the exclusion the 22q11.2DS-PD patient. PET assessment of a larger number of 22q11.2DS patients without a

diagnosis of PD but high bradykinesia scores could help clarify this relationship.

The functional consequences of elevated binding of ^{11}C-DTBZ in the context of other 22q11.2DS neurophenotypes remain to be determined but offer exciting new avenues for studying the cognitive and neuropsychiatric manifestations of the syndrome.[5,146] Cognitive deficits as well as neuropsychiatric disorders and behavioural disturbances are known to be common in patients with 22q11.2DS (e.g., schizophrenia, anxiety disorders, temper outbursts).[129,134] One or more of these features may be related to the excess presynaptic dopaminergic function identified in this study, given the involvement of the striatum in cognitive, emotion, and decision-making behaviours.[393] However, these features were not a focus of this motor-oriented study.[5,146] We observed a correlative trend between lower ^{11}C-DTBZ binding and worse global cognition scores as measured with the MoCA. There was no correlation of this binding, however with lifetime IQ scores. These results could be consistent with a possible pathophysiological relationship between dopaminergic disturbance and prodromal changes in cognitive functioning related to PD[73-75] in 22q11.2DS and/or the cognitive decline reported in some patients with 22q11.2DS.[394-397] Comprehensive longitudinal evaluation of cognition using more sensitive measures may help identify putative relationships between high ^{11}C-DTBZ binding levels and the possible effects of the onset of PD on cognitive function in 22q11.2DS.

Additional studies using larger samples will be necessary to determine if elevated ^{11}C-DTBZ binding in 22q11.2DS is related to neuropsychiatric symptoms. Schizophrenia was present in five of the 14 in the 22q11.2DS group. Two others had a psychotic mood disorder. The single study published to date that evaluated striatal ^{11}C-DTBZ binding in patients with treated idiopathic schizophrenia (n=12) showed no difference relative to controls, however,[398] in keeping with a recent meta-analysis of dopamine transporter binding studies that found no evidence of altered density of dopamine terminals in schizophrenia.[274] Also, we found here that ^{11}C-DTBZ binding levels were similar between 22q11.2DS patients with and without psychosis. The relationship to symptom severity and other psychiatric disorders in 22q11.2DS should be explored in future studies. There is a large animal literature showing no significant effects of standard antipsychotic, antidepressant or anticholinergic treatments, or of other monoaminergic manipulations, on ^{11}C-DTBZ binding.[48-50,322,373,375,376,399] Research into possible medication effects on VMAT2 has not been exhaustive, however, and we cannot exclude the possibility that

long-term use of psychotropic medications may have influenced ^{11}C-DTBZ binding levels in the 22q11.2DS patients.

Conclusions

Adults with 22q11.2DS ≥30 years, who are at elevated risk of developing early-onset PD due to hemizygosity of the 22q11.2 deletion region, commonly exhibit motor and olfactory deficits that are known prodromal features of PD. Elevated levels of striatal ^{11}C-DTBZ binding, a radioligand for presynaptic dopamine vesicles and index of dopaminergic innervation, in patients with 22q11.2DS indicate that this patient population is affected by a unique baseline dopaminergic abnormality that appears to precede the onset of the dopaminergic denervation of PD. Severity of bradykinesia may represent a possible clinical marker of early striatal neurodegeneration and increased risk of progression to PD in patients with 22q11.2DS. Longitudinal studies are needed to further elucidate these initial findings. Patients with 22q11.2DS represent a unique, clinically identifiable, population for the study of PD biomarkers, disease progression, and the potential for identifying effective neuroprotective interventions.

Chapter 6 Safety and efficacy of antipsychotic treatment in 22q11.2 deletion syndrome

6 Overview

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ABSTRACT

Background: Genetic testing in psychiatry promises to improve patient care through advances in personalised medicine. However there are few clinically relevant examples. **Aims:** To determine whether patients with a well-established genetic subtype of schizophrenia show a different response profile to the antipsychotic clozapine than those with idiopathic schizophrenia **Method:** We retrospectively studied the long term safety and efficacy of clozapine in 40 adults with schizophrenia, half with a 22q11.2 deletion (22q11.2DS-Schizophrenia) and half matched for age and clinical severity but molecularly confirmed to have no pathogenic copy number variant (Idiopathic-Schizophrenia). **Results:** Both groups showed similar clinical improvement and significant reductions in hospitalisations, achieved at a lower median dose for those with 22q11.2DS-Schizophrenia. Most common side effects were similarly prevalent between the two groups, however, half of the 22q11.2DS-Schizophrenia group experienced at least one rare serious adverse event compared with none of the Idiopathic-Schizophrenia group. Many were successfully retried on clozapine. **Conclusions:** Individuals with 22q11.2DS-Schizophrenia respond as well to clozapine treatment as those with other forms of schizophrenia, but may represent a disproportionate number of those with serious adverse events, primarily seizures.

Lower doses and prophylactic (for example anticonvulsant) management strategies can help ameliorate side effect risks. This first systematic evaluation of antipsychotic response in a genetic subtype of schizophrenia provides a proof-of-principle for personalised medicine and supports the utility of clinical genetic testing in schizophrenia.

6.1 Introduction

The advent of molecular diagnostics with the potential for clinical genetic testing in psychiatry has introduced the possibility of providing personalized treatments for patients with schizophrenia.[400] Through selection of effective and well-tolerated treatments, such advances promise to significantly reduce patient, family, and economic burden of illness. Pharmacogenomic studies of response to antipsychotic medications have been largely hampered, however, by the complex genetic etiology of schizophrenia.[213,400] Although initial studies have suggested common genetic variants that may mediate response to antipsychotic treatments, small effect sizes and difficulties in replicating across patient groups limit clinical utility.[400-402]

As an alternative, the well-established association between schizophrenia and 22q11.2 deletion syndrome (22q11.2DS) provides an opportunity to investigate the utility of a molecular diagnosis in studying treatment response in schizophrenia. The hemizygous 22q11.2 deletions associated with 22q11.2DS are found in up to one of every 100 individuals with schizophrenia,[214,403,404] representing the most highly replicated molecular subtype of schizophrenia to date.[149,214,219] Individuals with 22q11.2DS are clinically identifiable (e.g. other major features include dysmorphic features, congenital heart defects, and hypernasal speech), and standard testing for the 22q11.2 deletion is available at clinical laboratories.[146] The schizophrenia of 22q11.2DS (22q11.2DS-Schizophrenia) is essentially indistinguishable from the heterogeneous forms of schizophrenia found in the general population with respect to prodrome, age at onset, and core signs and symptoms.[149,215,219-221,405] Although previous case reports describe some challenges,[142,207-209,249,251,406] standard antipsychotic management and clinical practice guidelines for schizophrenia are currently recommended for patients with 22q11.2DS-Schizophrenia.[146]

Clozapine is an effective atypical antipsychotic with a low risk of extrapyramidal side effects; rare but serious side effects however largely restrict its use to treatment-resistant patients.[230,407,408] In this study, we compared the response and safety of clozapine treatment in individuals with 22q11.2DS-Schizophrenia and idiopathic schizophrenia (Idiopathic-Schizophrenia), where there was no 22q11.2 deletion or other clinically pathogenic copy number variation. We used a long-term observational retrospective design (median > 5 years) including comprehensive medical chart reviews and standard assessment methods.[409]

6.2 Methods

Participants

The initial 22q11.2DS cohort comprised 184 Canadian adults with 22q11.2DS. Participants were ascertained through adult congenital cardiac, psychiatric, and genetic services using active screening and/or clinical referrals.[148,261] Of the 66 with schizophrenia, we identified 21 (31.8%) unrelated individuals who had been treated with clozapine, of whom one patient was deceased but had ample data available for study. We excluded one individual who received two test doses of clozapine (total 18.75 mg) over a two day period immediately before death secondary to cardiovascular failure that was unrelated to clozapine initiation.[252] This yielded a sample of 20 patients with 22q11.2DS molecularly confirmed to have a chromosome 22q11.2 deletion using standard clinical genetic testing (n=19 by fluorescence *in situ* hybridization and probe from the deletion region, n=1 by clinical microarray).[147,156]

The Idiopathic-Schizophrenia group was ascertained from a Canadian community mental health sample, primarily recruited from a single clinic, who had direct clinical assessments for potential genetic syndromic features using a standardized protocol and high resolution genome-wide research microarrays.[121,148] Of 362 individuals, 42 (11.6%) were identified as ever having had a trial of clozapine, 31 of whom were potentially eligible for the current study (eight had no microarray results at study initiation and three were deceased; these 11 excluded individuals appeared comparable to those studied e.g., predominantly Caucasian males, median age at onset 22 years; clozapine initiated at median 31 years). A further 11 were excluded: four had a large (>500 kb) rare copy number variation (1q21.1 duplication, 8p23.1 deletion, 15q11-15q13 duplication, 16p11.2 duplication) independently deemed "pathogenic" by two clinical cytogenetic laboratory directors using standard criteria,[121,410] five had insufficient medical records, one was related to another patient, and one had syndromic characteristics.[121,148] This yielded a sample of 20 unrelated Idiopathic-Schizophrenia participants.

All participants met DSM-IV criteria[2] for schizophrenia or schizoaffective disorder (n=1 with schizoaffective disorder in the 22q11.2DS-Schizophrenia group, n=2 for Idiopathic-Schizophrenia).[390] Age at onset (AAO) was defined as the age at first treatment of psychosis. Intellectual disability was determined for the 22q11.2DS group using DSM-IV criteria.[2,220] None

of the Idiopathic-Schizophrenia group had a pediatric diagnosis of intellectual disability (Table 6-1); 11 had a history of non-specific learning difficulties or had failed at least one grade. Informed consent was obtained in writing, and the study was approved by local research ethics boards.

Assessment of clozapine efficacy and safety: To assess clozapine-related changes in psychiatric behaviors and functioning and adverse events, we used comprehensive lifetime clinical summaries compiled for each patient using medical records, extensive clinical histories, and semi-structured interviews. Patients and/or a caregiver or individual well-acquainted with the patient were contacted by telephone or in-person for follow-up regarding the patient's experiences with clozapine. We recorded: demographic data, psychiatric diagnoses, symptoms and hospitalizations, proxy measures of functioning (e.g. housing, employment, relationships, financial support), co-morbid medical conditions, smoking history, medications and doses, and adverse effects. Updated (to 2013) clinical information was available for the majority (n=35, 87.5%) of the 40 participants. One patient had died in the 22q11.2DS group and two individuals in each group were unavailable for follow-up.

The clozapine maintenance dose was defined as the most recent dose for patients still on clozapine. For patients who had discontinued clozapine, the last dose before tapering for cessation began, or before medication non-adherence was clearly documented, was considered the maintenance dose. For patients with more than one clozapine trial, the maintenance dose was determined for the trial of longest duration. Adjunct antipsychotics used during clozapine treatment at the maintenance dose were recorded at the most recent dose. The highest clozapine dose was recorded for the patient's lifetime experience with clozapine. Four 22q11.2DS-Schizophrenia patients were prescribed clozapine by study authors, ASB (n=2), WLAF (n=1), and EWCC (n=1).

The Clinical Global Impression (CGI) Scale was used to retrospectively evaluate mental illness severity and improvement following a clozapine trial, blind to group status.[409] For CGI evaluation, the 40 comprehensive clinical summaries were prepared using identical formatting and careful blinding to 22q11.2 deletion status, including the removal of all 22q11.2DS associated phenotypes (e.g. intellectual disabilities, congenital defects, speech therapy, hypocalcemia, etc.). The trial of longest duration was evaluated for patients with more than one

clozapine trial. The CGI-severity (CGI-S) scale was used to rate the severity of the patient's mental illness from 1 (normal) to 7 (among the most severely ill) at the time of clozapine initiation. The CGI-improvement (CGI-I) scale was used to assess total improvement compared with baseline, with 1 being very much improved, 2 much improved, 3 minimally improved, 4 no change, and 5–7 representing minimally, much, and very much worse, respectively. The CGI scales were completed by two psychiatrists (WLAF, ASB) for all 40 patients in randomized order and assessed for inter-rater reliability using intraclass correlation coefficients.[411] The CGI raters had no involvement in the patient chart reviews, data extraction, and patient/caregiver follow-up contacts conducted for the purposes of this study.

The average number of psychiatric hospitalizations per year was calculated for each person while on and off clozapine. Both psychiatric-related visits to the emergency room and hospital admissions were classified as a psychiatric hospitalization. Time off clozapine was considered the time from first psychiatric hospitalization until the start of the clozapine trial, and summed with other time periods off clozapine for patients with more than one trial or where the medication had been discontinued permanently. For patients where the precise start or end date of clozapine was unclear, the midpoint date between the last confirmed dates on or off clozapine was used.

Statistical analyses

To compare demographic and clinical variables, we used Fisher's exact tests for independent categorical variables and the non-parametric Mann-Whitney U test for independent continuous variables to limit bias from non-normal distributions. McNemar's exact test and the Wilcoxon signed rank sum test were used to assess paired categorical and continuous variables, respectively. Odds ratios for adverse events were calculated using Cochran-Mantel-Haenszel statistics. A 0.5 zero-cell correction was used, as necessary. Intraclass correlation coefficients were calculated using a two-way mixed model[411] using the "INTRACC" macro developed for SAS by Hamer.[412] An intraclass correlation coefficient equal to 0 represents inter-rater agreement equivalent to that expected by chance, while 1 represents perfect agreement. The following intraclass correlation coefficient interpretation scale was used:[413] poor to fair (≤0.4), moderate (0.41–0.60), excellent (0.61–0.80), and almost perfect (0.81–1). All statistical analyses

were two-tailed and performed with SAS version 9.2 software (SAS Institute, Cary, NC; Microsoft Windows XP), with statistical significance defined as $P<0.05$.

6.3 Results

Demographic and clinical characteristics are presented in Table 6-1. The 22q11.2DS-Schizophrenia group had significantly lower intellectual level and fewer smokers, and non-significantly fewer Caucasians and more women. All but three of the 40 patients were classified as markedly or severely ill at the time of initiation of a clozapine trial (Table 6-1). The median number of antipsychotics (treatment with a different antipsychotic medication and/or the addition of an adjunct antipsychotic) prior to clozapine initiation was high in both groups (22q11.2DS-Schizophrenia, 5, range 2-10; Idiopathic-Schizophrenia, 4.5, range 1-21; z=-0.62, p=0.54).

Dosing and therapeutic response

The median maintenance clozapine dose was significantly lower in the 22q11.2DS-Schizophrenia group (250, range 50-425 mg, n=19) compared with the Idiopathic-Schizophrenia group (400, range 50-600 mg, n=20, z=-3.08, p=0.002; Figure 6-1). The median dose of clozapine of the four smokers in the 22q11.2DS-Schizophrenia group was 268.75 mg. There were no significant differences between groups in the total time spent on clozapine, the length of the longest clozapine trial (median > 5 years for both groups), or frequency of adjunct antipsychotic treatment at maintenance dose (Table 6-1). Adjunct antipsychotic treatments for six individuals with 22q11.2DS-Schizophrenia were: chlorpromazine (50 mg) with methotrimeprazine (15 mg), flupentixol (4.5 mg), haloperidol (2.5 mg), quetiapine (37.5 mg), and risperidone (n=2, 0.5 mg and 4 mg). For five individuals in the Idiopathic-Schizophrenia group, adjunct antipsychotics were: flupentixol decanoate injection (150 mg/ml every four weeks), perphenazine (48 mg), risperidone (2 mg), and olanzapine (n=2, 10 mg and 20 mg).

The 22q11.2DS-Schizophrenia and Idiopathic-Schizophrenia groups responded well to clozapine treatment with the majority of both groups rated as much or very much improved (Figure 6-2). There was similar improvement (Fisher's exact test, p=0.33) as rated using the CGI-I with excellent inter-rater reliability (intraclass correlation coefficient=0.65). For those maintained on

Table 6-1 Demographics and clinical characteristics and response to clozapine of 20 adults with 22q11.2DS-Schizophrenia and 20 with idiopathic schizophrenia

	22q11.2DS-Schizophrenia (n=20)		Idiopathic-Schizophrenia (n=20)		Analyses	
	N	%	N	%	Fisher's exact test *P*	
Gender (male)	11	55.0	17	85.0	0.08	
Ethnicity (Caucasian)	15	75.0	20	100.0	0.05	
Serious congenital heart defect	2[a]	10.0	0	0.0	0.49	
Smoker	4	22.2[b]	16	94.1[b]	**<0.0001**	
Intellect					**<0.001**	
Average to borderline	9	45.0	20	100.0		
Mild intellectual disability	9	45.0	0	0.0		
Moderate to severe intellectual disability	2	10.0	0	0.0		
Clinical Global Impression severity (baseline rating)[c]					0.58	
Moderately ill	2	10.0	1	5.0		
Markedly ill	13	65.0	16	80.0		
Severely ill	5	25.0	3	15.0		
Treatment with adjunct antipsychotic	6	30.0	5	25.0	1.00	
	Median	Range	Median	Range	*z*	*P*
Age at last follow-up (years)	40.5	23-53	46	22-58	-1.41	0.16
Age at onset of schizophrenia (years)	21	14-29	21.5	17-39	-0.42	0.67
Clozapine						
Age at start (years)	31.1	18.7-42.0	33.0	20.0-46.0	-1.00	0.32
Total time on clozapine (years)	6.2	0.1-17.7	9.1[d]	0.09-18.2	0.41	0.68
Longest trial (years)	5.9	0.1-17.7	9.1[d]	0.09-18.2	0.80	0.42
Maintenance dose (mg)	250[d]	50-425	400	50-600	-3.08	**0.002**
Maximum dose (mg)	325[d]	50-575	425	112.5-600	-2.06	**0.04**

[a]Tetralogy of Fallot, n=1; Interrupted aortic arch type B, n=1
[b]Lifetime history; 22q11.2DS-Schizophrenia, n=18; Idiopathic-Schizophrenia, n=17
[c]Moderate inter-rater reliability (intraclass correlation coefficient=0.49)
[d]N=19

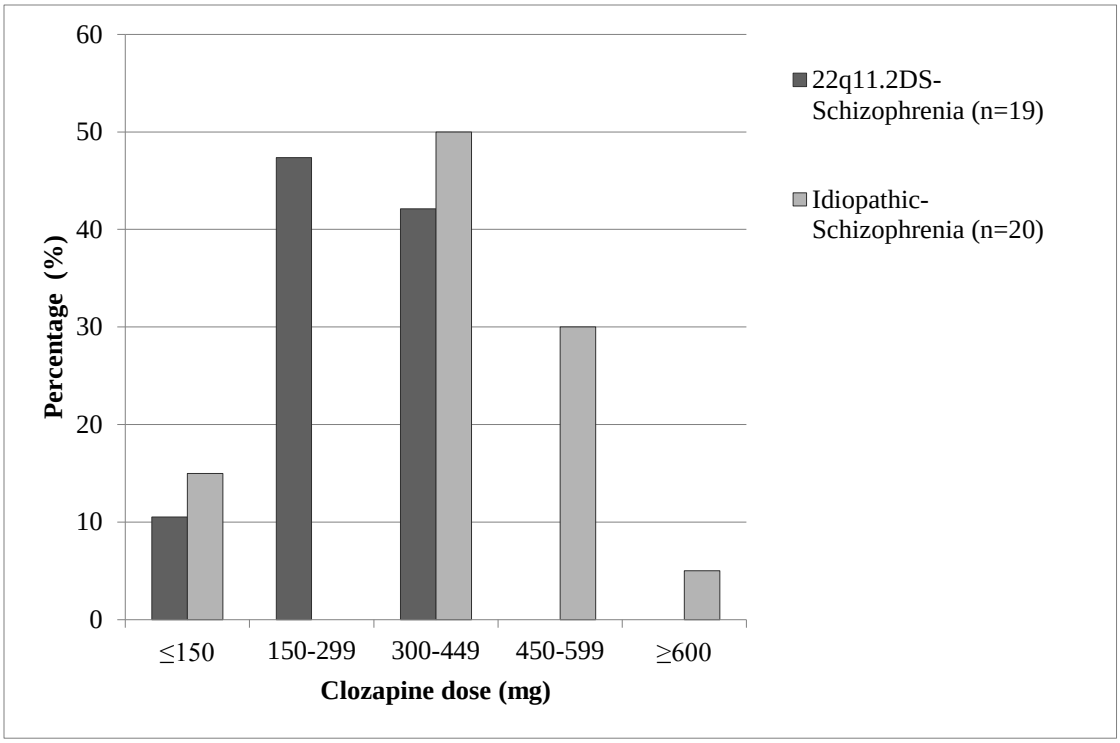

Figure 6-1 Patients with 22q11.2DS-Schizophrenia are maintained on a significantly lower therapeutic dose of clozapine than patients with idiopathic schizophrenia (*p*=0.002)

The two 22q11.2DS-Schizophrenia patients with doses ≤ 150 mg had been maintained in this range for at least one year; one was receiving adjunct antipsychotic flupentixol (4.5 mg) treatment. Of the three Idiopathic-Schizophrenia patients on a clozapine dose ≤ 150 mg, one was maintained on this dose for four years; the other two discontinued within nine and 28 weeks, respectively (the latter on adjunct antipsychotic perphenazine (48 mg) treatment) and were not titrated above this low dose range.

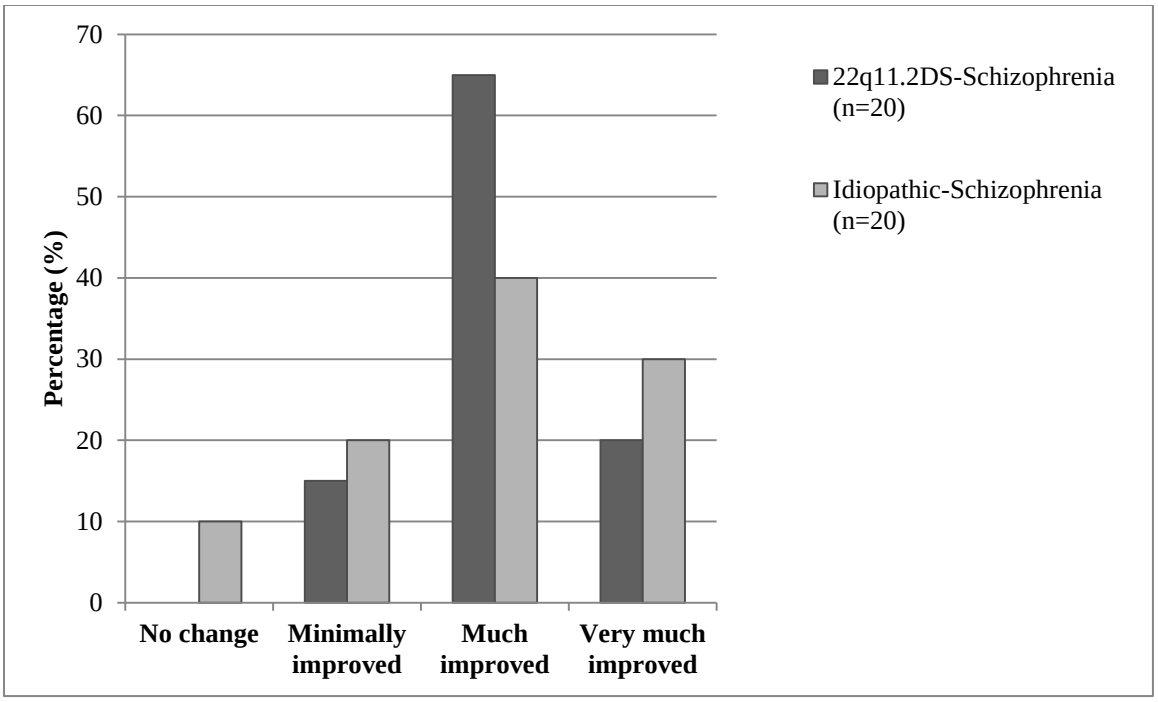

Figure 6-2 Clinical Global Impression-Improvement scale ratings for patients with 22q11.2DS-Schizophrenia and idiopathic schizophrenia

Therapeutic response to clozapine of patients with 22q11.2DS-Schizophrenia is similar to patients with idiopathic schizophrenia (p=0.33). One of the two patients with no change corresponds to a patient in the Idiopathic-Schizophrenia group with a clozapine dose ≤ 150 mg who discontinued within 28 weeks of treatment.

clozapine for at least 12 consecutive weeks, the median number of psychiatric hospitalizations standardized per year was significantly reduced for patients with 22q11.2DS-Schizophrenia (n=18; median, 0.0, range 0.0-1.90) compared with other antipsychotics (median, 0.73, range 0.15-6.35, Signed rank statistic (*S*)=66.5, *p*=0.002). Results were similar in the Idiopathic-Schizophrenia group (n=17; median, 0.09, range 0.0-4.15 on clozapine; median, 1.09, range 0.23-2.03 on other treatment; Signed rank statistic (*S*)=44.5, *p*=0.04). There was no significant between-group difference in reduction of hospitalizations (*z*=0.02, *p*=0.99).

Adverse effects

Common side effects of clozapine, including drowsiness/sedation, weight gain, and hypersalivation were reported in both groups in similar proportions (Table 6-2). However, even though clozapine treatment was at a higher dose and for a longer duration in the Idiopathic-Schizophrenia group overall than the 22q11.2DS-Schizophrenia group, the latter had a higher prevalence of uncommon side effects. Half of the 22q11.2DS-Schizophrenia group (n=10) experienced at least one serious adverse event compared with none of the idiopathic group (gender-adjusted OR=16.5, 95% CI=1.8-149.8), comprising myocarditis (n=1), severe neutropenia (n=3), and seizures (n=8).

One (5%) 22q11.2DS-Schizophrenia patient (25 year old Caucasian male) with no congenital heart defect developed myocarditis approximately three weeks after starting on clozapine (Table 6-2). Adjunct antipsychotic treatment included chlorpromazine and methotrimeprazine. A cardiologist diagnosed myocarditis following admission to hospital with dilated, akinetic cardiomyopathy and congestive heart failure with a two day history of chest tightness. The patient's symptoms resolved rapidly with discontinuation of clozapine (250 mg). A viral cause was considered unlikely and thus the discharge diagnosis was clozapine-associated myocarditis.

There were three confirmed cases of severe "red zone" neutropenia (15%), defined as a white blood count (WBC) $<2.0 \times 10^9$/L or absolute neutrophil count (ANC) $<1.5 \times 10^9$/L reported in mandatory monitoring, in Caucasian female patients aged 26 (Patient 1), 37 (Patient 2), and 39 (Patient 3) years with 22q11.2DS (Table 6-2). None were treated with other medications clearly associated with agranulocytosis (e.g. carbamazepine).[230,414] Documented concomitant

Table 6-2 Serious and/or common (≥25%) clozapine treatment-emergent adverse effects in adults with 22q11.2DS-Schizophrenia compared with idiopathic schizophrenia

Adverse effects (AEs)	22q11.2DS-Schizophrenia (n=20)		Idiopathic-Schizophrenia (n=20)		Novartis clinical trials[230]
	N	%	N	%	%
Serious AEs					
Seizures	8	44.4[a]	0	0	3
Red zone neutropenia	3	15	0	0	<3[b]
Myocarditis	1	5	0	0	0.06[c]
Other common AEs					
Drowsiness/sedation	15	75	14	70	39
Weight gain	10	50	7	35	31[d]
Hypersalivation	10[e]	50	9	45	31
Dizziness	9	45	3	15	19
Myoclonus	8[e,f]	40	1	5	1
Tremor	8[e,f]	40	1	5	6
Unsteady gait	6[e]	30	1	5	NR
Stiffness/rigidity	5[e,f]	25	0	0	3
Slurred speech	5[e]	25	0	0	1
Tachycardia	5	25	6	30	25

NR, not reported
[a]Excludes two patients with 22q11.2DS-Schizophrenia who had a prior diagnosis of epilepsy
[b]Includes leukopenia/decreased white blood cell count/neutropenia
[c]Post-marketing surveillance in Canada[230]
[d]InterCePT study[408]
[e]Includes one patient diagnosed with Parkinson's disease during the clozapine trial
[f]Includes pre-existing conditions that worsened on clozapine (myoclonus, n=1; stiffness/rigidity, n=2; tremor, n=3)

psychotropic medications at time of neutropenia included lamotrigine and clobazam for Patient 1, valproate for Patient 2, and lorazapam prn for Patient 3. Although there are rare case reports of neutropenia and agranulocytosis in patients taking valproate,[415,416] we note that Patient 2 received valproate both before and after clozapine with no neutropenia, and neutropenia resolved following discontinuation of clozapine. Available records for Patients 1 and 3 indicated that baseline WBC prior to clozapine initiation (4.8×10^9/L; 5.6×10^9/L) were within standard laboratory norms and consistent with baseline WBC results for the overall 22q11.2DS group (mean=5.8×10^9/L, SD=1.5×10^9/L; n=17). The mean baseline WBC results for the 22q11.2DS group was lower than that reported previously for 11,309 individuals monitored by the Clozaril Patient Management System (8.3×10^9/L, SD=2.7×10^9/L; t=-7.12, df=16, p<0.0001).[417] Notably, one of the three "red zone" 22q11.2DS-Schizophrenia patients (baseline WBC=5.6×10^9/L) exhibited persistent neutropenia on clozapine but was able to be successfully maintained for eight years with an approved lowered "red zone" alert zone. Such an alert, accompanied by a sore throat and fever, eventually led to clozapine discontinuation. The two other patients demonstrated further severe neutropenia episodes following a second trial of clozapine.

Seizures and other neurological side effects

Eight (44.4%; n=4 male) of 18 patients in the 22q11.2DS-Schizophrenia group experienced at least one seizure on clozapine (Table 6-2). Two patients with 22q11.2DS-Schizophrenia who had a prior diagnosis of epilepsy (one with seizures on clozapine), were not included in the analysis. No patients in the Idiopathic-Schizophrenia group had a seizure on clozapine. The occurrence of seizures in 22q11.2DS-Schizophrenia was significantly higher than reported for 1743 individuals during clinical testing of clozapine[418] (n=61; OR=22.1, 95% CI=8.4-57.9). In the 22q11.2DS-Schizophrenia group, non-significantly fewer (n=3, 16.6%) had seizures when they were treated with other atypical antipsychotics compared with clozapine (McNemar's test statistic (S)=3.57, p=0.059). Three individuals had one or two seizures each on a different atypical antipsychotic (risperidone with loxapine, quetiapine with low dose methotrimeprazine, and olanzapine), two of whom also had seizure(s) on clozapine.

Seizures on clozapine were typically primarily or secondarily generalized tonic-clonic seizures (EEG findings, Table 6-3). One patient also had complex partial and myoclonic seizures. Seizures occurred at relatively low doses (n=7; median 300, range 100-425 mg; Table 6-3).

Table 6-3 Clinical characteristics of the eight individuals with 22q11.2DS-Schizophrenia and seizures while on clozapine

Seizures before clozapine	Seizures on clozapine	Clozapine dose at seizure (mg)	Myoclonus	Electroencephalography findings	
				Interictal epileptiform activity	Background
GTCS	GTCS (n=3), CPS (n=1), MS (n=1)	N/A	Yes	Left temporal, generalized	Dysrhythmia grade IV, mild generalized disturbance
GTCS	GTCS (n=1)	125	No	Left parasagittal, temporal and right hemisphere spread	Normal
GTCS, FMS	GTCS (n=5)	300-425	Yes	None	Normal
-	GTCS (n>4)	100-400	Yes	Generalized	6-7Hz mild slowing
-	GTCS (n=2)	250, <325	Yes	Generalized	2-6 Hz polymorphic high amplitude mixed frequency slow wave activity, bi-hemispheric, severe slowing
-	GTCS (n=2)	300, 400	Yes	None	Generalized slowing
-	GTCS (n=2)	350	No	No EEG	No EEG
-	GTCS (n=1), MS	275	Yes	Generalized	Normal, generalized low amplitude 15-25 Hz

FMS, focal motor seizure; GTCS, generalized tonic-clonic seizures; IPGE, idiopathic primary generalized epilepsy; MS, myoclonic seizures; N/A, not available; CPS, complex partial seizures

Five (62.5%) of the eight individuals with 22q11.2DS-Schizophrenia who had seizures on clozapine had no prior history of seizures. Three had a prior history of one to two seizures each (primarily or secondarily generalized tonic-clonic seizures, one focal motor seizure) while on other antipsychotic medications. In two of these patients, the seizures occurred during antipsychotic management changes (changing from loxapine to risperidone, and on increase in loxapine dose).

Seizures during clozapine treatment appeared unlikely to be influenced by adjunct antipsychotic treatment; the majority of patients (n=7, 87.5%) had at least one seizure while on clozapine alone. Of the only two individuals who had a seizure while receiving adjunctive treatment (olanzapine, haloperidol), one also had multiple other seizures on clozapine when not on another antipsychotic. This patient was noted to have borderline-low calcium at the time of one of these other seizures. The second patient had a single seizure on clozapine and haloperidol, likely also in the context of hypocalcemia that had been diagnosed 11 days prior to seizure. Another patient who had two seizures on clozapine had hypocalcemia diagnosed four weeks prior to seizures.

The seizures of seven (87.5%) individuals who were continued on, or later retried on, clozapine were stabilized through both anticonvulsant treatments and management of hypocalcemia with Vitamin D and calcium supplements. In one notable patient where clozapine had originally been discontinued due to seizures, hypocalcemia was detected after the trial was discontinued and corrected with supplements. This occurred following diagnosis of 22q11.2DS and routine 22q11.2DS-indicated screening of ionized calcium levels.[146] Clozapine was then restarted following prophylactic treatment with an anticonvulsant (gabapentin) for seizure protection. This patient has subsequently been successfully treated with clozapine and concomitant anticonvulsant medications, and Vitamin D and calcium supplements for nine years. Another individual with treated hypocalcemia and no prior history of seizures was started prophylactically on valproic acid at clozapine initiation to reduce seizure risk and has been successfully managed for five years with no seizures.

Other neurological side effects that tend to be rarely associated with clozapine,[230] including myoclonus, tremor, unsteady gait, rigidity, and slurred speech appeared to be relatively common in the 22q11.2DS-Schizophrenia group (Table 6-2). Eight (40%; n=5 male) patients in the 22q11.2DS-Schizophrenia group but just one woman in the Idiopathic-Schizophrenia group had

myoclonus on clozapine (gender-adjusted OR=4.7, 95% CI=0.6-36.2). Myoclonus was defined by either explicit report of “myoclonic jerks” (n=5) or “myoclonus” (n=2) or description of abnormal movements consistent with myoclonic episodes (n=2; “jerky movements and twitching”, “jerking of limbs”). Two other patients in the 22q11.2DS-Schizophrenia group developed abnormal involuntary movements (“periodic orofacial movements” and “head-tilting”). Six of the individuals with myoclonus in the 22q11.2DS-Schizophrenia group had a seizure history (Table 6-3). Less frequent clozapine treatment-emergent side effects, including other neurological symptoms, are described in Table 6-4.

Discontinuation and re-trials of clozapine

At last follow-up, the majority of patients were still being treated with clozapine (n=13, 65% in each group). Clozapine was discontinued on initial trial in the 22q11.2DS-Schizophrenia group non-significantly more commonly (n=12, 60.0%) than in the Idiopathic-Schizophrenia group (n=9, 45.0%; X^2=0.90, p=0.34), and for different reasons, including rare serious side effects (Table 6-5). Eight (66.7%) of these 22q11.2DS-Schizophrenia patients had a retrial of clozapine. Five (62.5%) retrials were successful with clinical improvement and no further clozapine discontinuations (Table 6-5). The other three patients had second discontinuations due to recurrent “red zone” neutropenia (n=2) or Parkinson’s disease (n=1). This last patient was given a third trial of clozapine (maximum dose, 300 mg) upon relapse of psychosis but this was also discontinued, related to concerns about worsening parkinsonism. In contrast, the reasons for discontinuation in the Idiopathic-Schizophrenia group were primarily somatic complaints (Table 6-5). In three (33.3%) Idiopathic-Schizophrenia patients, there were retrials of clozapine; after which two showed clinical improvement.

6.4 Discussion

The results of this study demonstrate the potential for clinical utility and personalized psychiatric care with a molecular diagnosis of schizophrenia. While adults with 22q11.2DS-Schizophrenia responded as well to clozapine as those with idiopathic forms of schizophrenia, including significantly reduced hospitalizations, there were clinically relevant differences. Although a significantly lower dose was needed to achieve clinical improvement, there was a significantly higher proportion with a serious side effect in the 22q11.2DS-Schizophrenia group (seizures,

Table 6-4 Clozapine treatment-emergent adverse effects affecting <25% of patients with 22q11.2DS-associated schizophrenia and idiopathic schizophrenia

Adverse effects	22q11.2DS-Schizophrenia (n=20)		Idiopathic-Schizophrenia (n=20)		Novartis clinical trials[22]
	N	%	N	%	%
Central nervous system					
Bradykinesia	4[a,b]	20	0	0	NR
Fainting or drop attack	3	15	1	5	6
Dysphagia	3[b]	15	0	0	NR
Headache	3	15	0	0	7
Freezing gait	2[b]	10	0	0	NR
Restlessness	1	5	1	5	4
Concentration problems	1	5	2	10	NR
Weakness	1	5	1	5	1
Cardiovascular					
Hypertension	2	10	1	5	4
Hypotension	2	10	0	0	9
Chest pain/tightness	1	5	3	15	1
Metabolic disturbances					
Type 2 diabetes mellitus	2	10	3	15	3[c]
Hypercholesterolemia	1	5	2	10	NR
Hyperlipidemia	0	0	1	5	NR
Gastrointestinal and urogenital					
Constipation	3	15	4	20	14
Urinary incontinence	3[b]	15	1	5	1
Nausea	3	15	1	5	5
Vomiting	3	15	1	5	3
Urinary frequency	1	5	0	0	1
Bowel incontinence	1[b]	5	0	0	NR
Heartburn	0	0	1	5	<4
Liver test abnormality	0	0	1	5	1
Other					
Yellow zone neutropenia[d]	4	20	3	15	<3[e]
Falls	1	5	2	10	NR
Facial edema	1	5	0	0	NR
Dyspnea	1	5	1	5	1
Itchy feeling	0	0	1	5	NR

NR, not reported

[a]Includes one patient with pre-existing bradykinesia that worsened while on clozapine

[b]Includes one patient diagnosed with Parkinson's disease during the clozapine trial

[c]InterCePT study[24]

[d]White blood count (WBC)=2.0-3.5 x 10^9/L or absolute neutrophil count (ANC)=1.5-2.0 x 10^9/L or if a significant decline occurs in either count over a four week period e.g. a WBC decrease≥3.0 x 10^9/L or ANC decrease≥1.5 x 10^9/L

[e]Includes leukopenia/decreased white blood cells/neutropenia

Table 6-5 Reasons reported for initial discontinuation of clozapine treatment and success of clozapine retrials in adults with 22q11.2DS-Schizophrenia compared with idiopathic schizophrenia

22q11.2DS-Schizophrenia (n=12)		Idiopathic-Schizophrenia (n=9)	
Reason	**n**	**Reason**	**n**
Serious adverse effects	6	Non-adherence	2[a]
Red zone neutropenia	3	Unknown	2
Seizures	2[a]	Sedation	1
Myocarditis	1	Sedation/unsteadiness	1[a]
Insufficient clinical improvement[b]	2[a]	Heartburn	1
Non-adherence/weight gain	1	Other gastrointestinal complaints	1
Tachycardia/hypertension	1[a]	Venipuncture difficulties/"feeling funny"	1
Tachycardia/venipuncture difficulties	1[a]		
Venipuncture difficulties	1[a]		

[a]Denotes successful retrial (n=1) each: showed clinical improvement with retrial of clozapine and has had no further discontinuations.
[b]One patient responded well to clozapine before the development of Parkinson's disease and subsequent treatment changes (e.g. clozapine dose reduction to 100 mg with patient non-adherence and trials with adjunctive antipsychotics and electroconvulsive treatment). The other patient was treated with clozapine before discontinuing at approximately six months (maintenance dose=275 mg) and had a successful clozapine retrial (maintenance dose=300 mg).

severe “red zone” neutropenia, and myocarditis). Notably, our findings of low clozapine doses and serious side effects are consistent with previous case reports of clozapine treatment in individuals with 22q11.2DS.[142,207-209,249-251] Reports of seven patients show a low median therapeutic dose of clozapine (200 mg, range 75 to 350 mg).[142,207-209,249,250] Seizures were reported in four patients,[142,207-209] and agranulocytosis in another.[251] The results suggest that although patients with this under-recognized subtype account for up to 1% of all patients with schizophrenia[214,403,404] and have demonstrable efficacy of clozapine, they may be disproportionately represented in those with a rare serious adverse event on clozapine. Clinical testing for 22q11.2 deletions thus has significant implications in providing personalized management of schizophrenia.

Clinical implications

Studies of clozapine in schizophrenia consistently demonstrate its significant therapeutic benefits, including substantial clinical improvements and reductions in hospitalizations.[230,408] Findings from the current study indicate that clozapine treatment response in patients with 22q11.2DS-Schizophrenia is at least as good as in other forms of schizophrenia. Importantly, despite the increased risk of serious side effects, the majority of patients with 22q11.2DS-Schizophrenia in this study currently remain well managed on clozapine. Our experience and that of others with similar clinical situations indicates that a “start low, go slow” approach in the context of dose and dose titration increases the likelihood of successful outcome.[208] In addition, careful monitoring, and prophylactic anti-convulsant medication and calcium and Vitamin D supplementation may specifically help reduce associated side effects in the context of the lowered seizure threshold of 22q11.2DS.[146] Importantly, for patients in this study, changes such as these in management strategies largely made it possible for them to continue on clozapine, or have a retrial. Although we observed that seizures occurred in more patients with 22q11.2DS-Schizophrenia during clozapine treatment than when they were treated with any other atypical antipsychotic, this result did not reach statistical significance. Studies of the neurological side effects of other antipsychotic medications are needed for 22q11.2DS.

Standard blood monitoring protocols were sufficient to detect neutropenia, despite the increased risk observed in the 22q11.2DS-Schizophrenia group; notably, no patients progressed to agranulocytosis. The lower baseline WBC reported here and previously[146,419] in patients with

22q11.2DS-Schizophrenia may mediate a lowered threshold to clozapine-associated neutropenia. Larger studies with additional reports of individuals with 22q11.2DS-Schizophrenia treated with clozapine are needed to clarify the potential association of the 22q11.2 deletion with increased risk of severe neutropenia and the very rare occurrence of myocarditis.

In addition to seizures, the risk of other neurological abnormalities may also be elevated in 22q11.2DS-Schizophrenia.[146] Myoclonus has been reported previously in two female patients with 22q11.2DS treated with clozapine,[209,249] though in one patient myoclonic jerks were attributed to valproate.[249] Given that individuals with 22q11.2DS are susceptible to early-onset Parkinson's disease,[252] the emergence or worsening of motor symptoms such as tremor and rigidity, observed in some patients in this study, could indicate a progression of neurodegenerative disease or the unmasking of a vulnerable nigrostriatal system by any antipsychotic treatment.[238] Interestingly, there is one prior report of an individual with 22q11.2DS with early-onset parkinsonism whose muscle rigidity dramatically worsened following a change to clozapine from fluphenazine treatment.[142] Further studies are warranted to examine the side effects of clozapine and other antipsychotic medications on motor functioning in adults with 22q11.2DS, especially given clozapine's low binding affinity for dopamine receptors.[230]

Study Advantages and Limitations

To our knowledge, this is the first study to demonstrate that treatment outcomes and management can show clinically relevant differences in a genetic subtype of schizophrenia. We had access to a sample of adults with 22q11.2DS-Schizophrenia treated with clozapine with extensive lifetime medical records available and a matched comparison group molecularly screened to exclude pathogenic copy number variations. Replication in independent samples of well-characterized adults using comparable methods and ideally a prospective design with long term follow-up would be desirable. However, to our knowledge, our cohort remains the largest available (in this age range) on clozapine with long-term phenotypic data. The etiologic homogeneity of 22q11.2DS-Schizophrenia likely provided increased power to detect significant differences[220] in our modest sample size.

The main limitations of the current study are the retrospective, naturalistic design and unavoidable restrictions of available data. The frequency of side effects, especially those that are

mild and less likely to be documented in clinical records, may be under-estimated. The possible effects of polypharmacy, often necessary in managing adults with 22q11.2DS-Schizophrenia,[146] in mediating side effect risks during clozapine treatment requires additional study. We note that there were no serious side effects in the Idiopathic-Schizophrenia group, which demonstrated similar rates of antipsychotic polypharmacy. Clozapine was the only medication common to the individuals with 22q11.2DS-Schizophrenia who developed serious side effects. Notably, moderate to severe intellectual disability was rare in the 22q11.2DS-SZ group and developmental disabilities do not appear to impact the risk of serious side effects during clozapine treatment.[420,421]

Further studies will also be necessary to elucidate the mechanism underlying the increased risk of rare, serious side effects in 22q11.2DS-Schizophrenia. Our sample was underpowered to detect differences with small effect sizes between groups. We did adjust for possible gender effects on side effect risks (e.g. all three patients with 22q11.2DS-Schizophrenia who developed severe neutropenia were women) but not for ethnicity given that all subjects in the 22q11.2DS group who experienced serious side effects were Caucasian with the exception of two patients with seizures and this would therefore not be expected to weaken the findings. However, other possible differences would include genome-wide variants modifying drug response, such as CYP enzyme genotypes, or non-genetic factors (e.g. smoking). The latter two factors may affect clozapine metabolism but are unlikely to have an impact on serious side effects in 22q11.2DS-Schizophrenia that are not dose-dependent (e.g. myocarditis and severe neutropenia).[230,400] They could however mediate seizure risk. Other factors suggested to affect generally elevated seizure risk in 22q11.2DS include rare abnormalities of cortical development (e.g., polymicrogyria, periventricular nodular heterotopia, cortical dysplasia).[146] Other large copy number number variations do not appear to be a major factor in 22q11.2DS.[156]

Conclusions

The results suggest that the 22q11.2 deletion confers increased sensitivity to clozapine dosage and rare, serious side effects. The findings provide proof-of-principle of personalized medicine in psychiatry and evidence of the utility of clinical genetic testing in schizophrenia. Molecular diagnosis coupled with targeted management strategies could reduce adverse events and discontinuation rates of clozapine. Patients with 22q11.2DS could represent an identifiable and

more genetically homogenous population to investigate the molecular mechanisms mediating psychotropic treatment response and toxicity. Importantly, individuals with 22q11.2 deletions may account disproportionately for reports of rare, serious side effects associated with clozapine. Additional reports are needed. Studies of 22q11.2 deletions and other high penetrance genetic variants associated with schizophrenia promise to aid in our understanding of the complex etiology of schizophrenia and treatment response.

Chapter 7 Neurophenotypic mediators of baseline adaptive functioning in 22q11.2 deletion syndrome

7 Overview

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Butcher NJ, Chow EWC, Costain G, Karas D, Ho A, Bassett AS. 2012. Functional outcomes of adults with 22q11.2 deletion syndrome. *Genetics in Medicine.* 14:836-843.

ABSTRACT

Purpose: The 22q11.2 deletion syndrome is a common multisystem genomic disorder with congenital and later-onset manifestations, including congenital heart disease, intellectual disability, and psychiatric illness, that may affect long-term functioning. There are limited data on adult functioning in 22q11.2 deletion syndrome. **Methods:** We used the Vineland Adaptive Behavior Scales to assess functioning in 100 adults with 22q11.2 deletion syndrome (n=46 male; mean age=28.8, (standard deviation=9.7), years] where intellect ranged from average to borderline (n=57) to mild intellectual disability (n=43). **Results:** More than 75% of subjects scored in the functional deficit range. Although personal, vocational, and financial demographics confirmed widespread functional impairment, daily living skills and employment were relative strengths. Intelligence quotient was a significant predictor ($P<0.001$) of overall and domain-specific adaptive functioning skills. A diagnosis of schizophrenia was a significant predictor ($P<0.05$) of overall adaptive functioning, daily living skills, and socialization scores. Notably, congenital heart disease, history of mood/anxiety disorders, sex, and age were not significant predictors of functioning. **Conclusion:** Despite functional impairment in adulthood that is primarily mediated by cognitive and psychiatric phenotypes, relative strengths in activities of daily living and employment have important implications for services and long-term planning. These results may help inform expectations about outcomes for patients with 22q11.2 deletion syndrome.

7.1 Introduction

22q11.2 deletion syndrome (22q11.2DS) (OMIM #192430/188400) is the most common microdeletion syndrome in humans, with an estimated prevalence of at least 1 in 4,000 live births.[145,146] Penetrance of the associated hemizygous 22q11.2 deletion for any major feature is high, but expression is highly variable.[146] Commonly associated abnormalities include congenital heart disease (CHD) and intellectual disability (ID).[145-147,220,422] Psychiatric illnesses are prominent later onset conditions in 22q11.2DS; schizophrenia and anxiety disorders are particularly prevalent in adults.[147,150] Individually, each of these associated conditions can affect functioning of individuals in the general population.[423-426] Although most children with 22q11.2DS now live to adulthood,[427] there are limited data on adult functioning in 22q11.2DS, including the phenotypic traits most strongly predictive of functional outcome. Such knowledge is needed to inform expectations of families and clinicians and to facilitate the design of targeted interventions to optimize adaptive functioning in adulthood for patients with this common genomic disorder.

In this study, we assessed the functional abilities of 100 adults with 22q11.2DS using the Vineland Adaptive Behavior Scales and additional demographic proxies. We hypothesized the existence of widespread functional deficits that would have an impact on all major aspects of adult life. Secondary hypotheses of clinical interest, given the high inter-individual variability of 22q11.2DS,[146] were that ID, serious CHD, and psychiatric illnesses would be independent predictors of impaired functioning.

7.2 Methods

Subjects and phenotypic assessments

One hundred Canadian adults (aged 17 years and older) with 22q11.2DS (n=46 male, 46.0%) were included in the present study. The mean age of patients at time of adaptive functioning assessment was 28.8 years (SD=9.7 years). All subjects met clinical screening criteria for 22q11.2DS and 22q11.2 deletions were confirmed using standard methods.[147,390] The mean age of molecularly confirmed 22q11.2DS diagnosis was 23.0 years (SD=11.2 years). Nine had 22q11.2 deletions that were likely transmitted from parents (n=6 confirmed, n=3 probable by clinical diagnosis); the remaining 91 were known or presumed *de novo* mutations, depending on

the availability of parents for molecular testing. The majority of subjects were ascertained through genetic, adult congenital cardiac and psychiatric services using active screening and/or clinical referrals.[148,261] Detailed phenotyping is described elsewhere.[147,156,262,390] Briefly, lifetime psychiatric diagnoses were determined by research psychiatrists (ASB, EWC) according to the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV) using direct semi-structured interview, collateral information from family members, and medical records as previously described.[390] Subjects with schizophrenia or schizoaffective disorder (n=42) and psychotic disorder not otherwise specified (n=3), met the criteria for major psychotic disorders, collectively termed 'schizophrenia' for this study. The mean age at onset of schizophrenia was 21.3 years (SD=5.6 years). Nearly one third of the sample (n=30, 30.0%) had a lifetime history of a non-psychotic mood and/or anxiety disorder. Eight of these cases were co-morbid with a diagnosis of schizophrenia. None had a diagnosis of autism. One subject had a parent with schizophrenia. Subjects were classified using DSM-IV criteria as having average intellect (n=9, 9.0%), or borderline (n=48, 48.0%) or mild (n=43, 43.0%) ID.[220] Individuals with moderate to severe ID were not included in this study in order to minimize possible floor effects on the VABS scales; this level of intellect is uncommon in 22q11.2DS.[220] We classified CHD as before and considered only serious CHD (e.g., tetralogy of Fallot).[428] Of the 39 subjects (39.0%) with serious CHD, all but two had previously had corrective cardiac surgery. Most subjects had a degree of hypernasality[146] and the majority had received speech therapy (n=70, 70%). Of the 46 subjects with velopharyngeal insufficiency and/or submucous cleft palate, 32 had the defects surgically repaired. Another four had repaired overt cleft palate. Informed consent was obtained in writing, and the study was approved by local Research Ethics Boards.

Neurocognitive and functional assessments

Full-scale IQ was assessed using the Wechsler Adult Intelligence Scale-Revised (n=48, 48.0%; WAIS-R)[324] or the Wechsler Adult Intelligence Scale III (n=51, 51.0%; WAIS-III).[325] Historical IQ was used for one patient unavailable for testing. All psychometric tests were administered by a trained psychometrist.[220] To investigate adaptive functioning, an individual who was a direct caregiver, spouse, or otherwise well acquainted with the adult with 22q11.2DS was interviewed using the Vineland Adaptive Behavior Scales: Interview Edition, Expanded Form (VABS).[429] We previously reported VABS results for 58 individuals.[390] The VABS was initially created for use in pediatric populations but has since been used and validated in many studies of adults with

ID and/or genetic syndromes.[390,429,430-433] The VABS includes three main domains: Daily Living Skills (practical skills necessary for self-care, domestic tasks, and community functioning), Socialization (skills needed to get along with others, engage in leisure activities, and regulate emotions and behavior), and Communication (skills required for receptive, expressive, and written language). The Adaptive Behavior Composite (ABC) score, a measure of the individual's global adaptive functioning, is calculated from these three domains. The VABS provides standard scores (mean=100, SD=15). Higher scores indicate better functioning. The proportion of participants with abnormal scores on the VABS was determined using a 1.5 standard deviation cutoff, with scores below 78 indicating functional difficulty.[434] Demographic data on cross-sectional functioning were also collected through interviews with the caregiver and/or patient. We used the most current demographic data available for each subject in this study; the data were collected within two years of VABS assessment for 91 subjects.

Statistical analyses

Multivariate linear regression models for ABC and all three domains were used to identify phenotypic predictors of adult functioning in 22q11.2DS. Based on the major phenotypes of 22q11.2DS known to affect functioning in other general populations,[423-426] the selected clinical covariates considered in addition to age and sex were overall full-scale IQ, and diagnosis of CHD, schizophrenia, and non-psychotic mood and anxiety disorders. Post-hoc *t*-tests were used to assess the individual regression coefficients. Children with 22q11.2DS have been reported to have higher verbal IQ scores than performance IQ scores,[217,435] therefore we also tested verbal and performance IQ as predictors of functioning in place of overall IQ in our regression model.

In addition to overall descriptive statistics, we compared the adaptive functioning skills of subjects with average to borderline intellect (n=57) to those with mild ID (n=43). To assess functional outcomes and demographic and clinical variables we used chi-square tests for categorical variables and independent or paired *t*-tests for continuous variables. Pearson's correlation was used to assess the strength of the relationship between overall IQ and ABC score. To assess functioning in subjects with the mildest neuropsychiatric expression we repeated the analyses after restricting to subjects with average to borderline intellect who did not have schizophrenia (n=38). All statistical analyses were two-tailed and performed with SAS version

9.2 software (SAS Institute, Cary, North Carolina, USA), with statistical significance defined as $p<0.05$.

7.3 Results

The mean total IQ of the 100 adults with 22q11.2DS studied (71.7, SD=9.1) was in the lower range of borderline ID. There was no significant difference between mean verbal IQ (73.7, SD=9.4) and mean performance IQ (72.6, SD=8.8; t=1.6, df=95, p=0.1120). Those with average to borderline intellect (mean overall IQ=77.3, SD=7.7) did not significantly differ in age (t=0.8, df=98, p=0.4114), sex (X^2=0.1, df=1, p=0.7704), lifetime history of non-psychotic mood and/or anxiety disorders (X^2=0.1, df=1, p=0.7914) or presence of CHD (X^2=3.1, df=1, p=0.0770) from those with mild ID (mean overall IQ=64.3, SD=4.0). The mild ID subgroup had a significantly greater proportion of subjects with schizophrenia (60.5% vs 33.3%; X^2=6.2, df=1, p=0.0125). As expected,[156] mean overall IQ was significantly lower in patients with schizophrenia than in those who were non-psychotic [68.6 (SD=8.0), 74.3 (SD=9.1), respectively; t=-3.3, df=98, p=0.0013], as were verbal IQ [70.4 (SD=98.5), 76.2 (SD=9.2), respectively; t=-3.1, df=94, p=0.0024] and performance IQ [69.0 (SD=6.4), 75.4 (SD=9.3), respectively; t=-3.8, df=94, p=0.0003].

Adaptive functioning in 22q11.2DS

Consistent with our hypothesis, the VABS scores revealed widespread functional deficits in our adult cohort with 22q11.2DS. The mean scores for the Daily Living Skills, Socialization, and Communication domains were 81.3 (SD=19.2), 67.2 (SD=16.9), and 60.5 (SD=20.7), respectively. The mean ABC score was 65.2 (SD=17.3) and the majority of participants scored in the functional difficulty range on overall functioning (n=76, 76.0%).

A significant predictive model ($P<0.001$) emerged for the VABS ABC score (adjusted R^2=0.38) and all three of its domains: Daily Living Skills (adjusted R^2=0.32), Socialization (adjusted R^2=0.22), and Communication (adjusted R^2=0.35). As hypothesized, IQ and schizophrenia were significant predictors of overall adult functioning (Table 7-1). However, contrary to our hypothesis, neither CHD nor a history of mood or anxiety disorder had a significant impact on adaptive functioning. IQ and schizophrenia were also predictive of Daily Living Skills and Socialization scores, while IQ alone was a significant predictor of Communication scores (Table 7-1). No other variable achieved significance as a predictor for any of the three domains (Table

Table 7-1 Summary of multivariate regression analysis of Vineland Adaptive Behavior Scales scores of 100 adults with 22q11.2DS

	Vineland Adaptive Behavior Scales Domains							
	Adaptive behavior composite		Daily living skills		Socialization		Communication	
	B	*p*	*B*	*p*	*B*	*p*	*B*	*p*
IQ	1.02	**<0.0001**	1.05	**<0.0001**	0.66	**0.0006**	1.33	**<0.0001**
Schizophrenia	8.51	**0.0130**	8.55	**0.0299**	10.38	**0.0054**	4.32	0.2931
Mood/anxiety disorders	-0.02	0.9963	-2.37	0.5293	-0.46	0.8963	0.04	0.9927
CHD	-0.88	0.7755	-0.19	0.9566	-3.67	0.2733	0.27	0.9419
Age	-0.02	0.9020	-0.05	0.7656	0.22	0.1920	-0.23	0.2224
Sex	0.56	0.8437	-0.02	0.9949	-0.84	0.7835	1.70	0.6208

B, regression coefficient
p, *p*-value for regression coefficient post-hoc *t*-tests

IQ and age are continuous variables; all others are dichotomous categorical variables

7-1). The exploratory regression models including verbal or performance IQ in place of overall IQ showed similar results (data not shown). Also, an exploratory analysis comparing functioning of those with and without a palatal abnormality showed no significant differences between these subgroups (data not shown). Secondary analyses of the VABS standard scores showed the predominant effects of lower intellect on functioning across all domains. The average to borderline intellect subgroup performed significantly better overall and in all three domains than those with mild ID, and significantly fewer showed functional difficulty (Table 7-2). Restricting the analyses to the 38 individuals in the average to borderline intellect group who were non-psychotic showed only slightly higher mean VABS scores and half (n=19) exhibited overall functional difficulty (Table 7-2). Notably, Daily Living Skills appeared to be a relative strength regardless of neuropsychiatric subgroup (Table 7-2). No difference was found in the pattern of results when IQ was used to define the two subgroups (mild ID, IQ≤70; borderline to average intellect, IQ>70; data not shown).

Given the large variability in VABS scores observed in the two neurocognitive subgroups (Table 7-2), we examined more closely the relationship between IQ and overall functioning. Although IQ was well correlated with the VABS ABC score (all patients, r=0.60, p<0.0001; schizophrenia patients, r=0.60, p<0.0001; non-psychotic patients, r=0.52, p<0.0001) there was considerable variability in individual scores (Figure 7-1). For example, a small cluster of five individuals exceeded functional expectations compared with what would be predicted by IQ (Figure 7-1). Four were female and all five lived in a stable home environment with relatives or a spouse. Vocationally, they were students, employed, or had previously worked full time. Four had CHD, and four had an effectively treated mood/anxiety disorder. None had schizophrenia. Notably, all but two of the 24 individuals who functioned above the functional difficulty range on the ABC score had no psychotic illness (Figure 7-1).

Functional descriptive variables

Tables 7-3 and 7-4 show the details of specific functioning in personal relationships, vocations, and financial support for our sample. In total, 18% (n=18) of 22q11.2DS patients were or had previously been married, the majority of whom (n=14) had at least one child.[240] Over one in five had a driver's license (Table 7-3). Most patients who were married, had children or were licensed to drive had average to borderline intellect and no psychotic illness. More than half of

Table 7-2 Adaptive functioning as measured with the Vineland Adaptive Behavior Scales in neurocognitive subgroups of 100 adults with 22q11.2DS

	Intellectual subgroups															
	Mild intellectual disability (n=43)				Average to borderline intellect (n=57)				Analyses				Non-psychotic subjects from the average to borderline subgroup (n=38)			
	Scores		Functional difficulty		Scores		Functional difficulty		Scores		Functional difficulty		Scores		Functional difficulty	
Scores	Mean	(SD)	n	(%)	Mean	(SD)	n	(%)	*t*	*p* [a]	X^2	*p* [b]	Mean	(SD)	n	(%)
Adaptive behavior composite	53.8	(11.6)	41	(95.3)	73.6	(16.1)	35	(61.4)	6.8	**<0.0001**	13.7	**0.0002**	77.7	(16.2)	19	(50.0)
Daily living skills	68.7	(14.8)	32	(74.4)	90.8	(16.6)	14	(24.6)	6.9	**<0.0001**	22.6	**<0.0001**	94.0	(15.3)	6	(15.8)
Socialization	58.9	(12.2)	40	(93.0)	73.5	(17.3)	36	(63.2)	4.7	**<0.0001**	10.4	**0.0013**	72.8	(20.4)	21	(55.3)
Communication	47.7	(15.8)	41	(95.3)	70.1	(18.8)	32	(56.1)	6.3	**<0.0001**	17.2	**<0.0001**	77.4	(16.9)	17	(44.7)

[a] df=98
[b] df=1

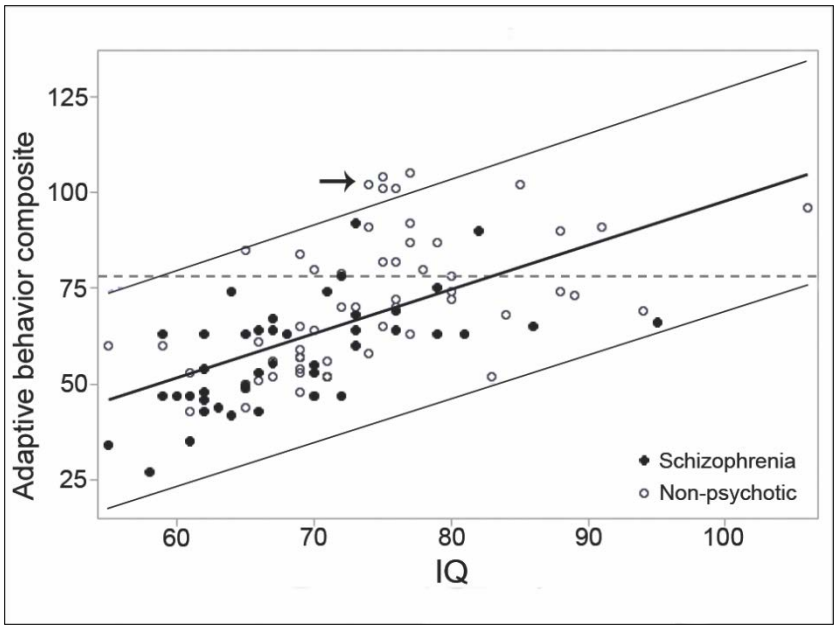

Figure 7-1 Full-scale IQ correlates with Vineland Adaptive Behavior Scales Adaptive Behavior Composite score (r=0.60, n=100) in adults with 22q11.2DS

Regression and predicted interval lines (95%) are shown. Subjects with an overall functioning score of less than 78 (dashed line) have functional deficits. Arrow denotes five subjects who have significantly higher functional scores than predicted by IQ.

Table 7-3 Social functioning of 100 adults with 22q11.2DS

	Mild intellectual disability (n=43)		Average to borderline intellect (n=57)		Non-psychotic subjects from the average to borderline subgroup (n=38)	
	n	(%)	n	(%)	n	(%)
Marital Status						
Married or common law	2	(4.7)	10	(17.5)	9	(23.7)
Separated, divorced, or annulled	1	(2.3)	5	(8.8)	3	(7.9)
Never married	40	(93.0)	42	(73.7)	26	(68.4)
Children	2	(4.7)	12	(21.1)	10	(26.3)
Home environment						
House or apartment	30	(69.8)	51	(89.5)	38	(100.0)
Boarding house/group home	11	(25.6)	4	(7.0)	0	–
Hospital/treatment facility	2	(4.7)	2	(3.5)	0	–
Living situation						
Alone	2	(4.7)	7	(12.3)	4	(10.5)
Spouse or partner	2	(4.7)	10	(17.5)	9	(23.7)
Parent(s) and/or other relative(s)	26	(60.5)	32	(56.1)	25	(65.8)
Unrelated roommates	13	(30.2)	8	(14.0)	0	–
Driver’s license ownership	1	(2.3)	21	(36.8)	19	(50.0)

Table 7-4 Employment and financial status of 100 adults with 22q11.2DS

	Mild intellectual disability (n=43)		Average to borderline intellect (n=57)		Non-psychotic subjects from the average to borderline subgroup (n=38)	
	n	%	n	%	n	%
Employment status of longest duration						
Employed	24	(55.8)	42	(73.7)	28	(73.7)
Student ± outside work	5	(11.6)	8	(14.0)	7	(18.4)
Unemployed or volunteer work	1	(2.3)	4	(7.0)	3	(7.9)
Sheltered workshop/day program	2	(4.7)	2	(3.5)	0	–
Disabled/unable to work [a]	11	(25.6)	1	(1.8)	0	–
Primary source of financial support						
Own job only	3	(7.0)	10	(17.5)	10	(26.3)
Own and spouse/partner income	1	(2.3)	6	(10.5)	5	(13.2)
Spouse/partner income	1	(2.3)	2	(3.5)	1	(2.6)
Parent(s) and/or other relative(s)	5	(11.6)	11	(19.3)	9	(23.7)
Fixed income[b]	33	(76.7)	28	(49.1)	13	(34.2)

[a]Includes intellectual, psychiatric, and/or physical impairments
[b]Includes pensions/retirement income, government social assistance, and disability pensions

all patients lived with their parents and/or another relative (mean age=24.6, SD=6.7, range 17.3-44.3, years), irrespective of intellectual functioning (Table 7-3). Thirteen (22%) of those who lived with family were aged 30 or older. They varied in severity of neuropsychiatric phenotype; there were roughly equal numbers with mild ID and psychosis (n=4) and average to borderline intellect, with (n=4) or without psychosis (n=5). Financial assistance from family and government sources was common (Table 7-4). Although over two-thirds of the total sample had most often been employed during adulthood (n=66, 66%), a minority was financially independent (Table 7-4). This was true even for individuals who had neither ID nor psychosis (Table 7-4). Parents and other relatives were the primary source of financial support for 16% (n=16) of the sample. Those who were married were typically supported at least in part by their spouse (n=10/12, 83.3%). The majority of the sample, however, was on fixed income (n=61, 61.0%). Fewer subjects with average to borderline intellect were on fixed income (X^2=6.7, *df*=1, *p*=0.0094) than those with mild ID (Table 7-4).

The types of jobs held by patients were diverse. Notably, few required previous experience or formal training. These included work in fast food restaurants and coffee shops, general labor (e.g., worker in a loading bay), or maintenance positions (e.g., janitorial or cleaning staff). Less commonly, patients held jobs requiring more advanced skills, such as working with children (e.g., child care), with the general public (e.g., receptionist, store clerk), or in technical positions (e.g., computer store staff, call centre worker, web designer). The sample also included a few trade workers (e.g., pipe fitter, wood worker, hair dresser). The highest professional designation attained was a school teacher.

7.4 Discussion

Consistent with our primary hypothesis, 22q11.2DS in adulthood is characterized by widespread functional deficits that have an impact on most major aspects of daily life. A substantial proportion of the 100 adults with 22q11.2DS studied were impaired in overall adaptive functioning as measured by the VABS and this corresponded with real-life deficits, reflected by the relatively low proportion of individuals who supported themselves financially, developed long-term romantic relationships, or lived on their own. Employability and skills necessary for activities of daily living appear to be areas of relative strength. There is however wide inter-

individual variability in functional abilities in adults with 22q11.2DS, which is mediated mainly by intellectual level and, to a lesser extent, by the presence or absence of schizophrenia.

Contrary to our hypotheses, other treatable psychiatric illnesses, such as mood and anxiety disorders, and CHD did not have a significant impact on functional abilities. This may be related to effective management and regular follow-up of these conditions.[146,427] This also appeared to be the case for major palatal anomalies. Consistent with the findings of a recent study of reproductive fitness in 22q11.2DS,[240] our results suggest that the relative impact of serious CHD such as tetralogy of Fallot and its surgical repair on functioning in adulthood for patients with 22q11.2DS is minimal. These results support a previous finding that neurodevelopmental deficits observed in children with CHD[424] may be driven in large part by the effects of associated genetic syndromes, like 22q11.2DS.[436]

As expected, IQ was highly correlated with the VABS scores but was not the sole predictor of functioning.[429] Our sample included a small group of adults who functioned at a higher level than would be predicted by their IQ suggesting that, at least for some individuals, effective long-term management of chronic conditions and appropriate social and vocational supports may promote the achievement of improved functional levels. Employment, for example, has been found to be associated with improved adaptive functioning skills in individuals with ID.[437,438] Employment rates have been reported to be low in adults with 22q11.2DS, however. A previous study including occupational data on 17 adults with 22q11.2DS reported that about 35% (5 men, 1 woman) were employed. [439] All others were homemakers. Notably, most in that sample were diagnosed as parents following the diagnosis of an affected child and had relatively mild manifestation of the syndrome and a high level of functioning.[439] Results were similar in another report of seven adults with 22q11.2DS.[152] Although we did not assess the cross-sectional vocational status of our subjects, we found that most adults with 22q11.2DS had been employed in some capacity for the majority of their adulthood, regardless of intellectual level. Relatively few, however, were financially independent. Future study is needed to address the reasons underlying this discrepancy but from our clinical experience, difficulties maintaining steady employment due to medical or psychiatric issues, the types of jobs in which they are employed (e.g. lower waged, part-time or temporary work) and poor money management skills may be contributing factors.

Daily Living Skills as measured by the VABS was an overall area of relative strength in adults with 22q11.2DS. This is consistent with previous reports using this assessment tool in other populations with various psychiatric illnesses and/or neurocognitive impairments.[430-433] Interestingly, studies of children with 22q11.2DS have generally not shown this.[440-442] The mean scores reported for children and adolescents in the literature on the Daily Living Skills domain were substantially (11 to 22 points) lower than the mean scores of adults in our sample, despite similar levels of intellect.[440-442] Children with 22q11.2DS and a relatively high IQ, however, tend to score similarly well on the Daily Living Skills domain as the adults in our sample with average to borderline intellect.[180] This suggests there may be gains in this domain later in development and with maturity in 22q11.2DS, particularly in patients with lower intellect. Such changes in adaptive functioning skills could parallel the reported differences between the cognitive profiles of children and adults with 22q11.2DS. Performance IQ has been reported to be lower than verbal IQ in children[217,435] but not in adolescents or adults with 22q11.2DS.[217,405] Alternatively, the higher Daily Living Skills scores we found in adults may be a function of differences in the 22q11.2DS populations sampled. For example, psychiatric conditions in our adults receiving psychiatric treatment may have had a modifying effect on functioning in this domain compared with children and adolescents where there may be emerging and untreated psychiatric symptoms.[441]

There may be other age-related changes in adaptive functioning in 22q11.2DS. Although one study reported that girls tend to have a higher level of cognitive functioning and performed better on the VABS than boys,[440] we found no effect of sex on functional abilities. Our results therefore suggest that any functional advantage females may have in childhood is not maintained into adulthood. There have been no systematic reports on functioning in older or elderly adults with 22q11.2DS to date, but there is some evidence from a case series of six patients with 22q11.2DS that cognitive abilities and adaptive skills decline with age, at least in some patients with treatment-resistant psychosis.[443] Large scale, longitudinal studies are needed to investigate changes in functioning in individuals with 22q11.2DS over the lifespan.

Study advantages and limitations

With improved pediatric survival in 22q11.2DS,[427] interests are shifting to understanding the long-term outcomes of this patient group. This is the first systematic study of adaptive

functioning in a large cross-sectional sample of adults with 22q11.2DS and the first to test phenotypic predictors of functioning. Enrichment for schizophrenia, serious CHD, mood/anxiety disorders, and ID in this sample enhanced power for examining the effects of each of these variables on adaptive functioning. The prevalence of serious CHD was lower in our cohort than in others, where ascertainment primarily occurs through cardiac clinics and includes all forms of CHD. Our ascertainment strategies meant that we avoided oversampling transmitting parents. No study, however, can ensure a straightforward generalization of its overall descriptive statistics on functioning to the total population of individuals with 22q11.2 deletions because the true prevalences of major features remain unknown.

There were limitations of our study. Our study design focused on major phenotypes of 22q11.2DS with prior evidence in other general populations of having an impact on functioning. The selected variables explained approximately 22-38% of the variance in the VABS domain and composite scores. Other variables that could contribute to adaptive functioning abilities in adults with 22q11.2DS remain to be identified. We did not examine the effect of psychiatric diseases other than schizophrenia and mood/anxiety disorders in 22q11.2DS. However, these other conditions are less common in our sample.[150] Small numbers of subjects with transmitted 22q11.2 deletions (n=9) or a parent with schizophrenia (n=1) precluded the possibility of assessing the potential impact of these factors on long-term functioning. Future studies may address the impact of other potentially important variables on adults functioning such as subtle brain malformations and other neuropsychiatric phenomenology.[146] The influence of early interventions on long-term outcomes in 22q11.2DS also warrants systematic investigation, but was outside the scope of the current study.

We found no effect of age on functioning in our sample. Longitudinal studies are needed to assess adaptive functioning across the lifespan of individuals with 22q11.2DS as our results and those in studies of children together suggest that the level of functioning and predictors of functioning could change over time. Many of our subjects were not formally diagnosed until adulthood and so future cohorts of adults with 22q11.2DS may have improved functional outcomes over those reported here due to earlier diagnosis and correspondingly, earlier access to optimal medical interventions and educational supports.[146] Exploratory analyses indicated that serious palatal anomalies did not appear to affect long term functioning, but the important issue of optimizing communication skills requires further, ideally longitudinal, study. As with all

interview-based assessments, the abilities of patients may have been over- or under-estimated in individual cases, however, this would be unlikely to systematically affect the pattern of overall findings. A small number of non-psychotic patients in this study may develop schizophrenia in the future. The effect of lifetime psychotic illness on functioning may thus have been slightly underestimated, however most adults in our sample were past the mean age of onset of psychosis.

Implications

These findings may help patients, their families, and health care providers in developing reasonable expectations and long-term goals, and inform interventions to improve functioning and increase level of independence. The results highlight the need for increased support and services for adults with 22q11.2DS that could build on relative strengths in daily living skills and employment and that remediate or help compensate for relative weaknesses in social and communication abilities. The use of modern tools, for example, could be considered. Receptive and expressive communication may be more effective using indirect methods (e.g. a computer, recorder, a smartphone, written text or pictures) compared with direct, verbal communication. Patients with mild ID are likely to face greater challenges in overcoming functional difficulties than those with higher intellect. As for others with ID, the development of cognitive rehabilitation strategies may help to remediate cognitive deficits and facilitate skill development.[437,444] In addition, optimized psychiatric care especially for psychotic illnesses (e.g. help with adherence to medications) will likely be of benefit.[146] Increasing social, communication, vocational skill sets and confidence in abilities may translate into improved employability and greater independence.

The relative strengths demonstrated by both the VABS and descriptive statistics suggest that many patients with 22q11.2DS may be most successful in occupations and recreational activities that utilize skills related to activities of daily living. These could include possibilities such as gardening, food preparation, cleaning or other domestic chores. Encouraging and assisting patients with 22q11.2DS with job placements in areas in which they are most likely to succeed may help to increase employment rates, independence and personal satisfaction. Individuals with 22q11.2DS tend to have executive functioning skills deficits,[220] but relatively strong rote memory,[217,435] and so may be well-suited to structured or repetitive tasks. Hands-on training,

demonstrations, and written instructions may also help patients succeed in the workforce, as we find that many adults with 22q11.2DS are better experiential than passive, auditory learners. Students with 22q11.2DS who struggle academically could consider programs with a co-op component, to encourage academic success and foster skill development. Proximity to home and access to facilities with public transportation should be considered for both vocational and recreational activities, given that most patients with 22q11.2DS do not have a driver's license. Given the high inter-individuality in functioning in adults with 22q11.2DS, vocational and social supports should be tailored according to individual needs and interests.

Conclusion

In summary, intellectual level and a diagnosis of schizophrenia are good but imperfect predictors of adult functioning, and the presence of non-psychotic mood disorders or serious CHD does not significantly affect outcome in 22q11.2DS. The characteristic wide inter-individual variability of clinical features associated with 22q11.2DS[146] extends to functional abilities. Future studies are needed to assess functioning across the lifespan and determine other remediable factors that predict better functioning. Nevertheless, the results suggest some strategies that may help the ever increasing cohorts of patients with diagnosed 22q11.2DS entering adulthood, and the families and clinicians who help care for them.

Chapter 8 Management of neurological and co-morbid multisystem conditions in adults with 22q11.2 deletion syndrome

8 Overview

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Fung WLA*, **Butcher NJ***, Costain G*, Andrade DM, Boot E, Chow EWC, Chung B, Cytrynbaum C, Faghfoury H, Fishman L, García-Miñaúr S, George S, Lang AE, Repetto G, Shugar A, Silversides C, Swillen A, van Amelsvoort T, McDonald-McGinn DM, Bassett AS. 2015. Practical guidelines for managing adults with 22q11.2 deletion syndrome. *Genetics in Medicine.* 17:599-609. ***Co-first authors**

ABSTRACT

22q11.2 deletion syndrome (22q11.2DS) is the most common microdeletion syndrome in humans, estimated to affect up to 1 in 2000 live births. Major features of this multisystem condition include congenital anomalies, developmental delay, and an array of early and later-onset medical and psychiatric disorders. Advances in pediatric care ensure a growing population of adults with 22q11.2DS. Informed by an international panel of multidisciplinary experts, and a comprehensive review of the existing adult literature, we present the first set of guidelines focused on managing the neuropsychiatric, endocrine, cardiovascular, reproductive, psychosocial, genetic counselling, and other issues that are the focus of attention in adults with 22q11.2DS. We propose practical strategies for the recognition, evaluation, surveillance, and management of the associated morbidities.

8.1 Introduction

22q11.2 deletion syndrome (22q11.2DS) (OMIM #188400/#192430) is the most common microdeletion syndrome in humans.[146,427,445,446] Survival to adulthood is now the norm.[427] In addition to the well known congenital and developmental features,[146,427] treatable later onset conditions are increasingly recognized as important components of 22q11.2DS. The multisystem nature and associated burden of morbidities means that adults with 22q11.2DS may be seen in virtually any medical practice.[146] Chromosome 22q11.2 deletions are absent in large populations of healthy controls, implying a high collective penetrance for at least one major phenotypic feature.[447] The growing numbers of affected children advancing to adulthood, and the advent of non-invasive prenatal testing for 22q11.2 deletions, have prompted demands for information about longer term issues. The existing clinical practice guidelines for 22q11.2DS are largely pediatric-focused.[146] We therefore present the first set of guidelines focused on managing the neuropsychiatric, endocrine, cardiovascular, reproductive, psychosocial, genetic counselling, and other issues pertinent to the adult with 22q11.2DS. A comprehensive review of the existing adult literature was complemented by the collective experience of professionals from various disciplines dedicated to caring for a total of over 500 adults with 22q11.2DS.

8.2 Methods

These guidelines were developed in two stages. First, we conducted a comprehensive review of the existing literature. The databases EMBASE (1947 to 2013 Week 28), MEDLINE (1946 to 16 July 2013), and PsycINFO (1806 to July Week 2 2013) were searched on 16 July 2013, using the OVID interface and the search term "[(22q* adj3 deletion) OR (velocardiofacial OR velocardio-facial OR velo-cardiofacial OR velo-cardio-facial OR VCFS) OR (DiGeorge syndrome) OR (conotruncal anomaly face syndrome)] AND adult". This search was limited to the human literature, and yielded 1785 potentially relevant articles (including journal articles, conference proceedings, book chapters, and books). Initial screening by scanning titles resulted in the elimination of 718 duplicates and 392 irrelevant articles, leaving 675 articles. Screening abstracts resulted in the exclusion of a further 176 articles. A hand search of the remaining 499 articles resulted in the elimination of 261 without a substantive focus on adult issues in 22q11.2DS. Ten articles of potential interest were unavailable (9 case reports and 1 case series involving three

patients). The search was repeated on 8 July 2014 and resulted in the identification of 25 additional articles, for a final total of 253.

Second, a draft consensus document was created by clinicians and researchers based in Toronto (Canada) having diverse expertise in managing adult issues in 22q11.2DS. The resulting manuscript was then circulated amongst seven clinicians/researchers experienced in the clinical care of and/or research on adults with 22q11.2DS from Asia, Europe, South America, and the United States for additional feedback. All authors met at least one of the following criteria: (i) substantial clinical experience working with adults with 22q11.2DS (operationalized as having seen at least 10 adult patients with 22q11.2DS in both consultation and follow-up); (ii) substantial research experience with adults with 22q11.2DS (based on peer-reviewed publications). The results of the literature search were used to guide the discussion, and to support consensus recommendations with scientific evidence when possible. However, a relatively limited literature exists for this complex condition in adulthood, particularly for management issues. Consequently, as for the general clinical practice guidelines for 22q11.2DS,[146] virtually all of the evidence would be levels III or IV (e.g., descriptive studies, case series, expert opinions etc.). Thus we have not formally graded the individual recommendations presented.

8.3 Review and practice guidelines

The phenotypes associated with 22q11.2DS are known to be highly variable in number and severity, even within families and between monozygotic twins.[439] This variability contributed to the historical naming and characterization of what were originally thought to be distinct clinical syndromes (e.g., DiGeorge syndrome, velocardiofacial syndrome) prior to the discovery of the common underlying microdeletion on chromosome 22q11.2. The available information on the adult phenotype and natural history of 22q11.2DS pertains primarily to neuropsychiatric, endocrine, cardiovascular, reproductive, and psychosocial issues.

Although a growing number of adults with 22q11.2DS received their diagnosis in childhood because of targeted testing prompted by typical syndromic features, the variable phenotype of 22q11.2DS often presents a significant diagnostic challenge to clinicians. Many of those adults reported in the literature and most familiar to geneticists were diagnosed following the birth of

an affected child.[439] Others were reported as a result of genetic screening studies in at-risk populations.[447] Multiple case reports in sub-specialty journals testify to the limited awareness in the general medical community of 22q11.2DS and its vast phenotypic spectrum. For this reason, many adults with a 22q11.2 deletion, especially those without typical congenital anomalies or who were born before confirmatory testing became available remain undiagnosed.[154] This is the greatest barrier to understanding the full spectrum of the condition, including course and outcome, and thus to providing the optimal anticipatory care that promises improved symptom management, quality of life, and functioning. The available data support the likelihood that all associated conditions in 22q11.2DS respond similarly to the idiopathic forms of these conditions - that is, to standard management strategies and treatments, whether these are surgical or medical. The caveat for 22q11.2DS is that the multisystem nature of the associated features and potential treatment side effects demand attention by all clinicians, regardless of their sub-specialty. These issues are outlined in Tables 8-1 through 8-4.

Neuropsychiatric manifestations in young to middle-aged adults

Neuropsychiatric diseases: (i) comprise the most common group of later onset conditions in 22q11.2DS,[147,150,215,217,395,448] (ii) are typically of greatest concern to the patient and family because of their seriousness and the associated stigma,[449,450] (iii) are most likely to bring the adolescent and adult patient (back) to medical attention and to affect the individual's daily functioning,[255,450] and (iv) may constitute a management challenge.[217,451,452]

Schizophrenia

There is a well established association between 22q11.2DS and schizophrenia.[149,214] Approximately 1 in every 4 to 5 adults with 22q11.2DS will develop this serious mental illness, usually in late adolescence or early adulthood.[150,215] Thus, individuals with 22q11.2DS have a greater than 20-fold increase in risk for schizophrenia.[149,150] Schizophrenia associated with 22q11.2DS is essentially indistinguishable from schizophrenia in the general population with respect to prodrome, age at onset, presentation, and cognitive profile, apart from lower mean IQ.[149,150,215,218-221,453] As for virtually all associated conditions in 22q11.2DS, standard management is recommended, i.e., according to clinical practice guidelines for schizophrenia, including antipsychotic medications.[146] Patients may benefit from a “start low, go slow”

Table 8-1 Recommendations for periodic assessments and health monitoring for adults with 22q11.2DS

Recommendations	Baseline work-up at adult diagnosis or initial assessment at transition from pediatric care		Annual or biennial follow-up as an adult	
	Complete	As needed	Complete	As needed
Genetic/general assessments and management				
Consultation with medical geneticist and/or clinicians experienced with 22q11.2DS	✓		✓	
Genetic counselling[a]	✓		✓	
Clinical genetic testing	✓[b]			
Family planning and prenatal counselling		✓		✓
Other clinical assessments				
Comprehensive medical history	✓			
Systems review	✓		✓	
Psychiatric assessment[c]	✓			✓
Cognitive and capacity assessments	✓			✓
Neurological assessment		✓		✓
Ophthalmological assessment		✓		✓
Orthopedic assessment		✓		✓
Family history	✓		✓	
Physical examination (including for hygiene and care)	✓		✓	
BMI/growth/nutritional assessment	✓		✓	
Other investigations				
22q11.2DS-relevant laboratory tests[d]	✓		✓	
Electrocardiogram (ECG)		✓		✓[e]
Transthoracic echocardiogram		✓ (if not previously performed)		✓[e]
Abdominal ultrasound[f]		✓		✓
Electroencephalogram (EEG)		✓		✓
Other management				
Contraception and safe sex counseling	✓		✓	
Counselling on internet safety	✓		✓	
Dental assessment	✓			✓
Audiology assessment	✓			✓
Other[g]	✓			✓

22q11.2DS, 22q11.2 deletion syndrome; BMI, body mass index

[a]Described in text. [b]Proband, offsprings, and parents (siblings if parents unavailable). [c]In addition to review for changes (Table 8-2), includes assessment of, e.g., substance use, gambling, risky behaviors. [d]Complete blood count (CBC) and differential, electrolytes, thyroid-stimulating hormone, pH-corrected ionized calcium, magnesium, parathyroid hormone, creatinine, liver function tests (especially alanine aminotransferase), lipid profile, glucose, HbA1c are examples. Consider checking CBC and calcium preoperatively and postoperatively, and regularly during pregnancy. [e]Especially for individuals with significant congenital heart disease. [f]Especially with regard to renal agenesis. [g]Including vocational counseling/training, supported employment and individual placement and support, diet and exercise counselling, life skills assessment, and financial management.

approach to antipsychotic dosing and prophylactic (e.g., anticonvulsant) management strategies to help ameliorate risk of associated side effects, particularly with respect to seizures during clozapine treatment.[256]

The elevated risk for psychotic illness in 22q11.2DS prompts questions about prevention, early signs, diagnosis, and treatment. Informed discussion about schizophrenia as a lifelong but treatable and manageable condition is essential. Placing the disease in the context of other chronic diseases like diabetes mellitus may be helpful. Promptly seeking expert help in diagnosis and effective treatment may improve prognosis.[149] Learning about early signs that may herald treatable psychiatric illness can facilitate this and may be empowering for families (Table 8-2). There are no proven preventions for psychotic illness. However, avoiding substance use, particularly early marijuana use, and lifelong general health measures such as good nutrition, and physical and mental exercise are reasonable recommendations.[454] Research is ongoing with respect to identifying predictors of future psychotic illness in 22q11.2DS.[163,453,455,456] Meanwhile, concerns, misconceptions and a sense of stigma should be solicited and addressed.[449,450,457]

Other psychiatric disorders and neurobehavioral features

Other, non-psychotic, treatable psychiatric illnesses are collectively more common in 22q11.2DS than is schizophrenia, and some may be higher even than the high rates of these conditions in the general population.[147,150,215,448] Anxiety disorders appear to be particularly common in adults with 22q11.2DS, sometimes persisting from childhood and sometimes arising later.[150] Notably, bipolar disorder, while sometimes seen in 22q11.2DS, has a similar prevalence to that in the general population, as do other more common disorders like major depression and substance use disorders.[147,156,215,448] Autism spectrum disorders and sometimes attention deficit disorder, with onset in childhood, remain important features in adulthood[448,458] but have no apparent relationship to the later appearance of schizophrenia.[458]

Challenges exist with respect to diagnosis and treatment of psychiatric disorders in the minority of adults with moderate or severe intellectual disability,[395,451] including communication limitations that may hinder recognition or assessment of treatment response using standards measures. In most cases, these challenges can be overcome with expert care. Emotional or temper outbursts, described in individuals with 22q11.2DS, may be a feature of untreated or undertreated anxiety or psychotic illness with multiple physical factors potentially

Table 8-2 Signs and symptoms representing a change from baseline that may suggest a treatable psychiatric illness

<table>
<tr><th colspan="2">New onset or exacerbation of problems in:</th></tr>
<tr><td>Thinking
Impaired memory, concentration, or attention
Preoccupations
Increased irrational statements or repetitive ideas
Misinterpretation of people’s motives, situations
Suspiciousness
Threatening suicide
Delusions and hallucinations (changed perception of reality; e.g., believe phone is tapped, hearing voices, new onset of imaginary friends)</td><td>Emotions
Increased anxiety, worry, nervousness, fear
Irritability, anger, hostility, resentment
Increased sadness, crying
Increased apathy, not as interested or enjoying life
Smiling or laughing for no apparent reason
Rapidly changing mood – from happy to sad to angry for no apparent reason
Hypersensitivity to perceived criticisms/insults (hurt feelings)</td></tr>
<tr><td>Behavior
Avoidance of people, social withdrawal (even from family)
Increased impulsive behaviors and/or emotional outbursts
Agitation (e.g., screaming, pacing, aggression)
Unusual/odd or self-injurious behavior
Neglect in self-care (e.g., hygiene, clothing, appearance)
Deterioration in functioning - at home, in social situations, at school or work</td><td>Physical/Somatic
Changes in amount of sleep (much less or much more)
Disruption of sleep patterns
Changes in energy level (e.g., increased fatigue)
Changes in appetite and/or weight
Increased motor disturbances (e.g., tremors, tics)
Increase in physical complaints, (e.g., gastrointestinal symptoms)</td></tr>
<tr><td colspan="2">Differential diagnosis/potential confounding or exacerbating factors:
Endocrine (e.g., thyroid, hypocalcemia) or other (e.g., infection, sleep apnea, excess water/carbonated beverages, Parkinson disease, emerging dementia) causing, e.g., metabolic disturbance or hypoxia
Substance use (e.g., caffeine, alcohol, street drugs such as marijuana)
Treatment related (medication side effects or undertreatment, e.g., secondary to poor/improper compliance)
Changes in physical environment (e.g., caregivers, co-residents, living space)
Hearing or other sensory deficits</td></tr>
</table>

Sources: Adapted from[149,454]

contributing.[146,149] Early diagnosis and prompt institution of standard effective management by a knowledgeable clinician are essential for all psychiatric illnesses. Routine monitoring for changes in emotions, thinking, physical state and behavior/functioning (Table 8-2) will facilitate this. Awareness of issues like suggestibility, the patient's intellectual level, and most importantly their baseline state and functioning, is essential.

Cognitive and adaptive functioning

Most patients with 22q11.2DS have an IQ in the borderline range (70–84), and 30-40% have mild intellectual disability (IQ 55-69).[218,220,455,459] More severe intellectual disability is rare,[147,220,460] but 22q11.2DS may be under-recognized in this sub-group.[395] A minority of individuals have intellect in the average range, although various learning difficulties may still be present, e.g., in arithmetic skills.[147,220,461] These may require accommodations in post-secondary education and workplace settings. Most affected individuals will require assistance with understanding and completing forms (e.g., to ensure benefits are received), managing money, and making complex life and work decisions. Structure and routine usually facilitate optimal functioning in all domains and can help reduce anxiety. Using visual reminders may help overcome deficits in verbal (auditory) learning and reduce frustrations for caregivers and patients alike.

On the other hand, deficits in receptive language and comprehension may be camouflaged by relatively good verbal skills, despite speech deficits. Coupled with a tendency to downplay, hide and/or deny existing problems, and difficulty engaging on a first meeting, medical history taking may be challenging. Patience, and collateral information from caregivers who know the patient well, can ameliorate these issues. However, more time and more investigations than for the average patient are often required. Similarly, the cognitive capability of patients with 22q11.2DS to describe or to understand the various medical conditions they have and the need for treatment for these conditions may be suboptimal, especially in the presence of intellectual disability and/or psychotic illness.[220,255] Standard measures to improve compliance with recommended management can include clear and careful explanations to the patient – together with caregivers – about their conditions and treatments. Simple, written instructions and monitoring of medication intake are often helpful.

Cognitive impairments may have an impact on many domains of functioning, including communication, living skills particularly in work settings, and management of finances.[220,255] The presence of a serious psychiatric disorder, such as schizophrenia, can also be an important mediator of functioning.[220,255] For many patients, the availability of supported employment opportunities and a close collaboration between the vocational team (employment specialists) and the clinical team can facilitate finding an appropriate job and job retention. Part-time employment may be preferred, and/or more accommodations provided (e.g., more breaks), especially with limitations such as fatigue and stress sensitivity, that may accompany medical or neuropsychiatric conditions. Areas of relative strength in adult functioning, and data that could help to inform vocational training and expectations, are described in more detail elsewhere.[255]

Deficits in socialization, comprehension, and executive functioning[218,220,255,462] can make some individuals with 22q11.2DS, even those with intellect in the average range, prone to poor social judgment and decision-making in everyday life. They may become involved in financially and/or emotionally exploitative friendships or romantic relationships, without realizing their abusive nature. Internet safety has also become a major issue (e.g., sharing intimate photos or other personal information). Lack of insight into limitations may lead to setting unrealistic goals. It may be a challenge to balance taking the individual's wishes and aspirations into consideration, while attempting to avoid repeated disappointments that are highly discouraging. Family members and professionals involved in the care of patients with 22q11.2DS should be cognizant of these potential problems in order to provide support accordingly.

Financial and other competency-related issues

Legally, individuals are generally presumed to be competent to make decisions in financial, medical, and personal matters when they reach the age of majority. In adults with low baseline intellectual level, specific cognitive deficits, and/or psychotic illness, however, there may be compromised competencies and particularly increased vulnerability to exploitation. With weakness in arithmetic skills common in 22q11.2DS,[220,461] financial competency may need to be assessed in early adulthood, followed up and steps taken to manage identified weaknesses in money management and/or determine formal financial incompetency. Extra lessons and guidance in shopping and budgeting may be beneficial, as may be supervised use of bank

accounts in patients with weak financial skills. In some cases, powers of attorney or legal guardianships may be needed to legally allow a competent parent or caretaker to manage the financial matters of an adult patient.

As for anyone, it would be prudent for patients with 22q11.2DS and their family members to discuss their medical wishes and designate a substitute decision maker or establish a power of attorney or an advance medical directive in preparation for any future development of incompetence because of accident, physical illness, and/or psychiatric illness.

Seizures

Single and recurrent seizures are common across the lifespan and may be unprovoked or related to identifiable factors, especially hypocalcemia.[147,189] The lifetime prevalence of epilepsy in patients with 22q11.2DS is in the 5-7% range,[147,189] far greater than in the general population (0.5-1.0%). There are limited data as to the type, localization, and cause of seizures in 22q11.2DS, particularly in adults.[211] Precipitating events can include fever, ischemia, hypoxia, surgery, medications (including antipsychotics[256]), hyperprolinemia, and hypocalcemia. Hypocalcemia can trigger seizures at any age,[186,204] even in patients with no prior history of hypocalcemia and/or seizures, due to the underlying parathyroid dysfunction that is part of the syndrome. Hypocalcemic seizures will generally resolve with appropriate supplementation[146] and monitoring alone, but anticonvulsant therapy may be indicated should seizures continue after ionized calcium levels have normalized.[463]

Some patients have generalized tonic clonic seizures, with or without myoclonic jerks, characterized as generalized epilepsy. Other seizures may present with minor symptoms such as brief episodes of behavioral arrest and loss of consciousness with or without automatisms. This form of dyscognitive (or complex partial) seizures may be interpreted as confusion or memory loss, potentially leading to underestimates with respect to the true prevalence of seizures in 22q11.2DS. Electroencephalography (EEG), and computed tomography (CT) and/or magnetic resonance imaging (MRI) of the brain, are indicated for all adults with 22q11.2DS with a history suggesting potential non-hypocalcemic seizures. In some cases, seizures may be related to cortical malformation such as polymicrogyria, periventricular nodular heterotopia, or cortical dysplasia.[186,211]

Published case reports indicate that response to standard treatment with anticonvulsant medications appears typical,[189,463] but there are as yet limited data. Standard cautions with respect to need for vitamin supplements with many anticonvulsants would apply.

Endocrine issues

Hypocalcemia (either overt or subclinical/latent) occurs in the majority of patients with 22q11.2DS, and may arise at any age, especially after puberty.[146,147,183-187] The largest study of adults reports 80% with a lifetime history of hypocalcemia,[187] including evidence that most cases of neonatal hypocalcemia had recurrence later in life.[187] Hypocalcemia in 22q11.2DS is typically attributable to hypoparathyroidism.[184,191] Recent data also suggest that in some cases hypothyroidism and hypomagnesemia may be associated findings.[204] Hypocalcemia can be associated with fatigue, emotional irritability, abnormal involuntary movements of any sort, seizures, and cardiac arrhythmias (ECG changes including prolongation of the QT interval), and may predispose to osteopenia/osteoporosis. There is an increased risk of hypocalcemia with any biological stress such as surgery, childbirth, or infection. Hypocalcemia may also be worsened by alcohol or carbonated beverages such as colas.

Regular investigations including pH-corrected ionized calcium, magnesium, parathyroid hormone (PTH), and creatinine levels are recommended.[146] Daily calcium and vitamin D supplementation are recommended for all adults with 22q11.2DS.[146,147] Magnesium supplementation for those with hypomagnesemia is indicated.[204] Treatment with hormonally active metabolites of vitamin D for more severe hypocalcemia usually requires consultation with an endocrinologist. Targeted monitoring of calcium level should be considered at vulnerable times, e.g., peri-operatively, perinatally, or with severe illness. Caution is advised with respect to over-correction, which can result in iatrogenic hypercalcemia, renal calculi, and renal failure. This can occur inadvertently, such as when treatment compliance with calcitriol improves after adequate management of psychiatric illness.

Routine clinical monitoring for autoimmune thyroid disease is also indicated.[147,204,464] Over one in four adults may develop hypothyroidism,[204] and one in 20 have hyperthyroidism.[147] As for hypocalcemia, symptoms of thyroid disease may be confused with those of psychiatric and other

conditions.[204] Thyroid function should be assessed on an annual basis.[146] Standard treatments appear effective.[204]

Obesity has been reported in up to 35% of individuals affected with 22q11.2DS,[147] often with onset in childhood or teenage years,[465] and sometimes with rapid weight gain. Obesity and antipsychotic medication use are known predisposing factors for the development of Type 2 diabetes mellitus, metabolic syndrome, and non-alcoholic fatty liver, the prevalences of which are as yet unknown in 22q11.2DS. Standard dietary and exercise measures are recommended, taking into account the other associated neuropsychiatric and medical conditions present.

Immune deficits

The severity and prevalence of immune deficits present in 22q11.2DS is highly variable, although complete thymic aplasia is very rare.[466] The immunocompromise found in children sometimes persists to adulthood including failure to mount an antibody response to vaccination.[467] Generally however, it is autoimmune disorders that are the major immune system issues in adults.[146] These represent the full range of possibilities, including the endocrine disorders above; others, such as adult arthritic conditions, await formal study.

Cardiovascular manifestations

Congenital cardiac anomalies are common in patients diagnosed as children with 22q11.2DS[427] and represent a chronic disease that requires regular follow-up as an adult. Although early studies reported serious congenital heart disease to be present in most patients with 22q11.2DS,[186,468] if ascertainment outside of cardiac clinics is taken into account, the prevalence appears to be about 40%.[147,469] Echocardiogram in adolescence or adulthood may also reveal aortic root dilation or other minor cardiac anomalies in small numbers of patients.[470]

The most common serious congenital cardiac lesions in adults with 22q11.2DS requiring ongoing care are conotruncal defects, especially tetralogy of Fallot. In developed countries, adults with tetralogy of Fallot have almost always undergone intracardiac repair in childhood. A significant proportion of these adults will require repeat cardiac interventions, such as pulmonary valve replacement for severe residual pulmonic regurgitation.[471,472] Cardiac determinants of outcome include the presence of residual valve lesions (regurgitation or residual obstruction),[473]

the systolic function of the right and left heart,[474] and the propensity of the patient to arrhythmias.[473] Morphological variants such as pulmonary atresia, double outlet right ventricle, atrioventricular septal defect, and branch pulmonary artery stenosis, are important factors in adverse late outcomes,[475] and these are more prevalent in patients with 22q11.2DS than in other patients. Management strategies are lesion specific and are discussed in detail elsewhere.[471,472] Other congenital cardiovascular conditions associated with 22q11.2DS include other conotruncal defects (e.g., interrupted aortic arch) as well as pulmonary stenosis, septal lesions (e.g., ventricular septal defect, patent ductus arteriosus), vascular rings, left-sided lesions and other aortic arch abnormalities. Ongoing follow-up may be warranted.[471,472]

Sexual health and reproductive issues in 22q11.2DS

Although reproductive fitness is reduced,[240] romantic partnerships, sexual activity, and pregnancy are important aspects of adult life for many individuals with 22q11.2DS. Developmentally appropriate sex education and routine reproductive healthcare (including contraceptive counselling for men and women, and Pap tests and sexually transmitted infection screening, as appropriate) are indicated.

General recommendations for managing pregnancies where one member of the couple has 22q11.2DS are provided in Table 8-3. Many common manifestations of 22q11.2DS, including endocrine, cardiac, and psychiatric disease, can pose risks to the mother and fetus during pregnancy and in the post-partum period. In an unpublished case series of pregnancies of women with 22q11.2DS, spontaneous fetal loss (miscarriage/stillbirth), pregnancy complications, prematurity, and low birth weight were common (Bassett, personal communication). There is evidence of elevated risks for fetal/neonatal complications for pregnancies where the fetus has a 22q11.2 deletion,[476,477] warranting consideration of high risk antepartum care. Specific pregnancy and contraception education is required for those women with congenital cardiac lesions because pregnancies in this group are associated with increased risks of maternal and fetal/neonatal complications.[472,478]

In some individuals, delayed maturity and poor decision-making abilities may increase the likelihood of engaging in high risk sexual practices.[240] Resources and guidelines for sexuality and relationship education for adults with intellectual disabilities or other genetic syndromes like trisomy 21 may be helpful when counselling individuals with 22q11.2DS. Notably, women with

Table 8-3 General recommendations for prenatal and perinatal care of adults with 22q11.2DS

General recommendation	Details
Discuss pregnancy options	• With both partners, and family members and health professionals as appropriate
Ensure provision of genetic counseling	• Including both partners, and other guardians/caregivers as appropriate • Discussion of: (i) recurrence risk (50% chance of having a child with 22q11.2DS), (ii) unpredictable intra-familial variability in expression, (iii) potential impact of maternal morbidities and associated treatments on maternal-fetal health, and (iv) challenges in caring for a child with (or without) the 22q11.2DS
Monitor for known and new onset endocrine conditions	• Monitor for and promptly treat gestational diabetes • Regular 22q11.2DS laboratory investigations, e.g., TSH, ionized calcium (Table 8-1) • Calcium and vitamin D supplementation
Optimize psychiatric care for mother and fetus, and partner if necessary	• Confirm medication safety (e.g., www.motherrisk.org) and necessity, and modify medications (only as required) • Monitor for signs of peri- and post-partum mood and psychotic disorders
Genetic diagnosis of 22q11.2 deletion in fetus/conceptus	• Standard prenatal testing using FISH, MLPA, or chromosomal microarray to analyse chorionic villus sample (beginning at 10-12 weeks gestation) or chromosome preparations from fetal cells obtained by amniocentesis (beginning at 15-18 weeks gestation) • Preimplantation genetic diagnosis and assisted reproductive techniques would be available in some jurisdictions for select cases • Non-invasive prenatal genetic testing is an emerging technology
Evaluate for potential fetal anomalies	• High-resolution ultrasound examination for polyhydramnios, and palatal, renal, and other anomalies (beginning at 18-22 weeks gestation) • Fetal echocardiogram (18-22 weeks gestation)
Provide and reinforce general recommendations	• Maintain good nutrition and physical activity • Standard preconception folate/vitamin supplementation • Avoid smoking, alcohol, and street drugs • Avoid known teratogens (e.g., retinoic acid)
Consider delivery at a tertiary care center and/or a center experienced in the care of neonates with 22q11.2DS and associated obstetric complications	• Women with significant congenital cardiac conditions should also be monitored by their cardiologists

22q11.2DS, 22q11.2 deletion syndrome; FISH, fluorescent *in situ* hybridization; MLPA, multiplex ligation-dependent probe amplification; TSH, thyroid-stimulating hormone. *Sources:* Adapted from [149,479]

22q11.2DS who have neither schizophrenia nor intellectual disability, regardless of the presence of cardiac anomalies, may have a similar number of offspring as their unaffected sisters.[240]

Outcomes in older adults

Information about long-term outcome and older age ranges is limited for 22q11.2DS; most reports involve individuals on average in their mid 30s. The substantial proportion of patients living into mid-adulthood presents a new set of challenges for long-term care. The aging parents of the current cohort of adults with 22q11.2DS are often faced with the complexities of managing their adult children in their home or elsewhere, which may present significant financial, physical, and emotional stress. A close partnership between families and appropriate professional agencies, including social work and occupational therapy, can help address these challenges. Spouses, siblings and other relatives may also play an important role.[439,450,480]

Early-onset Parkinson's disease and neurodegenerative diseases

There is accumulating evidence that 22q11.2DS is associated with an increased risk for early-onset (age <50 years) Parkinson's disease.[142,144,252] In one study, the occurrence of Parkinson's disease was significantly elevated over population expectations: 5.9% of 68 patients aged 36 to 64 years were diagnosed with Parkinson's disease.[252] All showed early-onset of motor symptoms, and typical symptom pattern, disease course, and treatment response. Periodic neurological assessment for signs of parkinsonism should thus be considered, especially for adults presenting with changes in motor functioning. Atypical antipsychotics, e.g., clozapine and quetiapine, that are associated with fewer extrapyramidal side effects will likely be preferable for patients who have both Parkinson's disease and schizophrenia.[252] Calcium levels should be monitored appropriately, as hypocalcemia may induce or aggravate existing tremors.[252] Functional imaging, where available, may help to distinguish Parkinson's disease from extrapyramidal side effects of antipsychotics.[144,252] There are as yet few studies of other neurodegenerative disorders in 22q11.2DS. In individuals with severe intellectual disability and psychotic illness may develop a form of dementia.[395]

Premature mortality in 22q11.2DS

Individuals with 22q11.2DS who survive childhood appear to be at increased risk of premature mortality as adults, not attributable to any single identifiable factor. In one study of 102 adults (mean 33.6 years) with 22q11.2DS, survival to ages 40 and 50 years was 89.9% and 73.9%, respectively.[262] The median age of death in 12 individuals was 41.5 years (range 18.1–68.6) and the causes of death reported were sudden death (most common), heart failure, stroke, suicide and postoperative complications.[262] Further studies, including essential post mortem data, are required to better define life expectancy, the mechanism responsible for sudden death, and the roles of major associated conditions.

Genetic counseling for adults with 22q11.2 deletion syndrome

Genetic counselling is an essential component in the ongoing management of adults with 22q11.2DS for all patients and concerned relatives at multiple time points, from late adolescence through adulthood.[146] Follow-up genetic counselling may also be necessary as knowledge about 22q11.2DS continues to expand. Although genetic counselling would ideally be provided by a trained genetic counsellor or medical geneticist, all clinicians involved in the ongoing care of an adult with 22q11.2DS should be in a position to reinforce the required information provided in genetic counselling sessions, particularly as it pertains to their medical specialty.

For adults who were diagnosed in childhood, a best case scenario would mean early interventions for developmental delay and learning difficulties,[481] anticipatory medical care that may have reduced morbidity,[147,149,204,479] and genetic counselling that provided updated information about the basic genetics and later onset features. However, the extent of genetic counselling will vary depending on the age, location, and presentation of the individual at diagnosis and follow-up, as well as the background of the clinician providing counselling. Children may not have been included in, or may not have understood, the genetic counselling provided. Furthermore, adolescents transitioning out of pediatric care often do not yet possess the maturity, curiosity, or educational preparation to fully appreciate the content and message of genetic counselling. Individuals diagnosed following birth of their child with 22q11.2DS may be more likely to have a milder clinical presentation,[154,240,439,482,483] but genetic counselling and a full systems review and standard investigations remain indicated and may reveal previously undetected medical conditions (e.g., hypocalcemia, renal abnormalities).[427,484]

Given the advantages of early detection and treatment of late-onset features of 22q11.2DS, all adolescents and adults should be informed of known clinical manifestations of the syndrome. Previously, there may have been concerns about “information overload” for the patient or apprehension about fully discussing the wide spectrum of possible phenotypes, especially the increased risk for psychotic illness.[449,457] A matter-of-fact approach with a competent and knowledgeable professional will help in all situations. Genetic counselling for adolescents and young adults especially should include discussion of, and preparation for, transition issues and reproductive decision-making.

Basic genetic counselling includes the fact that an affected individual, male or female, has a 50% chance of having an affected child at each pregnancy. In light of the variability of the syndrome, it is impossible to predict the range and severity of manifestations in the offspring. It can be challenging to explain to an adult with 22q11.2DS how their child may be "like them" (in having a 22q11.2 deletion) and yet not "like them" (in having a different phenotype). A diagram showing the contribution of a different intact chromosome 22 for a parent and offspring may be helpful. Notably, a parent who has experienced a life with 22q11.2DS may have a different perception of what the condition is compared with an unaffected couple discovered to have an affected fetus or infant.[469] When an adult is identified as having 22q11.2DS only after the birth of their affected child, the parent requires genetic counselling that focuses on his or her own diagnosis. This often includes the need for additional support in managing the care of their affected child.[439,469,479]

Traditional genetic counselling approaches must be modified to take into account the learning disabilities and other neuropsychiatric issues that are common in adults with 22q11.2DS (Table 8-4).[146,220,485] Finucane has presented sample dialogue from a genetic counselling session involving an adult with 22q11.2DS and intellectual disability to illustrate her technique.[485] In our experience, most adults with 22q11.2DS are able to learn simple facts about the genetics of their condition, and are satisfied with the standard genetic counselling process, provided there are the necessary modifications to traditional techniques.

Widespread use of genome-wide genetic diagnostic technologies, declining infant mortality with advances in pediatric surgery and care, non-zero reproductive fitness, and a 50% recurrence risk in 22q11.2DS predict a growing population of (diagnosed and undiagnosed) adults with

Table 8-4 Genetic counselling strategies for adults with 22q11.2DS with intellectual disabilities and/or psychotic illness

Traditional genetic counselling approach	Common characteristic of patients with 22q11.2DS	Alternative genetic counselling strategy for patients with 22q11.2DS
Assumption of average intelligence	Pervasive but extremely variable level of learning difficulties and cognitive impairment	Spend time gaining an understanding of the specific level of cognitive and practical functioning, and any specific learning disabilities Obtain neurocognitive testing or refer to existing test results
Discuss probability and risk	Difficulty understanding abstract concepts	Minimize discussion of probabilities and risk
Minimal use of visual aids	Visual memory may be a relative strength compared with auditory memory	Use simple diagrams and visual aids to illustrate all major points Provide handouts
Use analogies to illustrate concepts	Difficulty generalizing from one situation to another	Minimize use of analogies to illustrate concepts
A single genetic counselling session	Attentional dysfunction and other cognitive impairments	Ensure frequent repetition and reinforcement of information over the course of multiple genetic counselling sessions Provide written summary to patient and caregivers
Nondirectiveness	Expects to be told what to do	Based on discussion of patient's feelings and attitudes, summarize, rephrase, and verify patient's decision
Plan ahead to maximize prenatal testing options	Functions in the "here and now"	With patient's permission, involve his/her support system in making appointments, giving reminders and reviewing/reinforcing information discussed during genetic counselling
Identify patient's past decision-making strategies	Little or no experience in complex decision making	Elicit patient's feelings and attitudes related to the current decisions
Openly discuss psychosocial issues (e.g., abilities, reaction to stressful situations and level of responsibility, fears and concerns, suggestibility)	Denial of learning or social problems	Use nonjudgmental language to elicit history Use patient's preferred term for his/her own disability, even if not technically correct
Confidentiality, patient autonomy	Functions as part of a "system" which often includes family and professionals	Recognize that many genetic counselling tasks may be accomplished with involvement of the patient's support system
Use patient letter and literature to supplement counselling	Limited reading abilities	With permission, engage a support person to review summary letter with patient Use visual diagrams/handouts

Source: Adapted from [485]

22q11.2DS.[240,479] Increasing detection of 22q11.2DS is expected to place greater demands on healthcare providers, in particular with the introduction of non-invasive prenatal testing for microdeletion syndromes including 22q11.2DS. Studies are needed to assess impact on health care resources, given the likely high associated health care costs and use of medical services by adults with 22q11.2DS.

Conclusions

More information about adult outcomes in 22q11.2DS is urgently needed to assist accurate genetic counselling and to improve care and service planning. Many important conditions in the general adult population (e.g., cancer, chronic autoimmune conditions, hematologic issues) are understudied in 22q11.2DS. There is little evidence to support 22q11.2DS-specific management for associated conditions, and as yet there are few dedicated adult 22q11.2DS clinics. The issues identified herein with respect to 22q11.2DS may be common to multisystem genetic syndromes in adulthood more generally, and will increasingly challenge the traditional pediatric focus of clinical genetics services.

Chapter 9 General discussion and future directions

9 Overview

9.1 Review of research aims and hypotheses

The overarching goal of this thesis was to investigate hemizygous 22q11.2 deletions as a risk factor for early-onset PD (≤50 years), and to begin to characterize disease pathogenesis and the corresponding implications for patients with the associated multisystem syndrome, 22q11.2DS. In brief, the studies provided evidence that (i) hemizygous 22q11.2 deletions are a rare risk factor for early-onset PD that involves classic loss of midbrain dopaminergic neurons and variable Lewy body pathology; (ii) complex genetic mechanisms may be involved in the expression of PD in 22q11.2DS; (iii) adults with 22q11.2DS may exhibit motor and olfactory deficits relevant to the PD prodrome, as well as atypical pre-morbid nigrostriatal dopaminergic dysfunction; (iv) clozapine may be a good candidate to manage psychosis in patients with 22q11.2DS at risk of, or with, PD; and (v) pre-morbid cognitive level and severe psychiatric disorders mediate widespread functional deficits in adults with 22q11.2DS. Collectively, the findings of this thesis provide initial insights into the aetiology, pathogenesis, and features of the 22q11.2DS-PD phenotype, and some of the possible clinical implications of early-onset PD for patients with 22q11.2DS.

The first aim of this work was to evaluate a possible association between 22q11.2 deletions and early-onset PD, spurred by case reports of several individuals with 22q11.2DS and early-onset parkinsonism in the medical literature. The relevant data are presented in Chapter 3.[255] The results of this study revealed a significantly elevated risk of early-onset PD in the 22q11.2DS adult cohort assessed, relative to general population estimates. Importantly, neuropathology in available brain tissue in three cases confirmed the antemortem clinical diagnoses of PD and provided the first neuropathological description of 22q11.2DS-PD. Together these findings provided evidence of a pathogenetic link between 22q11.2DS and an early-onset form of PD.

The second aim of this thesis was to investigate possible genetic mechanisms and clinical features relevant to PD that might help inform PD risk within 22q11.2DS and provide a window on 22q11.2DS-PD pathogenesis. Evidence for reduced penetrance of PD in 22q11.2DS, shown in

Chapter 3,[255] suggested that other factors may influence expression of PD in combination with the 22q11.2 deletion. Whole-genome sequencing of the three cases with neuropathologically confirmed PD[255] provided preliminary evidence that an increased burden of rare mutations affecting genes in a functional PD-relevant network could potentially contribute to the development of PD in 22q11.2DS (Chapter 4). No evidence was found to suggest that PD was caused by an unmasked autosomal recessive locus on the intact 22q11.2 chromosome in these three cases. The data also did not show any contributing rare mutations in any known PD causative or risk genes elsewhere in the genome. Clinical and neuroimaging phenotypes were assessed as possible predictive or early markers of PD in adults with 22q11.2DS at age-related risk (≥30 years) of 22q11.2DS-PD. The results indicated that, relative to age and sex-matched controls, adults with 22q11.2DS commonly exhibit motor and non-motor symptoms possibly consistent with an early "pre-diagnostic" stage of PD (Chapter 5[254]). In particular, severity of bradykinesia may represent a putative clinical marker of early nigrostriatal neurodegeneration and increased PD risk in patients with 22q11.2DS. Evidence of a presynaptic (hyper)dopaminergic abnormality, identified using PET neuroimaging, suggested the potential of a novel mechanistic pathway to parkinsonism and/or PD in 22q11.2DS. A single 22q11.2DS patient diagnosed with PD showed typical findings, including evidence of severe striatal dopamine depletion, on PET imaging.

The third aim was to begin to explore the possible clinical implications of PD in the context of 22q11.2DS and associated neurophenotypes. This included (i) assessing patients with 22q11.2DS-associated schizophrenia for treatment response to clozapine, as compared with patients with idiopathic forms of schizophrenia (Chapter 6[256]); (ii) evaluating the pre-morbid functional abilities of 22q11.2DS adults to identify possible mediators of baseline functional capacity (Chapter 7[255]); and (iii) the development of guidelines to help inform cohesive management of the associated multisystem conditions in 22q11.2DS, including early-onset PD (Chapter 8[5]). The results provided evidence that treatment response to clozapine in 22q11.2DS is excellent, though a lower dose and prophylactic management of a lowered seizure threshold may be useful. Functional deficits that have an impact on most major aspects of daily adult life were found to be prevalent (even before the onset of any neurodegenerative disease) in the assessed cohort. These appeared to be primarily mediated by intellect and expression of schizophrenia in 22q11.2DS, and independent of 22q11.2DS-associated congenital heart defects or mood and

anxiety disorders. Finally, practical guidelines were developed to help guide overall management of adults with 22q11.2DS, informed in part by the results of this thesis and the increasing body of literature on adults with 22q11.2DS.

9.2 Advances and challenges in delineating the role of 22q11.2 deletions in Parkinson's disease

The starting point of this thesis was to evaluate the possible association between recurrent typical 22q11.2 deletions and early-onset PD (<50 years). The finding of a significantly increased occurrence of early-onset PD in unrelated 22q11.2DS patients (four of 68 patients above age 35 years; 5.9%) relative to general population estimates, together with supportive neuropathology and three previous case reports in the literature,[143,144,315] provided evidence of an involvement of the 22q11.2 deletion in early-onset PD.[252] A limitation of the work was the small number of 22q11.2DS patients with PD available for clinical and neuropathological characterization, and the lack of a replication study in an independent cohort of adults with 22q11.2DS. A challenge in studies involving 22q11.2DS adults is the relative rarity of the condition (1 in 2000-4000 live births[145,146]). This is further compounded by under-diagnosis of 22q11.2DS in the adult population[152-154] and premature mortality associated with the condition (median age at death is 40-45 years).[252,262] Larger-scale screening studies in 22q11.2DS populations are necessary to identify additional patients with 22q11.2DS-PD to permit further characterization of the prevalence, clinical features, disease course, and neuropathology of 22q11.2DS-associated PD. The recently established International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome, which includes 600 adults (≥18 years, including 127 subjects over age 35),[448] promises potential for initiating larger-scale studies of PD and other later-onset conditions in 22q11.DS.

Notably, since the 22q11.2DS-PD study[252] was published, additional 22q11.2DS-PD cases have emerged from around the world (Table 9-1). This provides some additional confirmatory support for a role of the 22q11.2 deletion in early-onset PD. In total, we are now aware of 15 cases of L-dopa responsive 22q11.2DS-PD, all with early-onset (Table 9-1). These include six unpublished cases (three with very young onset, <30 years). The clinical profile of PD in 22q11.2DS, based on the available limited data, appears to involve early-onset of typical motor symptoms and good

Table 9-1 Known unpublished and published cases of 22q11.2DS patients clinically diagnosed with L-dopa responsive early-onset Parkinson's disease (n=15) current to July 2015

Sex	Age at onset (y)	Supporting neuropathological or neuroimaging results (n=6)	Treated pre-morbid psychotic illness (n=6)	Source
N/A	Juvenile	Severe striatal dopamine depletion (DAT SPECT imaging)	N/A	Personal communication (Italy)
N/A	24	N/A	N/A	Personal communication (The Netherlands)
M	25	N/A	N/A	Personal communication (Germany)
M	30	No	No	Rehman et al. 2015[486]
M	36	N/A	Yes	Butcher et al. 2013[252]
M	~40	N/A	No	Personal communication (USA)
F	Early 40s	N/A	Yes	Personal communication (Chile)
M	42	N/A	No	Zaleski et al. 2008[143a]
M	*42	Severe striatal dopamine depletion (DAT SPECT imaging)	Yes	Booij et al. 2010[144]
M	44	Nigral cell death	No	Butcher et al. 2013[252] Zaleski et al. 2008[143a]
F	45	Severe striatal dopamine deletion (VMAT2 PET imaging)	No	Chapter 5 (this thesis)
F	45	Nigral cell death, Lewy bodies	Yes	Butcher et al. 2013[252]
M	45	N/A	Yes	Verhoeven et al. 2015[487]
M	48	Nigral cell death, Lewy bodies	Yes	Butcher et al. 2013[252]
F	48	N/A	No	Personal communication (Belgium)

Abbreviations: DAT SPECT, dopamine transporter imaging using single photo emission tomography; F, female; M, male; N/A, data not available; VMAT2 PET, vesicular monoamine transporter 2 neuroimaging using positron emission tomography

* denotes median age at onset of PD among these 15 known cases. Highlighting denotes cases described within this thesis. A study is in progress to gather additional cases and data to formally publish as a case series.

response to L-dopa treatment.[252] This initial data are in keeping with comparable results for other genetic forms of early-onset PD (e.g., involving *PARK2, PINK1, DJ-1* mutations).[120] 22q11.2DS-PD patients with atypical features (e.g., dystonic, oculomotor, or pyramidal abnormalities reported in some juvenile forms of PD[17,120]) may be yet to be described. Other 22q11.2DS-associated neurological phenotypes may contribute to the clinical picture and require consideration in clinical and research studies of PD in 22q11.2DS (Table 9-2).[5,7] Efforts to collate detailed phenotypic and supporting diagnostic information for a larger number of cases are ongoing and may help to further delineate the clinical spectrum of PD and related neurological disorders in patients with 22q11.2DS.

9.2.1 On expanding the genetic architecture of Parkinson's disease

The last two decades have marked a burgeoning field of genetics in PD, following the discovery of *SNCA* mutations as the first molecular genetic cause of PD in 1997.[85] A genetic cause can now be pinpointed in 10-15% of all PD cases, more frequently among patients with a family history of the disease and/or who have early disease onset.[17] These genetic advances have provided evidence that PD is the clinical endpoint of a heterogenous collection of genetic mutations, associated with a spectrum of overlapping clinical symptoms and pathologies.[488] The more than 20 rare disease-causing mutations and high risk genetic variants reported to be involved in PD (Table 1-2) may now be joined by the hemizygous 22q11.2 deletion. Here there is also early, and sometimes potentially juvenile, onset of the disease (Table 9-1), as there is for six of the ten genes (*PARK2, PINK1, DJ-1, ATP13A2, PLA2G6, FBXO7*) with strong confirmatory evidence for involvement in PD. The 22q11.2DS form of PD appears to be characterized by variable Lewy body pathology[252] reminiscent of that reported for autosomal dominant late-onset *LRRK2*-associated PD[99,489,490] and possibly also for autosomal recessive early-onset *PARK2* mutations.[489,491] This pathological variability may potentially suggest the involvement of a pathogenic mechanism that is upstream of alpha-synuclein, as proposed in the case of *LRRK2*.[125,492] All other early and juvenile-onset genetic forms of the disease reported to date have involved autosomal recessive mutations.[17,120] This led to the proposition that the 22q11.2 deletion may in fact be unmasking a recessive allele on the intact chromosome in 22q11.2DS-PD patients.[306] No evidence was found to support this hypothesis, however, in the three patients with 22q11.DS-PD and whole-genome sequencing results (Chapter 4).

Table 9-2 Co-morbid conditions in 22q11.2DS that may impact motor functioning and/or diagnostic assessment and treatment of neurological conditions

Co-morbid conditions[a]	**Clinical implications**
Congenital abnormalities	
Craniocervical spine anomalies	Cervical traction therapy, neurosurgery, as required
Patellar dislocation (10%)	Orthopedic surgery, as applicable
Scoliosis (45%)	Surgery, bracing, physical therapy, as necessary
Endocrine-related diseases	
Hypocalcemia (>60%)	Calcium and Vitamin D supplementation
Thyroid dysfunction and/or treatment	
Hypothyroidism (20%)	Initiate or adjust treatment
Hyperthyroidism (5%)	Initiate or adjust treatment
Seizures (40%)	
"Idiopathic" epilepsy (5%)	Anti-epileptic medications
Hypocalcemic seizures	Calcium and Vitamin D supplementation
Drug-induced seizures	Adjust dosage, reconsider medication choice
Other (e.g., fever, ischemia/hypoxia)	As applicable
Psychiatric disorders and treatments (60%)	
Psychotropic medication (e.g., antipsychotics, anticonvulsants)	Adjust dosage, reconsider medication choice, "start low, go slow" dosing approach
Parkinson's disease (6%)	Dopaminergic therapy; careful balancing with antipsychotics, as applicable

[a]Rates are estimates of lifetime prevalence of features for 22q11.2DS based on current data.[146,147] Estimates may vary depending on age of the patient and patient ascertainment sources, or change with increasing knowledge of 22q11.2DS.

Adapted with permission from John Wiley and Sons, from the following published journal article:

Boot E, **Butcher NJ**, Van Amelsvoort TA, et al. Movement disorders and other motor abnormalities in adults with 22q11.2 deletion syndrome. *Am J Med Genet A.* 2015; 167A:639-645.

The evidence that hemizygous 22q11.2 deletions, a large recurrent copy number variant (CNV) involving 46 protein-coding genes[6] may be a genetic risk factor for early-onset PD suggests that large rare structural variants, in addition to sequence-based (point mutations including nonsense and missense mutations) and other small structural variants (e.g., deletion or duplication of a single PD gene or its exons; Table 1-2), may contribute to the genetic architecture of PD.[252,493] Intriguingly, this might suggest that the 22q11.2 deletion confers an increased susceptibility to PD through the simultaneous disruption of multiple PD-related mechanistic pathways[307] through its single locus, consistent with a polygenic basis suspected for the majority of PD cases where no causative Mendelian mutation is found.[494] This suggests potential new avenues of genetic research in PD. CNVs are well-established genetic factors in developmental brain-based diseases such as autism and schizophrenia.[121,122] However, data are limited in PD with respect to the possible role of large CNVs, i.e., involving the gain (e.g., insertions or duplications) or loss (deletion) of a large (e.g., >500 kb) stretch of DNA sequence (Table 9-3).

To date, only five studies examining the possible contribution of genome-wide CNVs (rare or common) to PD have been reported (Table 9-3).[275-277,495,496] Only one of these employed a case-control approach that included the assessment of the burden of large rare CNVs in PD (Table 9-3). This study of 261 patients with PD (24.5% with early-onset <50 years, not analyzed separately), found that large (>500 kb) rare CNVs were significantly enriched in PD cases relative to controls (1.24-fold higher)[495] in an Ashkenazi Jewish cohort. The relatively limited scope of the CNV studies, including a lack of study of early-onset PD cases, performed to date likely explains why the recurrent 22q11.2 deletion associated with 22q11.2DS was not identified in these few previous studies. Patients with 22q11.2DS would be relatively unlikely to meet inclusion criteria for these studies. The reported demographics of the populations considered (Table 9-3) tend to focus on older age and/or later onset of PD,[276,495] positive family history of PD,[277] and/or exclusion of individuals with history of antipsychotic exposure.[275] In contrast, there is premature mortality associated with 22q11.2DS,[262] patients with 22q11.2DS would be unlikely to show a pattern of familial PD (~90% of 22q11.2 deletions are *de novo*[156]), and ~25% of adults with 22q11.2DS have schizophrenia treated with an antipsychotic medication.[5,150,156] Other large, rare, pathogenic CNVs with highly penetrant pathogenic features may also be less likely to be found in late-onset or familial cases for similar reasons.

Table 9-3 Studies reported to date involving genome-wide investigations of copy number variations in Parkinson's disease

Reference	Kim et al., 2008[275]	Simon-Sanchez et al., 2008[276]	Pankratz et al., 2011[277]	Pamphlett et al., 2012[496]	Liu et al., 2013[495]
N (PD/controls)	30/0	273/275	816/856	8/26	261/178
PD subject age (y)	Mean 64.6±8.8	55-84	Mean 62.1±10.4/61.4±11.6 (two cohorts merged)	63-81	Mean 60±12.2 (~25% with early-onset)
PD classification	Sporadic	Primarily sporadic (59 [21.6%] with family history)	Familial	N/A	Primarily sporadic (53 [20.3%] with family history)
Ethnicity	N/A	Caucasian	N/A	Caucasian	Ashkenazi Jewish
Platform	MACArray-Karyo 4K BAC-chip	Illumina Infinium Human-1 and HumanHap300	Illumina Human CNV370 array	Affymetrix Human SNP Array 6.0	Illumina Human 660 or 610-quad bead arrays
Major findings and remarks	Described copy number gains and losses in broad chromosomal regions	Homozygous PARK2 deletion identified in one PD case; 38 rare duplications and 44 rare deletions identified in PD cohort (not found in the control group or reference populations in DGV), all considered likely to be "benign" and not considered further	Assessed frequency of individual CNVs in PD cases compared with controls; only PARK2 CNVs met criteria for genome-wide significance	45 CNVs identified in 7 of 8 PD brains were not found in the controls; only 3 CNVs were rare (not in DGV) and these each overlapped a single gene. The median number of total CNVs per brain was similar in PD and control brains.	Large (≥500 kb) rare (≤1% in dataset) CNVs significantly associated with PD (1.24-fold higher in PD cases than controls)

None reported the recurrent 22q11.2 deletion associated with 22q11.2DS, see text for details.

Abbreviations: CNV, copy number variants; DGV, Database of Genomic Variants; N/A, not reported; PD, Parkinson's disease.

Patients with early-onset forms of the PD, where the genetic contribution to disease appears to be greater than that for patients with late-onset disease,[120,259] may be the most likely to be enriched for large rare CNVs. Such CNVs could potentially explain the development of PD in at least some cases of early-onset PD where no causative mutation has yet been identified. Currently, autosomal recessive *PARK2* mutations account for up to 15% of sporadic early-onset cases, while *PINK1* and *DJ-1* mutations are found in ~1-8 % and ~1-2% of cases, respectively.[120,133,259] Our targeted screening study of 225 early-onset PD patients for 22q11.2 deletions provided preliminary evidence that the 22q11.2 deletion is a rare (<1%) cause of early-onset PD.[252]

Future large well-powered studies (taking into account the ascertainment/inclusion criteria issues discussed above) demonstrating an enrichment in early-onset PD cohorts for the 22q11.2 deletion, and possibly other large rare CNVs,[495] would provide additional evidence of a putative contributory role of large rare CNVs to the genetic architecture of PD and potentially add to the growing collection of rare variants involved in PD pathogenesis.

9.2.2 Future possibilities using mouse models of the 22q11.2 deletion

Recapitulation of PD-relevant neurobehavioural (e.g., motor dysfunction and non-motor symptoms, including olfactory, cognitive, and autonomic disturbances) and neurodegenerative (e.g., degeneration of dopaminergic neurons and Lewy body pathology)[497] phenotypes in a mouse model of 22q11.2DS could help provide additional confirmatory evidence of the involvement of the hemizygous 22q11.2 deletion in PD. There are mouse models of 22q11.2DS available that have already proved useful for investigating the molecular function of 22q11.2 genes and their relationship to several 22q11.2DS-associated phenotypes.[498-500] These include models with deletions of large portions of the syntenic region on mouse chromosome 16.[165,498] For example, the well-established *Df1* mouse model involves a hemizygous 1.2 Mb deletion of 21 of the 32 coding genes of the human proximal deletion region.[501] These mice exhibit complex phenotypes similar to clinical manifestations of 22q11.2DS, such as variable expression of congenital heart defects.[501,502] Phenotypes relevant to PD have not been reported in mouse models of 22q11.2DS, as *Df1* and other mouse models of 22q11.2DS have primarily been studied in early developmental stages, in the context of congenital and neurodevelopmental 22q11.2DS-associated phenotypes.[167,245,503]

The neurodegenerative pathology of PD has been particularly difficult to achieve in currently available mouse models involving monogenic PD mutations (reviewed in Chesselet & Richter, 2011).[497] None develop Lewy bodies and few (e.g., some involving *SNCA* and *PARK2* mutations) even show the expected pattern of dopaminergic loss.[497] A recently developed mouse model with high overexpression of human mutant (A53T) α-synuclein specifically in dopamine neurons, via a "positive feedback" gene expression amplification system under a *DAT* promoter, showed evidence of increased dopamine neuron loss compared with previous *SNCA* mouse models.[504] These mice also exhibited mitochondrial abnormalities that preceded the onset of the degeneration of dopamine neurons. The ability to model key pathological features of PD is necessary to facilitate pre-clinical drug testing of preventative and therapeutic treatments for PD.[497] In a pilot study (Butcher & Kiehl; unpublished observations), we assessed ten aged (to mean 13 months) *Df1* mice for PD neurodegenerative pathology. As for most mouse models of PD,[497,505] we found no evidence of either nigrostriatal neurodegeneration or Lewy body pathology. It remains possible that strongly reduced penetrance of PD in 22q11.2DS requires sampling of a larger number of animals, or that the mice may go on to develop PD-like neuropathology at even older stages. No formal behavioural data were available for these aged mice, but there were no gross differences noted by the experienced handlers in Prof. Peter Scambler's laboratory at University College London. Future studies performing longitudinal assessments at different developmental stages using formal testing of PD-relevant motor and non-motor behaviors may reveal features in mouse models of 22q11.2DS supportive of the clinical association reported for 22q11.2DS and PD,[252] as for other PD mouse (monogenic) models despite the relative lack of typical neurodegenerative pathology.[497]

The use of additional physiological or genetic manipulations ('sensitization')[506-509] in mouse models of 22q11.2DS could potentially help reveal or enhance PD-associated phenotypes and facilitate the identification of the involved molecular pathways. For example, *DJ-1* knockout mice, a model of autosomal recessive DJ-1 (PARK7)-associated PD show increased susceptibility to striatal denervation following systemic injection of the mitochondrial neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).[510] This helped to identify a role for DJ-1 in the protection of neurons against oxidative stress, a condition in which the cellular antioxidant defense mechanisms are unable to keep the level of reactive oxygen species below a toxic threshold.[506,510] Impaired mitochondrial function increases oxidative stress, which

is strongly implicated in the pathophysiology of PD.[511] The 22q11.2 deletion region notably contains six genes involved in mitochondrial function.[6,245] It will be of interest to determine if 22q11.2DS mouse models exhibit increased sensitivity to MPTP that could implicate a neurodegenerative mechanism involving oxidative stress.

The introduction of additional genetic disruptions (outside the 22q11.2 deletion region) to a mouse model of 22q11.2DS could also be informative. Overexpression of mutant (A53T) alpha-synuclein in conjunction with wild-type or mutant (G2019S) LRRK2 under a *CaMKII* promoter, for example, provided evidence that these two proteins, alpha-synuclein and LRRK2, may function in a common molecular pathway.[125,507] These mice showed worsened neurodegenerative pathology than that observed in mutant (A53T) alpha-synuclein mice, including increased loss of neurons in the dorsal striatum and increased alpha-synuclein accumulation.[507] In a different *SNCA* mouse model with higher expression levels of human mutant (A53T) α-synuclein, mitochondrial abnormalities were worsened following genetic deletion of *PARK2* or *PINK1*. This provided the first *in vivo* evidence of a synergistic relationship between mutant α-synuclein and parkin or PINK1 deficiency in mitochondrial disruption.[504] Assessments of 22q11.2DS mouse models crossed with any of the primary monogenic mouse models of PD, if viable, could potentially help identify the mechanism and pathways underlying 22q11.2DS-associated PD. Such experiments however will likely await a more complete study of PD-relevant phenotypes under baseline conditions and at older ages in 22q11.2DS mouse models.

9.2.3 Reduced penetrance of the 22q11.2 deletion and other genetic mutations involved in Parkinson's disease

As a progressive age-related neurodegenerative brain disorder, the penetrance of mutations associated with PD is often incomplete and varies considerably with age. Reduced penetrance associated with disease-causing/high risk genes in PD poses challenges in our understanding of the aetiology of the disorder at the individual level, and how to best introduce clinical genetic testing with the provision of informative genetic counselling.[16,127,512] This has been well-illustrated in the case of *LRRK2* G2019S mutations carriers, the most common cause of both familial and sporadic PD.[18,124] The penetrance of PD in *LRRK2* G2019S mutation carriers ranged from approximately 20% at age 50 years to 75% by age 80 years in a large study of 1045 mutation carriers (133 families) from 24 populations worldwide.[124,127] Penetrance in the Ashkenazi Jewish population appears to be lower, with PD manifesting in ~25% of *LRRK2*

G2019S mutation carriers by age 80 years.[513] The penetrance of early-onset PD in 22q11.2DS requires further study with much larger samples of all age groups. Our initial findings however clearly indicate reduced penetrance: approximately 6% of 22q11.2DS adults aged 35 to 64 years had PD.[252] In contrast, autosomal recessive mutations in *PARK2*, *PINK1*, and *DJ-1* associated with early-onset PD appear to be fully penetrant.[120,514]

It has been hypothesized that reduced penetrance associated with PD mutations may be mediated by other genetic, and non-genetic, disease-modifying factors.[307,512,515] For example, common PD susceptibility variants (single nucleotide polymorphisms, SNPs)[134,136,137] and environmental risk factors,[10,19] identified in studies of idiopathic PD, could be involved. Individually, these factors would be expected to have a small effect (~OR<2) on modifying PD risk.[10,19,134,136,137] In contrast, rare coding sequence variants are enriched for deleterious alleles that may have a larger effect on disease risk.[284,285] An excess, or burden, of genome-wide rare protein code-altering variants in PD-relevant genes and pathways has recently been proposed to play a role in susceptibility to idiopathic forms of PD[516] and as a possible modifier of *LRRK2*-associated PD penetrance.[515]

As an initial assessment of the possible impact of rare genome-wide variants on the penetrance of PD in 22q11.2DS, we performed a pilot whole-genome sequencing (WGS) study in nine 22q11.2DS patients discordant for neuropathologically confirmed[252] early-onset PD. The results suggested that early-onset PD in 22q11.2DS, did not, in the subjects assessed, appear to be (i) the result of the unmasking of a recessive coding sequence mutation on the intact chromosome 22q11.2, or (ii) caused or mediated by rare sequence or structural variants in known PD causative or risk genes. The results supported the possibility that early-onset PD in 22q11.2DS may be mediated by the cumulative burden of rare mutations in genes outside the 22q11.2 region that perturb a functional PD-relevant network. Supporting the potential specificity of this PD-network finding to PD risk, no enrichment of rare variants involving other networks (e.g., schizophrenia or other unrelated disease gene-sets)[279] was found in the 22q11.2DS-PD cases. Similarly, there was no enrichment for rare variants involving any of the PD-relevant gene-sets in a WGS study using the same genotypic data and subjects but using schizophrenia as the phenotype for the analyses.[279] It is important to note the preliminary nature of these findings and the potential that the small sample assessed may have had limited power to detect difference between the 22q11.2DS-PD cases and the 22q11.2DS patients without a diagnosis of PD.

These findings provide an initial glimpse of the potential for WGS to reveal a more complete view of the genetic architecture of PD at the individual level. The results of this preliminary pilot study suggest that hemizygosity of the 22q11.2 deletion region, together with each individual's cumulative genome-wide burden of rare deleterious variants in PD-relevant pathways, may form a "multi-hit" pathway to the expression of PD in 22q11.2DS that could potentially be generalizable to other forms of PD.[307] These results appear to be consistent with the polygenic genetic architecture expected for a common, complex neurological disorder such as PD[279,517] and could potentially help to explain the reduced disease penetrance associated with other rare causative/high risk mutations.[16,494] Mutations in non-coding regions of the genome may also play a role in the aetiology of PD in 22q11.2DS and should be explored in future studies.[311] Advances in WGS bioinformatics methods will facilitate the analyses of non-coding mutations that could be involved in PD risk, such as splicing regulatory variants[312,313] and non-coding RNA mutations.[314,518] WGS studies of *LRRK2* mutation carriers discordant for PD are ongoing elsewhere that may add to these initial WGS findings for the 22q11.2 deletion form of PD.[515]

Perhaps most importantly, the results provided proof-of-principle that using WGS technologies in patients with PD may help inform our understanding of disease aetiology and penetrance. WGS provides a comprehensive method to reliably detect all classes and sizes of protein-coding and regulatory variants, rare and common, on a genome-wide scale in a single experiment.[519,520] This approach is thus well-suited to PD and other complex neurological disorders, where both sequence and structural variants are appreciated to be involved in disease risk[17] and the genetic contribution of the disease involves a continuum from Mendelian disease-causing mutations to multifactorial patterns of inheritance.[494]

Larger studies are needed to replicate these very preliminary findings and to investigate any possible interacting environmental or non-genetic effects on PD risk in 22q11.2DS. These studies may become possible as more patients with 22q11.2DS-PD are identified and are recruited into research studies. As a consequence of the small sample size, the analysis was necessarily limited to a candidate gene approach involving rare variants within coding regions of the genome. The sample was under-powered to investigate common SNPs that may play a minor modifying effect on PD risk.[134,136,137] The possible effects of rare and common variants in non-coding regions of the genome await advances in WGS bioinformatics methods to permit reliable analyses of non-coding regions in future studies.[311] Nevertheless, the finding of this pilot WGS

study appears to provide some initial insight into genetic mechanisms that may contribute to expression of PD in patients with 22q11.2DS.

Future studies may also consider the possible role of epigenetic factors that could be involved in the expression of PD in 22q11.2DS. There is growing interest in epigenetics, defined as alterations in gene expression or function without a change in DNA sequence,[521] as a potential mediator of the pathogenesis of PD and other complex diseases.[518,522-524] Epigenetic modulation of gene expression can be influenced by lifestyle, aging, and environmental and genetic factors.[521,522] Epigenetic mechanisms include DNA methylation at cytosine-phosphate-guanine (CpG) sites, post-translational histone modifications such as acetylation and methylation, and RNA-mediated changes in gene expression.[518,522-524] Though the field is still at a relatively early stage, there is increasing evidence to suggest that epigenetic mechanisms may regulate the expression of PD-relevant genes and moderate PD risk.[522-524] For example, DNA methylation appears to regulate expression of alpha-synuclein, encoded by the autosomal dominant PD gene *SNCA*.[525,526] Hypomethylation of *SNCA* intron 1, reported in patients with idiopathic PD, has been proposed as a putative disease biomarker.[525-528] Studies are needed to assess if there may be abnormal DNA methylation patterns in patients with 22q11.2DS (e.g., using genome-wide DNA methylation profiling such as methylation microarrays or whole-genome bisulfite sequencing) that could potentially be involved in mediating expression of PD or other 22q11.2DS-associated phenotypes.

Small non-coding microRNAs (miRNAs) have been emerging as important contributors in the pathophysiology of PD in recent years.[518] miRNAs bind to the 3'-untranslated region of target messenger RNAs (mRNAs), inducing either mRNA degradation or translational repression.[529,530] Abnormal miRNA expression profiles have been reported in blood[531] and brain tissue[516,517] from patients with PD.[532,533] Dysregulation of miRNAs may have an impact on the expression levels of key PD-relevant proteins.[533,534] For example, miR-34b and miR-34c appear to repress alpha-synuclein expression in human 'dopaminergic' (SH-SY5Y) cells. In contrast, the inhibition of these miRNAs increases alpha-synuclein level and leads to the formation of alpha-synuclein-containing aggregates.[534] These miRNAs are downregulated in the brains of idiopathic PD patients with PD, suggesting that this mechanism may contribute to PD pathogenesis.[533,534] Notably, miRNA dysregulation has also been proposed to mediate disease phenotypes such as schizophrenia in 22q11.2DS.[8,171,198] The typical ~2.5 Mb 22q11.2 region, in addition to

overlapping seven miRNAs, includes *DGCR8,* a critical gene in the biogenesis of brain miRNAs (Figure 9-1).[8] Data from mouse models implicate effects of *Dgcr8* deletion on global miRNA expression.[170,269,535-538] There is also preliminary evidence of a unique miRNA expression profile in blood from patients with 22q11.2DS relative to controls.[198,539] As the role of miRNAs dysregulation in 22q11.2DS and PD becomes better delineated, and validated gene targets are established, it should become possible to determine if there may be a shared miRNA mechanism between 22q11.2DS-PD and other forms of PD. It is conceivable that critical genes involved in PD-relevant pathways could be targets of miRNAs that are dysregulated in 22q11.2DS patients because of the *DGCR8* hemizygous deletion[269] and/or the effects of decreased dosage of the multiple 22q11.2 miRNA genes overlapped by this deletion. Delineating the role of epigenetic mechanisms such as abnormal miRNA function may help to identify novel therapeutic strategies for 22q11.2DS-PD and other forms of PD. For example, cobalt(III) protoporphyrin has recently been shown to be able to activate DGCR8 *in vitro* and compensate for miRNA processing deficiency.[540]

9.2.4 Opportunities for molecular insights into Parkinson's disease from within the 22q11.2 deletion region

The increasing clinical evidence that the 22q11.2 deletion is associated with early-onset PD (Table 9-1) raises the question of which genes within the 22q11.2 deletion region may be involved in mediating PD risk in 22q11.2DS. The typical 2.5 Mb recurrent 22q11.2 deletion encompasses about ~90 coding and non-coding genes (Figure 1-4; slight variability in the number of deleted genes may occur depending on the technology used and minor inter-patient variability).[6,156,159] Review of the literature showed that none of these genes have been identified as a disease-causing or high risk PD locus (Table 1-2), or are among the most significant and well-replicated PD susceptibility loci reported in genome-wide association studies (Table 4-3). This offers the exciting possibility of identifying novel PD risk genes in the 22q11.2 deletion region that may contribute to disease pathogenesis in other PD populations.

In an initial attempt to identify putative PD candidate genes within the 22q11.2 deletion region, the available clinical, animal, and molecular literature on the 46 protein-coding genes within the 22q11.2 deletion region was assessed.[6,252] Several possible candidates were identified based on their functional characteristics.[252] For example, mitochondrial dysfunction is strongly implicated in PD pathogenesis,[246] particularly in early-onset forms of the disease.[488] The 22q11.2 deletion

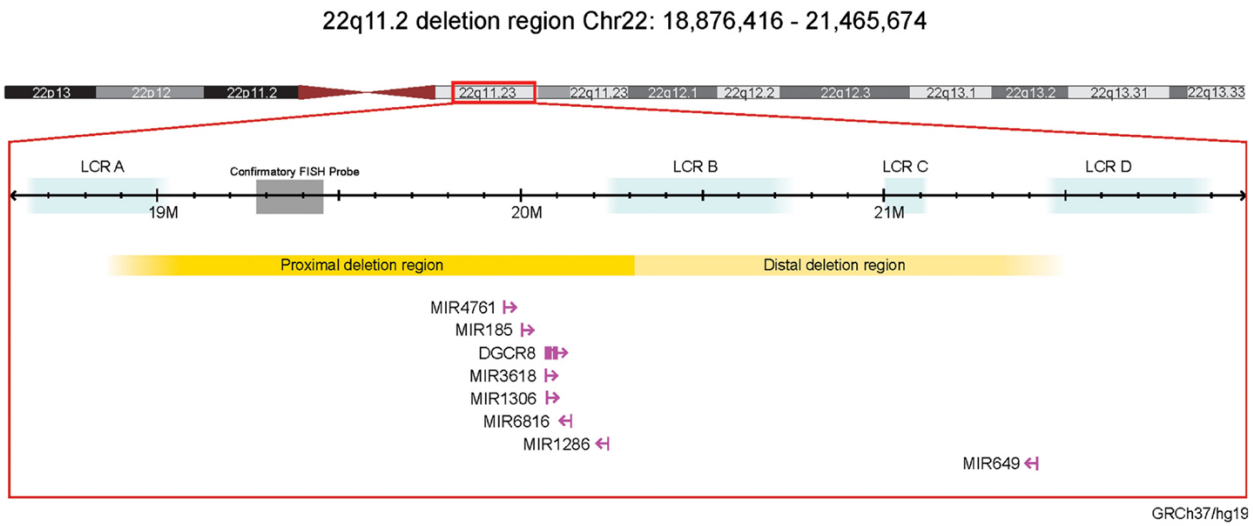

Figure 9-1 Schematic of miRNAs and the miRNA-processing gene *DGCR8* in the 22q11.2 deletion region

Affymetrix Human SNP Array 6.0 22q11.2 deletion breakpoints from 22q11.2DS patients are shown.[156] Gene and miRNA sizes are enlarged for illustrative purposes (not to scale).

Abbreviations: FISH, fluorescence *in situ* hybridization; LCR, low copy repeats.

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notably contains six genes with mitochondrial function (*MRPL40, PRODH, SLC25A1, TANGO2, TXNRD2,* and *ZDDHC8*).[245] There is some evidence from simple model organisms that *PRODH*[541] and *TXNRD2*[541] could be involved in motor functioning (discussed below).

The 22q11.2 deletion region also includes *COMT,* encoding catechol-*O*-methyltransferase, an enzyme that degrades dopamine and other catecholamines.[542,543] COMT is the therapeutic target of the COMT inhibitors, tolcapone and entacapone, that are commonly used in the management of PD to enhance and prolong the effects of L-dopa.[243] A common functional SNP in *COMT* (rs4680; Val$^{158/108}$Met) has been inconsistently associated with risk of developing PD.[543] Notably, a recent meta-analysis involving 9719 PD subjects and 14634 controls found no significant effect of the *COMT* rs4680 SNP on PD risk.[544]

The 22q11.2 deletion region gene, *SEPT5,* encoding septin-5, a substrate of the ubiquitin ligase parkin, the protein product of *PARK2,*[244] could possibly be involved in risk for PD in 22q11.2DS. Autosomal recessive early-onset PD mutations in *PARK2* are thought to disrupt the ubiquitination and degradation of parkin targets, leading to a potentially neurotoxic accumulation of proteins such as *SEPT5,* based on evidence from cell culture experiments.[244,545] Overexpression of *SEPT5* has been shown to cause rapid and selective neurodegeneration of rat nigral dopaminergic neurons.[546] In contrast, homozygous knockout of *SEPT5* in mice had no effect on brain development or synaptic functioning[547] and in mouse models of the hemizygous 22q11.2 deletion *SEPT5* expression is decreased by ~50%.[270] Expression levels of *SEPT5* in 22q11.2DS patients remain unexamined to date.[192-198] The hemizygous deletion of *SEPT5* in 22q11.2DS appears therefore unlikely to play a major role in mediating PD risk in 22q11.2DS, although more data are needed with respect to effects of this gene's expression.

The six mitochondrial genes, *COMT,* and *SEPT5* genes are all located in the proximal ~1.5 Mb proximal deletion region, potentially in keeping with the finding of this smaller deletion in one 22q11.2DS-PD patient.[252] Much remains to be known about the molecular function of 22q11.2 region genes, however, and their individual and collective roles in modulating 22q11.2DS disease phenotypes.[165,498,548] Other candidates may become apparent as knowledge of the functional roles of the individual 22q11.2 deletion genes increases. Investigating knockout or knockdown models of the 22q11.2 deletion region genes in simple model organisms could help better characterize their molecular roles and probe their involvement in the pathophysiology of

PD. For example, models of disease-causing PD mutations in the fruit fly, *Drosophila melanogaster*, have been able to recapitulate key neuropathological and behavioural phenotypes. These experiments have yielded critical insights into the pathological mechanisms of PD associated with these mutations,[549] particularly in defining the involvement of autosomal recessive PD genes in regulation of mitochondrial integrity and functioning.[488]

It was observed when examining the literature on 22q11.2 deletion genes for relevance to PD mechanistic pathways that simple model organisms have been largely unexploited in the study of 22q11.2 deletion region genes and associated phenotypes. As an initial step in determining the potential utility of simple model organisms in the study of 22q11.2DS-PD and other associated neurophenotypes, we investigated the evolutionary conservation status of genes within the 22q11.2 region in common model organisms, the fruit fly (*D. melanogaster*), the zebrafish (*D. rerio*), and the worm (*C. elegans*).[6] This included a comprehensive review of gene function and phenotypic alterations related to 22q11.2 gene disruptions and the development of an up-to-date resource of available knockout and knockdown models of putative 22q11.2 deletion region gene homologs (Guna, Butcher, & Bassett 2015, *Journal of Neurodevelopmental Disorders*).[6]

In brief, the results demonstrated that, of the human 22q11.2 region protein-coding genes, substantial proportions are conserved in simple model organisms. With respect to the typical 2.5 Mb deletion, 40 (87.5%) of the 46 protein-coding genes are conserved in the mouse, 37 (80.4%) in the zebrafish, 22 (47.8%) in the fruitfly, and 17 (37.0%) in the worm (Figure 9-2).[6] Assessment of the known phenotypes associated with available knockout and knockdown models of the 22q11.2 deletion region genes provided some insight into possible PD-relevant mechanisms, particularly with respect to mitochrondrial dysfunction.[6] *PRODH* encodes a mitochondrial enzyme that metabolizes L-proline, an amino acid involved in modulating glutamatergic and GABA-ergic transmission.[550] Knockout of *PRODH (slgA)* in the fruitfly was associated with severe locomotor defects and indecisive movement patterns compared with wild-type flies in an activity chamber assay.[550] These findings potentially suggest a role for *PRODH* in motor functioning. Interestingly, knockdown of another 22q11.2 mitochondrial gene, *TXNRD2* (implicated in the defense against oxidative stress, a mechanism with evidence of involvement in PD pathogenesis[246,488]) showed a modifying effect on motor phenotypes in *C. elegans.* Worms overexpressing human beta-amyloid peptide as a model for Alzheimer's disease were more susceptible to muscular dysfunction and paralysis when *TXNRD2* levels were reduced.[541]

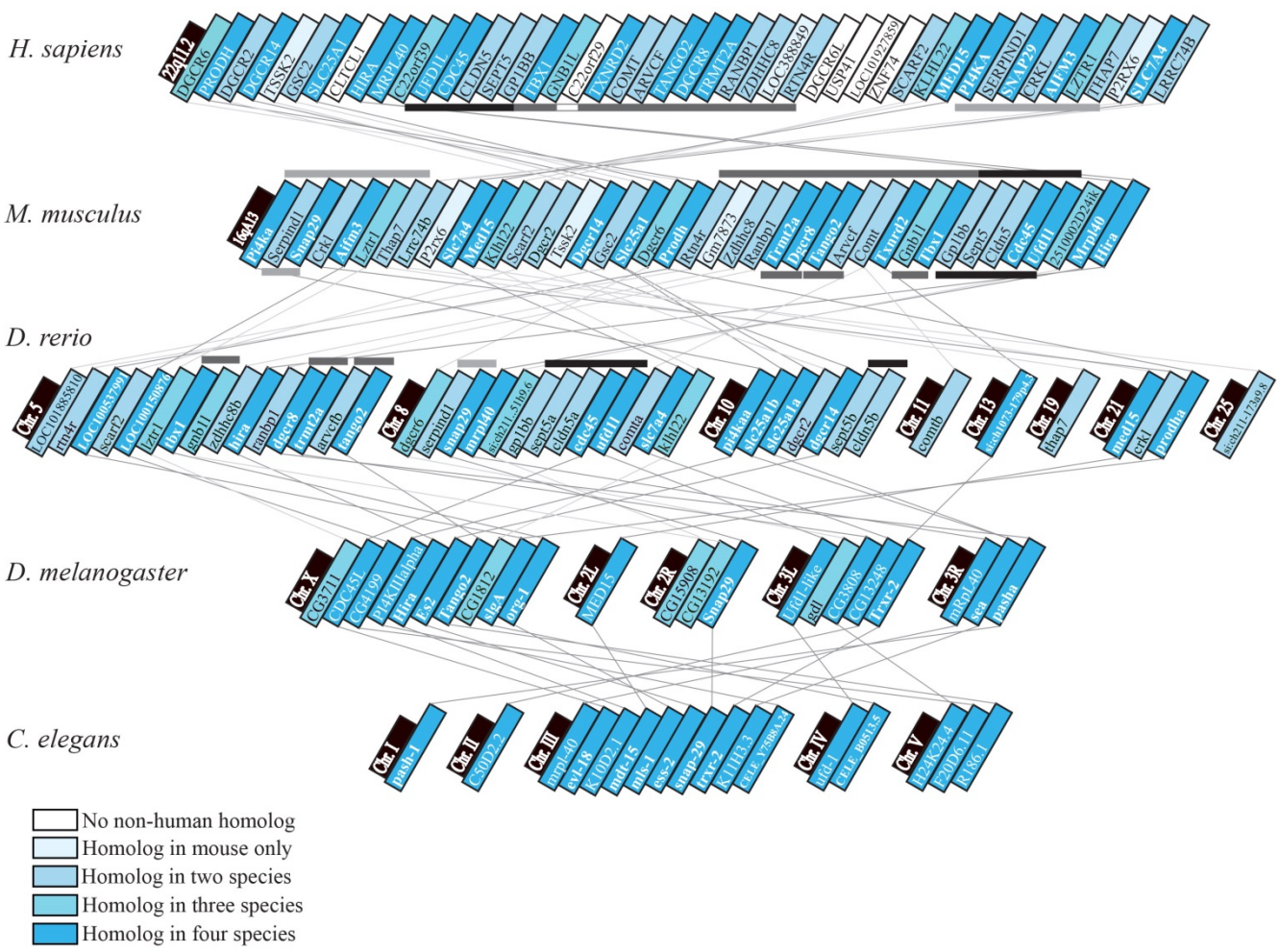

Figure 9-2 Comparative gene map of the human 22q11.2 region homologs

Protein Basic Local Alignment Search Tool (blastp) analysis on UniProtKB using the reciprocal best hits method (i.e., the protein products of genes in two different genomes represent the best hit in the opposite genome)[551,552] was used to compare protein sequences to identify putative homologs of the 46 human protein-coding genes in the mouse (*M. musculus*), 40 conserved genes; zebrafish (*D. rerio*), 37 conserved genes; fruit fly (*D. melanogaster*), 22 conserved genes; and worm (*C. elegans*), 17 conserved genes. Locations of human genes and putative homologs were mapped using NCBI Entrez Gene. Horizontal bars indicate syntenic clusters of two or more genes; fine lines otherwise join homologs between species.

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More comprehensive studies of neurodegeneration in lower organisms could shed light on the molecular function of these and other critical proteins. Future studies tailored to PD-relevant phenotypes and molecular mechanisms may provide further insights. Our development of this comparative multi-species resource of 22q11.2 genes could help facilitate studies using the three species studies examined as well as other model organisms, and serves to highlight their potential for investigating 22q11.2DS-associated neurophenotypes including PD.[6]

9.3 On the study of the natural history of Parkinson's disease in 22q11.2 deletion syndrome

The study of individuals at increased genetic or clinical (e.g., individuals with rapid eye movement behaviour disorder[28,32]) risk of developing PD provides opportunities to gain insight into the early prodromal stages of PD.[11,12] This may help improve our understanding of the natural history of the disease and correspondingly, facilitate the development of risk prediction and early detection strategies.[11,12] The possibility of identifying patients in an early pre-diagnostic stage of PD is an area of considerable interest given the implications for the development of neuroprotective treatments that could potentially slow or halt disease progression.[11,12] As yet, however, there are few systematic studies of patients at high genetic risk of PD prior to clinical onset. These include promising initial reports of individuals with *LRRK2* and *GBA* mutations (Table 9-4). Cohorts of individuals with mutations in these genes but without PD are enriched for clinical symptoms and neuroimaging markers associated with the PD prodrome (Table 9-4). Data from the initial investigation of adults with 22q11.2DS (≥30 years) for putative prodromal features of PD (Chapter 5) suggest that individuals with the 22q11.2 deletion may also provide opportunities to study the prodromal stages and early natural history of PD. The results provided evidence that, relative to healthy age and sex-matched controls, parkinsonian features and olfactory deficits possibly consistent with pre-diagnostic stages of PD, may be common in adults with 22q11.2DS who are at increased age-related (≥30 years) risk of 22q11.2DS-PD (Table 9-4). Positron emission tomography (PET) neuroimaging results using ^{11}C-DTBZ PET, a radioligand for the presynaptic vesicular monoamine transporter type 2 (VMAT2),[317-320] provided evidence of atypical pre-morbid striatal dopaminergic dysfunction in adults with 22q11.2DS (discussed below).[553]

Table 9-4 Clinical and neuroimaging features of patients with the hemizygous 22q11.2 deletion, LRRK2, and GBA mutations diagnosed with, or at risk of, Parkinson's disease

	Hemizygous 22q11.2 deletions	***LRRK2* mutations**	***GBA* mutations**[a]
Genetic features			
Mutation type	Copy number variant	Missense variants	Primarily missense variants (rare in/dels and complex alleles[b])
Reduced penetrance	Yes[252]	Yes[124]	Yes[554]
Major clinical characteristics of patients with Parkinson's disease			
Clinical phenotype	Early-onset, typical[252]	Late-onset, typical[126]	Late-onset, typical[c 555]
Neuropathology	Variable Lewy body pathology; nigral neuronal loss[252]	Variable Lewy body pathology; nigral neuronal loss[99]	Lewy body pathology; nigral neuronal loss[556]
Striatal dopaminergic neuronal imaging	Severe striatal dopamine loss	Severe striatal dopamine loss	Severe striatal dopamine loss
Method	VMAT2-PET (n=1) DAT-SPECT (n=1)[144]	VMAT2-PET[365,557] DAT-SPECT/PET[365,557,558] FDOPA-PET[365,557]	DAT-SPECT[558] FDOPA-PET[559,560]
Substantia nigra echogenicity	No (n=1)	Yes[561]	Yes[559,562,563]
Major clinical characteristics relevant to the Parkinson's disease prodrome in at risk cohorts			
Olfactory deficits	Yes	No[126,351,354]	Yes[349,350]
Subclinical parkinsonism	Yes	Yes[126,351-353]	Yes[349,350]
Striatal dopaminergic neuronal imaging	VMAT2-PET: Elevated DAT-SPECT/PET: Unknown FDOPA-PET: Unknown	VMAT2-PET: Reduced[364,365,557] DAT-SPECT/PET: Reduced[364,365,557] FDOPA-PET[d]: Unaffected[365]	VMAT2-PET: Unknown DAT-PET: Elevated in caudate (n=3)[553] FDOPA-PET: Reduced[560]
Substantia nigra echogenicity	No	Yes[354,564]	Yes[562,563]

Striatal dopaminergic neuronal imaging refers to presynaptic dopaminergic markers visualized using radioligands with SPECT (single photo emission tomography) or PET (positron emission tomography; Figure 1-1). Substantia nigra echogenicity was measured in all studies using transcranial sonography. Prodromal characteristics are defined by the enrichment for these features in individuals with these mutations relative to controls.
Abbreviations: FDOPA, fluorodopa; DAT, dopamine transporter; VMAT2, vesicular monoamine transporter 2
[a]Homozygous and heterozygous *GBA* mutations are similarly associated with Parkinson's disease, summarized here together for simplicity [b]Complex alleles result from recombination events with the *GBA* pseudogene (*GBAP*) [c]Associated with a slightly earlier mean onset of PD (~five years younger) and more frequent manifestations of associated cognitive impairment than idiopathic PD[140] [d]A study using an alternate scan protocol showed evidence of elevated dopamine turnover[364] (All other FDOPA studies noted employed FDOPA as a measure of presynaptic dopamine synthesis and capacity).

A primary limitation of the study was its cross-sectional design, and the availability of only one (living and able) patient with 22q11.2DS-PD to serve as a "positive control." Longitudinal follow-up of these 22q11.2DS patients may help determine which pre-diagnostic neurological features (or combination of features) may potentially be useful to help predict the probability and timing of "conversion" to PD in 22q11.2DS. Ideally, assessment of clinical and neuroimaging features relevant to PD (Table 9-4) would be performed at multiple time points across the lifespan (e.g., late adolescence/early adulthood, young adulthood, and middle age and older, if possible given the early mortality associated with 22q11.2DS[252,262]). This would help determine when these neurological signs develop in 22q11.2DS and help delineate their putative relation to PD. Data on the "typical" range of olfactory[68] and motor function[7] in 22q11.2DS adulthood are very limited. There have been no other studies assessing presynaptic striatal dopaminergic function in individuals with 22q11.2DS without PD. It cannot be ruled out that neurological abnormalities identified in this study, instead of being relevant to eventual PD expression, could be part of the variable clinical presentation of 22q11.2DS or related to other 22q11.2DS-associated conditions and/or their associated treatments (e.g., schizophrenia) in adulthood.[5,7,256] These possibilities are outlined in Table 9-2.

The results of the ^{11}C-DTBZ VMAT2 PET neuroimaging study in adults with 22q11.2DS at increased age-related (≥30 years) risk of 22q11.2DS-PD may serve to indicate the importance of considering baseline pre-morbid dopaminergic function in neuroimaging studies of genetic risk populations. The data provided evidence of increased striatal VMAT2 levels in 22q11.2DS relative to healthy controls. While alternative possibilities are described in Chapter 5, these results suggest an increased pre-morbid availability of presynaptic dopamine storage vesicles in 22q11.2DS that could potentially in turn be associated with excess striatal dopaminergic innervation. In this case, it is possible that for individuals with 22q11.2DS, early stages of dopaminergic degeneration could be masked by higher pre-existing presynaptic dopamine levels. This scenario could potentially explain the finding of a correlation between lower striatal VMAT2 binding and greater severity of bradykinesia in 22q11.2DS (a pattern predicted from idiopathic PD[319,367-369]), despite the fact that VMAT2 levels were not reduced relative to healthy controls. If this were to be confirmed, severity of bradykinesia could represent a putative clinical marker of early striatal neurodegeneration and increased risk of progression to PD in patients with 22q11.2DS. A possible implication of these findings is that the utility of presynaptic

dopaminergic neuroimaging in helping to distinguish between drug-induced parkinsonism and PD in patients with 22q11.2DS may be limited to later stages of the disease, i.e., when significant dopaminergic terminal loss has occurred.[45,144]

Notwithstanding these novel functional neuroimaging findings for individuals with 22q11.DS at risk for early-onset PD, the cumulative other evidence is reassuring about the expression of PD associated with a 22q11.2 deletion. The neuropathological[252] and neuroimaging data from the available 22q11.2DS-PD cases (Table 9-4) provide evidence that the dopaminergic dysfunction associated with clinically manifest PD in 22q11.2DS, at least, is typical. The findings were consistent with a profound loss of dopaminergic neurons that is the hallmark of idiopathic and other genetic forms of PD.[24,25,489] The mystery for 22q11.2DS, and likely other forms of PD, is what is happening in the brain before symptomatically confirmed PD.

The evidence of increased levels of VMAT2 in adults with 22q11.2DS appears to be a unique finding in patients at risk of developing PD. Relative to controls, asymptomatic *LRRK2* mutation carriers[364,365] and individuals with REM sleep behaviour disorder[366] who are known to be at risk for PD show evidence of reduced striatal VMAT2 levels using ^{11}C-DTBZ, consistent with a prodromal loss of striatal dopaminergic innervation.[364-366] Increased striatal VMAT2 levels in young/middle-aged adults with 22q11.2DS therefore perhaps appear more likely to be a general feature of 22q11.2DS rather than the consequence of a prodromal stage of PD. In keeping with the latter possibility however, one small PET neuroimaging study of three heterozygous *GBA* mutation carriers (age 47, 52, and 74 years with no signs of parkinsonism) using ^{11}C-CFT (2-beta-carbomethoxy-3 beta-(4-fluorophenyl)tropane) unexpectedly showed elevated mean levels of dopamine transporter binding (~19% increase) specific to the caudate nucleus of the striatum relative to that found in ten healthy controls (Table 9-4).[553] The reasons for this increased presynaptic dopaminergic binding were unclear, and were proposed to be potentially related to dopamine transporter upregulation or increased presynaptic dopamine levels.[553] In a separate PET imaging study,[560] the expected pattern of decreased fluorodopa levels consistent with prodromal PD was reported in two *GBA* mutation carriers in their 20s and 40s (exact ages not specified). The latter study thus provides some evidence against the possibility that all *GBA* mutation carriers at risk of PD may be characterized by a possible increased baseline presynaptic dopaminergic function involving the caudate. Additional studies, ideally also assessing VMAT2

levels using ^{11}C-DTBZ, will be needed to further clarify pre-morbid dopaminergic function in *GBA* mutation carriers and determine any similarities in 22q11.2DS.

Replication of elevated ^{11}C-DTBZ binding in a younger cohort of individuals with 22q11.2DS (e.g., before the onset of possible early neurological changes related to PD and ideally in the absence of major neuropsychiatric disease) could help to clarify if elevated VMAT2 levels is a general feature of 22q11.2DS. Post-mortem studies in patients with 22q11.2DS without PD could potentially confirm if the observed elevated VMAT2 levels are reflective of increased presynaptic dopamine vesicle density and/or increased striatal synaptic innervation. Brain tissue of adults with 22q11.2DS, however, remains an extremely rare resource.[210,252] In functional neuroimaging studies, increased striatal dopaminergic innervation in 22q11.2DS would be expected to be reflected by increased binding using any of the various radioligands specific to the presynaptic plasmalemma dopamine transporter (e.g., ^{11}C-CFT, ^{11}C-MP). Dopamine transporter levels could also be further elevated, through upregulation, in an attempt to reduce (putatively) elevated synaptic dopamine levels. The pattern of binding expected for neuroimaging studies using fluorodopa, a measure of dopamine synthesis and capacity,[39] is less clear. Fluorodopa is generally well-correlated with VMAT2 and dopamine transporter levels[39,47] and thus may also show elevation. Increased dopamine synthesis capacity related to schizophrenia[565] could also contribute to a finding of elevated fluorodopa binding in 22q11.2DS patients with schizophrenia. On the other hand, dopamine synthesis could be down-regulated in response to the putatively elevated density of presynaptic dopamine vesicles and/or dopaminergic terminals.

The finding of elevated VMAT2 levels, a presynaptic marker of dopamine storage vesicles, in adults with 22q11.2DS (≥30 years) is potentially consistent with hyperdopaminergic function in 22q11.2DS. Abnormalities in peripheral dopaminergic markers (e.g., elevated levels of dopamine in urine) in patients with 22q11.2DS without PD are supportive of central dopaminergic dysfunction in this genetic population.[566,567] Increased levels of dopamine in the brain have been hypothesized previously in 22q11.2DS, related to the high prevalence of neuropsychiatric disorders in 22q11.2DS that are thought to be related to dopaminergic dysfunction (e.g., schizophrenia, attention deficit disorder).[566-569] A popularly proposed mechanism has involved the effects of missing one copy of the *COMT* gene that is located in 22q11.2 deletion region.[566-569] *COMT* encodes an enzyme important in dopamine degradation,

and is particularly important in the prefrontal cortex because of the relative scarcity of the dopamine transporter in this region.[542,543,570] *COMT* contains a common functional single nucleotide polymorphism, a valine-to-methionine substitution (Val$^{158/108}$Met).[543] The Met allele has less thermostability and about 40% lower activity than the Val allele based on human post-mortem data from the prefrontal cortex.[543]

One might speculate that the increased availability of presynaptic dopamine in the striatum suggested by the finding of elevated ^{11}C-DTBZ binding, coupled with a putative deficient dopamine clearing mechanism related to reduced COMT function, may lead to abnormal striatal dopaminergic signaling in 22q11.2DS. Notably, chronic exposure to the neurotoxic properties of dopamine and its metabolites has been proposed to be involved in PD pathogenesis.[571,572] Under normal conditions, COMT plays a relatively minor role in dopamine clearance in the striatum (responsible for <15% of dopamine turnover).[542] Striatal dopamine levels in *COMT* knockout mice have been shown to be normal.[573,574] The role of COMT in striatal dopamine degradation may be more important under challenged conditions, however.[575] For example, rats treated with a COMT selective inhibitor showed larger increases in striatal dopamine when challenged with L-dopa compared with controls.[576] Similarly, COMT inhibitors only appear to be effective in the clinical management of PD during L-dopa treatment.[243,577]A study using [^{123}I]IBZM single-photon emission computed tomography in 15 non-psychotic adults (18-43 years) with 22q11.2DS without PD found that the ten participants with the *COMT* Met allele (Met hemizygotes) had significantly lower mean striatal dopamine D_2 receptor binding than the five with the Val allele.[575] This finding was consistent with lower striatal COMT activity in Met hemizygotes that would expected to be accompanied by less synaptic dopamine clearance and thus higher synaptic dopamine levels (and consequently, lower dopamine D_2 receptor binding).[575]

The possible interacting effect of the *COMT* Val$^{158/108}$Met polymorphism on striatal presynaptic dopaminergic function in 22q11.2DS could be investigated in future neuroimaging studies.[566,575] For example, if there is a negative feedback loop between synaptic dopamine levels and presynaptic dopamine function in 22q11.2DS, one might expect Met hemizygotes to show decreased presynaptic dopamine synthesis (as assessed with fluorodopa in PET neuroimaging), and increased dopamine transporter levels, relative to Val hemizygotes. Though expression of VMAT2 does not appear to be easily altered,[373-375] evidence of higher ^{11}C-DTBZ binding levels

in Met hemizygotes could suggest that striatal COMT deficiency in 22q11.2DS may lead to upregulation of VMAT2 and/or synaptic vesicle number in an attempt to remove and sequester excess (toxic) dopamine from the cytosol into synaptic vesicles.

9.4 Consideration of clinical and research implications for patients with 22q11.2 deletion syndrome

9.4.1 Screening and diagnosis of movement disorders in 22q11.2DS

In childhood, motor disturbances are commonly reported in 22q11.2DS, including delayed gross motor milestones, low muscle tone (hypotonia) and weakness, fine and gross motor deficits, and poor balance and coordination difficulties relative to their peers.[172-178,181] The data from the studies presented in this thesis represent an initial attempt to characterize the manifestations and aetiology of motor abnormalities in adulthood in 22q11.2DS. Evidence was found to support the involvement of early-onset PD,[252] mild parkinsonism and action/postural tremor (Chapter 5), and side effects from antipsychotic treatments[7,256] in mediating motor functioning in adults with 22q11.2DS. These collective results have potential implications for the screening and diagnosis of PD and other movement disorders in adults with 22q11.2DS.

The phenotypic range of 22q11.2DS-related PD/parkinsonism and motor dysfunction requires further study to better characterize the possible spectrum of neurological abnormalities in adulthood and inform early diagnosis and treatment strategies. Causes of movement problems may be multifaceted in 22q11.2DS and require careful systematic investigation to help facilitate timely diagnosis and treatment.[5,7,146] For example, the use of antipsychotics in 22q11.2DS to manage associated psychiatric symptoms appeared to delay diagnosis and treatment of PD by up to 10 years in the cases studied.[252] Adults with 22q11.2DS are also commonly treated with psychotropic medications including antidepressants[578,579] and anticonvulsants[580,581] which can more rarely cause adverse motor effects. Musculoskeletal abnormalities, seizures, hypocalcemia, and/or abnormal thyroid levels could also potentially contribute to neurological symptoms (Table 9-2).[7] Periodic neurological assessment for signs of parkinsonism and other motor disturbances may be considered for adults with 22q11.2DS, especially for those presenting with changes in motor functioning (e.g., worsening of existing, or emergence of new motor, symptoms).[5]

9.4.2 Psychosis and Parkinson's disease in 22q11.2DS

9.4.2.1 Issues in managing psychosis in adults with 22q11.2DS at high risk of, or diagnosed with, Parkinson's disease

Evidence that adults with 22q11.2DS are at risk of developing early-onset PD[142-144,252,486,487] (Table 9-1) suggests that the choice and dose of antipsychotic medication in the 25% of 22q11.2DS adults with schizophrenia[5,146,149,150] may require extra consideration in this context.[5,252,487] A similar treatment issue may arise for 22q11.2DS-PD patients with no history of schizophrenia but who may develop PD-related psychosis. PD-related psychotic symptoms occur in about 30% of patients with idiopathic forms of PD.[78,80] These are proposed to result from complex interactions between disease and dopaminergic treatment-related effects.[79,80] Psychotic symptoms and PD can be difficult to manage when they co-occur as the typical treatments of each tend to have opposing effects on the dopamine system.[69,248,582] Standard antipsychotic treatments induce a functional dopamine deficiency through the blockade of post-synaptic dopamine receptors,[234,235] while treatments for PD act to increase dopamine levels.[60]

The atypical antipsychotic medication, clozapine, has a relatively low binding affinity for striatal dopamine receptors[230,583] and would be expected to have good efficacy and be well-tolerated by patients with 22q11.2DS at risk of, or diagnosed with, PD, based on clinical studies of the management of psychosis in other forms of PD.[69,248] The literature on the treatment of patients with schizophrenia who develop PD is limited to case reports. Among these, successful management using clozapine has been reported.[582,584-586] A retrospective assessment of treatment response to clozapine in patients with 22q11.2DS-Schizophrenia provided preliminary evidence that patients with 22q11.2DS-Schizophrenia show excellent therapeutic response to clozapine, equivalent to those with idiopathic forms of schizophrenia, though at significantly lower doses (Chapter 6).[256] Data showing an increased frequency of treatment-emergent serious side effects in 22q11.2DS, most commonly seizures, suggested that clozapine treatment in 22q11.2DS may require appropriate caution and careful management with respect to dosing and side effect risks, however.[256] Replication of these results remains necessary to help confirm these findings. Notably, the finding of a lower therapeutic dose and lowered seizure threshold appears to be consistent with previous case reports describing clozapine treatment effects in patients with 22q11.2DS with no history of PD[142,207-209,249-251] and a recent case series of 28 22q11.2DS-Schizophrenia patients including one diagnosed with L-dopa responsive early-onset PD.[487]

A primary limitation of the study examining clozapine treatment response in 22q11.2DS[256] was the absence of an age-matched 22q11.2DS control group not treated with any antipsychotic medication that could have helped distinguish between clozapine-associated side effects and 22q11.2DS-associated symptoms. For example, the results showed that extrapyramidal symptoms that typically tend to be (nearly) absent during clozapine treatment[230,408,587-589] frequently emerged or worsened during long-term clozapine treatment in adults with 22q11.2DS with no prior diagnosis of PD.[256] These included tremor, rigidity, unsteady gait, and slurred speech.[256] It remains unclear if these motor symptoms could unexpectedly be related to clozapine treatment effects, or if they were related to early neurodegenerative processes in 22q11.2DS[142-144,252,487] or other 22q11.2DS-associated motor abnormalities[7,252] (Table 9-2). Notably, the single patient with 22q11.2DS-PD treated with clozapine was discontinued from treatment by his neurologist due to concerns that clozapine treatment was related to signs of worsening parkinsonism.[256] There is a prior report of an individual with 22q11.2DS with early-onset parkinsonism whose muscle rigidity worsened following a change to clozapine from fluphenazine decanoate treatment.[142] These observations serve to illustrate the possible clinical complexity in disentangling progressive neurodegenerative symptoms from antipsychotic-induced parkinsonism in 22q11.2DS, even in patients treated with clozapine where parkinsonian side effects are not expected.[230,408,587-589]

The results of the study of clozapine treatment in 22q11.2DS represent an initial step in examining the safety and efficacy of clozapine in this genetic population. Additional studies are needed to inform the optimal treatment of schizophrenia in individuals with diagnosed 22q11.2DS-PD or 22q11.2DS-PD patients including those who subsequently develop PD-related psychosis. The small number of patients diagnosed with 22q11.2DS-PD available for study precluded any systematic study of antipsychotic treatment response among these patients. Of the four 22q11.2DS-PD cases treated for psychosis, there were three with schizophrenia who developed PD. Three of these patients were treated with clozapine (250 mg),[252,256] quetiapine (400 mg),[252] and risperidone (0.5 mg; with 1 mg of benztropine),[252] respectively. The fourth patient, with no history of psychosis, developed delusions and agitation at age 58 years, approximately 14 years after onset of PD and L-dopa treatment, managed with olanzapine (5 mg).[252] Another case with schizophrenia with early-onset (age 45 years) 22q11.2DS-PD treated with low-dose clozapine (150 mg) who exhibited persistent psychotic symptoms and functional

decline was recently reported.[487] Optimal treatment strategies for this unique patient population will be better informed as more patients with 22q11.2DS-PD are identified.

Although clozapine is recommended for the co-management of PD and schizophrenia/psychosis,[69,248,582,585] clozapine treatment will not be suitable for a subset of patients e.g., due to the onset of adverse effects that require discontinuation of treatment such as severe neutropenia/agranulocytosis or myocarditis, or where the burden of regular bloodwork, or metabolic side effects are deemed unacceptable.[230,231,256,589,590] Among the other atypical antipsychotics, quetiapine has shown some evidence as a safe and effective treatment of psychosis in PD patients.[69,248] A review of the few 22q11.2DS cases treated with quetiapine in the literature[7,487] indicates that these involve patients without PD. The results suggest that there is good efficacy and tolerability of quetiapine at doses (200 to 700 mg) similar to those used for idiopathic schizophrenia.[591] Psychiatric symptoms in our single 22q11.2DS-PD case with schizophrenia treated with quetiapine (350 mg to 375 mg) were well-managed for about the first three years of L-dopa treatment.[252] A relapse of psychotic and depressive symptoms of schizophrenia then prompted an increase to 400 mg, and then a proposed change to clozapine, only days before his unexpected death (unrelated to clozapine). Systematic studies of the effects of quetiapine should be considered to delineate its safety and therapeutic profile in 22q11.2DS.[592]

9.4.2.2 Schizophrenia and the risk for Parkinson's disease in 22q11.2DS

The 22q11.2 deletion represents the strongest known molecular risk for schizophrenia.[5,146,149,150] One in four individuals with 22q11.2DS develop schizophrenia, typically during late adolescence or young adulthood.[149,150] The evidence that individuals with 22q11.2DS are also at increased risk of developing early-onset PD[142-144,252,486,487] raises the question of whether schizophrenia and/or chronic treatment with dopamine D2 receptor blocking antipsychotic agents could be involved the expression of PD in 22q11.2DS. Based on the most current data available (Table 9-1), the proportion of 22q11.2DS-PD patients with and without a treated pre-morbid psychotic disorder appears to be similar. Of the 12 22q11.2DS-PD cases where the psychiatric status is known, six are ≥30 years with no history of psychosis (Table 9-1). The identification of a larger number of 22q11.2DS patients who have PD will be necessary to determine if 22q11.2DS

patients with schizophrenia may be more likely to develop early-onset PD among patients with 22q11.2DS.

From a diagnostic perspective, antipsychotic treatment in 22q11.2DS patients may lead to an earlier unmasking and/or exacerbation of motor symptoms due to a vulnerable nigrostriatal system.[252] It is less clear if 22q11.2DS-Schizophrenia and associated antipsychotic treatment may be involved in PD pathogenesis. A few studies using DAT neuroimaging to assess striatal terminal density have reported that up to half of idiopathic schizophrenia patients with drug-induced parkinsonism show signs of nigrostriatal degeneration.[232,238,593] However, the majority of neuroimaging studies have not reported altered striatal dopamine terminal density in patients with treated and untreated idiopathic schizophrenia. A meta-analysis of 13 PET and SPECT DAT neuroimaging studies including 202 patients with idiopathic schizophrenia and 147 controls showed no evidence of altered density of dopamine terminals. There was no significant effect of antipsychotic medication on DAT density.[274] A PET neuroimaging study using ^{11}C-DTBZ similarly showed no difference in striatal binding in 12 treated idiopathic schizophrenia patients relative to age- and sex-matched healthy controls, providing evidence of intact striatal dopamine terminals in these patients.[398] These results are consistent with the data presented in Chapter 5 that the seven patients with 22q11.2DS-Schizophrenia do not show significantly different striatal ^{11}C-DTBZ binding from the five 22q11.2DS patients with no history of psychosis. Importantly, post-mortem studies of patients with 22q11.2DS-Schizophrenia[252] and of patients with idiopathic schizophrenia[272,273,594] with no history of PD, treated with antipsychotic medications, do not show the severe nigrostriatal dopaminergic cell loss and Lewy body pathology that are the hallmark of PD. These findings collectively suggest that schizophrenia and antipsychotic treatment in patients with 22q11.2DS are unlikely to play a major role in 22q11.2DS-PD pathogenesis.

9.4.3 Functional capacity in adulthood

PD is associated with significant functional decline and disability, particularly in the more advanced stages of the disease. Functional deficits (e.g., in activities of daily living, and in socialization and communication abilities) in patients with PD are related to the progression of the associated motor, cognitive, and psychiatric symptoms, which may vary substantially between individuals.[595-597] Individuals with 22q11.2DS are commonly affected by conditions

associated with these symptoms, such as schizophrenia and intellectual disability, even prior to the onset of PD.[131,132,158] The initial study of the functional abilities of adults with 22q11.2DS (pre-morbid to PD) showed evidence that broad functional impairments are common in individuals with 22q11.2DS in adulthood, even in the absence of PD, related to the manifestation of cognitive dysfunction and schizophrenia associated with 22q11.2DS (Chapter 7).[255]

These findings could serve as a baseline for future prospective studies of the impact of PD in the assessed 22q11.2DS cohort, and potentially may aid in the development of reasonable expectations and long-term goals for patients with 22q11.2DS.[255] Though adults with 22q11.2DS represent an increasing population,[148,239,240] there have been few studies to inform expectations about the future and to guide long-term planning.[146,450] Direct assessments at multiple time-points in future studies (e.g., before and after onset of PD) would help delineate the functional impact of PD on patients with 22q11.2DS. Such studies are needed to inform patient, family member, and clinician expectations for the future following a diagnosis of PD in 22q11.2DS. The finding that level of intellect and the presence of schizophrenia affect pre-morbid adult functioning in 22q11.2DS helps to illustrate the importance of considering pre-morbid functional capacity in clinical studies of individuals with 22q11.2DS-PD. In the absence of longitudinal assessments, careful history from family members and/or caregivers may be useful to help disentangle symptoms of PD that are impacting daily functioning from that of lifetime 22q11.2DS-associated features such as cognitive impairment and psychiatric disorders.

9.4.4 Introducing Parkinson's disease into genetic counselling for 22q11.2DS

The evidence of an association between 22q11.2DS and early-onset PD[142-144,252,487] may have clinical, psychosocial, and practical implications that may be important to individuals with 22q11.2DS and their families. For example, knowledge of the possibility of developing PD in early to mid-adulthood could be helpful for planning finances, family structure, vocational goals, etc. On the other hand, such knowledge may increase patient burden and cause emotional distress. Importantly, symptoms of PD are treatable.[60,61,66] Awareness of the apparent increased risk of PD in 22q11.2DS may increase vigilance in monitoring for neurodegenerative symptoms that may help reduce potential diagnostic and treatment delays for these complex patients.[5,252] This may be particularly relevant to 22q11.2DS patients treated with an antipsychotic medication who appear to experience significant delays in diagnosis and symptomatic treatment.[142,144,252]

Patients with 22q11.2DS represent a notable population in terms of provision of genetic counselling for a neurodegenerative disease. They comprise a clinically identifiable population known by definition to carry the 22q11.2 deletion, which appears now to be a risk factor for early-onset PD.[142-144,252,487] Genetic counselling for the myriad of clinical features associated with 22q11.2DS is already an essential component in the management of 22q11.2DS.[5,146] Counselling is recommended to be performed at multiple developmental time points as the spectrum and severity of the associated conditions often evolve with age, and our knowledge of the syndrome continues to grow.[5,146] Discussion of the risk of PD could potentially be incorporated into these sessions, the extent of which may vary depending on the age and presentation of the individual at diagnosis and follow-up. Traditional genetic counselling approaches modified to take into account the cognitive deficits and other neuropsychiatric issues that are frequent in adults with 22q11.2DS may be useful (Table 8-4).[146,220,485] Improved knowledge of the penetrance of PD in 22q11.2DS, the range of age at onset, and disease course remain necessary to better inform possible genetic counselling practices for individuals with 22q11.2DS with respect to PD risk.

9.5 Future directions

The above sections provide details about several possible future directions arising from the findings of this thesis. These include avenues for epidemiological (Section 9.2.1), animal (Section 9.2.2, 9.2.4), genetic (Section 9.2.3), neuroimaging (Section 9.3), and clinical (9.4.2.1, 9.4.3) research studies. Importantly, these could help confirm and extend the presented findings with respect to the manifestation of PD and associated neurophenotypes in individuals with 22q11.2DS.

The most pressing experiment to be performed involves obtaining further confirmatory evidence of the association between the 22q11.2 deletion and early-onset PD. Screening PD cohort(s) for the 22q11.2 deletion (and ideally also other rare copy number variants e.g., using high-resolution genome-wide microarray platforms) is an essential next step to help evaluate the contribution of the 22q11.2 deletion and rare copy number variation to the genetic architecture of PD. The demonstration of an enrichment of the hemizygous 22q11.2 deletion in an early-onset PD cohort, together with the presented data on the enrichment of early-onset PD in a cohort of adults with 22q11.2DS, would help provide a strong rationale to perform animal and molecular studies to

investigate the possible underlying pathogenic mechanism(s). These may include, for example, the use of 22q11.2DS mouse models, and other simple model organisms involving manipulation of 22q11.2 deletion region genes, to investigate their putative function in PD-relevant pathways. Such studies importantly could also help inform the pathophysiology of more common idiopathic forms of the disease.

The results of this thesis also indicate the potential value of performing longitudinal follow-up studies of the assessed adult 22q11.2DS cohort. Regular (e.g., biannual) neurological screening assessments could be conducted to track the development of new PD cases and to better delineate the early signs and age-specific penetrance of PD in 22q11.2DS. This may facilitate improved clinical monitoring and genetic counselling for PD for patients with 22q11.2DS. This could also help expand on the preliminary findings of thesis that suggest that motor dysfunction of varying phenomenology and severity is common in adults with 22q11.2DS. The identification of a novel putative presynaptic hyperdopaminergic state in adults with 22q11.2DS (≥30 years) at risk of PD using PET neuroimaging illustrates the need for neuropathological studies and other functional neuroimaging studies in 22q11.2DS patients to probe dopaminergic function in those who have not yet developed PD. Studies such as these could help further our understanding of the role of the dopamine system in neurodegenerative and neuropsychiatric phenotypes in 22q11.2DS. The identification of the pathogenic mechanisms underlying the development of PD and associated neurophenotypes in 22q11.2DS may help lead to the discovery of novel therapeutic targets and treatments for the respective diseases that are prevalent in the general population.

9.6 Conclusion

The collective findings of this thesis provide evidence that the hemizygous 22q11.2 deletion is involved in the pathogenesis of early-onset Parkinson’s disease, further advancing understanding of the complex genetic architecture and the clinical presentation of Parkinson’s disease. Patients with 22q11.2DS represent a clinically identifiable population amenable to the study of disease pathogenesis from its earliest stages. Longitudinal assessments of patients with 22q11.2DS may reveal genetic and clinical biomarkers of disease risk that may also inform other forms of Parkinson’s disease. These findings have important clinical implications for patients with 22q11.2DS and their families.

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