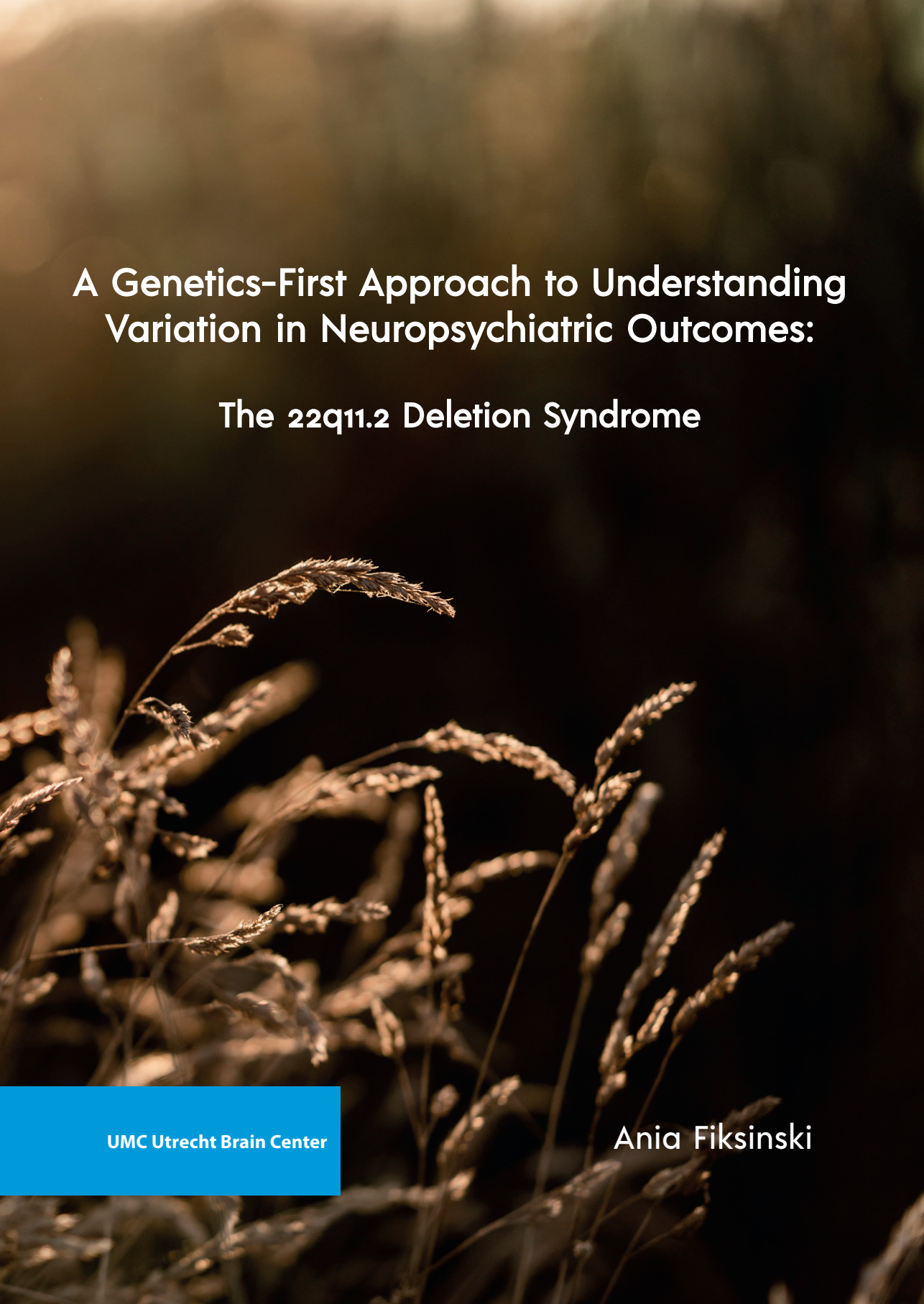
A Genetics-First Approach to Understanding Variation in Neuropsychiatric Outcomes:

The 22q11.2 Deletion Syndrome



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A Genetics-First Approach to Understanding Variation in Neuropsychiatric Outcomes: The 22q11.2 Deletion Syndrome

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A Genetics-First Approach to Understanding Variation in Neuropsychiatric Outcomes: The 22q11.2 Deletion Syndrome

Het Begrijpen van Variatie in Neuropsychiatrische Beelden:
het 22q11.2 Deletie Syndroom als Genetisch Model
(met een samenvatting in het Nederlands)

Proefschrift

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CHAPTER 1

1

General Introduction

1. The 22q11.2 deletion syndrome

A specific genetic condition forms the backbone of the research comprising this dissertation: individuals, both children and adults, with the 22q11.2 deletion syndrome (22q11DS). 22q11DS results from a missing piece of DNA, specifically a deletion involving the long arm ("q) at locus 11.2 on chromosome 22; i.e., the 22q11.2 deletion[1,2]. In ~90% of individuals, this structural genomic variant occurs as a *de novo* event, meaning that the deletion is not inherited from either parent but rather occurs spontaneously in the sperm or egg before conception of the individual carrying the deletion[3]. A 22q11.2 deletion is estimated to occur in 1 in 2000-4000 live births, and in up to 1 in 900 pregnancies[4]. The 22q11.2 deletion typically encompasses a ~3Mb region, involving ~50 protein-coding (functional) genes (**Figure 1**). "Velocardiofacial syndrome", "DiGeorge syndrome", and several other names were previously used to describe the collection of symptoms that was eventually discovered to be associated with this particular genetic variant. Currently, the term "22q11.2 deletion syndrome" (22q11DS) is widely used and accepted among clinicians and researchers worldwide [5,6].

The 22q11.2 deletion is a *pathogenic rare variant*: *pathogenic* indicating that it is related to disease outcomes- both physical and neurodevelopmental-; *rare* defined as occurring in less than 1 in 2000 individuals in the general population. However, among other such rare pathogenic variants, the 22q11.2 deletion is relatively common in the population and was discovered earlier. Indeed, its genetic identification in the 1980's has preceded by several decades the identification of most other comparable pathogenic structural genetic variants with impact on neurodevelopment [5,7,8].

The manifestation, or phenotypic expression, of 22q11DS is characterized by a broad range of potential physical and neuropsychiatric problems, varying greatly in number and severity [5]. Some of the most common physical features include – but are not limited to - congenital heart and/or palatal abnormalities (in ~50% of individuals), and a typical facial appearance [9]. Virtually all individuals with 22q11DS also experience some neurodevelopmental difficulties, which may vary in type and severity. A wide spectrum of neuropsychiatric problems is associated with 22q11DS, including below average level of cognitive functioning (intellectual disability in ~40%), and schizophrenia (in ~20-25%) [9,10] (**Figure 1**). The large differences in observed neuropsychiatric outcomes in this population, i.e., the *large phenotypic variability*, poses a significant challenge for patients, caregivers, and (mental) health professionals [5,11,12]. The relatively well-defined group-level risk is not directly translatable to individual risk prediction, which is likely dependent on additional individual genetic and non-genetic factors. For example, while we now know that any individual with 22q11DS has a baseline, *a priori*, risk of ~20-25% to develop schizophrenia, there is no way to identify those individuals before this psychotic illness fully manifests. Consequently, while being informed of the risk of schizophrenia associated with 22q11DS is important, this also means that for ~75% of parents, the psychological burden of this

considerable risk will turn out to be unsubstantiated[13-15]. Similarly, a question that almost invariably comes up for parents after having received the genetic diagnosis of a 22q11.2 deletion in their child is: "will my child be able to function and live independently?" At present, one can only answer this question at the global level of baseline risk of intellectual disability, which for this specific population is ~45%, with no way to direct the answer towards a more specified outcome for an individual.

This very significant clinical challenge is mirrored in a research context: **while it is understood that the 22q11.2 deletion increases the risk of various neuropsychiatric outcomes substantially, the factors that determine an individual's outcome (i.e., whether or not someone will develop schizophrenia), as well as the trajectories that precede it and the mechanisms that drive outcomes, are largely unknown**. This question is most notable in the context of schizophrenia, among the most severe of mental illnesses, for which the increased risk is substantial and which has been the first neuropsychiatric phenotype to be identified as associated with 22q11DS [16,17]. However, the challenge of outcome uncertainty is equally relevant for other neuropsychiatric outcome influenced by this genetic variant, including cognitive functioning and social difficulties.

Problem 1 = While we know which neuropsychiatric manifestations are associated with 22q11DS, there is no way to predict type and severity of such outcomes for an individual. Among the most prominent of these uncertain outcomes are schizophrenia and level of cognitive functioning.

Figure 1. Overview of the 22q11.2 deletion: chromosomal location and associated phenotypes.

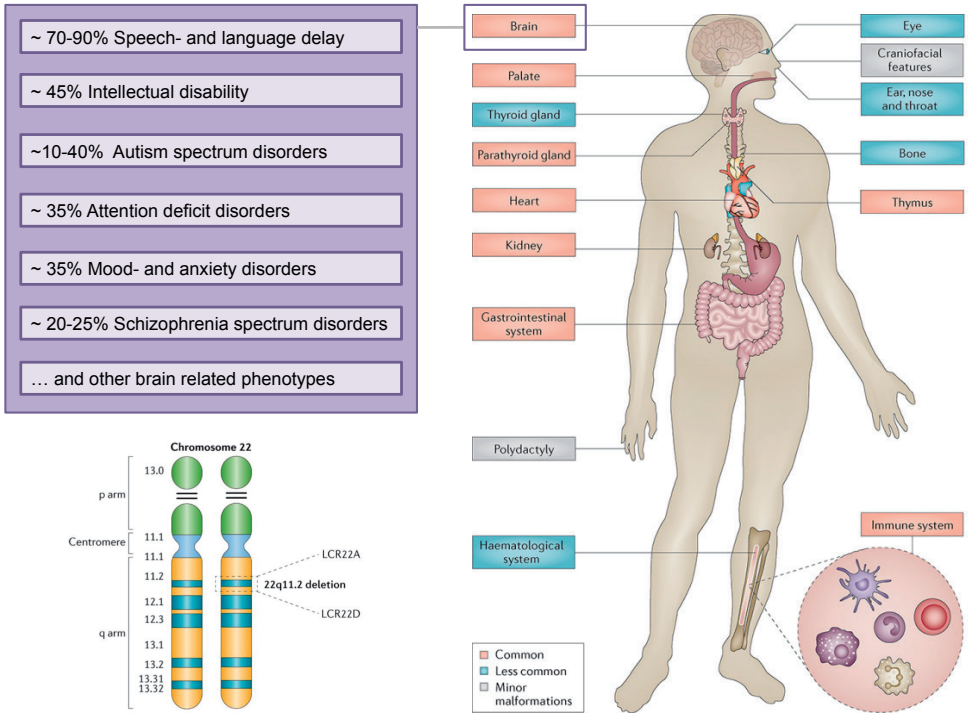


Figure adjusted from McDonald-McGinn et al., 2015 (Nat Rev Dis Prim)

The following sections will introduce concepts that are related to the study of individuals with 22q11DS and relevant to the research comprising this dissertation.

2. Genetics

Our genetic architecture can be considered the roadmap for the formation and functioning of the human body, including the brain. The human genome (i.e., the totality of our DNA) consists of ~20.000 genes. A gene is a sequence of DNA building blocks named "base pairs", of which humans have ~3 billion. Genes encode for specific proteins, which have certain functions in the human body. With the exception of genes on the sex chromosomes in males, each individual has two copies of each of the ~20.000 genes; one inherited from the mother and one from the father. The DNA is distributed over 46 chromosomes, arranged in 23 pairs, including 22 autosomes which are numbered 1-22 for reference, and one pair of sex chromosomes (XX for females; XY for males)[18].

The last few decades have witnessed an exponential growth in technical possibilities to investigate the genome, and these have allowed for an emerging array of scientific findings and insights [18-22]. Investigations of the structure and functioning of the human genome have indisputably revealed the role of genetics in shaping outcome in the broadest sense, including neuropsychiatric outcomes such as mental illness or level of cognitive functioning. Variation in the human genome is substantial and exists across multiple levels [23]:

- Genetic variation may be inherited (from mother or father) or occur as a "*de novo*" event; the latter means that it is a new event, not inherited from either parent.
- Genetic variation can occur at a structural level or at the level of single base pairs. The most common type of structural variants are referred to as structural copy number variants (CNVs). CNVs can involve a *missing* part of a chromosome (a "deletion" such as the 22q11.2 deletion); or an *extra* part of a chromosome (a "duplication" such as the 22q11.2 duplication). At the *whole* chromosome level, there may be an extra chromosome (e.g., Trisomy 21 or Down's syndrome). Variations at the level of single base pairs are referred to as single nucleotide variants (SNVs) and involve a change in only one nucleotide, i.e., comprising a single base pair.
- Genetic variation can be rare or common (i.e., occurring in >1% of the population)[24]. Variants both at the structural and at the sequence level can be rare or common, and in the latter case may be referred to as single nucleotide polymorphisms (SNPs).

Given that our genes contain the "blueprint" of our body, variations in genes can lead to changes in the formation and function of various components of our organism. Consequently, genetic variation in various forms can be associated with outcome, including clinically relevant neuropsychiatric outcome. In other words: *genotype* (the term used to describe the genetic characteristics of an individual, usually with respect to a particular aspect) may be associated with *phenotype* (the term used to describe any observable trait of an individual, usually with respect to one specific trait). It was the observation of clustering of certain phenotypes in certain families (indicative of "heritability") that was historically the first to point towards the association between genotype and phenotype. A well-known and well-established genotype-phenotype association is that between mutations in the BRCA1 gene ("185delAG" or "5382insC") and breast cancer: about 50% (dependent on ethnicity and other factors) of females with the BRCA1 mutation will develop breast cancer[25]. In this case, the mutation affects a single gene, is usually inherited, and is relatively rare.

An example of a method that has contributed greatly to our understanding of genotype-phenotype associations is that of genome wide association studies (GWAS) [26,27]. Generally, GWAS compare common sequence-based DNA between individuals with the

goal of identifying those SNPs that vary with respect to a certain phenotype. For example, SNPs associated with the risk of schizophrenia can be identified with a GWAS comparing individuals with schizophrenia ("cases") and individuals without schizophrenia ("controls"). When certain alleles (a version of the genetic variant) are identified in the case-group significantly more frequently than in the control-group, this is taken to indicate that that particular variant is associated with risk for the phenotype; in this case schizophrenia. Since these studies principally involve common variants, each of which typically only exert a minutely small effect on the phenotype, they require very large samples in order to identify significant associations. This is a challenge considering the relative rarity of the phenotypes examined (e.g., ~1% for schizophrenia). While such "phenotype-first" studies are promising, for neuropsychiatric phenotypes it is the rule rather than exception that individual common variants associated with a certain disorder only elevate disease risk by a very limited amount [22,28,29].

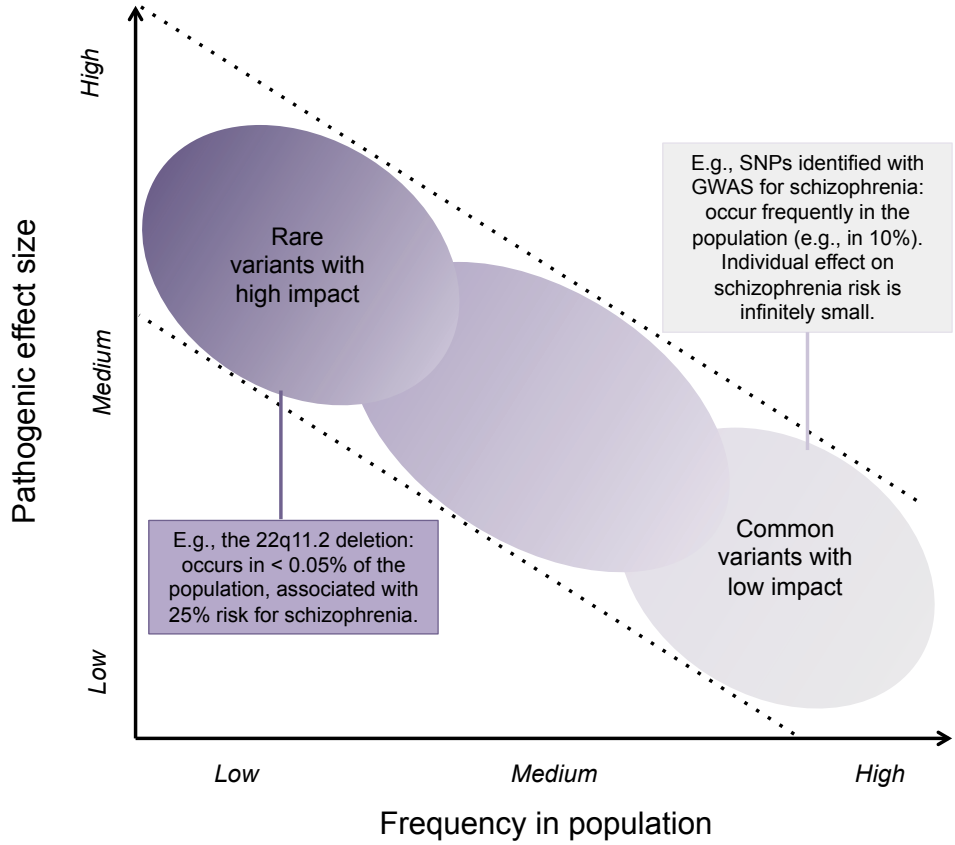
An advance to this GWAS approach is to calculate disease risk from the common genetic risk alleles *collectively*. In other words, each identified risk allele can be attributed a certain weight that corresponds to its contributing risk for a certain illness, and the total "sumscore" of the individual's variants on the relevant risk loci collectively comprises the so called "polygenic score" or "polygenic risk score" (PS, PRS respectively)[30]. PS holds promise with respect to explaining variation in neuropsychiatric traits at the population level. For example, the schizophrenia PS accounts for up to 13% of schizophrenia risk at the population level[29,31], and the PS for global cognitive functioning accounts for ~4% of population variation in IQ[32,33]. Very large samples are required to enable such findings. Indeed, the largest GWAS for schizophrenia now includes ~37.000 individuals with schizophrenia (*cases*) and ~113.000 individuals without schizophrenia (*controls*), however still "only" accounts for up to ~13% of population variance[29]. As the sample size increases, so does the explanatory power of a polygenic score[28,29]. Despite these promising, and ever-increasing, explanatory values at the population level, they do not account for much risk at the level of the individual, which is in part consequential to the low baseline-risk in the general population[24]. Even in the hypothetical scenario where a certain genetic risk factor would elevate schizophrenia risk by 50% - seemingly an impressive metric-, given the population-based risk of ~1%, risk for an individual carrying this variant would still only be 1,5%: a negligible difference with the 1%. In other words; for any randomly selected individual from the general population, knowledge of their individual polygenic score for schizophrenia, does close to nothing in terms of predicting whether the individual will or will not develop schizophrenia. Thus, **while the field has been able to identify common genetic variants that can collectively (calculated as a polygenic score) account for a substantial proportion of variance in neurodevelopmental outcome at the population level, these variants, individually or collectively, have very little explanatory power in terms of phenotypic outcome prediction at the level of the**

individual (Figure 2). None of these in and of themselves are at the level of clinical relevance for schizophrenia, for example.

The study of rare structural genomic variants, in contrast, provides a different perspective. When such rare, usually large, recurrent CNVs are present in an individual, they typically confer a substantial risk with respect to certain neuropsychiatric phenotypes[19,34,35]. Therefore, these are referred to as high-impact or "pathogenic" (related to illness) CNVs. The 22q11.2 deletion is an example of such a pathogenic high-impact (rare) CNV. **Given the rarity of such pathogenic CNVs, they have very low explanatory power in terms of phenotypic outcome prediction at the population level. At the individual level, however, they often explain a significant proportion of phenotypic outcome variability**, in contrast to what is observed for common SNVs (**Figure 2**). For example, anyone born with the 22q11.2 deletion, has an *a priori* risk of ~20-25% to develop schizophrenia. Nonetheless, a significant problem for most individuals who have such a pathogenic CNV remains that there is no way to predict the type and severity of outcomes at the individual level beyond the baseline risk estimates for certain phenotypes (see *Problem 1*). Even a high individual risk of 25% for schizophrenia implies that three out of every four individuals with 22q11DS do *not* develop this illness. Phenomena, consistently observed in CNVs with neurodevelopmental impact, that contribute to the challenge of outcome uncertainty in the context of a pathogenic variant include:

- different neuropsychiatric phenotypes can be (independently, or largely independently) associated with the same pathogenic variant. This phenomenon is referred to as *pleiotropy*: e.g., some individuals with 22q11DS have intellectual disability, others have schizophrenia, and some (but not most) may have both;
- not every individual with the same variant will express a certain phenotype. This is referred to as *variable penetrance*: e.g., while ~25% of individuals with the 22q11.2 deletion will develop schizophrenia, the remaining ~75% will not;
- and there are varying degrees of severity of neurodevelopmental phenotypes, indicative of *variable expressivity*: e.g., while most individuals with 22q11DS have some form of developmental delay, interindividual variability in cognitive functioning still ranges from severe cognitive impairment, to average cognitive functioning.

Figure 2: Schematic representation of the relationship between population frequency and pathogenic effect size of genetic variants implicated in neuropsychiatric outcomes.



In general, there is a negative correlation between population frequency and pathogenic effect size for genetic variants implicated in neuropsychiatric outcomes. I.e., rare variants likely have larger pathogenic effects, while common variants likely exert smaller pathogenic effects. All of these can occur at the structural level (e.g., CNV) or at the single nucleotide level (SNV).

Figure adjusted from Manolio et al., 2009, Nature[36].

The reality is that neither common SNVs nor rare CNVs exist in isolation. It is likely that multiple small and larger; both common and rare, variants, in concert and in interaction with one another contribute to risk for certain outcomes [37]. As of yet, our knowledge of how common genetic variation interacts with larger structural genetic variants with respect to neurodevelopmental outcomes is limited[24]. Only very recently have there been studies investigating the role of common genetic (background) variation (for example the PS for a certain phenotype) in the context of a structural pathogenic variant, such as the 22q11.2 deletion[38].

Problem 2: Pathogenic CNVs, such as the 22q11.2 deletion, confer a substantial neuropsychiatric risk at the individual level. However, given their rarity, they hardly have explanatory power at the population level. Common genetic variation, e.g., captured in the polygenic score, on the other hand performs poorly at the level of individual risk prediction, even though it explains a substantial portion of variance at the population level. Our understanding of the role of common genetic variation in the context of a pathogenic structural variant, such as the 22q11.2 deletion, is limited.

3. Psychopathology

When an individual's behaviors, thoughts, emotions or experiences are significantly deviant and cause distress, dysfunction, and sometimes danger in daily life functioning, one can speak of mental illness or psychopathology. The Diagnostic and Statistical Manual of Mental Health (DSM, currently in edition 5) is one of the most widely used tools to categorize mental illness: it provides mental health workers and researchers with a shared vocabulary (i.e., formal diagnoses) to describe and categorize observed clinical phenomena. There are various and many (n = 157) psychiatric disorders included in the DSM-5, that may occur during various stages of the lifespan[39]. One domain of disorders of particular relevance for the work presented in this dissertation comprises schizophrenia and schizophrenia spectrum disorders; ~20-25% of individuals with 22q11DS develop schizophrenia. **Schizophrenia** is among the most severe and chronic psychiatric conditions, with substantial impact on wellbeing[40] and long-term outcome[41], including life expectancy[42]. It is characterized by disturbances in thought (e.g., delusions where one has a firm and fixed belief that is out of sync with reality), perception (e.g., auditory hallucinations where one hears voices in the absence of an audible stimulus), and behavior (e.g., unpredictable, inappropriate, or grossly disorganized behavioral responses). Increasingly, the **prominent role of cognitive (dys)function has become evident in understanding and defining schizophrenia**. For example, it has now been well established that changes in thinking processes (i.e., cognitive deficits) are one of the earliest manifestations of the disorder, amongst the most difficult to treat, and have the strongest impact on long-term outcome and functioning [43,44]. While schizophrenia is typically diagnosed in early adulthood (around the age of 20-25), when the most obvious symptoms of the disorder become manifest (hallucinations, delusions), there is evidence that the illness process, including cognitive changes, precedes this moment by at least several years[45-47]. However, the early developmental trajectories of, and mechanisms driving, schizophrenia, as of yet, remain insufficiently understood. Given the advances in the understanding that the first observable changes attributable to schizophrenia occur long before the first psychotic episode, schizophrenia is increasingly viewed by some as a "neurodevelopmental disorder"[48].

Neurodevelopmental disorders represent a domain of psychiatric illness that is typically characterized by an onset very early in life (i.e., early childhood). Two examples of particular relevance to this dissertation are **intellectual disability** and **Autism Spectrum Disorders** (ASD). Intellectual disability is primarily defined by early-onset poor cognitive functioning (with an Intelligence Quotient (IQ) of <70), in addition to impaired daily life functioning. ASD typically manifests in young children (with symptoms observable before the age of three), and is characterized by impairments in communication and social functioning, as well as rigid and/or stereotyped behaviors.

All mental health conditions, collectively referred to as neuropsychiatric disorders, including schizophrenia and other neurodevelopmental disorders such as ASD, are currently understood and defined mostly by their observable manifestation. In other words, their DSM 5 categorization provides little insight into causes (etiology), pathways, or disease mechanisms. Consequently, while there are biological and non-biological treatments available for some psychiatric conditions, with varying degrees of effectiveness, an understanding of underlying causes and mechanisms is largely lacking. This hampers the development of new, targeted and effective intervention strategies. Similarly, despite advances in descriptions of neuropsychiatric conditions and their trajectories, we are currently unable to provide adequate disease prognosis or outcome prediction, posing significant challenges to patients, caregivers, clinicians, and researchers[49,50]. **This lack of insight into etiology and disease mechanisms constitutes a key challenge for the mental health field.**

Several issues hamper the advancement of a better understanding of neuropsychiatric disease etiology, mechanisms, and even definitions. First; **the difficulty of identifying individuals *at risk* for certain neuropsychiatric conditions.** Patients with neuropsychiatric disorders often only come to clinical attention at an advanced stage of the illness, hence hindering the study of disease trajectories. Schizophrenia is an example of a neuropsychiatric illness in which the study of (early) disease trajectory is particularly difficult: precisely because patients typically only come to clinical attention after their first psychotic episode, it is difficult to advance insights with respect to early risk markers, trajectories, and early disease mechanisms. The study of such early risk- or disease phenotypes is further complicated by the relatively low prevalence rates of neuropsychiatric conditions at the population level (e.g., ~1% for schizophrenia): population-based studies would require unfeasibly large sample sizes in order to longitudinally follow individuals before full-blown disease manifestation[48].

It is in this context that the **potential of studying individuals with a substantially increased risk** to develop a certain neuropsychiatric disorder is being increasingly recognized. Such "high-risk" individuals can be identified before, or at an early stage of, disease onset, and their development monitored over time [48,51]. One approach

to this comprises family-studies, e.g., where family members of an individual with a neuropsychiatric illness are studied[52-54]. Another method is the identification and investigation of "clinical high-risk" groups: individuals who are selected based on certain traits or markers that have been associated with increased psychiatric risk[55,56]. Another approach, whose value is increasingly recognized, is the (longitudinal) study of individuals who have a genetically determined elevated neuropsychiatric risk, because they carry a high-impact pathogenic CNV[50]. Indeed, after decades of questioning the validity of a neuropsychiatric diagnosis such as schizophrenia in the context of a 22q11.2 deletion, *22q11DS is now viewed as a valuable genetic model to study early trajectories and disease mechanisms related to schizophrenia* [17,48,57]. Specifically, these individuals can be identified very early in life, or even before birth, and the manifestation of schizophrenia converges with observations of individuals with idiopathic schizophrenia[58]. The 22q11.2 deletion is the strongest known molecular genetic risk factor of schizophrenia[5], with ~20-25% of patients developing schizophrenia. In turn, the 22q11.2 deletion can be found in ~1 in 100-200 individuals with schizophrenia[59].

Second, **the large etiological heterogeneity of neuropsychiatric disorders**: Both collectively and individually, neuropsychiatric disorders are likely to have a wide variety of causes, which in turn can interact in various ways with normal and abnormal developmental and environmental factors to result in varying manifestations of different and possibly multiple neuropsychiatric conditions[49,60]. This large etiological heterogeneity, combined with the lack of etiologically informed definitions of neuropsychiatric conditions, significantly complicates or obstructs the identification of causal pathways, (non-) biological mechanisms, and consequently, an understanding of effective intervention strategies.

Therefore, the **potential of studying populations that are etiologically more homogeneous** (i.e., share the same causal or risk factor with respect to a certain mental illness), such as individuals with 22q11DS, is increasingly recognized in the field[48,49,61,62].

Third, **the categorical approach based on clinicians' observation that is largely used to define neuropsychiatric outcomes.** This categorical approach is exemplified historically by the DSM, where neuropsychiatric disorders are placed into categories, and an individual receives a diagnosis (yes/no) based on presenting symptoms. This categorization provides a useful common framework and language for mental health professionals and researchers, and has an important role in how clinical populations are managed. However, it does little to aid in understanding underlying etiology and pathways and does insufficient justice to the clinical reality of varying degrees of severity

of different neuropsychiatric symptoms observed in the population, that may occur in concert or independent of one another, dependent on the underlying cause.

This problem contributes to a gradual shift in conceptualization in the field of neuropsychiatric conditions: the **importance of a dimensional, or quantitative, approach to understanding neuropsychiatric traits**, in addition to the categorical perspective on mental illness, is now being progressively recognized[63,64]. A dimensional approach, where mental health is viewed as a conglomerate of domains of behavioral, emotional, cognitive, and social functioning, may do more justice to the complexity and diversity of psychopathological manifestations observed in the population. Consequently, this approach holds promise for advancing insights into etiology and mechanisms and, by extension, for developing targeted and effective intervention strategies. This conceptual shift towards a dimensional approach of mental illness pertains both to how disorders are defined (and this is already reflected in the DSM 5, which allows for a more quantitative approach than previous versions[65]) and to how mental health parameters are understood and assessed [66-68].

Problem 3: There is a disproportional lack of insight into disease etiology, mechanisms, and early developmental trajectories in the field of neurodevelopmental disorders, including schizophrenia, intellectual disability, and ASD. Important contributing challenges are:

1. The difficulty of (early) identification of individuals at risk for neuropsychiatric conditions.
2. The large etiological heterogeneity of neuropsychiatric conditions.
3. The categorical conceptualization of neuropsychiatric conditions.

It is in the context of these challenges that the study of individuals with the 22q11.2 deletion may substantially contribute to furthering the understanding of neuropsychiatric conditions, given the strongly elevated risk for various neuropsychiatric disorders, the relative etiological homogeneity, and the typically early identification of this population.

4. Cognitive functioning

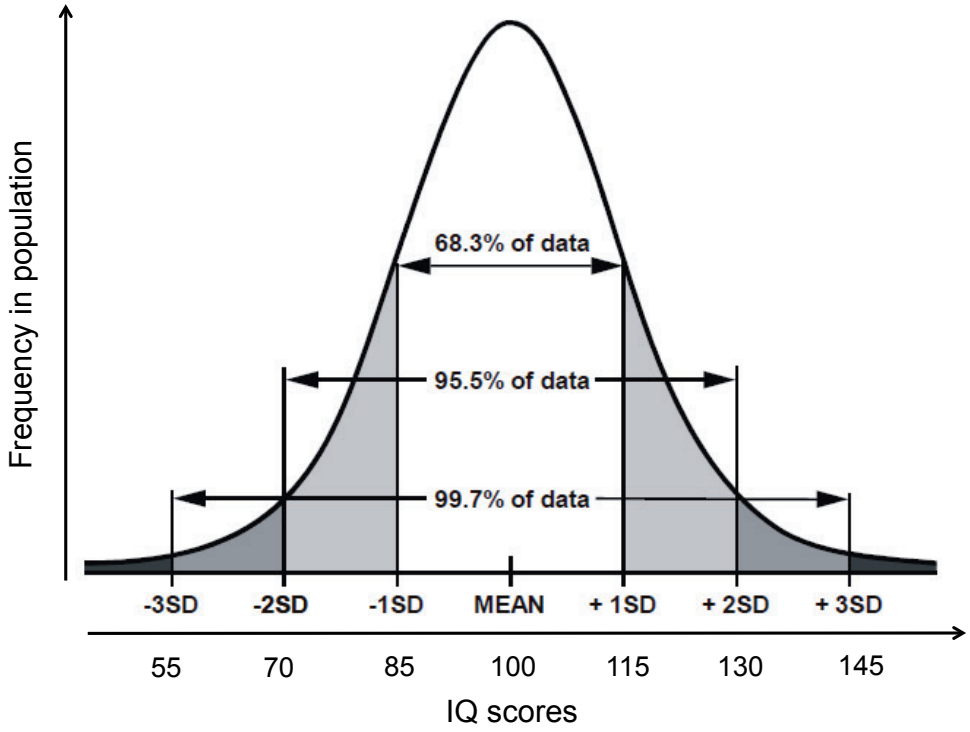
Overall cognitive functioning refers to human thinking and reasoning abilities and it encompasses various and wide-ranging skills, including verbal and perceptual, as well as concrete and abstract reasoning capacities. **Overall cognitive functioning is a key determinant for human day to day functioning**; it plays an important role in how well we can understand and organize the world around us, communicate with others, and hence, more specifically, how well we do in school, how we can organize our lives and what professional activities we can pursue[69,70].

Human cognitive functioning can be reflected in an IQ-score: Intelligence Quotient. IQ can be measured through standardized, validated and normed assessment instruments of intelligence. The most widely used tests are the Wechsler scales of Intelligence (e.g., [71,72]). Ever since the first Wechsler Scale of Intelligence in 1939[70], various versions have been developed over the years to be applicable to all age groups, and to be commensurate with (then) current times. There are also other, less commonly used, instruments to measure IQ, including the Stanford-Binet[73]. Regardless the specifics of an IQ-test, the overall IQ (frequently referred to as Full Scale IQ: FSIQ) comprises a few key components, i.e., **the global level of cognitive functioning arises from a combination of cognitive domains**. While the specific names of these domains may vary (and are theory- and assessment instrument-dependent), the following components represent what are generally believed to be the core domains of global intellectual functioning[71]. Verbal IQ (or verbal comprehension) refers to an individual's ability to access one's mental vocabulary, to express oneself in a meaningful way, and to one's reasoning skills, both concrete and more abstract. Performance IQ (or perceptual reasoning) refers to the ability to think and reason using visual information, to mentally organize information and structure one's surroundings; the ability to "see" what is being asked. Working memory refers to the ability to store simple information and retrieve and manipulate it – on a short-term ("the RAM memory" of human brain functioning). Processing speed refers to the speed with which one can process an external stimulus, and get the output of an appropriate action to it. Apart from the components that comprise global cognitive functioning, i.e., IQ, there are more specific domains of neurocognition. These include domains such as executive functioning, complex attention, and learning and memory[74]. These domains are more specific than the overall IQ, and while generally related to global cognitive level, can reveal specific domains of neurocognitive difficulties that may be important for an individual's day-to-day functioning. Moreover, these more specific domains of neurocognition, as opposed to IQ, may be more susceptible to amelioration from intervention, for example cognitive remediation strategies, as investigated in individuals at clinical high risk for schizophrenia[75,76]. Awareness of one's cognitive profile, including individual strengths and difficulties, is important in optimizing the balance between individual capacities and environmental demands, for example in school-, work-, or home-settings.

Generally, the different components of IQ are highly interrelated in one individual; while there are intra-individual differences between components, one's level of verbal reasoning skills, for example, is likely similar to one's level of visuospatial reasoning skills[71]. Moreover, **cognitive functioning, reflected in IQ, is generally stable over the lifespan,** bar the growth and demise of cognitive capacities typical of young and old age respectively. As IQ-scores are standardized and adjusted to age, an individual's IQ-score at age 6 is likely to remain the same as the individual develops throughout childhood, adolescence, adulthood, and into older age[71].

In the general population there is **natural variation in cognitive functioning among humans**[69]. This is reflected in that IQ-scores follow a normal distribution, where most people have an IQ-score around the mean (general population mean IQ = 100), but that there are also individuals with much less common IQ-scores that can be either high or low. The standard deviation of IQ is 15 (SD = 15), which, given the normal distribution of IQ, means that about 68% of people from the general population will have an IQ-score between 85 (-1 SD) and 115 (+1 SD); about 95% of people will have IQ scores between 70 and 130 (-2 SD - +2 SD). Only less than 2,5% of people will have IQ scores lower than 70 (-2 SD), and only less than 2,5% of people have IQ-scores higher than 130 (+2 SD)[71] (**Figure 3**).

Figure 3: Normal distribution of IQ in the general population



Even with this information, the number comprising an IQ-score is relatively abstract and difficult to translate to a concept useful for daily living situations. The construct of "mental age" is helpful here: **an IQ-score in fact corresponds to one's mental age**[73]. An approximation of the mental age equivalent, regardless assessment instrument used, can be calculated with the following formula:

Mental Age (MA) = (Chronological Age (CA) x IQ-score) / 100 *

** This formula works up to a maximum chronological age of 17. So for individuals aged 18 or older, 17 always needs to be inserted as the chronological age.*

For example, if an IQ value of 70 is measured in an 8-year old, the corresponding mental age (MA) is 5.6 years. (*CA = 8, IQ = 70 —> (8 x 70) / 100 = 5.6)*. Therefore, although the child is 8 years old, he or she can be said to function cognitively approximately at the level of a *typically developing* child of 5.6 years old. Commonly and naturally, people receiving their IQ-scores, as well as their caregivers or clinicians, have a more accurate intuition of what mental age represents than when limiting feedback to only the IQ scores.

The role of cognition, and specifically, aberrant cognitive functioning and cognitive development, in neuropsychiatric conditions is increasingly recognized. A notable example is schizophrenia, where deviations in cognitive development are identified as one of the earliest manifestations of the disorder[44]. The role of genetics in shaping cognitive outcomes is also becoming increasingly evident. Global cognitive functioning is among the most highly heritable neurobehavioral traits[77], and common genetic variation associated with IQ, accounts for ~4% of IQ variation at the general population level[78]. Moreover, an increasing number of pathogenic genetic variants that are associated with deviant cognitive functioning, including high prevalence rates of intellectual disability, is being identified[79]. The 22q11.2 deletion is strongly associated with aberrant cognitive outcomes: about 45% of individuals with 22q11DS have a formal intellectual disability (defined largely by an IQ < 70)[80,81]. In addition to the previously identified difficulty of predicting degree of severity of cognitive difficulties in individuals with pathogenic variants such as the 22q11.2 deletion, there is another challenge. In studies of cognition in populations of CNV carriers, indices of cognitive functioning are used that are derived from studies in the general population. However, given the deviant level (often lower) and development (often slower) of cognitive functioning in such populations, **general population indices of cognitive development may not be entirely applicable to individuals with pathogenic CNVs**, which may hamper advances with respect to understanding cognitive outcomes in these individuals[82-84]. In addition, as is the case for a categorical approach with respect to neuropsychiatric disorders, the conceptualization of cognitive functioning in a dichotomous manner (i.e., intellectual disability yes/no) does not adequately represent the reality of the (normal) distribution of levels and trajectories of cognitive functioning.

Problem 4 = Indices of cognitive functioning and development derived from the general population may not be entirely applicable and sufficiently informative in populations of individuals with pathogenic variants, such as the 22q11.2 deletion.

5. Main objectives and outline chapters of this dissertation

The four problems outlined in the preceding sections all motivate the research comprising this dissertation, with each chapter addressing directly or indirectly one or several of these

problem(s). Specifically, the overall aim of the research comprising this dissertation is the following:

To contribute to the understanding of the variable expression of, and mechanisms driving, neuropsychiatric phenotypes in individuals with the 22q11.2 deletion.

The rationale is to ultimately contribute to:

- Furthering the understanding of mechanisms involved in pathways underlying neuropsychiatric disorders in individuals with this, and other high-impact variants.
- Advancing insights into trajectories and mechanisms of neurodevelopmental phenotypes such as schizophrenia and cognitive functioning in the general population.
- Improving clinical care and management practices for individuals with 22q11.2 deletions and their families.

Specifically, in the research review in **Chapter 2**, we provide an overview of insights into the neurodevelopmental and early psychiatric manifestations of 22q11DS. We provide a discussion of important considerations in the context of understanding the pediatric neuropsychiatric phenotype in 22q11DS, including its specificity, phenomena such as pleiotropy, gene-environment interactions, the age-dependency of phenotypes, and the impact of assessment and ascertainment bias.

In **Chapter 3**, we employ a prospective longitudinal study design in the Utrecht 22q11DS cohort to investigate a potential early phenotypic risk marker of schizophrenia outcome in individuals with 22q11DS. Specifically, we examine whether among children with 22q11DS, those with an autism spectrum diagnosis and/or ASD-like symptoms, operationalized quantitatively, are more likely to subsequently develop a schizophrenia spectrum disorder than children without ASD (*relevant to Problems 1 and 3).*

In **Chapter 4**, we investigate various specific domains of neurocognitive functioning as well as level of adaptive (daily life) functioning in adults with 22q11DS from the Toronto cohort, with and without schizophrenia. Specifically, we examine whether there is an association between any of the neurocognitive domains and subsequent functional outcome, while accounting for the effects of schizophrenia and global cognitive functioning. In addition, we explore the profile of neurocognitive strengths and weaknesses in individuals with 22q11DS, both with and without schizophrenia (*relevant to Problems 1 and 4).*

In **Chapter 5** we address the problem that indices of cognitive functioning (IQ) are derived from the general population and may not be entirely applicable and/or useful to individuals with 22q11DS. We present normative data on IQ and IQ-trajectories from the largest sample of 22q11DS individuals available to date; the IBBC, and explore the utility

of a 22q11DS-specific normative chart for IQ and IQ-development (*relevant to Problems 1, 3, and 4)*.

In the final two research chapters of this dissertation, we broaden our focus from investigating outcomes related to neuropsychiatric conditions in individuals with 22q11DS, to exploring potential underlying mechanisms. Simultaneously, these chapters mark a step towards improving individual risk and outcome prediction, beyond the level of baseline risk. In **Chapter 6**, we study families consisting of adults with a *de novo* 22q11.2 deletion and their unaffected parents from the Toronto cohort. We investigate whether parental functioning on important, quantitatively assessed, neurobehavioral traits; cognitive, social and motor functioning, is associated with the level of functioning in their offspring. Our genotype-first approach, combined with the within-family design, allowed us to investigate patterns of influence of parental phenotypes, the 22q11.2 deletion itself, and schizophrenia on the neurobehavioral quantitative phenotypes (*relevant to Problems 1, 2, and 3)*.

While we indirectly explore the impact of shared (common) genetic variation by investigating parent-proband associations in Chapter 6, in **Chapter 7** we continue this examination by using polygenic scores, derived from the general population, in the IBBC sample. Here, we aim to better understand the role of common genetic variation in the context of a high-impact genetic variant. Specifically, we investigate whether, in the context of a 22q11.2 deletion, the polygenic scores for schizophrenia and IQ are associated with not only schizophrenia and IQ, but also with early schizophrenia-related phenotypes, including cognitive decline and subthreshold psychotic symptoms. Moreover, we explore the potential of using polygenic scores in the context of a high-impact variant such as the 22q11.2 deletion for improving phenotypic risk prediction, specifically for schizophrenia and intellectual disability (*relevant to Problems 1, 2, and 3)*.

Text box: Brief background to the samples of participants included in this dissertation

The Utrecht cohort

The study of genetics and psychopathology in individuals with 22q11.2 deletion in Utrecht started in 2001. Since then, we have seen ~250 children with 22q11DS, more than half of whom we have followed longitudinally (i.e., they have visited us two, three, or even four times over the years). Patients are referred to the outpatient 22q11DS psychiatry clinic as part of standard clinical care, and subsequently asked to participate in our research study. Ever since 2001, the focus of the research has been to better describe and understand psychiatric and cognitive outcomes in these children. Therefore, standardized psychiatric and cognitive assessments are not only part of routine clinical care, but also the principal study parameters. Such assessments are conducted by trained and experienced clinicians (psychiatrists and psychologists). In addition, blood samples for DNA and RNA extraction have been drawn from the majority of research participants. The majority of the Utrecht participants are also included in the IBBC database (see below). Studies that have outlined in more detail the Utrecht cohort and study methods include [85] and [86].

The Toronto cohort

Since beginning the study of adults with 22q11DS in Toronto in the 1990's, over 350 adult patients have been followed clinically and participated in research. Patients are ascertained mainly through a congenital cardiac clinic, medical genetics sources, and psychiatric sources. Over the decades of research, severe psychiatric illness such as schizophrenia has always been a key study parameter, and in addition various standardized assessment methods have been used for other neurobehavioral phenotypes, including IQ-testing and batteries of specific neurocognitive tests. For a majority of the Toronto cohort there are genetic and/or phenotypic data available for patients' family members. About half of the individuals with 22q11DS from this cohort are included in the IBBC as well (see below). Studies that have outlined in more detail the Toronto cohort and study methods include [87] and [88].

The 22q11DS International Consortium on Brain and Behavior (IBBC)

The IBBC is an international collaboration of 22 "phenotyping-sites"; i.e., centers that specialize in phenotypic assessment of individuals with 22q11DS, including neuropsychiatric traits and outcomes, and several "genotyping-sites"; i.e., centers that specialize in genetic analyses and that helped process the genotyping in the consortium. The collaboration naturally evolved over the last ~20 years, with clinicians and researchers specializing in 22q11DS reaching out to one another. Given the rarity of conditions such as 22q11DS, it is highly valuable to collaborate internationally to advance scientific research, in particular with respect to genotype-phenotype or genotype-genotype analyses. This importance, as well as the potential of studying 22q11DS as a neurogenetic model of schizophrenia, was recognized and financial support for a retrospective study provided by funding from the National Institutes of Mental Health (NIMH). This marked the formal formation of the, already informally existing, 22q11DS IBBC. This has resulted in a database of 1789 individuals with 22q11DS, varying in age from 3 to 68 years, most with extensive neuropsychiatric phenotyping

data and genetic data available. Studies that have outlined in more detail the IBBC cohort and research rationale include [89] and [38].

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CHAPTER 2

2

Understanding the Pediatric Psychiatric Phenotype of 22q11.2 Deletion Syndrome

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Abstract

Aim. The purpose of this article is to provide an overview of current insights into the neurodevelopmental and psychiatric manifestations of 22q11.2 deletion syndrome (22q11DS) in children and adolescents.

Recent findings. The pediatric neuropsychiatric expression of 22q11DS is characterized by high variability, both inter-individual and intra-individual (different expressions over the lifespan). Besides varying levels of intellectual disability, the prevalence of autism spectrum disorders, attention deficit disorders, anxiety disorders, and psychotic disorders in young individuals with 22q11DS is significantly higher than in the general population, or in individuals with idiopathic intellectual disability. Possible explanations for this observed phenotypic variability will be discussed, including genetic pleiotropy, gene-environment interactions, the age-dependency of phenotypes, but also the impact of assessment and ascertainment bias as well as the limitations of our current diagnostic classification system.

Implications. The implications inferred by these observations mentioned above bear direct relevance to both scientists and clinicians. Observations regarding the neuropsychiatric manifestations in individuals with 22q11DS exemplify the need for a *dimensional approach* to neuropsychiatric assessment, in addition to our current categorical diagnostic classification system. The potential usefulness of 22q11DS as a *genetic model* to study the early phases of schizophrenia as well as the phenomenon of neuropsychiatric pleiotropy observed in many CNV's will be delineated. From a clinical perspective, the importance of *regular neuropsychiatric evaluations* with attention to *symptoms* not always captured in diagnostic categories and of *maintaining equilibrium* between individual difficulties and competencies and environmental demands will be discussed.

Keywords: 22q11DS, psychiatry, pleiotropy, pediatric psychiatry, clinical implications, schizophrenia.

1. Introduction

Ever since the first reports of psychotic disorders in individuals with the 22q11.2 deletion (22q11DS) were published, now some 25 years ago, there has been increasing interest in this remarkable association[1-4]. These initial findings have been replicated in several studies, confirming an approximately 25-fold increased risk of developing schizophrenia in patients with 22q11DS compared to a ~1% lifetime risk in the general population[5]. Understandably, this observed association continues to receive much attention from both clinicians and investigators. From a clinical perspective the increased risk mandates careful monitoring of patients, in particular during adolescence and early adulthood, when the risk of psychotic development is highest. From a research perspective the association represents the strongest known genetic risk for schizophrenia conferred by a single genetic variant[6]. In addition, the phenotypic expression of schizophrenia in 22q11DS has been described as indiscernible from schizophrenia in the general population[7,8]. These observations have spurred the research community to examine 22q11DS as a unique genetically homogeneous model for schizophrenia [9], and, in the words of Thomas Insel: initiate prospective studies of this population that will provide "important insights into the trajectory from risk to disorder"[10].

While the emphasis on schizophrenia and associated psychotic disorders (commonly referred to as "schizophrenia spectrum") in 22q11DS is clearly justified[11], a potential downside of a highly specific focus may be that the occurrence of other neuropsychiatric phenotypes in individuals with this genetic disorder can be easily overlooked. Multiple independent studies indicate significantly increased rates of a number of psychiatric and other neurodevelopmental disorders (including anxiety, autism spectrum, attention deficit disorders) in addition to schizophrenia in individuals with 22q11DS [12-16]. This article will review the current knowledge of these phenotypes in 22q11DS, with a focus on childhood and adolescence. In addition, potential pitfalls regarding these findings will be examined, including the effect of ascertainment bias and possible limitations of categorical diagnostic classifications. Furthermore, both research and clinical care implications of the neuropsychiatric phenotypes in 22q11DS will be discussed.

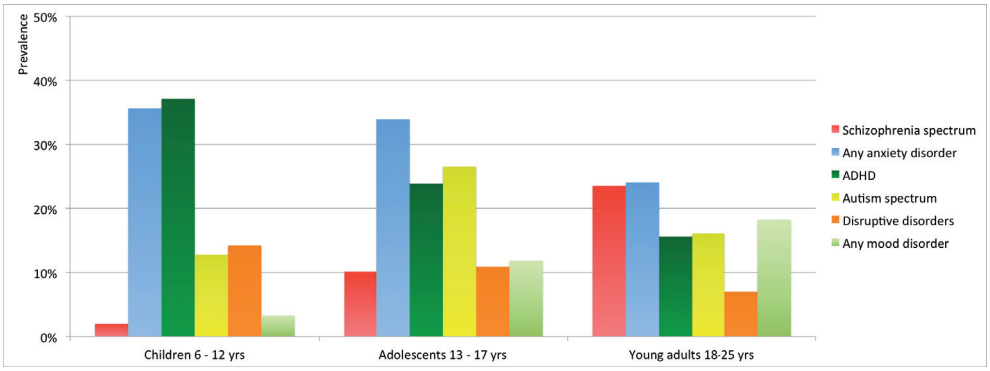
2. Neurodevelopmental disorders in childhood and adolescence

2.1.1 Overview of the neuropsychiatric and cognitive phenotype

The neurodevelopmental and psychiatric phenotype of children and adolescents with 22q11DS is highly diverse. From infancy onward, delayed and/or impaired speech and language development are frequently observed [17]. Moreover, intellectual functioning in the borderline range (FSIQ between 70-85) is most common, followed by mild intellectual

disability (FSIQ 55-70). More severe levels of intellectual disability are uncommon in children, but more frequently observed in adults with 22q11DS[17-19], suggesting that cognitive abilities may not be stable in all individuals with 22q11DS. Indeed, a recent longitudinal study found that, overall, individuals with 22q11DS showed a modest but significant decline in IQ between the ages of 8 and 24[20]. Notably, in those who developed a psychotic disorder, the decline, most pronounced in verbal IQ, was significantly steeper compared to those without a psychotic disorder. These findings provide evidence that cognitive decline might be a useful early clinical marker of psychotic disorders in people with 22q11DS[20].

Figure 1. Prevalence of psychiatric disorders in 22q11DS from childhood to young adulthood.



The international brain and behavior consortium (IBBC) on 22q11DS has provided the most comprehensive overview of psychopathology in individuals with 22q11DS to date [12]. This first study from this multicenter consortium reported on the psychopathology of 1401 individuals with 22q11DS, recruited across multiple sites, aged 6-68 (mean = 18,78, SD = 10,66)[9,12]. **Figure 1** presents a summary of the findings across three different age groups between 6 and 25 years. Developmental disorders such as attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) are reported more frequently in the younger age groups as compared to the older groups (see also: [13-15]). Disruptive disorders are relatively less frequent overall, and their prevalence declines with increasing age. Mood disorders are frequently observed, and notably their prevalence increases significantly over time, particularly for major depressive disorder. Anxiety disorders are frequently reported across all age groups, but are especially prevalent in children and adolescents (see also: [16]). The prevalence of schizophrenia spectrum disorders increases significantly over time, with prevalence rates of 24% in emerging adults and about 41% in individuals over 25 years old (see also: [4,21]).

2.1.2 The specificity of the psychiatric and neurodevelopmental profile in 22q11DS

Observations from epidemiological studies indicate that the overall rate of psychopathology is increased in youth with idiopathic intellectual impairment [22]. One particularly salient question therefore is to what extent the prevalence of psychiatric disorders in 22q11DS deviates from what is reported in unselected cohorts with intellectual impairment. Table 1 lists the prevalence rates of developmental and psychiatric disorders in individuals with 22q11DS (6 through 17 years) compared to observations in individuals with idiopathic intellectual impairment (n=641)[23] and the general population.

Table 1. Prevalence rates of psychopathology in youth with 22q11DS compared to youth with idiopathic intellectual impairment and the general population

	Youth with 22q11DS[a]	Youth with idiopathic intellectual impairment[b]	Youth in general population[c]
ADHD	32.1%	8.3%	5%[I 24]
Disruptive disorders[d]	13.0%	20.5%	6%[II]
ASD	21.5%	8.0%	1 – 1.5%[III, IV]
Any mood disorder	7.0%	1.4%	10%[II, V]
Any anxiety disorder	34.9%	11.4%	12%[VI]
Any psychotic disorder, including schizophrenia	5.5%	not reported	<< 1% [VII, VIII, IX]

[a] Data from [12] (805 individuals, note that percentages deviate from figure 1 since we collapsed data obtained in the age range 6 to 17 years, most individuals assessed at a level of mild intellectual disability)

[b] Data from [23] (641 individuals, age range 5 to 16 years, intellectual impairment based on parental/ teacher report, most individuals estimated at a level of mild intellectual disability, the numbers represent point prevalence (i.e. symptoms present during the month – half year preceding the assessment)

[c] Data from cohorts including both children and adolescents were used to obtain these estimates; exact age ranges vary between the different studies.

[d] Oppositional defiant disorder, conduct disorder.

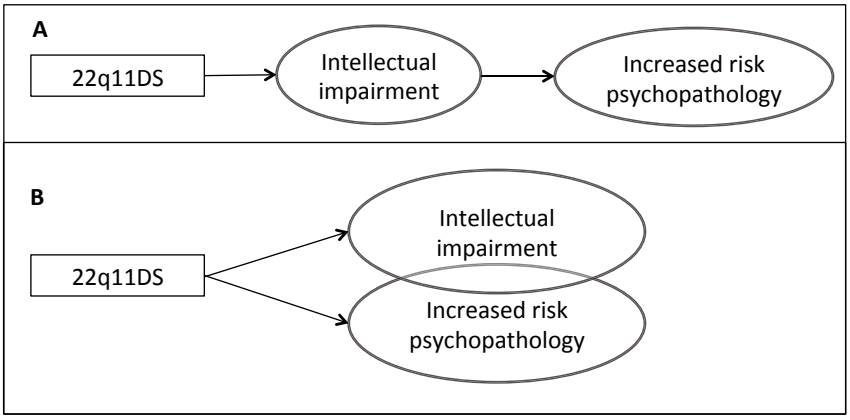
I 24, II 25, III 26, IV 27, V 28, VI 29, VII 30, VIII 31, IX 32.

It is important to note that prevalence rates reported in **Table 1** are all to some extent influenced by variable degrees of ascertainment and/or assessment biases and should therefore be compared with caution. For instance, individuals in the idiopathic intellectual impairment group were not recruited through a clinical site and intellectual impairment was established based on parental / teacher report. In contrast, many studies contributing to the overview of 22q11DS findings were conducted at a clinical site (see discussion below) and in many individuals IQ was obtained through formal testing. Rates reported in the general population are not specific for youth.

Notwithstanding these precautions, some preliminary insights can be gained from table 1. First, from this young age onwards, psychotic disorders occur in the 22q11DS

population (between age 13 and 17 years the rate is already 10%). Many individuals fall into the diagnostic category "psychotic disorder not otherwise specified", likely due to the young age at assessment. Second, the prevalence of other developmental disorders (ASD and ADHD) and mood/anxiety disorders is increased in 22q11DS youth over what is observed in a population with idiopathic intellectual impairment. Third, the rate of disruptive disorders in people with 22q11DS is well *below* the rate in the idiopathic intellectual impairment population. These findings suggest that the 22q11.2 deletion has a specific impact on the behavioral phenotype and therefore that the risk of psychopathology cannot be solely explained as an unspecific consequence of intellectual impairment (**Figure 2**, scenario A).

Figure 2. Two possible associations between intellectual impairment and the increased risk for psychopathology in 22q11DS.



Further support for the proposition that the observed increased rates of psychopathology are specific to 22q11DS (**Figure 2**, scenario B), as opposed to a non-specific effect of broad intellectual impairment, is provided by studies that show no correlation between cognitive level and the risk for psychiatric disorders in individuals with 22q11DS [33,34]. Accordingly, higher rates of psychopathology are reported in 22q11DS individuals compared to IQ-matched controls[35]. Inversely, the lower than expected prevalence rates of both disruptive disorders (albeit higher than in the general population, see **Table 1**) and substance use disorders in 22q11DS[36], compared to both general and idiopathic intellectually impaired populations [37,38] provide further evidence for a specific genetic effect. These findings suggest that scenario B (**Figure 2**) is likely - although it does not exclude some effect by the mechanism of scenario A altogether. Furthermore, findings suggest that 22q11DS increases the risk of some psychiatric disorders, but not of others. Indeed, similar to observations in cohorts with other structural pathogenic variants, emerging evidence

indicates that specific profiles of psychopathology can be distinguished when comparing 22q11DS to idiopathic, unselected populations[39,40].

2.2 Understanding the high degree of phenotypic variability

2.2.1 Neuropsychiatric pleiotropy

The psychiatric phenotypic expression of 22q11DS is highly variable. Many different psychiatric disorders are associated with this CNV, phenotypes can differ considerably between individuals, and differences exist across age groups. This variability is consistent with the phenomenon of phenotypic pleiotropy observed in many rare CNV's [41,42], whereby one specific genetic variant can result in independent phenotypic expressions. For example, in the context of 22q11DS, the presence or absence of congenital cardiac problems does not seem to be associated with an altered risk for psychotic disorders [43]. Therefore, congenital cardiac defects and psychotic disorders can be considered as true pleiotropic manifestations of the 22q11.2 deletion.

Observations from numerous studies suggest that in 22q11DS different *psychiatric* phenotypes can also emerge independently, as pleiotropic conditions. The distinction between true pleiotropy and "pseudopleiotropy" is important in this regard. The latter refers to phenotypes that are observed as separate manifestations, whereas in reality they represent the same pathological process, for instance at different developmental stages [44]. Prospective longitudinal studies can provide insight in this regard. For example, a recent study found evidence for true pleiotropy regarding ASD and psychotic disorders, given that individuals with 22q11DS with ASD at a young age were not at an increased risk for developing psychotic disorders later in life compared to those without ASD [45] [44]. On the other hand, cognitive decline [46] and psychotic disorders in 22q11DS were initially reported as two different phenotypes, while subsequent prospective studies indicated that these two phenotypes represent, at least in a subset of individuals, the same pathological process but at different developmental stages [20], thereby providing an example of pseudopleiotropy. Such findings underline the importance of considering additional factors that may influence the observed phenotypic variability. Here, we will briefly discuss such factors, including the phenomena of gene-environment interactions, cross-site ascertainment and assessment differences, age-dependent phenotypes, and diagnostic classification artifacts that impact the observed comorbidity.

2.2.2 Environmental factors as a source of inter-individual variability

Several studies point towards the impact of environmental factors on the observed behavioral phenotype in individuals with 22q11DS. For example, studies have shown that lower parental socio-economic-status (SES) and intrusive parenting style [47] were associated with worse social functioning and other clinically significant problems in children with 22q11DS [48,49]. Additional evidence is provided by a study examining the effect of *Sept5* deficiency (one of the genes in the 22q11.2 region) on social functioning in a mice model

of 22q11DS[50]. This study showed that *Sept5* deficient mice had decreased affiliative social interactions compared to wild type mice. Interestingly, *Sept5* deficient male mice exposed to individual housing were characterized by reduced anxiety and increased affiliative social interactions compared to mice exposed to group-housing, thereby showing a significant gene-environment interaction. Although more research is needed in this regard, such emerging evidence points to complex interactions between genetic and environmental factors as a source of phenotypic variability among individuals with 22q11DS.

2.2.3 Ascertainment and assessment bias

The IBBC studies[12] that contributed to the overview in **Figure 1** varied considerably in terms of ascertainment. Some sites are child psychiatry clinics, others are non-clinical research settings and still others function primarily as adult specialty clinics. Studies conducted in clinical sites may bias against individuals with 22q11DS who function well, while clinical treatment may also reduce the "true natural" occurrence of psychiatric phenotypes in an untreated population. A recent epidemiological Danish nation-wide study of 22q11DS and 22q11.2 duplication syndrome offers a different perspective, as a population-based study. This study included 244 adult individuals with 22q11DS identified through case registration in a population of 3,768,943. The reported patterns of developmental and psychiatric disorders in 22q11DS, compared to 24,400 age- and gender-matched population controls, were similar to what is described in clinically ascertained 22q11DS cohorts, but, as expected, with somewhat lower prevalence rates [51].

The IBBC studies also varied with regard to the diagnostic assessment tools they employed and comorbid mental health problems screened [12]. For example, not all sites used instruments to assess at risk status for psychotic disorders or standardized assessment for autism. Consequently, some of the differences in prevalence rates reported from different sites may be, at least partly, explained by differences in ascertainment and assessment (biases) across sites.

2.2.4 Age-dependent phenotypes

In individuals with 22q11DS, the variability observed across age groups can be inherent to phenotypic characteristics such as typical age of onset, consistent with observations in the general population. For example, in patient cohorts under the age of 18, one would not expect to find a high prevalence of schizophrenia spectrum disorders, as the first psychotic episode typically emerges in late adolescence/early adulthood [52]. Differences in typically conducted psychiatric assessments across age groups may also contribute to the phenotypic variability reported across different ages. Standard adult psychiatric assessment often does not include screening for developmental disorders such as ASD or ADHD. In cohorts assessed as adults, one would therefore not expect to find a high prevalence of such developmental disorders, while in reality a portion of these individuals might have been diagnosed as such, had their psychiatric assessment – either as children or

adults – included screening for these developmental disorders. With increasing awareness of ASD and ADHD in adults and associated clinical service developments however [53], the reported prevalence of ASD and ADHD in adults with 22q11DS may change in future reports.

Not only can psychiatric conditions emerge as an individual matures, certain psychiatric disorders or neurodevelopmental presentations may also improve over time. Indeed, our clinical impression suggests that in some individuals with 22q11DS with a previous childhood neurodevelopmental diagnosis, improvement occurs to such extent that a diagnostic classification may no longer be justified in adulthood (e.g. ADHD or ASD), which is consistent with what is observed in idiopathic populations with such neurodevelopmental disorders (e.g.[54,55]). The finding that in a sample of 70 individuals with 22q11DS 30% of those previously diagnosed attention deficit disorder (ADD) did not manifest sufficient symptoms justifying an ADD diagnosis at follow-up assessment [56] provides preliminary evidence in this regard. Although such late-maturation trajectories are observed only in a minority of individuals with 22q11DS, they are relevant and warrant further study.

2.2.5 The pitfalls of a categorical approach to the psychiatric phenotype in 22q11DS

From both a clinical and a research perspective, employing a categorical diagnostic classification system has merits, as it provides clinicians and researchers with a shared vocabulary. However, a too stringent adherence to such a categorical, dichotomous approach to psychopathology also has substantial limitations. While this is relevant to all psychiatric populations, several observations in the 22q11DS population render the consideration of such potential limitations particularly salient for these individuals. On the one hand, it may result in the application of several diagnostic labels to account for a relatively small set of symptoms, while on the other hand clinically relevant but isolated symptoms may not readily fit in any diagnostic category and thus be overlooked [57].

From a categorical perspective, a substantial portion of 22q11DS individuals is diagnosed with more than one psychiatric disorder [12,16,58]. In addition, categorical classifications may be influenced by different interpretations of the same symptom domains. For instance, repetitive behaviors may be classified as an obsessive-compulsive disorder by one clinician, while the same symptoms in the same patient may be considered as part of an autistic spectrum disorder by another. The same diagnostic ambiguity may exist with regard to anxiety symptoms, which can justify an anxiety disorder but may not always be considered as such when occurring in the context of a psychotic disorder. Such ambiguities are inevitable as symptoms belonging to different diagnostic categories are frequently observed within the same individual with 22q11DS [57]. Moreover, even in those children not meeting formal criteria for a psychiatric disorder, clinically relevant psychiatric symptoms are often present. Even *within* one symptom domain the use of categorical diagnoses may fall short in describing the reality of psychiatric symptoms with clinical

relevance. For example, much higher prevalence rates of psychotic *symptoms* (25%) than of psychotic *disorders* (10%) are observed in adolescents with 22q11DS [13,59]. Importantly, such symptoms, though not reflected in an individual's psychiatric diagnosis, may still be relevant in understanding an individual's profile of difficulties and competencies, as well as in implementing adequate treatment and preventive strategies.

3. Implications for research

3.1 22q11DS as a genetic model for schizophrenia

Several aspects of 22q11DS make this genetic disorder a highly appealing model to investigate neuroscientific questions, particularly the etiology of schizophrenia[6]. The highly increased risk for this illness in 22q11DS patients, and the opportunity to identify individuals early in life based on their genetic diagnosis has allowed investigators to study clinical and biological correlates of the developmental trajectory of schizophrenia. Moreover, a unique and easily overlooked aspect of these studies is that they are conducted in a genetically relatively homogeneous context, i.e. all individuals share the same 22q11.2 deletion, which can be assumed to be the cause of their high vulnerability for schizophrenia. In contrast, the broad and largely unknown genetic heterogeneity of schizophrenia hampers studies in unselected general population cohorts. Recent studies confirm the usefulness of 22q11DS as a human genetic model to unravel the gene x environment interactions leading to schizophrenia[10]. Importantly, the psychopathological path leading to transition to psychosis in 22q11DS [59,60] is broadly comparable to that observed in other clinical high risk samples[61]. More specifically, the sub-threshold psychotic symptoms and the Clinical High Risk (CHR) for psychosis criteria, previously developed in the general population[61], are reliable and also applicable in the 22q11DS population.

3.2 22q11DS exemplifies the need for dimensional and repeated assessment approaches

Up until now, subclassifying individuals with 22q11DS by psychiatric diagnoses has not proven particularly useful in delineating underlying neurogenetic mechanisms. Indeed, it has been proposed that despite the divergence in diagnostic classifications, observations from different studies in 22q11DS converge into a limited number of symptom domains[57]. Furthermore, preliminary associations have been described between genetic factors and symptoms dimensions that cut across existing diagnostic categories[62-64]. These and other studies underscore the potential added value of broad dimensional, quantitative and repeated assessments as a means towards a more dimensional perspective on mental health equilibrium and the risk of psychopathology[65].

3.3 22q11DS as a model for neuropsychiatric pleiotropy in rare copy number variants

The high variability of neuropsychiatric phenotypes observed in 22q11DS represents another unique research opportunity. The past decade has witnessed the discovery of a growing list of pathogenic genetic variants, including Copy Number Variants (CNVs)[66] and rare Single Nucleotide Variants (SNVs)[67]. Typically, the prevalence of each of these pathogenic variants is rare, but when present in an individual they can confer a substantial risk. The picture emerging from neuropsychiatric studies in individuals carrying these rare high impact variants is remarkably similar to what is observed in 22q11DS: a high degree of neuropsychiatric variability with increased rates of different disorders including schizophrenia, anxiety, ADHD, ASD and affective disorders and varying levels of cognitive impairment[42]. Examples include, but are not limited to, the 3q29 deletion, which is associated with various neuropsychiatric disorders including anxiety and mood disorders and schizophrenia [68], and the 16p11.2 deletion, associated with intellectual disability, ASD, and schizophrenia [69]. Quantifying the risk of the different associated disorders and understanding how they emerge across the lifespan is essential to improve counseling and clinical care for individuals carrying these rare but high impact genetic variants[70]. To this end, studies are needed to investigate for each of these rare pathogenic genetic variants to what extent there is true neuropsychiatric pleiotropy, the age-dependent emergence of certain phenotypes, and the possible influence of classification artifacts and ascertainment bias. In addition, it will be key to identify factors, both genetic and environmental, that modulate the different neuropsychiatric outcomes. Similarly, early phenotypic characteristics should be studied as potential markers for poor functional outcome. However, such studies are severely hampered by the fact that each of these variants occurs at extremely low rates in the population, impeding the collection of sufficiently powered samples.

It is in this context that the 22q11.2 deletion syndrome provides a unique research opportunity. Its prevalence is high (estimated 1/2,000-4,000)[11] and its genetic description in the early 1980s[11] has preceded by approximately two decades the much more recent discovery of the majority of other rare pathogenic genetic variants associated with psychiatric phenotypes[66]. This has afforded 22q11DS studies a significant advance over studies on other pathogenic variants. Indeed, at present, multiple cohorts of several hundreds of individuals with 22q11DS are examined in research institutes across the world. Findings of 22q11DS studies show the potential of using early phenotypic characteristics to identify subgroups with poor functional outcome[71], and indicate several early potential biomarkers of psychotic disorder[63,72-76]. The IBBC [9] has pooled phenotypic data from over 22 sites which together amount to well over 1,800 individuals with 22q11DS. This sample size, unprecedented in any other study on rare genetic variants with neuropsychiatric impact, allows the investigation of aforementioned questions with sufficient statistical power.

4. Implications for clinical care

4.1 Need for regular psychiatric assessments

International guidelines for clinical care for individuals with 22q11DS mandate regular psychiatric and cognitive assessment[77], understandably so, considering the overall high rates of psychopathology in this patient population. The observations reviewed in this article provide several directions in this regard. Psychiatric symptoms and disorders in 22q11DS may either remain constant over time, they may emerge or intensify (e.g. psychotic disorders), or they may be outgrown and no longer be valid as individuals mature. Cognitive abilities may not be stable in a subgroup of patients and a decline in verbal IQ may indicate increased risk of developing a psychotic disorder[20]. In order to maintain accuracy in describing an individual's neuropsychiatric profile (and thereby allowing individualized mental healthcare) this time-related phenotypic variability needs to be considered and, consequently, repeated psychiatric and neuropsychological assessments are required.

Emerging mental, rather than physical, health concerns are more likely to bring adolescents and young adults with 22q11DS to medical attention[11,59,78,79]. Despite increasing awareness of the importance of planned transitions for young people with neurodevelopmental disorders to adult health and social care[80-82], there is limited investigation of how best to do this[83,84] and future research is warranted. However, best practice guidelines for young people with 22q11DS should include a planned transition of mental health care (including psychiatric and cognitive assessments if resources allow) across different life stages and stressors. Examples include when moving from primary to secondary school and from adolescent to adult health care.

With respect to all clinical recommendations for individuals with 22q11DS, including the need for regular psychiatric assessments and the importance of a guided transition to adulthood, there is the necessity to acknowledge, investigate and work towards overcoming the obstacles to implementing such recommendations. While there is consensus regarding the importance of discussing risk for psychiatric disorders in these individuals repeatedly throughout different stages of life [77,85], studies have reported that, for various possible reasons including stigma, other medical issues requiring attention and a young age at time of counseling, at present such discussions are insufficiently implemented in most clinical settings [86,87].

4.2 Need to look beyond diagnostic categories

Full-blown developmental and psychiatric disorders occur and may necessitate pharmacological or cognitive-behavioral therapeutic interventions in individuals with 22q11DS. However, the myriad of clinically relevant symptoms in these individuals is not always sufficiently captured in diagnostic classification categories. When symptoms are under-recognized or occur in isolation (i.e. in the absence of usually co-occurring

symptoms critical for a diagnostic classification), they will not be reflected in an individual's psychiatric diagnosis. Such symptoms may have significant impact on an individual's daily functioning, but will remain unrecognized if only diagnostic categories are taken into account. In psychiatric assessments of individuals with 22q11DS, careful attention should therefore be paid not only to psychiatric diagnoses, but also to symptomatology as this may be important for lifestyle adaptations and professional care and (symptomatic) treatment. Taking symptom domains into consideration is pivotal in understanding an individual's profile of competencies and difficulties[57,88]. Delineating such a profile is highly informative in finding and/or creating an environment that is optimally adapted to the individual.

4.3 Emerging symptoms may represent imbalance abilities and demands; the importance of stress

In all instances where severe psychopathology becomes manifest, adequate psychiatric treatment, often including pharmacological treatment, is required. In some instances where new symptoms emerge in a developing child, such as anxiety, depression, or psychosis, they appear to be related to an emerging discrepancy between the individual's competencies and difficulties, and environmental demands. Clinically relevant psychiatric symptoms of mood, anxiety or psychosis may be indicative of a mismatch between an individual's abilities and environmental demands and symptoms may improve when this balance is recovered. In some instances an accurate understanding of an individual's neuropsychiatric phenotype, taking into consideration subclinical symptoms and fluctuations over time, allows for adequate and timely environmental adaptations, without exposing individuals to psychopharmacological compounds and their related side-effects.

If a mismatch between an individual's capacities and difficulties and their environmental demands exists for a prolonged period of time, the individual is likely to experience chronic stress. Stress has been identified as a risk factor for psychopathology in the general population[89] as well as a trigger to manifestation of a psychotic episode in idiopathic schizophrenia populations[90]. Indeed, high levels of anxiety in youth with 22q11DS have been proposed as a predictor of transition to psychosis[91], which supports the hypothesized importance of stress. In light of the 20-25% risk for developing schizophrenia and related psychotic disorders that individuals with 22q11DS have, optimal care should be taken to avoid stress. Creating and maintaining a balance between their neurocognitive, social and behavioral profile and environmental demands is essential in this regard.

5. Conclusion

The 22q11.2 deletion exemplifies the fast emerging novel class of rare pathogenic genetic variants as identifiable etiologies in the field of psychiatry, which raises important new challenges with immediate relevance for both researchers and clinicians. The early discovery of this rare CNV in the 1980's however, has allowed a time advantage over the majority of the other, more recently identified pathogenic rare variants. Consequently, implications from studies on 22q11DS are not limited to this genetic disorder, but can also contribute to the understanding of phenomena observed in other rare pathogenic variants, including the high variability and variable expression of associated phenotypes.

In addition to the well-established risk for schizophrenia, individuals with 22q11DS are at increased risk for a wide range of psychopathology from early childhood onwards. To some extent, this phenotypic variability may be an artifact of forcing the observed symptoms into our current diagnostic classifications. In addition, variable assessment and ascertainment methods are bound to further contribute to differences between studies. However, notwithstanding these caveats, several observations from 22q11DS studies begin to stand out.

First, the prevalence of some pediatric psychiatric phenotypes, in particular anxiety, ASD, ADHD, and mood disorders, clearly exceeds what is observed in idiopathic ID. Together with the increased risk of schizophrenia in adolescents and (young) adults with 22q11DS, these observations strongly suggest a phenotypic effect that is specific to this genetic variant. Second, some neuropsychiatric phenotypes in 22q11DS are independent of others, indicating true pleiotropy, while others may represent time-dependent expressions of the same disease trajectory. Third, the disparity of reported phenotypes is mostly manifested in the limited framework of categorical diagnostic classifications. When focusing on the observed symptom domains, regardless of the classification used, a stronger coherence between different studies becomes readily apparent.

The clinical implications follow from these observations. In *any* child with 22q11DS a thorough psychiatric evaluation is mandated, regardless of intellectual level. Furthermore, expression of psychiatric phenotypes may vary over time even within the same individual, such as the decline in cognitive functioning observed in a subgroup of 22q11DS individuals. Taken together, these observations underline the importance of *repeated* clinical evaluations in this population. In the context of a genetic predisposition for developing schizophrenia, it is important to maintain an optimal balance between individual abilities and environmental expectations. A global low cognitive level (IQ), but also specific (and sometimes covert) relative weaknesses in cognitive domains and neurodevelopmental functions (e.g. attention, information processing, social and communicative abilities and sensitivity to sensory input) can contribute to chronic stress due to demands that exceed abilities. The clinical importance is twofold. First, such a situation is undesirable in itself as it causes discomfort and stress to any person. Second,

numerous studies indicate that stress may play a role in the course of schizophrenia. While such evidence is not yet robustly available in the 22q11DS population, it is possible that in the context of a high genetic risk, high levels of stress may contribute to the expression of schizophrenia.

The genetic predisposition for psychotic disorders conferred by 22q11DS provides strong impetus to obtain detailed insight into an individual's cognitive and neurodevelopmental profile to avoid or reduce (chronic) situations of stress. Interventions to correct such situations may have direct clinical impact but can also serve as a model to study the feasibility of primary and secondary intervention strategies in a population at risk for schizophrenia. However, the full scope of clinically relevant symptoms may often not be accurately represented in diagnostic classifications. Findings from studies in the 22q11DS population indicate that a dimensional, quantitative symptom assessment of psychopathology may be required in order to obtain the most accurate picture, both for clinical care and for scientific research.

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CHAPTER 3

3

Autism Spectrum and Psychosis Risk in the 22q11.2 Deletion Syndrome. Findings from a Prospective Longitudinal Study

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Background: Individuals with 22q11.2 deletion syndrome (22q11DS) have a 25% risk for schizophrenia and related psychotic disorders. Some have hypothesized that Autism Spectrum Disorders (ASD) diagnosed in children with 22q11DS may actually represent the social-communicative defects often observed during the early developmental stages of schizophrenia.
Methods: We prospectively studied 89 children with 22q11DS to test this hypothesis. At baseline, the Autism Diagnostic Interview was used to assess ASD, evaluating both current and early childhood behaviors. At follow-up, the Schedule for Affective Disorders and Schizophrenia for School-age Children (K-SADS) was used to determine development of a psychotic disorder or psychotic symptoms.
Results: The average age (±SD) at first and last assessments was 14.3±1.9 and 19.0±3.0 years, respectively. Nineteen (21.3%) children developed a psychotic disorder. Contrary to our hypothesis, there was no significant difference in the proportion that developed a psychotic disorder, comparing those with (n=9, 17.3%) and those without ASD at baseline (n=10, 27%; OR = 0.500, 95% CI = 0.160 – 1.569, p = 0.235). Similar results were obtained using autistic symptom severity as quantitative predicting variable, psychotic symptoms as the outcome, and when correcting for age, gender and full scale IQ.
Conclusion: Results indicate that in children with 22q11DS, early childhood autistic features are not associated with an increased risk for subsequent development of psychotic disorders or symptoms, replicating previous retrospective findings in adults with 22q11DS. These results indicate that ASD and psychotic disorders can emerge independently, as pleiotropic phenotypes in the context of 22q11DS.

Keywords: Schizophrenia, comorbidity, 22q11DS, velocardiofacial syndrome, high risk, genetic.

Abbreviations: 22q11DS: 22q11.2 deletion syndrome; ASD: autism spectrum disorder; ADI: autism diagnostic interview; FSIQ: full scale intelligence quotient

Abstract

1. Introduction

Over the past two decades, the 22q11.2 deletion syndrome (22q11DS) has consistently emerged as the strongest single genetic risk factor for schizophrenia and related psychotic disorders [1]. Individuals with this microdeletion have a 25-fold increased risk for developing a psychotic disorder [2-6], and account for 0.5-1% patients with schizophrenia in the general population [1,7]. 22q11DS offers an appealing model to examine the developmental trajectory of schizophrenia [8,9].

In children and adolescents with 22q11DS, a range of neurodevelopmental and psychiatric disorders are reported, including attention deficit hyperactivity disorder (ADHD), anxiety disorders and autism spectrum disorders (ASD) [10]. Regarding the latter, several authors have proposed that the repetitive behaviors and social-communicative deficits observed in children with 22q11DS may be early prodromal symptoms of schizophrenia [1,5,11,12]. However, in a retrospective study in adults with 22q11DS, no significant association between childhood ASD and later onset of schizophrenia was found [13].

In the current study, we used a prospective longitudinal study design to investigate the hypothesis that among children with 22q11DS, those with a diagnosis and/or symptoms of ASD are more likely to subsequently develop a psychotic disorder than those without ASD.

2. Methods

2.1. Participants and procedures

The participants were children with 22q11DS, confirmed by either fluorescence in situ hybridization (FISH) or multiplex ligase-dependent probe amplification (MLPA[14]) using standard probes, who were referred to our specialized psychiatric 22q11DS clinic as part of standard clinical care[15]. The study protocol is part of a larger ongoing longitudinal behavioral and genetic study on 22q11DS patients that has been approved by the local research ethics board (Dutch Central Committee on Research Involving Human Subjects; C.C.M.O). Written informed consent was obtained from participants and their parents or guardians. Our clinical follow-up program implies that approximately 3 to 4 years after the baseline measurement (T0) the child is invited for a follow-up visit, regardless of the presence or absence of any behavioral concerns. In the interim, parents can contact our center at any time in case of emerging concerns.

2.2. Psychiatric and cognitive assessments

All psychiatric and cognitive assessments were performed by the same multidisciplinary team. The baseline measurement included a semi-structured assessment of DSM-IV items,

the Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime Version (K-SADS-PL [16]) mood disorder and psychosis sections, and the Autism Diagnostic Interview – Revised (ADI-R [17]) scored by certified interviewers. Demographic variables and psychotropic medication use were recorded. All instruments except the ADI-R were re-administered at follow-up.

ASD, including Autistic Disorder (n = 5, 5.6%) and Pervasive Developmental Disorder Not Otherwise Specified (n = 47, 52.8%) and psychotic disorders, including Schizophrenia (n = 4, 4.5%), Brief Psychotic Disorder (n = 2, 2.2%) and Psychotic Disorder Not Otherwise Specified (n = 13, 14.6%) were defined according to DSM-IV criteria and ascertained based on direct patient observation, interview of patient and caregivers, and collateral information from school or residence. ASD symptom severity was quantified by the raw ADI-R score, using the sum of the scores obtained on the 37 algorithm items[18]. The ADI-R, an instrument with established reliability in a population with mild to moderate intellectual disability [19], yields information on early childhood behaviors (age 4 – 5 years) as well as on current behaviors. Psychotic symptoms were recorded as present if the child obtained a score of 2 or higher on one or more items of the positive psychotic symptoms subscale of the K-SADS. To assess intellectual level (IQ), we used the Dutch versions of the entire Wechsler scales[20]: WISC-III (n=73), WISC-R (n=3), or WAIS-III (n=5).

2.3. Statistical analyses

All statistical analyses were conducted with SPSS version 22 statistical analysis software (APSS, Chicago, IL). Power analysis[21] indicated that our sample size (n = 89) provides a power of 80% to detect a moderate effect size (OR > 2 (2.1 or higher)) with respect to the association between ASD and the development of a psychotic disorder in 22q11DS (α= 0.05, two-sided, expected rate of psychotic disorder 25%). The ASD group and the non-ASD group were compared on the possible confounding variables age, gender, interval (time between first and last measurement), full scale IQ (FSIQ) and medication use (any psychopharmacological medication, or any antipsychotic), by means of chi-square and one-way ANOVA analyses.

To test the primary hypothesis that ASD at baseline is associated with a psychotic disorder at follow-up, we initially conducted a chi-square analysis. To allow for the consideration of possible confounders we used a binary logistic regression model (model 1), with predictor variables a diagnosis of ASD, age, gender and FSIQ and with outcome variables either psychotic disorder at follow-up (model 1) or persistent psychotic symptoms at follow-up, regardless of whether formal diagnostic criteria for a psychotic disorder were met (model 2).

To investigate the association between ASD symptom severity (dimensional) at baseline and the presence of a psychotic disorder at follow-up, another binary logistic regression model was used, using the raw ADI-R scores as a predictor variable, and adding

possible confounders to the model (model 3). The same analysis was conducted to investigate the association between ASD symptom severity at baseline and the presence of psychotic symptoms at follow-up (model 4).

Post-hoc, we compared the use of any psychopharmacological medication or antipsychotic medication (during the interval time between first and last measurement) between the ASD and non-ASD groups, and added this to the regression models, to test if medication use affected the predictive effect of ASD symptoms/diagnosis on psychotic symptoms/diagnosis.

3. Results

At baseline, there were 52 (58.4%) participants in the ASD and 37 (41.6%) in the non-ASD group. There were no significant differences between the ASD and non-ASD groups on any of the variables examined (Supplement Table 1). The average interval (±SD) between first and last measurement was 56.6±29.6 months.

The results revealed no significant predictive effect of a diagnosis of ASD on the subsequent development of a psychotic disorder (p = 0.270), see **Table 1**. The findings remained similar when age, gender and FSIQ were added to the regression model (OR = 0.500, 95% CI = 0.160 – 1.569, p = 0.235). In fact, in the ASD group the proportion of individuals who developed a psychotic disorder (17.3%, n=9) was lower than in the non-ASD group (27%, n=10), albeit not statistically significantly. Accordingly, there was no significant difference in the proportions of patients who developed psychotic symptoms, regardless of a diagnosis of a psychotic disorder, between the ASD-group and the non-ASD group (model 2; OR = 0.977, 95% CI = 0.362 – 2.634, p = 0.963).

Table 1: Distribution of patients with and without ASD at baseline who developed a psychotic disorder at follow-up (model 1)

Total N = 89 (100%)	**Psychotic disorder** n = 19 (21.3%)	**No psychotic disorder** n = 70 (78.7%)	**Analysis**			
	n (%)	n (%)	**OR**	**95% CI**	***P*[a]**	***P*[b]**
ASD n = 52 (58.4%)	9 (17.3%)	43 (82.7%)	.500	.160 – 1.569	.270	.235
Non-ASD n = 37 (41.6%)	10 (27%)	27 (73%)				

Abbreviations: ASD: Autism Spectrum Disorder; FU: Follow-up, OR: Odds Ratio, CI: Confidence Interval.
[a] Chi-square.
[b] Binary logistic regression. Corrected for age, gender, FSIQ.

Next, we investigated the association between the severity of autistic symptoms - regardless of whether or not a formal ASD diagnosis was present- and the subsequent development of psychotic disorders. There was no significant difference in the total raw score on the ADI-R between the group that did not develop a psychotic disorder at follow-up (mean = 24.2; SD = 12.9) and the group that did develop a psychotic disorder (mean=21.0; SD=16.0; model 3; OR = 0.968, 95% CI = 0.922 – 1.016, p = 0.188; see **Supplement Figure 1**). Thus, consistent with results from the categorical analyses (models 1 & 2), there was no support for a predictive effect of symptom severity in the domains of social interaction, communication or repetitive behaviors on the development of subsequent psychotic disorders. Comparing ASD symptom severity at baseline between those with psychotic symptoms at follow-up (mean=22.8, SD=15.6) and those without (mean=23.9, SD=12.7) provided similar results (model 4; OR = 0.996, 95% CI = 0.995 – 1.038, p = 0.835).

A between-group comparison of the use of psychopharmacological medication, and just antipsychotics, during the interval time between first and last measurement between both groups revealed no significant differences (ASD vs. non-ASD; 30.8% and 16.2%, p = 0.117; 19.2% and 13.5%, p = 0.478 respectively) and post-hoc addition did not alter the results for any of the four regression models.

Thirteen subjects were already diagnosed with a psychotic disorder at T0 (see Supplement Figure 2). Therefore, we reran the analyses post-hoc, defining psychotic disorder (or symptoms) at *any* time point, i.e. including T0, as the main outcome. The results of these analyses (both categorical and dimensional) were similar to those of the main analyses.

4. Discussion

Our results reveal no association between ASD in early childhood and the subsequent development of psychosis in individuals with 22q11DS. Both phenotypes were analyzed at the level of symptoms and at the level of diagnosis, generating similar results. The findings of this prospective study replicate those of a previous retrospective study investigating this issue in an independent cohort [13]. They indicate that ASD and psychotic disorders should be considered as relatively independent neuropsychiatric consequences of 22q11DS and that symptoms characteristic of ASD are unlikely to represent a prodromal stage of schizophrenia.

These results reflect both the incomplete penetrance and pleiotropy of the neuropsychiatric phenotype in 22q11DS patients; not all patients develop a psychotic disorder (indicative of incomplete penetrance), and other neuropsychiatric phenotypes (i.c. ASD) can occur independently, in patients with the 22q11.2 deletion (indicative of pleiotropy). This is consistent with the high degree of phenotypic heterogeneity observed in other

pathogenic copy number variations [22,23], indicating that 22q11DS may be a useful genetic model through which the associations between different neuropsychiatric phenotypes in the context of the same CNV can be better understood [24]. Possibly, (non-) genetic risk factors in addition to the high-impact CNV modulate which neurobiological pathways are affected and therefore which psychiatric phenotype is manifested[23,25,26]. In such a model, a high-impact CNV (such as a 22q11.2 deletion) could act as a first hit that renders certain neurobiological pathways vulnerable to the effect of additional risk factors [27 28 22,26].

4.1 Advantages and limitations

The available sample size (n = 89) provided 80% power to detect a moderate effect size (OR >2) regarding the association between ASD and subsequent psychotic disorder in 22q11DS. The fact that the results replicate those of a comparable, retrospective study using an independent cohort [13], provides further confidence in the conclusion that there is no clinically relevant association between ASD and subsequent psychotic disorders in 22q11DS.

The main analysis was conducted in a prospective way; i.e. to assess to what extent ASD, determined at T0, is associated with the subsequent development of psychosis. Given that we were primarily interested in the predictive effect of early autistic symptomatology on psychotic disorders at any point in life, we performed post-hoc analyses with psychotic disorder at *any* time point as the outcome variable, and these showed similar results.

The relatively young average age of our sample at the time of the last measurement (19.0 years) can be considered a limitation of this study as individuals in the non-psychotic group may still develop schizophrenia or related psychotic disorders. However, given the average age of onset of the first psychotic episode in 22q11DS (estimated around 18 years for samples ascertained as children [29,30]), this effect is expected to be modest. In addition, broadening our outcome measure to psychotic symptoms instead of a formal psychotic disorder generated similar results.

The relatively large proportion of children diagnosed with ASD in our cohort, the majority of whom were diagnosed with PDD-NOS, might imply a potential clinical bias to these diagnoses in our sample. We therefore added a dimensional measure of autism-like symptom severity, regardless of a formal ASD diagnosis, to the study design (i.e. the raw ADI-R score). Findings from this dimensional analysis did not differ from the results using the categorical approach (i.e. ASD diagnoses). This indicates that early impairments in social and communicative functioning and repetitive or stereotyped behaviors, regardless of whether formal ASD diagnostic criteria were met, are not associated with later psychotic disorders in 22q11DS.

In conclusion, the results of this prospective study, together with similar findings in an independent cohort [13], indicate that in 22q11DS, autistic symptoms and/or a diagnosis of ASD are not predictive of developing a psychotic disorder or persistent psychotic symptoms later in life.

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Supplemental materials

Supplement Table 1: Demographic and clinical characteristics of 89 children with 22q11DS

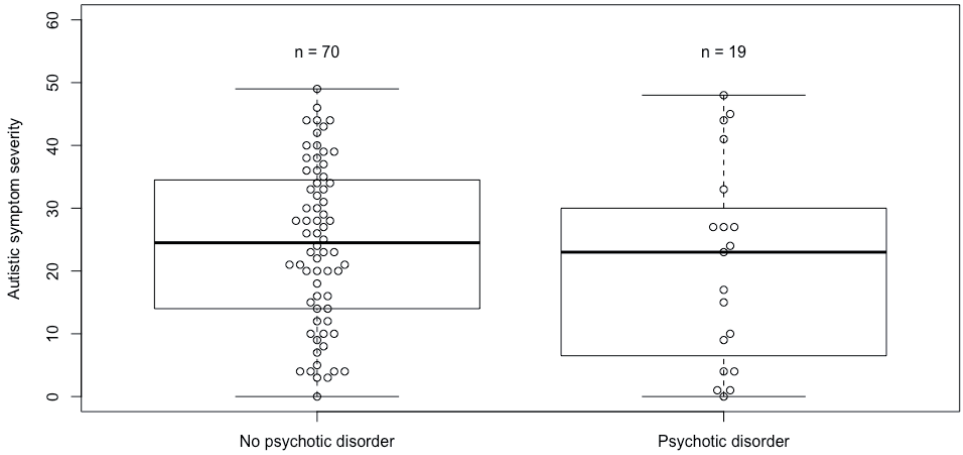
	Total		**ASD**		**Non-ASD**		**Analysis**		
	n (%)	*mean±SD (range)*	*n (%)*	*mean±SD (range)*	*n (%)*	*mean±SD (range)*	*statistic*	*df*	*p*
Age (years) at T0	89 (100%)	14.3±1.9 (11.3 - 18.9)	52 (58.4%)	14.1±1.8 (11.3 - 17.8)	37 (41.6%)	14.5±2.0 (11.3 - 18.9)	F=.808	1, 87	.371
Age (years) at TL	89 (100%)	19.0±3.0 (14.1 – 27.9)	52 (58.4%)	18.7±3.0 (14.1 - 24.8)	37 (41.6%)	19.4±3.0 (14.9 – 27.9)	F=1.290	1, 87	.259
Gender (males)	36 (40.4%)		24 (46.2%)		12 (32.4%)		c^2=1.690	1	.194
Follow-up-interval (months)	89 (100%)	56.6±29.6 (16.7 – 132.1)	52 (58.4%)	54.7±30.6 (16.6 - 132.1)	37 (41.6%)	59.2±28.3 (25.9 – 121.3)	F=.489	1, 87	.486
FSIQ at T0	80 (89.9%)	64.1±12.1 (45 – 92)	45 (50.6%)	63.0±11.7 (45 – 91)	35 (39.3%)	65.5±12.7 (48 – 92)	F=.841	1, 78	.362
Age at onset of psychotic disorder	19 (21.3%)	16.7±2.8 (12.4 – 23.2)	9 (17.3%)	15.5±1.9 (12.5 - 17.9)	10 (27.0%)	17.8±3.0 (12.4 – 23.3)	F=3.909	1, 17	.064
Use any psychotropic medication[1]	22 (24.7%)		16 (30.8%)		6 (16.2%)		c^2=2.460	1	.117
Use of antipsychotic medication[2]	15 (16.9%)		10 (19.2%)		5 (13.5%)		c^2=.504	1	.478

Abbreviations: ASD: Autism Spectrum Disorder; T0: first measurement; TL: last measurement; FSIQ: Full Scale Intelligence Quotient

[1] Including: methylphenidate (n=2) , carbamazepine (n=1), fluoxetine(n=1), topiramate (n=1), sertraline (n=1), paroxetine (n=1), venlafaxine (n=1), biperiden (n=1), temazepam (n=1), citalopram (n=1), and the antipsychotics listed below.

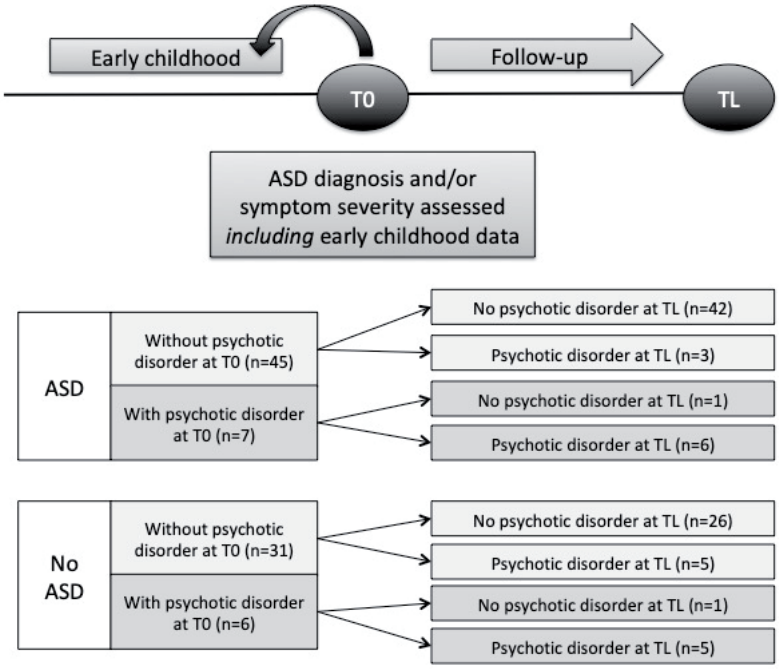
[2] Including: risperidone(n=7), pipamperone (n=3), aripiprazole (n=1), and flupentixol (n=1).

Supplement Figure 1: Autistic symptom severity in the psychotic versus non-psychotic group



This figure represents the distribution of autistic symptom severity at baseline in those who developed a psychotic disorder during follow up and those who did not. Each point represents an individual's raw ADI-R score at baseline, which is indicative of the severity of autistic symptomatology. The boxplot specifies the medians, the first and third quartiles, and the minima and maxima of all data points.

Supplement Figure 2. Study overview



Abbreviations: ASD: Autism Spectrum Disorder; T0: First measurement, TL: Last measurement

Supplement Table 2. Demographics for patients with psychosis

Case	Age at last assess-ment	Gender	FSIQ (at T0)	Psychotic disorder	Age at onset psychotic disorder	Autism spectrum diagnosis	Comorbid psychiatric diagnosis
1	20.6	Female	50	Psychotic disorder NOS	15.0	No	No
2	23.1	Male	48	Psychotic disorder NOS	20.0	No	ADHD, inattentive type; ODD; combined language disorder
3	20.2	Male	91	Schizophrenia, paranoid type	20.1	No	No
4	23.2	Female	68	Psychotic disorder NOS	17.0	No	Dysthymic disorder
5	26.0	Male	59	Schizophrenia, paranoid type	18.9	No	No
6	16.6	Female	64	Brief psychotic disorder	15.9	No	Depressive disorder
7	23.1	Female	63	Psychotic disorder NOS	23.3	No	No
8	16.0	Female	60	Psychotic disorder NOS	18.0	No	No
9	18.1	Female	69	Psychotic disorder NOS	17.5	No	No
10	14.9	Female	64	Psychotic disorder NOS	12.4	No	No
11	23.8	Female	59	Schizophrenia, paranoid type	14.2	ASD	No
12	24.8	Female	51	Brief psychotic disorder	17.4	ASD	No
13	20.0	Female	61	Psychotic disorder NOS	15.6	ASD	No
14	18.3	Female	59	Psychotic disorder NOS	17.9	ASD	No
15	16.0	Female	-	Psychotic disorder NOS	12.5	ASD	No
16	19.8	Male	61	Psychotic disorder NOS	17.5	ASD	ADHD, combined type
17	19.3	Male	47	Psychotic disorder NOS	16.3	ASD	ADHD, combined type
18	18.4	Male	-	Schizophrenia, paranoid type	14.3	ASD	No
19	17.9	Female	71	Psychotic disorder NOS	13.5	ASD	No

Abbreviations: ASD: Autism Spectrum Disorder; FSIQ: Full scale IQ, T0: First measurement.

CHAPTER 4

Neurocognition and Adaptive Functioning in a Genetic High Risk Model of Schizophrenia

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Background. Identifying factors that influence functional outcome is an important goal in schizophrenia research. The 22q11.2 deletion syndrome (22q11DS) is a unique genetic model with high risk (20-25%) for schizophrenia. This study aimed to identify potentially targetable domains of neurocognitive functioning associated with functional outcome in adults with 22q11DS.

Methods. We used comprehensive neurocognitive test data available for 99 adults with 22q11DS (n=43 with schizophrenia) and principal component analysis to derive four domains of neurocognition (Verbal Memory, Visual and Logical Memory, Motor Performance, and Executive Performance). We then investigated the association of these neurocognitive domains with adaptive functioning using Vineland Adaptive Behavior Scales (VABS) data and a linear regression model that accounted for the effects of schizophrenia status and overall intellectual level.

Results. The regression model explained 46.8% of the variance in functional outcome ($p < 0.0001$). Executive Performance was significantly associated with functional outcome ($p = 0.048$). Age and schizophrenia were also significant factors. The effects of Executive Performance on functioning did not significantly differ between those with and without psychotic illness.

Conclusion. The findings provide impetus for further studies to examine the potential of directed (early) interventions targeting Executive Performance to improve long-term adaptive functional outcome in individuals with, or at high-risk for, schizophrenia. Moreover, the neurocognitive test profiles may benefit caregivers and clinicians by providing insight into the relative strengths and weaknesses of individuals with 22q11DS, with and without psychotic illness.

Keywords: adaptive functioning, neurocognition, schizophrenia, 22q11DS, high-risk.

Abstract

Introduction

Identifying domains of neurocognition (e.g., executive functioning, motor performance, and visual and non-visual memory) that influence functional outcome is an important goal in schizophrenia research[1-7]. Neurocognitive domains represent more specific abilities than global IQ [8], and have the advantage of being largely independent of other symptoms, present before the onset of illness, and relatively stable over time [7,9,10]. These domains are therefore considered important components of risk for schizophrenia and potential targets for early interventions that could prevent or reduce poor functional outcomes [11,12]. This is the case not only for individuals with full expression of schizophrenia but also for individuals deemed to be at high risk for schizophrenia because of observable symptoms ("clinical high risk") or positive family history [12-14]. As of yet however, little is known in this regard about individuals at genetic high risk for schizophrenia because of a 22q11.2 microdeletion.

The 22q11.2 microdeletion is considered both the strongest and most prevalent single genetic risk factor for developing schizophrenia [15,16]; individuals with this deletion have an approximately 25-fold increased risk for developing schizophrenia over that of the general population [15-17]. Potentially identifiable from the prenatal period to adulthood, the associated 22q11.2 deletion syndrome (22q11DS) is a valuable genetic model to study schizophrenia and high risk for schizophrenia [12,15,18,19].

The current study aimed to identify domains of neurocognitive functioning that are associated with functional outcome in individuals with 22q11DS, while accounting for the effects of full scale IQ (FSIQ) and the presence or absence of a psychotic disorder [20]. We hypothesized that at least one domain of neurocognitive functioning would contribute to functional outcome in a regression model.

Methods

Subjects and phenotypic assessments

A total of 99 adults (age ≥ 17 years; 43 males) with 22q11.2DS were included in this study. Inclusion criteria were: (1) molecularly confirmed 22q11.2 deletion using standard methods [21-23], (2) completion of a comprehensive battery of neurocognitive tests [24] (described below), and (3) absence of moderate or more severe intellectual disability (i.e. FSIQ < 54) and/or clinical history of, Parkinson's disease (PD) or other major neurological disorder (e.g. stroke, head injury associated with persistent neurological loss of function). The majority of participants were ascertained through genetic, psychiatric or adult congenital cardiac services by active screening and/or clinical referrals [20,21,24,25]. Written informed consent was obtained for all study participants and/or their guardians and the study was approved by the local research ethics board.

Psychiatric diagnoses were determined by a psychiatrist (A.S.B., E.W.C.C) according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV), based on the results from the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (SCID-IV), in addition to all information obtained from direct observation, collateral history from family members, and available information from lifetime medical records, as previously described [20,22,25,26]. There were 43 individuals (43.4%) who had a lifetime diagnosis of a psychotic disorder (schizophrenia, n=40; schizoaffective disorder, n=3; average age at onset 21.2 (SD=5.2) years) and 56 (56.6%) with no history of psychotic illness. Detailed demographic and clinical characteristics of the study participants are presented in Supplementary Materials 1.

Neurocognitive assessments

Overall cognitive functioning, operationalized by FSIQ, was assessed using the Wechsler Adult Intelligence Scale-Revised [27] (WAIS-R, n = 41 (41.4%) or Wechsler Adult Intelligence Scale III [28] (WAIS-III, n = 58 (58.6%). In addition, a comprehensive battery of 15 neurocognitive tests involving motor, learning and memory, language, visual spatial and executive skills (see Supplementary Materials 2) was administered [24]. All subjects were assessed during a stable or remitted phase of their psychiatric illness. Neurocognitive test scores were converted to Z-scores using standardized norms. All psychometric tests were administered by a trained psychometrist [24]. The average age at neurocognitive assessment was 26.6 years (SD = 8.6).

Assessment of functional outcome

To assess functional outcomes, a direct caregiver, spouse or other close relative was interviewed using the Vineland Adaptive Behavior Scales (VABS): Interview Edition, Expanded Form [29]. 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 global adaptive functioning of the individual, is calculated from these three domains. The VABS provides standard scores (mean = 100, SD = 15), with higher scores indicating better functioning and scores below 78 indicating functional difficulty [30]. Due to incomplete or missing VABS assessments, analyses regarding functional outcome included a total of 84 individuals at average age 27.5 (SD = 8.3) years; on average 1.7 (SD = 1.9) years after the neurocognitive assessment. We have reported previously on VABS data for 76 of these 84 individuals [20].

Statistical analyses

All statistical analyses were performed using SPSS 22 (IBM SPSS Statistics). We obtained sample characteristics and data regarding neurocognitive functioning and functional

outcome using descriptive statistics, using t-tests, chi-squares, or ANOVAs including Bonferroni correction, as appropriate.

To derive domains of neurocognitive functioning from the 15 tests we conducted a data-driven principal component analysis (PCA). An oblique *promax* rotation with Kaiser normalization was performed on each solution to facilitate the interpretation of results. Components meeting the Kaiser criterion (eigenvalues >1) were considered significant. We identified four significant components that, based upon the distribution of loadings of the individual neurocognitive tests, were named "Visual and Logical Memory", "Verbal Memory", "Motor Performance", and "Executive Performance" (see the Results section).

To investigate the association between the PCA derived domains of neurocognitive functioning and functional outcome we conducted a regression analysis. As we were interested in examining the added explanatory power of these domains while accounting for the effects of psychotic illness and FSIQ, these variables were included in the regression model, together with age at VABS assessment and sex. The primary outcome variable was the total VABS ABC score. We also investigated the same model using the three subdomains of the VABS as outcome variables.

Post-hoc, we investigated whether the effects of neurocognitive functioning on adaptive functioning were different for those with and without a psychotic disorder. To this end, we added an interaction variable (neurocognitive functioning on relevant domain * psychotic disorder) to a univariate analysis of variance that also included the relevant neurocognitive domain, psychotic disorder, and FSIQ, age at VABS assessment and sex as covariates.

The number of neuropsychological tests is fairly large given the number of participants [31-35], thus risking the possibility that the PCA results could be artefactual if any of the components thus derived were as they related to adaptive functioning. Therefore, in post-hoc analyses we forced the model to derive a single principal component of all 15 neuropsychological tests, then performed a linear regression analysis using the same factors as above but with this single component in place of the original four PCA-derived components. This allowed us to assess whether this unifactorial model could better explain variance in functional outcome than the original four-factor model.

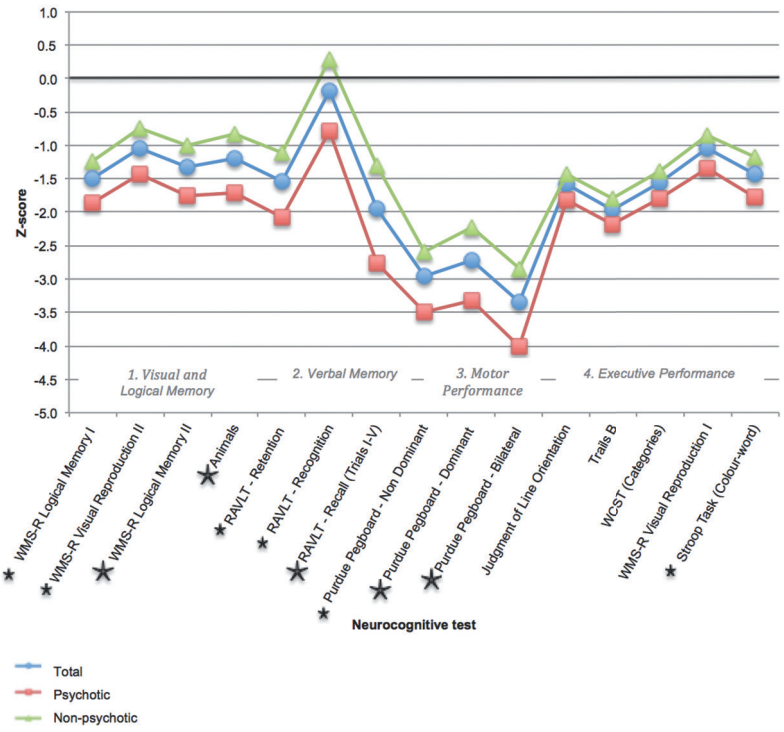
As a final post-hoc step, we conducted a *stepwise* linear regression analysis including the four PCA-derived factors, psychotic illness expression, FSIQ, age and sex to assess whether this alternative statistical approach would support the results of our main regression model.

Results

Neurocognitive performance

The mean FSIQ was 71.8 (SD = 8.6) for the 99 individuals with 22q11DS studied. As expected,[15-17] on average the FSIQ was lower in the psychotic group (mean = 68.7, SD = 6.5) than in the non-psychotic group (mean = 74.2, SD = 9.3; p = 0.001). Although mean z-scores were below zero on all 15 neurocognitive tests, indicating lower than average performance (compared to population-based norms) in individuals with 22q11DS, there was substantial variability amongst the tests and between those with and without a psychotic illness (Supplementary Materials 3, **Figure 1**). Overall, participants obtained highest mean z-scores on the Rey Auditory Verbal Learning Test (RAVLT)–Recognition test (Z = -0.19, SD = 1.37) with those in the non-psychotic group showing mean performance slightly above norms. Performance on average was otherwise relatively better on tasks related to visual than verbal memory (**Supplementary Materials 3**). As expected [24,36-38], performance was worst on motor tasks (**Supplementary Materials 3**).

Figure 1: Mean Z-scores on 15 neurocognitive tests for 99 adults with 22q11DS, ordered by the magnitude of difference between those with (n = 43) and (n = 56) without psychotic illness within each of four neurocognitive domains.



★ : Difference between those with and without psychotic illness significant at the <0.01 level
★ : Difference between those with and without psychotic illness significant at the <0.0001 level

Principal component analysis of neurocognitive tests

The principal component analysis indicated four components with eigenvalues > 1 that together explained 65.4% of the variance of the 15 neurocognitive tests. **Table 1** shows the factor loadings and corresponding coefficients after rotation and Kaiser normalization. Investigating which neurocognitive tests loaded most strongly on each individual component allowed us to recognize common themes representing four domains of neurocognitive functioning. We named the components accordingly: Visual and Logical Memory, Verbal Memory, Motor Performance, and Executive Performance. **Figure 1** shows the relative mean performance profile overall and for subjects with schizophrenia and with no psychotic illness for the component tests within these cognitive domains. Differences were greatest ($p = 0.0004$ to < 0.0001) between these clinical subgroups of 22q11DS on individual motor (Purdue Pegboard) tests, RAVLT-Recognition and Recall, WMS-R Logical Memory II, and Animals (**Supplementary Materials 3; Figure 1**)[12].

Table 1. Rotated factor solutions for the principal component analysis using 15 neurocognitive tests for 99 individuals with 22q11DS

	Component 1 Visual Memory		Component 2 Verbal Memory		Component 3 Motor Performance		Component 4 Executive Performance	
Eigenvalue[1]	5.886		1.512		1.282		1.125	
% variance explained[1]	39.2		10.1		8.5		7.5	
	Loadings	**Coefficients**	**Loadings**	**Coefficients**	**Loadings**	**Coefficients**	**Loadings**	**Coefficients**
WMS – LM II	.898	.339	.458	.020	.346	-.028	.356	-.032
WMS – LM I	.875	.350	.386	-.022	.327	-.019	.310	-.045
WMS –VR II	.680	.160	.612	.133	.391	-.008	.496	.072
Animals	.566	.177	.336	.029	.499	.166	.136	-.150
RAVLT – Retention	.398	-.024	.893	.378	.272	-.044	.392	.026
RAVLT – Recall	.538	.030	.879	.319	.370	-.026	.509	.069
RAVLT - Recognition	.354	-.028	.812	.351	.375	.063	.228	-.094
Purdue bilateral	.324	-.037	.269	-.029	.869	.378	.422	.028
Purdue non- dominant	.397	-.008	.333	-.007	.866	.358	.433	.015
Purdue dominant	.453	.014	.408	.031	.864	.348	.402	-.028
Stroop color-word	.429	.034	.401	.032	.334	-.032	.725	.323
WMS – VR I	.630	.142	.391	-.017	.428	.004	.692	.248
Trails B	.221	-.050	.238	-.016	.368	.049	.647	.317
JLO	.562	.195	.070	-.170	.270	-.039	.608	.279
WCST	.114	-.093	.270	.041	.228	-.016	.604	.329

[1]Principal component analysis with Promax rotation using Kaiser normalization. Rotated components with eigenvalues >1 were considered significant. Together these four components accounted for 65.4% of the variance of the total 15 neurocognitive tests.

Adaptive functioning

As expected [20], the majority of participants scored in the functional difficulty range on overall functioning (n = 66; 78.6%), with mean VABS scores of 63.0 (SD = 19.6) for the composite (ABC) score, representing overall adaptive functioning (where population norm = 100), and 78.0 (SD = 23.5), 64.8 (SD = 18.2), and 58.6 (SD = 22.8) for the Daily Living Skills, Socialization, and Communication subdomains, respectively. The psychotic group had significantly lower mean scores than the non-psychotic group on global adaptive functioning and the three subdomains of the VABS (see **Supplementary Materials 4**).

Regression analyses

Consistent with our hypothesis, the regression model was significant, explaining 46.8% of the variance in global functional outcome ($p < 0.0001$), and the neurocognitive domain Executive Performance was significantly associated with overall functional outcome ($B = 4.849$, $t = 2.014$, $p = 0.048$), while accounting for other factors (**Table 2**). Psychotic illness status and age were also significant factors in the model (**Table 2**). The other three neurocognitive domains, FSIQ and sex were non-significant factors. VABS adaptive functioning scale scores were higher in those with better performance on Executive domain tests, no psychotic illness and older age. **Figure 2** offers a visual representation of the main results related to the neurocognitive domains.

Table 2. Relationship between neurocognitive domains and other factors to global adaptive functioning (Vineland Adaptive Behavior Scales, VABS) in 84 adults with 22q11DS.

Model: R^2: 0.468, F = 8.250, p-value = 0.000					
Variable	**Coefficient**	**SE (coefficient)**	**Standardized beta**	**t-ratio**	**p-value**
Visual and Logical Memory (1)	-0.502	2.362	-0.025	-0.213	0.832
Verbal Memory (2)	2.773	2.096	0.145	1.323	0.190
Motor Performance (3)	0.325	2.018	0.017	0.161	0.872
Executive Performance (4)	**4.849**	**2.407**	**0.249**	**2.014**	**0.048***
Schizophrenia expression	**-11.740**	**4.185**	**-0.299**	**-2.805**	**0.006***
FSIQ	0.386	0.308	0.166	1.254	0.214
Age (at VABS assessment)	**0.703**	**0.215**	**0.296**	**3.268**	**0.002***
Sex	1.796	3.455	0.046	0.520	0.605

Linear regression model, dependent = VABS

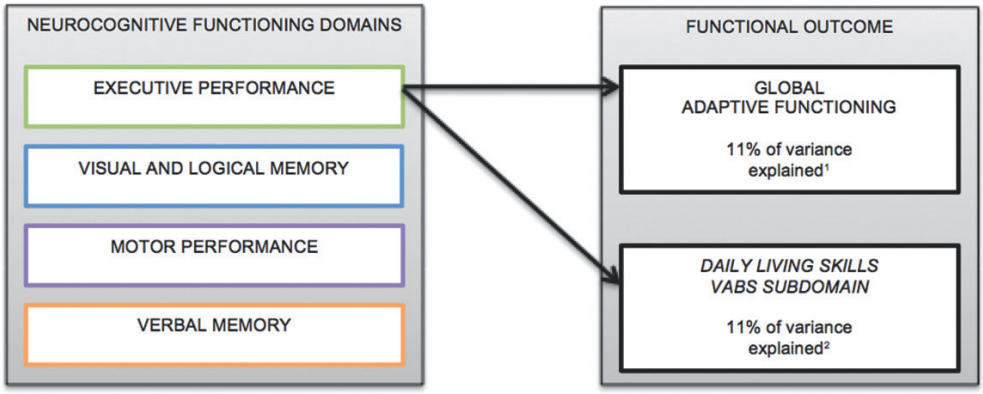
S.E. = standard error

* Indicates significance at the <0.05 level (factors shown in bold font). Better functioning is associated with better Executive Performance, absence of schizophrenia, and older age in this 22q11DS sample.

The regression model for the VABS daily living skills subdomain was similarly significant (explained 47.7% of the variance; $p < 0.0001$), with Executive Performance ($B = 6.473$, $t = 2.264$, $p = 0.026$) and age ($B = 0.918$, $t = 3.593$, $p = 0.001$) the only significant factors.

Although the regression models for VABS Socialization and VABS Communication scores were also significant ($p < 0.0001$, explaining 38.3% and 37.7% of the variance, respectively), only age remained a significant factor in these models.

Figure 2. Visual representation of main findings related to neurocognitive domains.

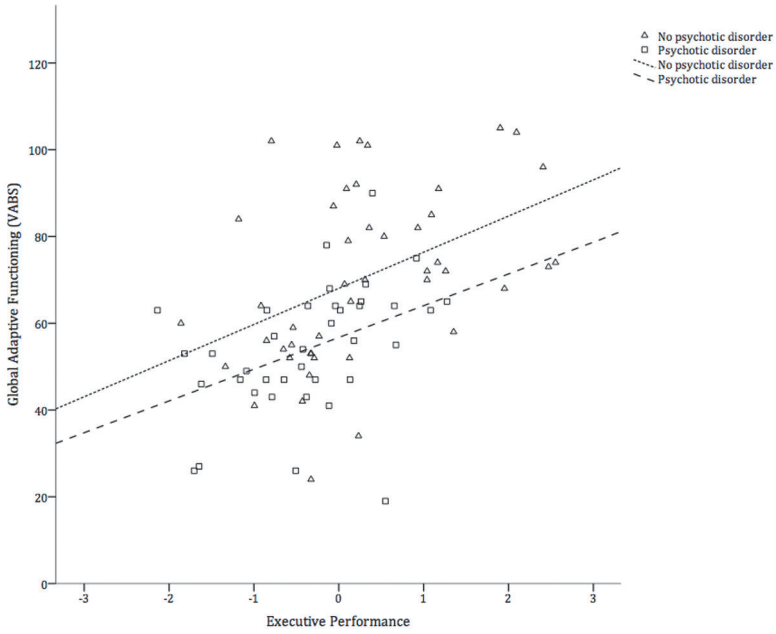


[1]The full regression model (see regression results and Table 2) explains 46.8% of the variance in global adaptive functioning. In addition to the 11% explained by Executive Performance, 16% is explained by age (at Vineland assessment), 10% by psychotic illness status, 5% by FSIQ ($p>0.05$), and 4% by the domain Motor Performance ($p>0.05$).
[2]The full regression model (see regression results) explains 47.7% of the variance in the daily living skill VABS subdomain. In addition to the 11% explained by Executive Performance, 15% is explained by age (at Vineland assessment), 6% by psychotic illness status, 3% by FSIQ ($p>0.05$), and 11% by the three remaining neurocognitive domains combined ($p>0.05$).

Post-hoc analyses

Post-hoc analyses showed that, for the main outcome VABS overall functioning scores, the interaction term Executive Performance*psychotic disorder had no significant effect ($F = .151$ (1), $p = 0.699$), indicating that the effect of the neurocognitive domain Executive Performance on global functional outcome was similar for those with and without a psychotic disorder (see **Figure 3**). Comparable interaction analysis showed similar results for the VABS daily living skills scores ($F = .541$ (1), $p = 0.464$).

Figure 3. Correlation of Executive Performance and Global Adaptive Functioning (VABS ABC score) by psychotic disorder expression.



Post-hoc, after forcing the PCA to create a single factor from the neurocognitive tests, we found that this single component accounted for 39.3% of the variance in the 15 individual tests, in contrast to 65.4% for the original four-component model (**Table 1**). The regression model using this single neurocognitive factor explained 44.4% of the variance in adaptive functioning, just less than the 46.8% of the variance explained in the model using the original four neurocognitive components. However, in contrast to the significant effect found for Executive Performance, the single neurocognitive factor was not significantly associated with adaptive functioning. These results therefore indicate that the Executive Performance component was not artefactually selected as related to functioning. The four-component solution appears somewhat better suited than a global deficit model to account for the variability in the individual neurocognitive tests, and the variability in adaptive functioning.

Finally, the post hoc stepwise linear regression model confirmed the added predictive value of the domain Executive Performance on adaptive functioning. A regression model including psychotic illness status, FSIQ, age at VABS assessment, and Executive Performance provided the best fit ($F = 16.140$ (79), $p < 0.000$).

Discussion

In this study we investigated the relationship between domains of neurocognitive functioning and functional outcome in individuals with high risk of schizophrenia conveyed by a 22q11.2 deletion. We found that higher performance on the neurocognitive domain Executive Performance was significantly associated with better adaptive functioning, after accounting for the effects of FSIQ and expression of schizophrenia. The results indicate that Executive Performance contributes significantly to the variability in adaptive functioning in individuals with 22q11DS, both in those who express schizophrenia and in those who do not. Consistent with studies of other high risk and schizophrenia samples [11,13,14], the findings support the potential for early interventions that target neurocognitive performance to improve functional outcome.

Schizophrenia risk and functional outcome

Individuals with 22q11DS showed impaired performance on the tests that comprise the domain Executive Performance, regardless of whether or not they suffered from psychotic illness. This is consistent with findings from studies of other high-risk groups, including clinical high risk [12 13]. The results for 22q11DS not only indicate a relationship of neurocognitive performance to functional outcome, but also suggest that Executive Performance in 22q11DS may be a core expression of the underlying genetic risk of schizophrenia, as for other genetic high-risk groups (positive family history of schizophrenia) [8,39-41]. The comparability of results is notable given differences for the various high-risk groups in the specific neurocognitive tests and functioning outcome measurements used.

Strengths and limitations

Domains of neurocognitive functioning are more resilient to "noisy fluctuations" and clinically more useful than performance on individual neurocognitive tests [42-44] and the data-driven approach to derive these domains was an advantage of this study. To take into account the suboptimal participant to variable ratio in our PCA we conducted post-hoc analyses that suggested that the four components and the association of Executive Performance with adaptive functioning were not artefactually identified, and supported the added explanatory value of Executive Performance in addition to previously identified factors such as FSIQ and psychotic illness status [20]. Indeed, stable and reproducible PCA results can be obtained with small samples provided that the data used are qualitatively strong[42 45 46]. Nonetheless, we acknowledge that the stability and replicability of our results may be limited by the low participant to variable ratio. Replication studies that aim for larger sample sizes of well-characterized adults with 22q11.2DS with and without schizophrenia, and/or include fewer neurocognitive tests, would be optimal.

Although a data-driven approach creating unbiased domains may be considered an advantage, a possible disadvantage of this approach is that the derived domains may be inconsistent and may not fully coincide with a priori theoretically derived expectations. While most of the neurocognitive tests loaded most strongly on domains consistent with expected, theoretical constructs, some (e.g., 'animal naming') did not. Also, PCA results in general may reveal principal components that include individual tests that do not load clearly on just one domain (e.g., WMS – VR I), in which case the domain with the higher loading is selected in the data-driven approach. Such results are relatively common in the field of neurocognition [47 48]. Methodological differences such as these may contribute to the lack of similar results reported in a previous study that used a 22q11DS sample comprising mostly adolescents and few (n = 8) individuals with psychotic illness and where different executive functioning tasks were selected in a non-data-driven method [49].

Multiple indices selected from global single tests are likely to be more highly correlated with one another than variables between tests, thereby potentially artificially distorting the PCA factor loadings (e.g. the three variables from the Purdue pegboard test that formed the Motor domain). However, the Executive Performance domain would have been the least of the four domains to be affected by this limitation.

The potential directionality of the association between Executive Performance and subsequent adaptive functioning is tentatively supported by the observation that in all but three cases, the neurocognitive assessments were conducted before the VABS assessment. Further prospective follow-up would allow the assessment of longer-term effects on adaptive functioning. The regression model used in the current study explained a greater proportion of the variance in overall adaptive functioning than a previous model that did not include neurocognitive domains [20]. Nonetheless, a substantial proportion of the variance remains unaccounted for and a larger sample may have allowed us to detect additional significant associations with other neurocognitive domains such as verbal memory [13].

Potential implications

The results of this study further support 22q11DS as a genetic model of schizophrenia and schizophrenia high-risk [12,15,18,19] that provides an appealing opportunity to study the developmental trajectory and the potential of early-life interventions [12,15-17]. Several studies have shown positive impact on neurocognitive and functional outcomes in trials of cognitive remediation strategies targeting various domains of cognitive functioning in individuals with, and those at high risk for, other forms of schizophrenia [12 13 50-52 14,53]. These initial findings provide impetus to potentially extend such research to 22q11DS.

In addition, to our knowledge, this is the largest study of adults with 22q11.2DS and schizophrenia comparing neurocognitive profiles between those with and without psychotic illness. Our findings for these profiles are globally similar to those for general population forms of schizophrenia [38,54], and in some respects to a recent study of 22q11DS using a different testing battery and domain composition that reported those with and without psychotic illness to be most similar on "complex cognition" [55]. The profiles may be helpful for genetic counseling of individuals with 22q11DS and their families, emphasizing average relative strengths and weaknesses yet the substantial variability between individuals and the necessity to balance individual capabilities and environmental demands in day-to-day situations [16,56]. Notably, neither the tasks nor domains showing the best or worst performance on average, or greatest difference between those with and without schizophrenia, were those found to be most related to functional outcome. Other findings of possible clinical relevance include the fact that best performance for adults with 22q11DS was on the Daily Living Skills subdomain of the VABS [20], and that older age was significantly associated with better functional outcomes. The latter would be in accordance with the overall slower pace of development often observed in 22q11DS [20,57] and other individuals at high risk for schizophrenia [58].

Conclusion

In this study we report that the neurocognitive domain Executive Performance was significantly associated with overall adaptive functioning as well as daily living skills in 22q11DS, a high-risk model of schizophrenia. For the 22q11DS population, as for the general schizophrenia (high-risk) population, these findings provide a rationale for further studies to examine the potential of directed (early) interventions targeting Executive Performance to improve long-term adaptive functional outcome in individuals with, or at high-risk for, schizophrenia. Caregivers, clinicians and researchers may benefit from further insights into the pattern of relative neurocognitive strengths and weaknesses of adults with 22q11DS.

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Supplemental Materials

Supplementary Materials 1. Demographic and clinical characteristics of 99 adults with 22q11DS, with and without psychotic illness

	Total N = 99 (100%)		Psychotic N = 43 (43.4%) [I]		Non-Psychotic N = 56 (56.6%) [II]		
	n	**%**	**n**	**%**	**n**	**%**	***P*[a]**
Sex (Male)	43	43.4	21	48.8	22	39.2	0.34
Ethnicity							
European	89	89.9	35	81.4	54	96.4	0.02*
Other	10	10.1	8	18.6	2	3.60	
Handedness (Right)	84	84.9	36	83.7	48	85.7	0.78
Deletion Origin							
De novo[b]	83	83.8	37	86.1	46	82.1	0.92
Inherited	9	9.10	3	7.00	6	10.7	
Unknown[c]	7	7.10	3	7.00	4	7.20	
Deletion Extent							
Typical[d]	96	96.0	41	95.3	55	96.5	0.99
Atypical	3	0.03	2	0.05	1	0.02	
Unknown[e]	1	0.01	0	0.00	1	0.02	
Congenital Heart Defect (CHD)							
None	41	41.4	28	65.1	13	23.2	<0.0001*[f]
Simple	16	16.2	6	14.0	10	17.9	
Complex *(requiring surgery)*	42	42.4	9	20.9	33	58.9	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	***P***
Age at onset psychosis	-	-	21.1	5.2	-	-	-
Age at neurocognitive testing (y)	26.6	8.6	28.2	6.6	25.5	9.6	0.111
FSIQ	71.7	8.6	68.7	6.5	74.2	9.3	0.001*
VIQ	73.6	8.5	70.3	6.6	76.2	9.0	0.001*
PIQ	72.8	8.7	69.6	6.1	75.5	9.7	0.001*
Age at Vineland assessment (y)	27.5	8.3	29.8	6.9	25.5	8.8	0.016*
Interval time neurocognitive <> Vineland (y)	1.6	2.2	1.9	2.8	1.2	1.6	.145

* Indicates significance ($p<0.05$), [a] X^2 for sex, CHD, and handedness variables; Fisher's Exact Test for ethnicity and deletion origin variables; simple ANOVA's for Age at assessment, interval time, FSIQ (Full-scale IQ), VIQ (Verbal IQ), PIQ (Performance IQ), [b] includes probable de-novo, [c] Parental 22q11.2 deletion status and/or testing results unavailable, [d] deletion overlaps the A-B region, [e] neither typical nor atypical, [f] Difference due to ascertainment differences, e.g., non-psychotic patients ascertained through adult congenital cardiac clinics, [I] All 43 individuals in this group (100%) were receiving antipsychotic medication, [II] 27 individuals (48.2%) in the non-psychotic group were diagnosed with a mood- (25%) , or anxiety (37.5%) disorder; consistent with expectations of previous studies [59].

Supplementary Materials 2. 15 Neurocognitive Tests.

Neurocognitive test	Abbreviation used	Description
Purdue pegboard – bilateral[60]	Purdue - Bilateral	Measures how fast metal pegs can be placed in the holes along the board within a 30-second time period. *Using both hands.*
Purdue pegboard – dominant[60]	Purdue - Dom	Same as for Purdue – Bilateral, except only *Using the dominant hand*
Purdue pegboard – Non dominant[60]	Purdue – Non Dom	Same as for Purdue – Bilateral, except only *Using the non-dominant hand*
Stroop Task (color-word)[61]	Stroop (c-w)	Measures relative speed of reading the colour names that are printed in another colour. Involves cognitive interference, requiring inhibition of a reading response.
Rey Auditory Verbal Learning Test - Recall[62]	RAVLT - Recall	Measures immediate memory recall, learning curve, susceptibility of interference, retention ability and recognition memory. Fifteen words are read aloud for five consecutive trials (List A). A new list of unrelated words (List B) is read aloud which tests for interference effects. *Asked to recall words from List A after all five trials*
Rey Auditory Verbal Learning Test - Recognition[62]	RAVLT - Recog	Same as RAVLT – Recall, but *Asked to identify words (from List A) from a list of filler words and words from List B and List A*
Rey Auditory Verbal Learning Test - Retention[62]	RAVLT - Retention	Same as RAVLT – Recall, but *Asked to recall words from List A after learning interference List B*
Wechsler's Memory Scale- Revised, Logical Memory I[63]	WMS-R LM I	Measures the ability to recall concepts in two orally presented stories. *Immediate recall of events*
Wechsler's Memory Scale- Revised, Logical Memory II[63]	WMS-R LM II	Same as WMS-R LM I, but with *Delayed recall of events*
Wechsler's Memory Scale- Revised, Visual Reproduction I[63]	WMS-R VR I	Measures the ability to reproduce and draw the geometric designs that were showed briefly. *Immediate recall and drawing of shapes*
Wechsler's Memory Scale- Revised, Visual Reproduction II[63]	WMS-R VR II	Same as WMS-R VR I, but with *Delayed-recall and drawing of shapes*
Animals[64]	Animals	Measures how many items belonging to a semantic category (e.g. animals) can be recalled within 60 seconds.
Judgment of Line Orientation[65]	JLO	Measures the ability to evaluate angles of line segments by matching line pairs.
Trails B[66]	Trails B	Measures how fast 25 encircled numbers and letters can be connected in an alternating and progressive order.
Wisconsin Card Sorting Task - Categories[67]	WCST (cat)	Measures the ability to form abstract concepts and to process feedback to shift or maintain mental processes. Four stimulus cards with different shapes and colours are placed in front, asked to match every card (from a given deck) to one of the four stimulus cards. Feedback is given to every move.

Supplementary Materials 3. Mean Z-scores on the 15 neurocognitive tests for 99 adults with 22q11DS, with (n = 43) and without psychotic illness (n = 56).

Neurocognitive test	Total sample		Psychotic		Non-psychotic				
	Mean	SD	Mean	SD	Mean	SD	t	p	Effect size
Visual and Logical Memory									
WMS-R Logical Memory I	-1.50	0.91	-1.87	0.83	-1.23	0.87	3.70	**0.0004**	0.75
WMS-R Visual Reproduction II	-1.04	1.13	-1.44	0.95	-0.74	1.19	3.17	**0.002**	0.64
WMS-R Logical Memory II	-1.32	0.92	-1.75	0.80	-1.00	0.88	4.35	**<0.0001***	0.89[a]
Animals	-1.20	1.06	-1.71	1.09	-0.82	0.88	4.51	**<0.0001***	0.91[a]
Verbal Memory									
RAVLT - Retention	-1.53	1.52	-2.08	1.40	-1.10	1.50	3.31	0.001	0.67
RAVLT – Recognition[b]	-0.19	1.37	-0.78	1.73	0.28	0.75	3.76	**0.0004***	0.83[a]
RAVLT Recall (Trials I – V)	-1.94	1.60	-2.76	1.31	-1.30	1.53	4.99	**<0.0001***	1.01[a]
Motor Performance									
Purdue Pegboard – Non Dominant	-2.96	1.31	-3.50	1.10	-2.59	1.30	3.69	**0.0004***	0.75
Purdue Pegboard - Dominant	-2.71	1.31	-3.33	1.35	-2.22	1.07	4.54	**<0.0001***	0.93[a]
Purdue Pegboard – Bilateral[b]	-3.35	1.30	-4.02	1.18	-2.84	1.17	4.99	**<0.0001***	1.00[a]
Executive Performance									
Judgement of Line Orientation	-1.58	0.94	-1.81	0.77	-1.44	1.00	2.00	0.048	0.41
Trails B	-1.96	1.01	-2.18	0.82	-1.80	1.12	1.94	0.05	0.38
WCST (Categories)	-1.56	0.88	-1.80	0.93	-1.38	0.81	2.43	0.02	0.49
WMS-R Visual Reproduction I	-1.05	1.14	-1.34	1.07	-0.85	1.15	2.20	0.03	0.44
Stroop task (Colour-word)	-1.43	0.98	-1.78	0.76	-1.17	1.06	3.37	**0.001***	0.65

Bolded values indicate significance with Bonferroni adjustment ($p < 0.0028$)

* Indicates significance ($p < 0.05$) after analysis of covariance (ANCOVA) with ethnicity, CHD and FSIQ

[a] Large effect size ($d \geq \geq 0.8$) (Cohen, 1988) for the comparison between psychotic and non-psychotic subgroups of 22q11DS

[b] Best (RAVLT-Recognition) and worst (Purdue Pegboard-bilateral) average neurocognitive test results for the sample overall, as for all of these results, would not necessarily reflect expectations for any individual, given substantial variability (high SD) of all tests. The relative strength on RAVLT-Recognition is consistent with the effort these individuals with 22q11DS were making and the fact that this subtest requires little verbal expressive abilities and relies on immediate recognition of simple auditory stimuli, reflecting perhaps the benefit of prompts.

Supplementary Materials 4. Adaptive functioning for 84 adults with 22q11DS, with and without psychotic illness.

	Total sample		Psychotic (n=38 (45,2%))		Non-psychotic (n=46 (54,8%))		
Vineland Domains	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**P[a]**
Adaptive Behaviour Composite *(overall functioning)*	63.0	19.6	54.1	14.8	70.3	20.2	0.000
Daily Living Skills	78.0	23.5	68.8	22.9	85.6	21.4	0.001
Socialization	64.8	18.2	57.1	14.0	71.2	18.9	0.000
Communication	58.6	22.8	51.2	16.9	64.6	25.2	0.006

[a] ANOVA

CHAPTER 5

5

A Normative Chart for Cognitive Development in a Genetically Selected Population

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Abstract

Certain pathogenic genetic variants impact neurodevelopment and cause deviations from typical cognitive trajectories. Understanding variant-specific cognitive trajectories is clinically important for informed monitoring and identifying patients at risk for comorbid conditions. Here, we demonstrate a variant-specific normative chart for cognitive development for individuals with 22q11.2 deletion syndrome (22q11DS). We used IQ data from 1365 individuals with 22q11DS to construct variant-specific normative charts for cognitive development (Full Scale, Verbal, and Performance IQ). This allowed us to calculate Z-scores for each IQ datapoint. Then, we calculated the change between first and last available IQ assessments (delta Z-IQ-scores) for each individual with longitudinal IQ data (n = 708). We subsequently investigated whether using the variant-specific IQ-Z-scores would decrease required sample size to detect an effect with schizophrenia risk, as compared to standard IQ-scores. The mean Z-IQ-scores for FSIQ, VIQ, and PIQ were close to 0, indicating that participants had IQ-scores as predicted by the normative chart. The mean delta-Z-IQ-scores were equally close to 0, demonstrating a good fit of the normative chart and indicating that, as a group, individuals with 22q11DS show a decline in IQ-scores as they grow into adulthood. Using variant-specific IQ-Z-scores resulted in 30% decrease of required sample size, as compared to the standard IQ-based approach, to detect the association between IQ-decline and schizophrenia ($p<0.01$). Our findings suggest that using variant-specific normative IQ data significantly reduces required sample size in a research context, and may facilitate a more clinically informative interpretation of IQ data. This approach allows identification of individuals that deviate from their expected, variant-specific, trajectory. This group may be at increased risk for comorbid conditions, such as schizophrenia in the case of 22q11DS.

Key words: Cognitive development, IQ, high-risk, pathogenic genetic variant, 22q11DS, schizophrenia, normative chart.

1. Introduction

Over the past two decades, a growing list of genetic variants associated with clinical phenotypic outcomes has emerged, including cognitive trajectories that deviate from what is typical in the general population[1-3]. In the general population, the age-adjusted level of cognitive functioning is generally stable over the lifespan; i.e., the IQ curve, where obtained scores are age-adjusted, is expected to be a virtually constant line over the years [4]. A divergent trajectory may be part of the developmental impact of an underlying pathogenic genetic variant. Examples include early cognitive decline and loss of acquired skills in the case of Rett's syndrome [5,6], or early onset dementia in the case of Down's syndrome [7-10]. General cognitive functioning is the term we use in this article to reflect the important human quantitative trait that accounts for much of the variation in diverse cognitive abilities, including intellectual functioning, and can be operationalized as the commonly used Intelligence Quotient (IQ) [11-13].

Populations of carriers of pathogenic variants that impact neurodevelopment would benefit from a better understanding of variant-specific cognitive trajectories. To that end, ideally variant-specific (age-) normative reference data are obtained, allowing for the comparison of an individual's performance to the group's indices over time and potentially helpful in setting realistic expectations regarding (future) performance. This is analogous to the significantly improved accuracy and clinical relevance of monitoring physical growth in an individual with Down's syndrome when using normative physical growth data from studies of individuals with Down's syndrome [14,15]. When using norm data obtained from the general population, a child with Down's syndrome may be considered growth-delayed, whereas in reality their growth trajectory may be as expected for someone with this genetic condition.

In a similar way, genetic subgroup-specific normative data on *cognitive* development may be highly informative. Such cognitive norm charts may be relevant for both research and clinical purposes as they allow the identification of individuals who deviate from what is a typical trajectory given the genetic variant and potentially, monitoring effects of interventions over time. For example, when an individual does not follow their expected IQ trajectory; i.e., deviates from their IQ curve, this may indicate underlying brain-related pathology, warranting additional examinations. A parallel may be drawn to how in a child who deviates from their expected physical growth curve a diagnostic work-up is warranted that could help identify the cause (e.g., endocrine problems), and potentially inform treatment strategies (e.g., growth hormones) [16].

The 22q11.2 deletion syndrome (22q11DS) is a genetic condition associated with aberrant neurodevelopmental outcomes [17]. It is the most common chromosomal microdeletion

disorder, estimated to result from (in ~85% of cases *de novo)* non-homologous meiotic recombination events occurring in approximately 1 in every 1,000 fetuses [18]. 22q11DS has a highly variable phenotypic expression [19-22], including various levels of cognitive functioning with differing developmental trajectories that, on average, appear to display a mild downward trend [17,23]. Individuals with 22q11DS also have a 25-fold increased risk for developing schizophrenia, making it the strongest single molecular genetic risk variant for psychotic disorders [24]. We have previously reported that the subgroup of individuals with a cognitive decline steeper than average in this population had an even further elevated risk for schizophrenia [25]. Here, we aim to generate a 22q11DS-specific normative chart for IQ to be used as a reference in both clinical and research settings. We will demonstrate how a normative chart for cognitive development in a genetically defined population can be reliably established and provide potential directions for its future utility.

2. Methods

2.1 Participants and instruments

Data on 1789 individuals with a confirmed 22q11.2 microdeletion were collected from 22 different sites as part of the international Brain and Behavior Consortium on 22q11DS [26 27]. For this study, we included individuals who had at least one Wechsler IQ assessment available and were between the ages of 6 and 38 years, resulting in a total number of participants of 1365 (76.3%). Of those, 657 individuals (48.1%) had one assessment available, and we refer to this subgroup as the *baseline* sample. 708 (51.9%) individuals had two or more IQ assessments available, and we refer to this subgroup as the *longitudinal* sample (see also **Figure 1**). All individuals, and when appropriate their legal guardians, provided informed consent and the study was approved by the local institutional research ethics boards of each site.

Level of overall intellectual functioning (IQ) was assessed using age-appropriate Wechsler scales (see also **Table 1**) [28-34] and all IQ-data underwent extensive quality control. Clinical diagnoses of schizophrenia spectrum disorders were made by experienced clinicians in accordance with the Diagnostic and Statistical Manual of Mental Disorders, Fourth [35] or Fifth edition [36]. Positive psychotic symptoms were assessed using standardized clinical interviews, including the Positive and Negative Syndrome Scale (PANNS [37]), the Comprehensive Assessment of At Risk Mental States (CAARMS [38]), the Structured Interview for Psychotic Syndromes (SIPS [39]), and the Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime Version (K-SADS-PL [40]).

2.2 Data analysis

The data analysis for this study consisted of two steps. First, we constructed the normative charts for IQ and second, we used the available longitudinal data to calculate the difference

(delta) scores between the first and last available IQ assessments (see also **Figure 1**). All data quality control and statistical analyses were conducted in R 3.6.2 GUI 1.70.

2.2.i Normative chart

To construct the normative chart for IQ, we used all available IQ datapoints (n = 2512) from all participants with at least 1 IQ-assessment available (n = 1365; all 22q11DS individuals from the IBBC). We used polynomial regression models of the 1st, 2nd, 3rd and 4th order and we used the Akaike and the Bayesian Information Criterion (AIC and BIC) to determine the best fit. Furthermore, we checked basic assumptions for polynomial regressions, including multivariate normality and homoscedasticity, by examining the distribution of the residuals and the residual variance of the final model.

Subsequently, we used the coefficients derived from the best fit to determine the normative IQ chart. This normative chart enabled us to calculate a (standardized) Z-score for each individual IQ-point, and thereby identify how much individuals deviated from the average IQ in this population at a certain timepoint, given their age. We applied the same strategy for all basic summary scores: Full Scale IQ (FSIQ), Verbal IQ (VIQ), and Performance IQ (PIQ).

2.2.ii Delta Z-scores

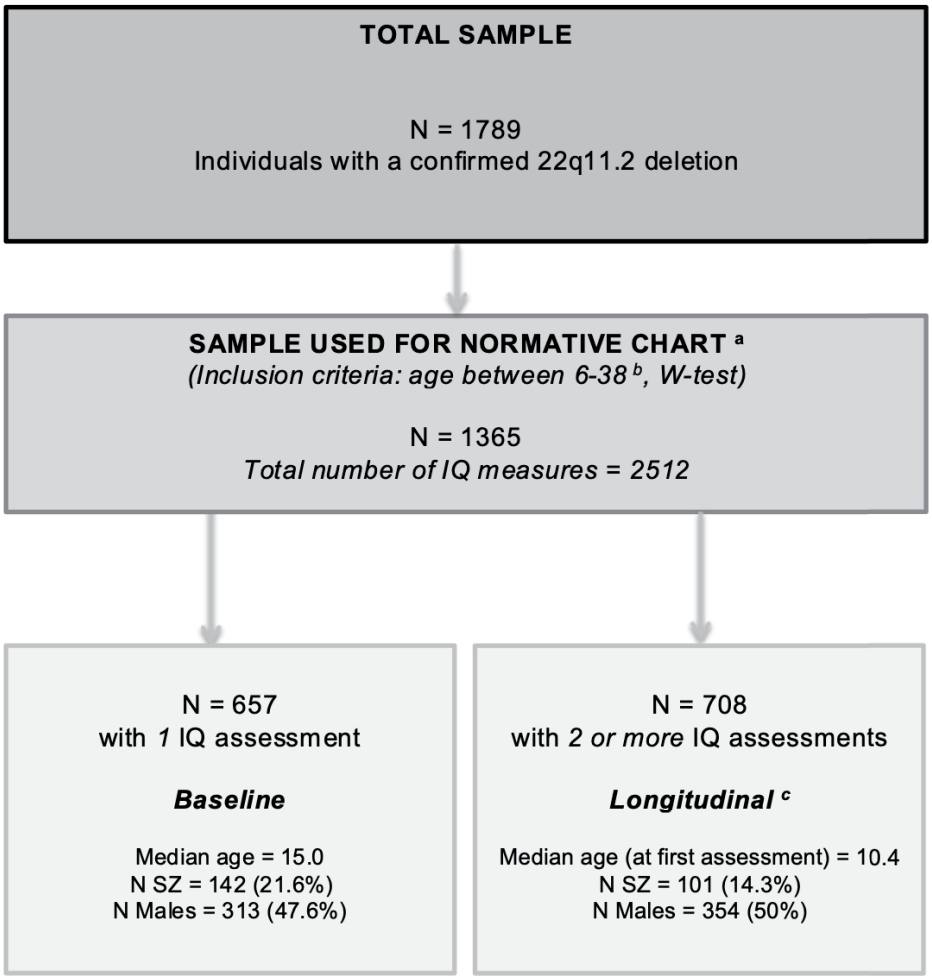
For those individuals with more than one IQ point available (n = 708, 51.9%), we determined delta-Z-scores by calculating the difference between the Z-score corresponding to the first available IQ measurement and that of the last IQ-measurement (*Last IQ Z-score – first IQ Z-score = delta-Z-score*). The average delta-Z score across all participants provides an indication of the extent to which individuals follow, on average, their expected trajectory as predicted by the normative chart. In addition, we examined the distribution of the delta-Z-scores.

2.2.iii Post-hoc

Post-hoc, we investigated whether using the delta-Z-score, as compared to the standard (population-normed) IQ-scores would result in a decrease of required sample size to detect the previously reported association between IQ-decline and schizophrenia risk [25]. To this end, we used odds ratios (ORs) from two regression models: both models had schizophrenia status (yes/no) as the dependent variable and baseline VIQ (we chose to focus on VIQ and VIQ-decline as this component of IQ had the strongest association with schizophrenia risk [25]), sex, age, and time-interval as covariates. In the (variant-specific) Z-based model the main (binary) independent variable was VIQ-Z-decline (yes/no; based on a cut-off of -0.5 SD in delta Z-score). In the parallel model the main independent variable was VIQ-decline (yes/no; based on a cut-off of -7.5 IQ-points (i.e., -0.5 SD) in absolute (population normed) VIQ-difference scores). To obtain a measure to compare

both strategies, we calculated sample sizes needed in both models to obtain sufficient power to detect the association with increased schizophrenia risk.

Figure 1. Flowchart of participants with 22q11DS for inclusion in the current study.



[a] Data (cross-sectional and longitudinal) from these 1365 individuals were used for the construction of the normative chart (Methods section 2.2.i)

[b] For the current study we limited the age range to 6-38 years, as the main purpose of the study is to create one easily applicable normative chart. Above the age of 38 years, the number of participants in each age-year was too small ($n < 10$) to obtain a reliable normative value for that particular year. Below the age of 6 years, the number of participants in each age-year was also small and scores showed disproportionally greater variability (consistent with greater testing effects observed in IQ-tests in younger children).

[c] Data (longitudinal) from these 708 individuals were used for calculating the delta-z-scores (Methods section 2.2.ii).

3. Results

3.1 Participants and instruments

Figure 1 provides a schematic depiction of the participants included in this study. **Table 1** provides descriptives for all participants, as well as separately for those with only one IQ-assessment (baseline) and those with two or more IQ-assessments available (longitudinal). Importantly, there were no differences in mean FSIQ, VIQ and PIQ scores (on the first available assessment) between the baseline- and longitudinal-samples (**Table 1**).

3.2.i Normative chart statistics

The 3rd order polynomial regression provided the best fit for the FSIQ, VIQ and PIQ data, as indicated by the AIC and BIC, and the normative charts were constructed based on this. The parameters for the model for FSIQ were $R^2 = 0.03$, $F(3,2508)=24.01$, $p<0.001$; for VIQ $R^2 = 0.03$, $F(3,2439)=19.19$, $p<0.001$; and for PIQ $R^2 = 0.03$, $F(3,2336)=26.35$, $p<0.001$. **Supplemental Table 1** provides the coefficients of the regressions. The residuals of the model were normally distributed and constant over the age range, indicating accurate prediction of the trajectory by the normative chart. Further, the distribution of the Z-scores confirmed that the normative chart provided a good fit for the data. The mean Z-scores were close to 0 for FSIQ (-0.03), VIQ (-0.02) and PIQ (-0.03), indicating that on average, individuals with 22q11DS had an IQ-score as predicted by the model considering their age. In addition, there was no difference of the mean Z-scores between the *baseline*- and the *longitudinal*-samples. The standard deviations (SD) of the z-scores were close to 1 for FSIQ (1.02), VIQ (1.01) and PIQ (1.04) (an SD of 1 is the equivalent of 15 IQ-points). **Figure 2** displays the normative growth chart for FSIQ, VIQ, and PIQ (including data points in **Supplemental Figure 1)**.

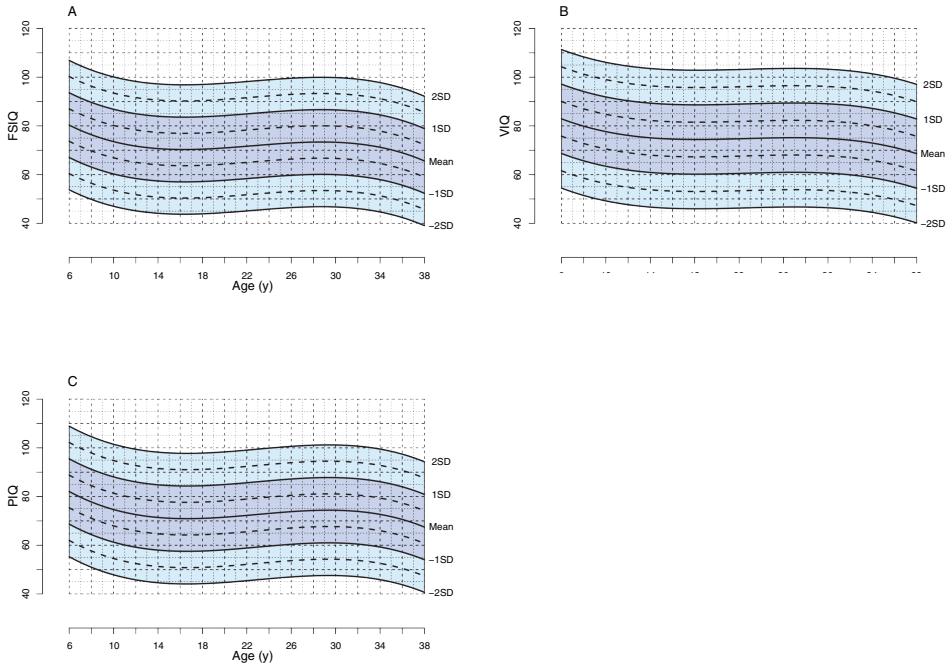
In addition, as an illustration to aid in understanding the IQ decline observed on average in individuals with 22q11DS, **Supplemental Figure 2** represents the approximate corresponding raw score trajectory for 22q11DS, compared to raw IQ score change in the general population.

Table 1. Sample descriptives for total sample (n = 1789) of individuals with a 22q11.2 deletion, baseline subset (n = 657) and longitudinal subset (n = 708).

	Total sample *N = 1789*	**Subset: *baseline*** *N = 657*	**Subset: *longitudinal*** *N = 708*	
Age in years at first assessment	-			
Mean (SD)		17.1 (8.0)	11.6 (4.8)	
Median (Range)		15.0 (6.0 – 37.8)	10.4 (6.0 – 35.2)	
Age in years at last assessment	-	-		
Mean (SD)			17.9 (5.8)	
Median (Range)			17.1 (7.4 – 38)	
Sex (% males)	868 (48.5%)	313 (47.6%)	354 (50%)	
Psychotic illness expression				
Psychotic illness	332 (18.6%)	142 (21.6%)	101 (14.3%)	
Control (age >25 y)	295 (16.5%)	99 (15.1%)	63 (8.9%)	
Putative control	850 (47.5%)	323 (49.2%)	385 (54.4%)	
Control combined	*1145 (64%)*	*422 (64.2%)*	*448 (63.3%)*	
Putative subthreshold	268 (15%)	74 (11.3%)	146 (20.6%)	
Affective psychosis	33 (1.8%)	14 (2.1%)	13 (1.8%)	
Unknown	11 (0.6%)	5 (0.8%)	0	
Age in years at last psychiatric assessment				
Mean (SD)	21.3 (11.4)	19.9 (10.0)	19.0 (6.8)	
Median (Range)	18.0 (2 – 71)	17.0 (5 – 56)	18.0 (7 – 61)	
IQ-test used (first assessment)				
WPPSI	-	49 (7.5%)	32 (4.5%)	
WPPSI-R	-	5 (0.8%)	18 (2.5%)	
WISC-III	-	153 (23.3%)	323 (45.6%)	
WISC-IV	-	96 (11.6%)	100 (14.1%)	
WISC-R	-	24 (3.7%)	56 (7.9%)	
WAIS-III	-	139 (21.2%)	49 (6.9%)	
WAIS-IV	-	50 (7.6%)	9 (1.3%)	
WAIS-R	-	61 (9.3%)	7 (1.0%)	
WASI	-	80 (12.2%)	114 (16.1%)	
				p [a]
Mean baseline FSIQ (SD)	-	72.0 (14.3)	73.3 (13.1)	0.1048
Mean baseline VIQ (SD)	-	76.3 (14.5)	76.9 (14.6)	0.4236
Mean baseline PIQ (SD)	-	73.2 (14.9)	74.0 (13.4)	0.2741

[a] p-value of difference statistic (t-test) between baseline and longitudinal subsets.

Figure 2. 22q11DS-specific normative chart for FSIQ (A), VIQ (B), and PIQ (C) over time.



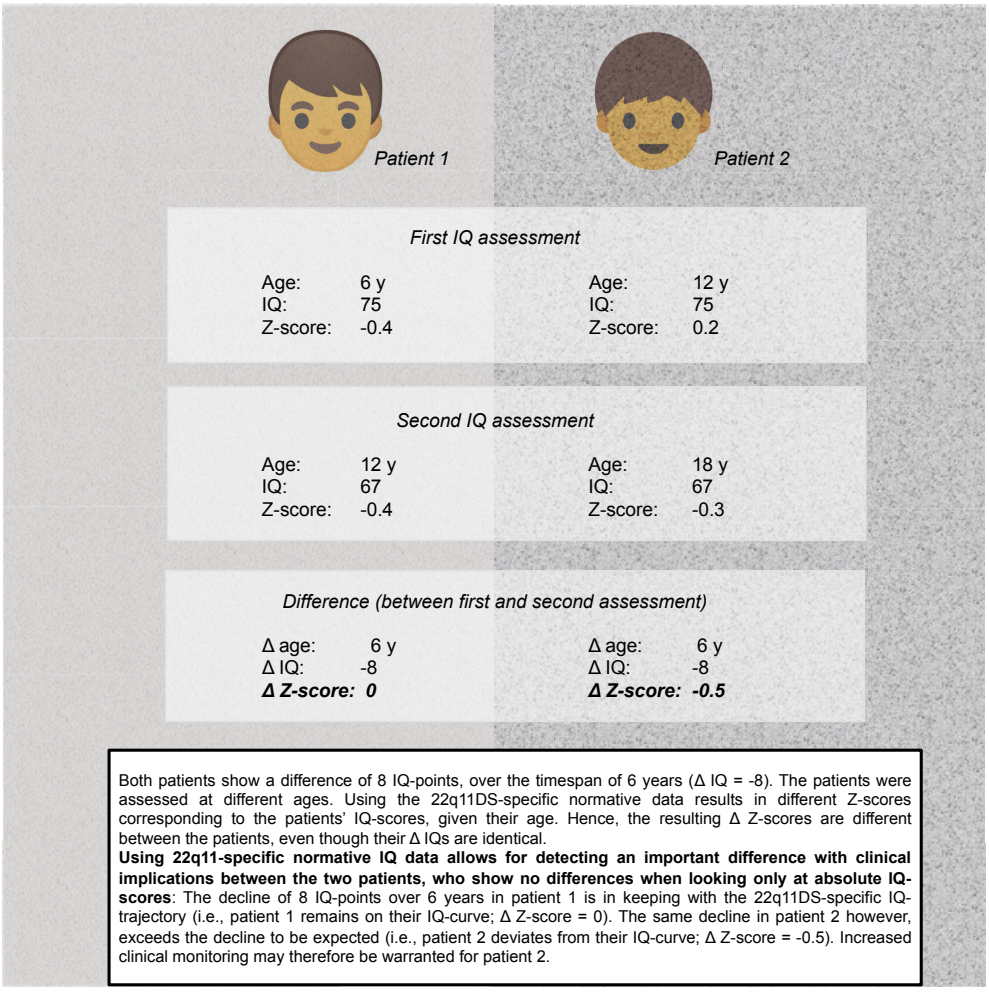
These figures represent the normative charts for IQ development in individuals with the 22q11.2 deletion (A: FSIQ, B: VIQ, C: PIQ). The lines represent the observed average IQ trajectories ("Mean"), and the observed trajectories that deviate +/- 1 or 2 SDs from the mean. The normcharts are derived from 2512 IQ assessments in 1365 individuals with the 22q11.2 deletion between the ages of 6 and 38 years.

3.2.ii Delta Z-scores statistics

For the 708 individuals with longitudinal IQ-data were, we calculated delta-Z-scores; i.e., the difference between the Z-scores corresponding to the first and last available IQ-measurements. A model with a good fit would be expected to result in mean delta-Z-scores of around 0, as this would indicate that, on average, individuals stay on their trajectory. **Supplemental Figure 3** displays the distribution of the delta-Z-scores for FSIQ, VIQ and PIQ. The means were close to 0 (0.064, 0.069, and 0.089 respectively) and the standard deviations were 0.637, 0.679, and 0.720 respectively. Of the 708 individuals, 58% (FSIQ and VIQ) and 55% (PIQ) were between -0.5 and 0.5 SD. This indicates that on average, individuals stay on their trajectories as predicted by the normative IQ charts. **Figure 3**, presenting IQ data of two hypothetical individuals, serves to illustrate the enhanced impact of using delta-Z-IQ-scores (referenced to 22q11DS-specific norms) compared to general-population delta-IQ-values.

Supplemental Materials 1 provides the calculator which allows for obtaining the expected IQ-score given a certain age, and hence the corresponding Z-score for an individual given their age and observed IQ-score. When multiple IQ-assessments for one individual are available, the delta-Z-scores can be calculated. This can be done for FSIQ, VIQ, and PIQ.

Figure 3. Two hypothetical cases that illustrate the advantage of 22q11DS-specific normative IQ-data over only (general population-based) IQ-data.

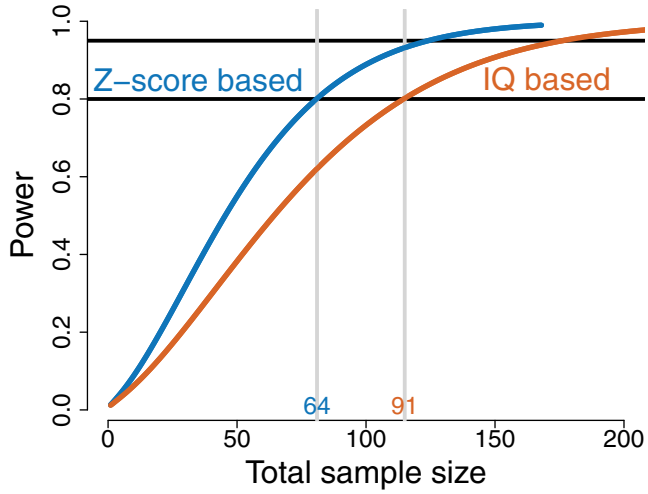


3.2.iii Post-hoc analyses

Post-hoc, we compared the Z-IQ-scores model to the population-based IQ model in terms of required sample size to detect the previously reported association between IQ-

decline and schizophrenia risk [25]. As expected, both models reveal a significant association between VIQ-decline and schizophrenia. Importantly, the OR derived from the Z-based regression model was larger compared to the IQ-based model (Z-based OR = 2.84, 95% CI = 1.595 – 5.025, p = 3.405e-04; versus IQ-based OR = 2.09, 95% CI = 1.231 – 3.533, p = 5.862e-03). Based on these OR's we calculated sample sizes needed to obtain sufficient power to detect the association between IQ-decline and schizophrenia. To obtain 80% power, the Z-based model required a sample size of 64, while a sample size of 91 was needed using the untransformed IQ values (**Figure 4**). In other words, using the Z-scores-based approach resulted in a 30% decrease of needed sample size, as compared to the untransformed IQ-based approach, to detect the association between IQ-decline and schizophrenia illness expression with 80% power.

Figure 4. Sample sizes of individuals with 22q11DS required to detect a significant effect (between VIQ-decline and schizophrenia) with 80% power: Z-score based approach versus IQ-based approach.



4. Discussion

In this study we constructed a variant-specific normative chart for cognitive development from the largest sample of individuals with the 22q11.2 deletion available to date. Our findings suggest that in this population, a variant-specific normative IQ-chart can be reliably constructed and our discussion offers the rationale for how other pathogenic variants may benefit from a similar strategy. We propose that this approach allows for more accurate and informative interpretation of individual IQ-scores and trajectories, compared to using (untransformed) population-based IQ norms.

The findings further demonstrate that using variant-specific normative IQ data can significantly reduce the sample size needed to detect a certain effect (i.e., VIQ-decline and schizophrenia risk in 22q11DS), compared to population-based normative IQ data. From a research perspective, this is an important discovery. It is challenging to assemble adequately large datasets to provide sufficient power for phenotype-phenotype, or genotype-phenotype analyses, in particular with respect to longitudinal (deep-phenotyping) data. In populations with high-impact variants associated with neurodevelopmental outcome this challenge is even further magnified, given the low population-wide prevalence rates of such conditions.

22q11DS, IQ, and schizophrenia

Using data from 1365 individuals with the 22q11.2 deletion, our findings corroborate several important observations regarding IQ in this population. First, the data confirm the previously reported lower baseline IQ in individuals with 22q11DS [41,42], and show that the deletion thus appears to shift the IQ-distribution in carriers to the left (~-2 SD) as compared to non-carriers, but does not alter the characteristics of the distribution. This is in line with a recent study which reported that while FSIQ, VIQ, and PIQ were ~30 IQ-points lower in 22q11DS patients compared to their unaffected parents, the distribution was normal and significantly associated to the parental distribution [submitted manuscript Fiksinski *et al*.].

Second, our data reiterate that in individuals with 22q11DS there is, on average, a decline in IQ over the lifespan [25] [23]. This observation underscores the impetus for regular and comprehensive cognitive assessments in individuals with 22q11DS [43-45]. We posit that in childhood and adolescence, the observed typical decline in 22q11DS mostly reflects a slower pace in cognitive development in individuals with 22q11DS, compared to their typically developing peers [46] (see also **Supplemental Figure 2**). In adulthood, however, this decline suggests that individuals with 22q11DS are losing cognitive capacities at a faster pace compared to the general population [31]. Importantly, using the 22q11DS-specific normative IQ-data allows for plotting an individual's IQ-score or IQ-trajectory against expectations for this specific population.

Third, as previously reported [25], individuals with 22q11DS who show a VIQ decline that is steeper than what is expected based on the variant-specific trajectory are at a further increased risk for subsequently developing a psychotic disorder. This is in contrast to individuals with 22q11DS who do not deviate from their expected trajectory, but may still show a VIQ decline when compared to general population norms. These findings corroborate longitudinal studies in the general population, which report that individuals who later developed a psychotic disorder showed increasing cognitive impairments over time, especially during adolescence. In individuals from the general population who are at high risk for psychotic disorders, similar but milder delays in cognitive trajectories have been reported [47,48] [13,49,50].

Implications for this and other pathogenic variants

The often atypical and complex cognitive profile in carriers of pathogenic variants, such as the 22q11.2 deletion, adds complexity to the challenge of finding equilibrium between an individual's profile of strengths and weaknesses on the one hand, and environmental demands on the other [43]. Realistic daily-life expectations given an individual's capabilities are key in optimizing the fit between their individual profile and demands, and this is *particularly* important in populations with increased neurodevelopmental and psychiatric vulnerability [43,51].

Variant-specific normative IQ-data allow for "plotting" an individual's IQ-score against norms *given* their specific variant, and, by extension, a likely projection into future performance. In other words, they allow for setting more realistic expectations and more informative monitoring of individual carriers of a pathogenic variant. For example, our data suggest that for a child with 22q11DS, a decline of 7 IQ-points between the ages of 7 and 13 is not unlikely. This is relevant in and of itself to the child's day-to-day functioning with respect to environmental demands such as in school. The main relevance of knowing that this observed IQ-decline is in keeping with expectations given that this child has the 22q11.2 deletion is twofold. First, it aids in setting more realistic expectations in terms of future functioning and, potentially, taking proactive measures accordingly. Second, it may help to avoid unnecessary concern (e.g., in parents, teachers) as in fact, this child's cognitive development is in line with the norms given their genetic variant and overall skills may not necessarily be deteriorating; but, rather, just demonstrating a slower rate of growth that is in line with the phenotypic performance for the condition.

Further, variant-specific normative IQ data enable the identification of those individuals who deviate more than what can be expected *given* the genetic variant; i.e., who deviate from their (adjusted) curve. This may be helpful in interpreting the observed IQ-decline and distinguishing between individuals who cannot keep up with increasing environmental (social, academic) expectations, and those who display an actual loss of abilities. While in both scenarios a decline in absolute IQ-scores can be observed, the underlying mechanisms and clinical implications may be very different [17,23,25,52]. Future studies could include raw IQ data (i.e., not standardized and norm-referenced) to further elucidate these different underlying mechanisms of IQ-decline, as well as to allow for further improved specificity and greater variance at the extremes end of the IQ-distribution [53].

Variant-specific normative IQ data may also allow for improved risk stratification for comorbid conditions. This strategy applied to 22q11DS facilitates the identification of those individuals with 22q11DS with a VIQ-decline in excess of what it typical for this population and that may be a significant risk factor for developing schizophrenia [25]. The clinical implication is that increased (early) monitoring for signs of psychotic development may be warranted in this subgroup. Vice versa, while still at increased risk of

psychosis compared to the general population, the individuals who do not deviate from their expected trajectory (but may still show an IQ decline when compared to general population norms) could receive care as usual for 22q11DS patients [44,45]. Also, the stress experienced by patients and caregivers due to this genetically determined *a priori* risk for schizophrenia [54] may be somewhat mitigated in this group.

As is the case for 22q11DS, the variability in (degree of) expressed phenotypes with any rare pathogenic variant can still only be described in terms of group prevalence rates. Our current inability to provide individualized outcome prediction causes uncertainty for caregivers with respect to individual needs and daily life expectations [55,56], and undermines the potential for prevention or early intervention strategies. Although variant-specific normative data for IQ provide an important step towards improved outcome prediction at a group level, the identification of factors influencing *individualized* outcome prediction is needed. Recent studies are making progress in this regard in carriers of various high-impact genetic variants including 22q11DS, for example by investigating the impact of parental functioning on patient functioning on several phenotypes [57,58].

Similarly, more research is needed to further improve individualized risk stratification with respect to comorbid conditions and, subsequently, to elucidate how to potentially implement this in clinical practice. A recent IBBC study shows promising progress in this area by demonstrating that the use of polygenic scores, in the context of a population with an a priori increased risk (22q11DS), can significantly improve the positive predictive value with respect to a particular phenotype; in this case schizophrenia [59].

Strengths and limitations

The main strength of this study is that we used IQ-data from the largest database of individuals with 22q11DS currently available. The multi-site collected data underwent extensive quality control, as described elsewhere[26]. We provide an easy-to-use normative IQ chart for the three main IQ constructs, which is readily accessible both to the clinical and research communities.

Limitations are that the available data did not allow for using independent samples in the two main parts of the analyses: creating the normative IQ chart (using all available data), and calculating the z-scores (using only longitudinal data), which would have been methodologically preferable. The results, however, provided confidence that our data were unbiased and indeed normative. Importantly, the data revealed no differences in IQ parameters between the subsets with longitudinal data available and the subset with only cross-sectional IQ-data (see also **Table 1**).

Our normative IQ-chart is limited to individuals with the 22q11.2 deletion between the ages of 6 and 38 (see also Footnote **Figure 1**), and the sample was not stratified for other key variables typically used in the development of normative tables (e.g., socioeconomic

status, region of country). Future studies could include both younger and older individuals to expand coverage of the normchart to the entire lifespan of individuals with 22q11DS.

Finally, it is important to note that there are four key components of overall IQ that formally or informally permeate all versions of the Wechsler scales. Working Memory and Processing Speed are assessed independently from VIQ and PIQ and reflect key neuropsychological processes. Specific abnormalities in these domains may be associated with specific psychiatric or neurodevelopmental outcomes [31]. At the time of the current study, available data were limited to VIQ and PIQ, in addition to FSIQ. However, future studies that aim to elucidate Working Memory and Processing Speed data and trajectories in individuals with 22q11DS are important to further our understanding of the complete cognitive profile in individuals with this high-impact variant.

Conclusion

Here, we have discussed the rationale and methodology for using a normative chart for IQ and IQ development specific to a population with a specific pathogenic variant. Using the 22q11.2 deletion as a model, we demonstrate that a variant-specific IQ normative chart can be reliably constructed and offers important advantages over using only standard (general population) IQ norms. It allows for more informed interpretation and monitoring of cognitive performance in carriers of the pathogenic variant. It also contributes to the identification of individuals who deviate from their expected trajectory and may be at increased risk for clinically relevant comorbid conditions; e.g., in individuals with 22q11DS and a VIQ-decline steeper than what is expected in this population, the risk for schizophrenia is further elevated. We also demonstrated that using variant-specific normative IQ-data significantly reduces required sample size to detect relevant effects in a research context. The development of this normative chart, based on the largest sample of individuals with 22q11.2DS in the world, should provide additional opportunities to study the cognitive phenotypic presentation of this population specifically, but also provides a proof of principle regarding the identification of cognitive developmental trajectories in groups of individuals affected by other pathogenic variants. We expect that such knowledge will be valuable for clinical researchers and, ultimately, facilitate advances in clinical practice for these individuals and their families.

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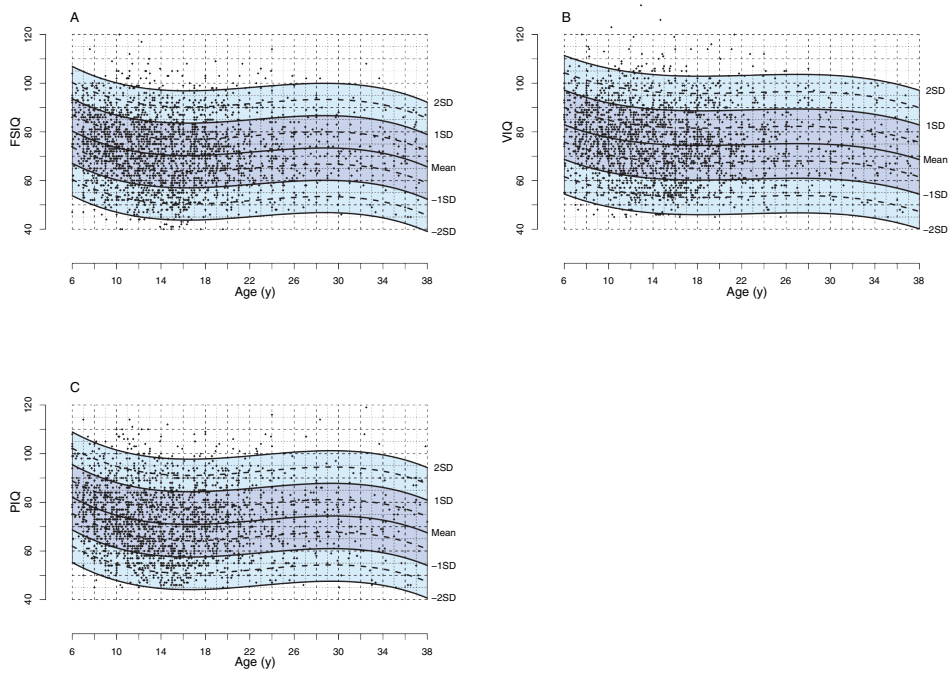
Supplemental Materials

Supplemental Table 1: The coefficients for the regression models that provide the best fits for the normative charts for FSIQ, VIQ, and PIQ in individuals with 22q11DS.

	cf1	cf2	cf3	cf4	cf5 (SD)
FSIQ	97,95171307	-4,085538635	0,193413442	-0,002838645	13,254038208
VIQ	96,13751	-3,004919	0,1368446	-0,002021919	14,21834
PIQ	101,0576726	-4,409169005	0,20642429	-0,002984025	13,3761346

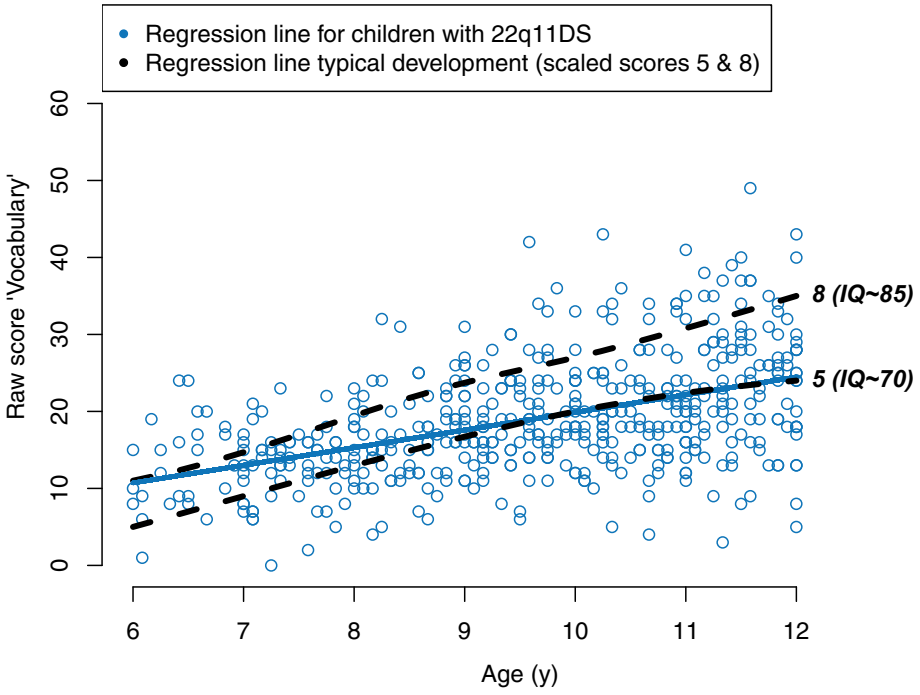
* Formula for calculating the expected IQ score: E = cf[1] + (AGE * cf[2]) +(AGE^2*cf[3]) + (AGE^3*[cf4])
** Formula for calculating respective Z-score is: (O – E) / SD (whereby O = observed IQ; E = expected IQ; SD = cf5.

Supplemental Figure 1. 22q11DS-specific normative chart for FSIQ (A), VIQ (B), and PIQ (C) over time including datapoints. Derived from 2512 IQ assessments in 1365 individuals with the 22q11.2 deletion syndrome between the ages of 6 and 38 years.



These figures represent the normative charts for IQ development in individuals with the 22q11.2 deletion (A: FSIQ, B: VIQ, C: PIQ), including the data points from the current study. Each black dot corresponds to one of the 2512 IQ assessments in 1365 individuals with the 22q11.2 deletion between the ages of 6 and 38 years. The lines represent the observed average IQ trajectories ("Mean"), and the observed trajectories that deviate +/- 1 or 2 SDs from the mean.

Supplemental Figure 2. Illustration of raw IQ-score development (for subtest Vocabulary) corresponding to the average trajectory in 22q11DS.



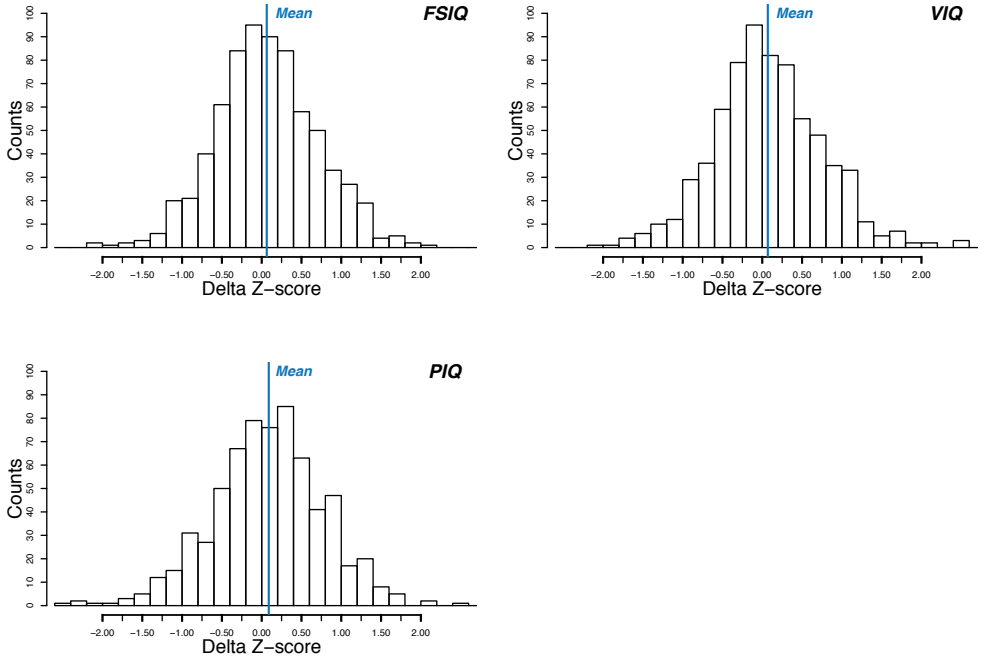
This figure displays (1) the black intermitted line: the estimated trajectory of raw scores on Vocabulary corresponding to a scaled subtest score of 5 and 8. These scaled scores correspond, approximately, to an IQ of 70 and 85 respectively*; (2) the blue dots: the data points from the current study corresponding to the raw scores on the subtest Vocabulary (WISC-III and WISC-IV) for individuals with 22q11DS aged 6-12 years; (3) the blue line: the observed regression line that best fits these data.

This figure illustrates that, for a child with 22q11DS, a decline in IQ that is in keeping with the expected 22q11DS-specific IQ-trajectory (i.e., delta Z-IQ = 0) corresponds to a n upward development in raw scores, but at a slower pace than what is observed in the general population (and would be required to retain the same absolute IQ-score over the years). The on average observed downward trend in cognitive development over the years in individuals with 22q11DS, most prominent in childhood, thus corresponds to a slower development of cognitive capacities, here represented by raw subtest scores.

The regression model that results in the fit for this particular example (the blue line) is a 1st order polynomial regression with $R2 = 0.22$, $F(1, 485)=140.2$, $p<0.001$.

* The IQ-scores that would correspond to the specific raw and scaled scores on a particular subtest of an IQ-test can only be estimated by approximation: the IQ-score is ultimately calculated from a conglomerate of subtests, that each contribute in a unique way to the final IQ-construct.

Supplemental Figure 3. Distributions of the delta-Z-scores for FSIQ, VIQ and PIQ.



These figures represent the distributions of delta-Z-scores in individuals with the 22q11.2 deletion. For FSIQ: n = 708, mean (SD) = 0.064 (0.637); for VIQ: n = 688, mean (SD) = 0.070 (0.639); for PIQ: n = 654, mean (SD) = 0.088 (0.720).

CHAPTER 6

6

Within-Family Influences on Dimensional Neurobehavioral Traits in a High-Risk Genetic Model

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Background: Genotype-first and within-family studies help to elucidate factors that contribute to psychiatric illness expression. Combining these approaches, we investigated the patterns of influence of parental phenotypes, a high-impact variant, and schizophrenia on dimensional neurobehavioral phenotypes implicated in major psychiatric disorders.

Methods: We quantitatively assessed cognitive (FSIQ, VIQ, PIQ), social, and motor functioning in 82 adult individuals with a *de novo* 22q11.2 deletion (22 with schizophrenia), and 148 of their unaffected parents. We calculated within-family correlations and effect sizes of the 22q11.2 deletion and schizophrenia, and used linear regressions to assess contributions to the neurobehavioral phenotypes.

Results: Proband-parent intra-class correlations (ICC) were significant for cognitive measures (e.g., FSIQ ICC=0.549, $p<0.0001$), but not for social or motor measures. Compared to biparental scores, the 22q11.2 deletion conferred significant impairments for all phenotypes assessed (effect sizes -1.39 to -2.07 SD), strongest for PIQ. There were further decrements in those with schizophrenia. Regression models explained up to 37.7% of variance in IQ, and indicated that for proband IQ, parental functioning had larger effects than schizophrenia expression.

Conclusions: This study, for the first time, disentangles the impact of a high-impact variant from the modifying effects of parental background and schizophrenia on important dimensional neurobehavioral phenotypes. Results suggest that, independent of effects of the 22q11.2 deletion and schizophrenia, there are parental modifying effects on cognitive functioning, in contrast to the pattern for social and motor functioning. The findings set the stage for studies to elucidate the contributing genetic factors, their overlap with schizophrenia risk, and sharing between major risk groups.

Keywords: Genetics, variable expression, quantitative traits, parental phenotypes, 22q11.2 deletion syndrome, schizophrenia.

Abstract

Introduction

The dimensional study of clinical and behavioral phenotypes can further our understanding of the etiologies of major neuropsychiatric disorders,[1,2] with 'genotype-first' strategies involving specific genetic variants increasingly recognized as successful routes to this end.[3-6] Selecting a cohort based on a high-impact genetic variant that confers increased risk of psychiatric disorders can provide increased etiologic homogeneity and reduce phenotypic ascertainment bias.[3,4,7-9]

In the general population, to understand how shared genetic background can shape the expression of neurobehavioral traits, a standard approach involves measuring the averaged outcome of both parents (the biparental mean).[10,11] For individuals with a high-impact genetic variant, emerging findings using a comparable within-family design suggest that parental functioning may also play a role in explaining the variable phenotypic expressivity of such variants;[12,13] data on high-risk variants for schizophrenia however are lacking.

In the current study we combined genotype-first and within-family approaches to disentangle the relative impact of a *de novo* 22q11.2 deletion, from the modifying effects of parental outcomes on neurobehavioral phenotypes. The 22q11.2 deletion confers the highest known molecular risk for developing schizophrenia (~25-fold) and is therefore considered a valuable genetic model for studying factors involved in psychotic illness.[4,8,9,14] Importantly, individuals with a 22q11.2 deletion, with and without schizophrenia, often show impairments in the same heritable traits of cognitive, social and motor functioning[15-17] as do patients with idiopathic schizophrenia and at-risk groups.[18-20]

The primary goal was to investigate whether parental variability on dimensional neurobehavioral phenotypes would account for a significant proportion of their variability on phenotypes in adult probands with a *de novo* 22q11.2 deletion. We estimated effect sizes, anticipating a deleterious effect of the 22q11.2 deletion on all phenotypes, with additional impairment in those with schizophrenia, relative to parental phenotypes.

Methods and Materials

Procedure and participants

The families included were recruited through participants in a longitudinal study of adults with 22q11.2 deletion syndrome (22q11DS) (**Supplemental Methods**).[8] Written informed consent was obtained for all participants and the study was approved by local research ethics boards.

Study participants comprised 230 individuals: 82 adult probands with a *de novo* 22q11.2 deletion (mean age 27.2 (9.0; range 18-55) years; 41 (50.6%) male), and 148 unaffected parents (77 mothers, 71 fathers; n=79 probands) for within-family analyses

(**Supplemental Figure 1**). Mean age of parents was 57.6 (SD 9.0; range 39-83) years. We aimed to include as many complete trios as possible, in order to use biparental mean scores of neurobehavioral measures for analyses. However, if data for one parent were unavailable, we included the proband-parent dyad.

Clinical genetic testing confirmed the molecular diagnosis of a typical 22q11.2 deletion for all probands, and its absence in all participating parents (details in **Supplemental Methods**).[14]

Standard diagnostic assessment (**Supplemental Methods**)[8] placed the majority of probands (n=52, 63.4%) in the no psychotic illness subgroup; of these, at assessment n=22 (42.3%) were aged ≥25 and n=30 (57.7%) 18-25 years. The schizophrenia subgroup comprised 22 (26.8%) probands diagnosed with schizophrenia/schizoaffective disorder, mean (SD) age at onset 19.8 (4.2), duration of illness 8.9 (9.4), years; none in an acute psychotic phase of illness at assessment. The remaining n=8 (9.8%) probands had a mood disorder with psychotic features, or history of psychotic symptoms, thus were excluded from analyses using the main diagnostic subgroups. No parent had psychotic illness.

Assessment instruments

We used the same assessment instruments for all participating probands and parents. All dimensional trait assessments were administered by trained psychologists. The number of families for whom both proband and parental data were available differed per instrument, resulting in maximum sample sizes of n=78 families for cognitive (FSIQ, VIQ n=77; PIQ n=78), n=61 families for social, and n=72 for motor, functioning (**Supplemental Figure 1**). Proband-only analyses included three individuals for whom no parental data were available.

To assess level of cognitive functioning we used the Wechsler Abbreviated Scale of Intelligence, second edition (WASI-II).[21] The WASI-II provides Full Scale IQ (FSIQ), Verbal Comprehension Index (VCI, equivalent to verbal IQ (VIQ)) and Perceptual Reasoning Index (PRI, equivalent to performance IQ (PIQ)) scores, each with general population mean 100, SD 15.[21]

To assess level of social functioning, we used the Social Responsiveness Scale-II (SRS)[22]; a 65-item measure assessing overall social impairment. Raw scores (mean 30, SD 20) were used for analyses, as these provide optimal differentiation at the lower and higher ends of the scale.[22]

To assess motor functioning (dexterity), we used the Purdue Pegboard Test (PPT) [23]. We used t-scores (mean 50, SD 10) from the bilateral condition for analyses. (Details in **Supplemental Methods**.)

Statistical analyses

Primary analyses examined the association between parental and proband functioning by intraclass correlation analyses (ICC). Where ICC results identified a significant association

between parental and proband phenotype we proceeded with a linear regression analysis to investigate the effects of parental phenotype on the respective proband phenotype, while accounting for possible effects of schizophrenia,[24] proband sex and proband age.

We also compared scores between probands and parents, and between probands with and without schizophrenia, using related samples t-tests to investigate the deleterious effects of the 22q11.2 deletion and schizophrenia for all dimensional neurobehavioral domains. We calculated effect sizes, expressed in SDs, of the respective difference scores for all phenotypes in a standardized way to allow for cross-phenotype comparisons.

Further, we investigated whether the effect of parental phenotype on proband functioning was different for probands in the schizophrenia and no psychotic illness subgroups. Within the schizophrenia subgroup we assessed possible influence of age at onset or illness duration, and for the no psychotic illness subgroup we repeated analyses restricting to probands aged ≥25 years, likeliest to be through age at risk for schizophrenia.[4,9]

To examine potential parental sex effects we repeated analyses separately for mothers and fathers. In trios, we performed ICC analyses to evaluate the association between parental scores within-families. We examined correlations among the three phenotypes, and where appropriate, assessed whether taking this correlation into account made a difference to results.

All data quality control/preparation and statistical analyses were conducted in R 3.6.2 GUI 1.70.[25]

Results

Impact of the *de novo* 22q11.2 deletion, and expression of schizophrenia, on dimensional phenotypes

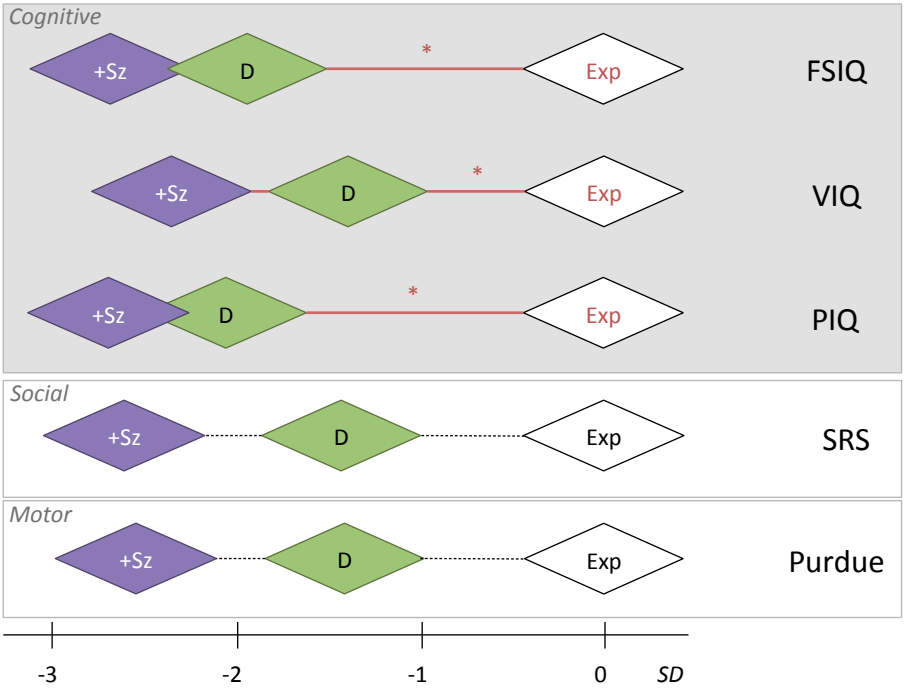
Compared to their unaffected parents, on all measures assessed there was significantly impaired functioning for adults with a *de novo* 22q11.2 deletion (**Table 1, Supplemental Figures 2A, 2B**). As expected,[18-20] mean scores indicated better functioning in the no psychotic illness than the schizophrenia subgroup, for all parameters (**Table 2, Supplemental Figure 2C**).

Examining the dimensional trait results within these main subgroups allowed us to estimate the relative effect sizes of the *de novo* 22q11.2 deletion and of expression of schizophrenia, anchored by parental expectations. For those with no psychotic illness, the differences between proband and parental mean scores indicated that the 22q11.2 deletion exerts a deleterious impact of -1.93 SD (FSIQ), -1.47 SD (VIQ), -2.07 SD (PIQ), -1.39 SD (SRS), and -1.39 SD (Purdue), i.e., substantial for all phenotypes but greatest for PIQ (**Figure 1**).

Within the schizophrenia subgroup, the decrements were greater. Estimated from the results for the non-psychotic subgroup, expression of schizophrenia added a further -0.78 SD (FSIQ), -0.84 SD (VIQ), -0.62 SD (PIQ), -1.23 SD (SRS), and -1.20 SD (Purdue) to the deleterious impact of the 22q11.2 deletion on the phenotypes assessed (i.e., least for PIQ) (**Figure 1**).

Importantly, the mean (SD) parental scores did not differ significantly between the non-psychotic and schizophrenia proband subgroups on any of the phenotypes assessed (FSIQ 104.4 (13.27) vs. 102.84 (15.68); VIQ 102.05 (12.89) vs. 103.77 (17.80); PIQ 105.9 (13.08) vs. 100.55 (12.06); SRS 30.53 (19.75) vs. 32.56 (22.40); Purdue 42.50 (10.62) vs. 40.63 (10.88), respectively).

Figure 1. Relative effect sizes of the *de novo* 22q11.2 deletion and schizophrenia in the context of within-family expectations on five dimensional neurobehavioral traits.



For each of five dimensional neurobehavioral constructs studied, the within-family biparental mean, indicated by the white diamonds, was set at standard deviation (SD) of 0, representing the expected [Exp] level of functioning for adult proband offspring with a de novo 22q11.2 deletion. Red lines with an asterisk indicate significant effects of biparental values on the respective dimensional phenotype expressed in probands (details in **Tables 1 and 3**). Green diamonds are centered at the average estimated effect size (in SDs) of the de novo 22q11.2 deletion [D] on each phenotype, using results for affected adult offspring with 22q11.2DS and no psychotic illness. Purple diamonds are centered at the average estimated effect size (in SDs) of 22q11.2DS and schizophrenia [+Sz], on each phenotype. Details about these effect size SDs are presented in the manuscript text. For simplicity sake, the sizes of all diamond shapes were kept consistent; while pictorially representing general inter-individual variability, they do not represent confidence intervals. Individual within-

family results that indicate the preserved relationship between probands and parents, regardless of cognitive level, are shown for FSIQ in **Figure 2**.

Phenotypic impact of parental phenotypes within families

Within-family analyses showed highly significant correlations between proband and parental values for each of the three IQ parameters (**Table 1, Figure 1, Figure 2**), with effects evident within both the non-psychotic and schizophrenia subgroups (**Supplemental Table 1**).

A linear regression model explained 37.7% of the variance in proband FSIQ (p<0.001, **Table 3**), with parental FSIQ and schizophrenia showing independent significant contributions. Results for VIQ were similar with the model explaining 37.7% of the variance for probands (**Table 3**). For proband PIQ, the model explained a somewhat lower proportion of the variance (25.5%) and schizophrenia did not reach significance (**Table 3**). For all three models, parental IQ had the largest effect on proband IQ, and proband sex and age were non-significant factors (**Table 3**).

In contrast to the cognitive traits studied, the within-family proband-parent correlation results for the social and motor phenotypes, although in the expected direction, did not reach significance (**Table 1; Supplemental Table 1**).

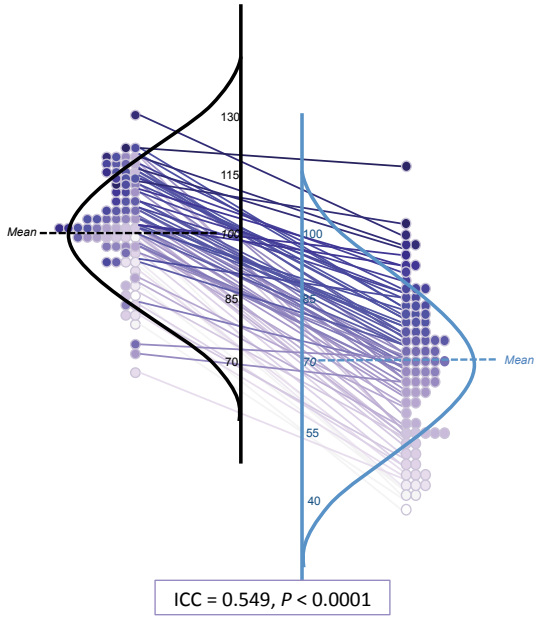


Figure 2. Within-family proband-parent correlation of FSIQ for adults with a *de novo* 22q11.2 deletion

Details of within-family FSIQ data for adult probands with a de novo 22q11.2 deletion, each purple colored circle representing one family (total n=77), ordered by proband FSIQ level (deepest intensity, highest FSIQ), with the same colored circle used for the corresponding biparental FSIQ result and within-family connections indicated by straight lines between each proband-parent pair. These data are superimposed on schematic depictions of their normalized distributions, for adult probands (blue, right) and their unaffected parents (black, left). While the results for those with a 22q11.2 deletion are on average lower, the individual datapoints and connector lines indicate the overall preserved relationship of FSIQ within families, regardless of proband FSIQ level. **Supplemental Figures 2A-2C** show the scales shifted so that idealized curves mirror each other, and present schematic representations of FSIQ distributions for the no psychotic illness and schizophrenia subgroups.

Additional analyses

For all phenotypes assessed, within-family parental (i.e., mother-father) values were significantly correlated, most strongly so for FSIQ and VIQ. The ICCs between available maternal and paternal values were: FSIQ 0.640 ($p<0.001$, n=59 pairs), VIQ 0.733 ($p<0.001$), PIQ 0.332 ($p=0.005$), SRS 0.319 ($p=0.009$, 53 pairs), and Purdue 0.368 ($p=0.003$, 53 pairs); **Supplemental Table 1** shows biparental ICC results for non-psychotic and schizophrenia subgroups.

There were no significant parental sex effects on any of the proband phenotypes, nor interaction effects between the predictor variables (probands age, sex, and schizophrenia) in regression models. Limiting the analyses to non-psychotic individuals aged ≥25 years did not impact results. Of the phenotypes assessed in probands, only IQ and Purdue results were significantly correlated with each other (FSIQ r=0.463; VIQ r=0.404; PIQ r=0.484; $p<0.001$ for each). Incorporating this into ICC analyses did not alter results.

Discussion

In this study, we combined a genotype-first approach with a within-family analysis to evaluate the relative impact of parental phenotypes, a *de novo* 22q11.2 deletion, and schizophrenia on relevant neurobehavioral traits. We chose cognitive, social and motor functioning as dimensional traits that are heritable in the general population,[10,11] implicated in major psychotic disorders,[18-20] and affected by high-risk variants including the 22q11.2 deletion.[15-17] This approach allowed us to estimate that the deleterious impact of the 22q11.2 deletion ranges in effect size from 1.39 to 2.07 SD on the phenotypes examined, and that schizophrenia exerts a further negative impact of 0.62 to 1.23 SD. These estimates are consistent with reports for idiopathic schizophrenia and risk groups.[18-20] Notably, for the cognitive measures, within-family analyses revealed that parental IQ scores maintained a significant and robust correlation to IQ scores in probands that was independent of the effects of the 22q11.2 deletion and of schizophrenia. The modifying effect of the parental phenotypes on the dimensional phenotypes of adults with a 22q11.2 deletion did not reach significance for social or motor functioning, suggesting possible differences in genetic architecture for these traits within this etiologically homogeneous high-risk population.

Relative to within-family parental expectations, the impact of the 22q11.2 deletion was strongest for PIQ, while the outcomes affected most by schizophrenia expression were VIQ, and social and motor functioning. These results are consistent with previous findings that PIQ is differentially impaired in 22q11DS from a young age, and that VIQ declines over development, but especially so in individuals who go on to develop schizophrenia.[15] Interestingly, there is other evidence from population-based data in support of a genetic

risk relationship between schizophrenia and lower PIQ. Hubbard et al. reported that the strongest polygenic risk score correlation between any quantitative cognitive phenotype and schizophrenia expression was for PIQ,[26] and Lowther et al. reported that rare structural variants were enriched in individuals with schizophrenia and differentially impaired PIQ.[27]

In the context of parental results that were comparable to general population expectations,[21,22,28] the deleterious effects of the 22q11.2 deletion on cognitive, social and motor functioning identified in the current study are in line with previous findings for the 22q11.2 deletion, in the absence of this parental context.[15-17] When accounting for the impact of the 22q11.2 deletion, the within-family proband-parent correlations for IQ approach those observed in the general population for first degree relatives.[10,29] The results are broadly consistent with, but extend those of, studies of 22q11DS with parental data that used a proxy for IQ or did not correct for within-family effects.[30,31]

In comparing our findings to those for another *de novo* pathogenic variant, the 16p11.2 deletion,[12] several observations stand out (**Supplemental Table 2**). First, although the 22q11.2 deletion and the 16p11.2 deletion both exert deleterious effects across the dimensional phenotypes assessed, the impact of the 22q11.2 deletion appears overall somewhat stronger on cognitive functioning, especially PIQ. The findings are also broadly in line with a study that modeled effect sizes of CNVs on IQ. [32] Second, the significant within-family effects of parental PIQ on proband PIQ, but not for social functioning assessed using the SRS, differ from findings for the 16p11.2 deletion.[12] This may be related to the differential impact of the 22q11.2 deletion on risk of schizophrenia and of the 16p11.2 deletion on risk of autism spectrum disorders. [33] Methodological differences could also play a role, including diverse cognitive assessment tools from the Simons autism project, younger age at assessment, and higher parental and proband IQs in the 16p11.2 deletion study.[12] Nonetheless, the findings collectively suggest that high-impact variants may have differential patterns of relative effect size on expression of neurobehavioral phenotypes, that may be related to risk of major neuropsychiatric illness, and to the degree of modification by parental background factors.

Potential implications

The findings have potential implications for clinical care and research. The robust modifying effect of parental cognitive functioning on proband cognitive functioning, regardless of the major effects of the 22q11.2 deletion or of schizophrenia expression, suggests that parental measures, and factors relevant to these measures, could be valuable in developing predictive algorithms for outcomes of individuals with this, and other, high-impact variants. Eventually, such research could be translatable to clinical settings to refine individualized predictions for patients, and possibly to suggest ameliorating strategies.[12,32,34]

The comparability of the results to those for the general population, supports the likelihood that inherited common and rare genetic variants help shape the variable expression of the cognitive phenotype in individuals with the 22q11.2 deletion [35], as they do for the schizophrenia phenotype.[36,37] Studies that include parental data could thus help determine the extent to which common variant polygenic risk, and rarer inherited or *de novo* variants explain the association between parental and proband cognitive functioning, and – importantly – the relationship of these dimensional phenotypes to schizophrenia risk.[36-38] The findings could thus inform hypotheses about shared genetic mechanisms that may underlie expression of schizophrenia and key component dimensional phenotypes, not only in the context of the threshold-lowering 22q11.2 deletion but in other at-risk populations.[4,9]

The fact that the observed within-family correlations for social and motor functioning were not significant may suggest that additional inherited (shared) variants, at least in the context of a 22q11.2 deletion, exert lesser effects on these phenotypes than in the case of IQ, where heritability is high.[29,38] Additional non-shared factors, perhaps including rare variants,[36,37,39] may play a more prominent role for motor and social traits.

For optimal predictive algorithms, future studies that incorporate the potential roles of shared and non-shared genetic and non-genetic, e.g., environmental, factors in individuals from this and other high-risk groups, are warranted [35,40]. Collectively, these will be critical for implementing precision-medicine and promise to eventually contribute to care for individuals with, and at risk for, schizophrenia and other neuropsychiatric disorders.[5]

Advantages and limitations

Simultaneously assessing multiple dimensional traits in adult probands with a *de novo* 22q11.2 deletion and their unaffected parents, and thereby combining a genotype-first approach with quantitative within-family phenotypic assessments, enabled us to disentangle the impact of this high-impact variant from the modifying effects of parental background, and of schizophrenia expression, on the phenotypes assessed. Standardized estimates of effect sizes not only allowed for comparing patterns of relative impact across phenotypes, but also for the possibility of comparisons with other samples.[12,31,41,42] The potential generalizability of the results is supported by studying probands with a *de novo* 22q11.2 deletion, only a minority of whom had schizophrenia, with results for probands similar to those for other high-risk groups,[18-20] and results for parents similar to general population expectations.[21,22,28]

The main limitation, analogous to that of similar studies,[12] relates to sample size. *De novo* proband-parent samples are challenging to recruit, especially for adults with a

high-impact variant, thus a minority of the sample comprised proband-parent dyads. Based on the statistical power available to detect the significant effects observed for IQ-parameters, we estimate the minimum detectable effect size of the sample to be ~0.3. Thus proband-parent correlations for social and motor functioning, observed to be in the expected direction but non-significant, would be predicted to be small, i.e., less than 0.3. Differences in psychometric properties could also have played a role: the SRS and PP both capture constructs that are narrower and potentially more prone to 'ceiling-effects' compared to global cognitive functioning, as assessed with the WASI-II.[6,21,22] Nonetheless, a larger number of trios could possibly have allowed the detection of smaller effect sizes.

Conclusion

The results of this study help to distinguish the major impact of the 22q11.2 deletion from the modifying effects of parental phenotypes and schizophrenia expression across heritable, dimensional neurobehavioral phenotypes that are broadly implicated in schizophrenia. Novel findings include effect size estimates of these relative effects for probands with a 22q11.2 deletion, and significant within-family correlations for cognitive, but not for social or motor, functioning. The results suggest that shared familial variation contributes to shaping the expression of the cognitive phenotype in individuals with a 22q11.2 deletion, with potential implications for schizophrenia risk for this and other high-risk groups. Improved understanding of the variable phenotypic expression of such a high-impact pathogenic variant promises to aid delineation of the genetic architecture of schizophrenia in general. Future studies using genome sequencing data will be needed to elucidate the relevant genetic mechanisms involved and which of the shared (inherited) variation identified for cognitive factors overlaps with, and which is separable from, the risk of schizophrenia. Detailed genomic and phenotypic data in the context of a known high-impact variant will complement studies of more heterogeneous samples, helping to converge on mechanisms and pathways to inform precision medicine for all.

Table 1. Dimensional neurobehavioral functioning domains in adults with a de novo 22q11.2 deletion and their unaffected parents.

Domain of functioning	Probands with 22q11DS [a]	Parents [b]	Proband–parent differences [c]					Proband–parent correlation statistics [c]	
				Paired samples t-test			Estimated effect size (SD) of the 22q11.2 deletion [c]	Intraclass correlation coefficient (ICC)	
	Mean (SD)	*Mean (SD)*	*Mean (SD)*	*t*	*df*	*p*		*ICC*	*p*
Cognitive [d]									
FSIQ	72.84 (16.61)	104.46 (13.71)	-32.03 (14.56)	-19.30	76	**5.0e-31**	- 2.14	0.549	**9.9e-08**
VIQ	78.31 (16.90)	103.06 (14.12)	-25.19 (15.65)	-14.12	76	**6.0e-23**	- 1.68	0.496	**1.9e-06**
PIQ	71.37 (16.04)	104.72 (12.81)	-33.75 (14.61)	-20.40	77	**8.7e-33**	- 2.25	0.500	**1.3e-06**
Social [e] (SRS)	67.80 (27.92)	31.43 (20.78)	37.66 (32.08)	9.17	60	**5.2e-13**	- 1.88	0.161	0.106
Motor [f] (Purdue)	24.85 (12.39)	41.60 (10.51)	-16.39 (15.05)	-9.24	71	**8.5e-14**	- 1.64	0.127	0.141

[a] For the descriptive statistics presented here, all available data for probands were used: FSIQ and VIQ n=81; PIQ n=82; SRS n=66; Purdue n = 77 (**Supplemental Figure 1**).

[b] For the descriptive statistics presented here, all available data for parents were used: FSIQ, VIQ and PIQ n=137; SRS n=116; Purdue n=128 (**Supplemental Figure 1**).

[c] For the proband-parent difference and correlation analyses, data from families where scores were available for both proband and one or both parents were used (see below and **Supplemental Figure 1** for details). Overall effect size estimates of the 22q11.2 deletion are based on within-family parental expectations (regardless of expression of schizophrenia in the proband). Figure 1 shows relative effects in non-psychotic and schizophrenia proband subgroups; details of effect sizes are presented in the text.

[d] For cognitive functioning, FSIQ and VIQ data were available for 77 families: n=58 (75.3%) complete trios (thus biparental mean was used), n = 16 (20.8%) proband-mother dyads, and n=3 (3.9%) proband-father dyads. PIQ data were available for 78 families: 59 (75.6%) complete trios (thus biparental mean was used), n=16 (20.5%) proband-mother dyads, and n=3 (3.8%) proband-father dyads.

[e] For social functioning, SRS data were available for 61 families: n=51 (83.6%) complete trios (thus biparental mean was used), and n=10 (16.4%) proband–father dyads.

[f] For motor functioning, Purdue data were available for 72 families: n=52 (72.2%) complete trios (thus biparental mean was used), n=15 (20.8%) proband-mother dyads, and n=5 (6.9%) proband–father dyads.

Bold font indicates significance at the $p < 0.001$ level.

Table 2. Dimensional neurobehavioral functioning domains in adults with a *de novo* 22q11.2 deletion with and without schizophrenia

Domain of functioning	22q11DS No psychotic illness subgroup [a]	22q11DS Schizophrenia subgroup [b]	t-test
	Mean (SD)	*Mean (SD)*	*p*
Cognitive			
FSIQ	75.51 (14.73)	63.86 (16.47)	**0.007**
VIQ	80.94 (14.37)	68.36 (17.13)	**0.005**
PIQ	73.73 (15.50)	64.50 (14.87)	**0.02**
Social (SRS)	59.19 (25.82)	83.88 (24.62)	**0.002**
Motor (Purdue)	27.69 (10.23)	15.70 (11.46)	**0.0002**

[a] Of the total n=52 probands in the no psychotic illness subgroup, data for FSIQ and VIQ were available in n=51; PIQ n=52; SRS n =43; and Purdue n=49.
[b] Of the total n=22 probands in the schizophrenia subgroup, data for FSIQ, VIQ, and PIQ were available in n=22; SRS n=16; and Purdue n=21.
Bold font indicates significance at the $p < 0.05$ level.

Table 3. Relationships between biparental IQ and proband IQ in adults with a 22q11.2 deletion, accounting for schizophrenia status, age and sex in linear regression models [a].

Dependent variable	Variable in model	Coefficient	Standard error (coefficient)	Standardized beta	t-ratio	*p*
Proband FSIQ [b]	**Biparental FSIQ**	**0.62547**	**0.111**	**0.547**	**5.658**	**3.6e-07**
	Proband schizophrenia status	**-9.651**	**3.360**	**-0.280**	**-2.872**	**5.5e-03**
	Proband age	-0.078	0.179	-0.043	-0.434	0.666
	Proband sex	2.391	3.102	0.075	0.771	0.443
Proband VIQ [c]	**Biparental VIQ**	**0.6034**	**0.111**	**0.541**	**5.454**	**7.9e-07**
	Proband schizophrenia status	**-12.121**	**3.377**	**-0.349**	**-3.589**	**6.3e-04**
	Proband age	-0.200	0.186	-0.109	-1.077	0.285
	Proband sex	3.569	3.111	0.111	1.147	0.255
Proband PIQ [d]	**Biparental PIQ**	**0.582**	**0.129**	**0.477**	**4.507**	**2.7e-05**
	Proband schizophrenia status	-5.672	3.643	-0.167	-1.557	0.124
	Proband age	0.091	0.189	0.050	0.480	0.632
	Proband sex	1.386	3.341	0.044	0.415	0.680

[a] Total of n=71 families were available for the linear regression analyses for FSIQ and VIQ, i.e., FSIQ and VIQ data for probands (no psychotic illness n=49 (69.0%); schizophrenia n=22 (31.0%)) and their parent(s). Total of n=72 families were available for the linear regression analysis for PIQ, i.e., PIQ data for probands (no psychotic illness n =50 (69.4%); schizophrenia n=22 (30.6%) and their parent(s). Mean (SD) age of the 72 probands was 26.6 (8.8) years; n=37 (51.4%) were males.

[b] The overall model for proband FSIQ was significant: R2=0.377, F = 11.6, p=3.4e-07.

[c] The overall model for proband VIQ was significant: R2=0.377, F = 11.6, p=3.5e-07.

[d] The overall model for proband PIQ was significant: R2=0.255, F = 7.1, p=8.1e-05.

Bold font indicates significance at the p< 0.01 level.

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Supplemental Materials

Supplemental Methods

Participants and procedure. All individuals with a molecularly confirmed 22q11.2 deletion and the potential availability of both biological parents were eligible for this study. Despite the potential biases to such family studies where complete trios or even dyads are challenging to recruit, especially for adult patients, the data suggest that our sample is largely representative of the overall population of individuals with the 22q11.2 deletion. Historically, the focus of the clinic (and longitudinal study) through which the participants were recruited was congenital cardiac abnormalities, medical genetics sources, and psychiatric illness. Over time, the center has evolved into a nation-wide specialty clinic for adults with the 22q11.2 deletion, regardless of the phenotypic expression. The participants in this study, therefore, vary in terms of phenotypic expression, including level of overall cognitive functioning (ranging from IQ 44 – 128), as is characteristic of the 22q11DS population.

The *de novo* status of the 22q11.2 deletion was confirmed through genetic testing of both parents for 74 probands; status was deemed probable *de novo* for the remaining 8 probands given that the unavailable co-parent had no features consistent with 22q11DS [1]. Of the 79 families, 73 probands had a confirmed de novo status, and the remaining 6 probands were deemed de novo.

Clinical research diagnoses including schizophrenia spectrum disorders were made by experienced clinician-scientists [2], using DSM criteria and information from the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (SCID-IV), direct observation, collateral history from family members, and available lifetime medical records, as previously described [2,3]. For the current study, we derived DSM-5 diagnoses.

Assessment instruments. The Wechsler Abbreviated Scale of Intelligence, second edition (WASI-II) is designed to provide a brief and accurate assessment of IQ in individuals ranging in age from 6 to 90 years. It has sound psychometric properties and is used for general population and clinical samples, including those with intellectual disabilities or neurodevelopmental disorders.

For the Social Responsiveness Scale-II (SRS), answers on specific items can range from 1 (not true) to 4 (almost always true). Parents first completed the SRS reporting on their offspring with 22q11DS, and then on the other parent. The raw scores of the SRS can be used to yield standardized scores that indicate social functioning ranging from normal, to mild, moderate or severe impairment.

In the Purdue Pegboard, individuals are presented with 2 cups filled with pins and two vertical rows of 25 holes. Individuals are instructed to place as many pins as possible in 30 seconds down the row on the side of their dominant hand, then their non-dominant hand, and then both hands simultaneously.

R-packages. All statistical analyses were performed using R 3.6.2 GUI 1.70, and we made use of the additional R-packages "plyr", "psych", "ICC", "irr", "pwr", "lme4", and "lm.beta".

Supplemental Table 1. Within-family correlation analyses in the non-psychotic and schizophrenia subgroups of adult probands with *de novo* 22q11.2 deletions.

	Intra-class correlation (ICC) statistics							
	Non-psychotic 22q11.2 deletion proband subgroup [a]				Schizophrenia 22q11.2 deletion proband subgroup [b]			
	Proband-parent		Biparental (mother-father)		Proband-parent		Biparental (mother-father)	
	ICC	*p*	ICC	*p*	ICC	*p*	ICC	*p*
FSIQ	0.526	**4.4e-05**	0.583	**5.0e-05**	0.668	**2.5e-04**	0.782	**1.7e-04**
VIQ	0.456	**4.4e-04**	0.655	**3.1e-06**	0.684	**1.6e-04**	0.860	**9.8e-06**
PIQ	0.475	**2.1e-04**	0.335	**0.019**	0.506	**6.8e-04**	0.251	0.174
SRS	0.124	0.223	0.420	**0.005**	0.112	0.340	-0.130	0.664
Purdue	0.043	0.387	0.394	**0.009**	0.332	0.071	0.246	0.209

[a] Of the total n = 52 probands in the non-psychotic subgroup, proband-parent data were available for ICC analyses for n = 49 families for FSIQ and VIQ; n = 50 families for PIQ; n = 39 families for SRS; and n = 46 families for Purdue. Biparental (mother-father) ICC analyses included n = 38 pairs for FSIQ, VIQ, and PIQ; n = 35 pairs for SRS and Purdue.

[b] Of the total 22 probands in the schizophrenia subgroup, proband-parent data were available for ICC analyses for n = 22 families for FSIQ, VIQ, and PIQ; n = 15 families for SRS; and n = 20 families for Purdue. Biparental (mother-father) ICC analyses included n = 15 pairs for FSIQ, VIQ, and PIQ; n = 12 pairs for SRS and Purdue.

Bold font indicates significance at the $p < 0.05$ level.

Supplemental Table 2. Parent-proband functioning results across five dimensional neurobehavioral phenotypes – Current and previous studies of *de novo* 22q11.2 deletion and *de novo* 16p11.2 deletion.

	FSIQ				VIQ				PIQ			
	Parental scores Mean (SD)	Proband scores Mean (SD)	Deletion impact (SD)	Proband-parent correlation	Parental scores Mean (SD)	Proband scores Mean (SD)	Deletion impact (SD)	Proband-parent correlation	Parental scores Mean (SD)	Proband scores Mean (SD)	Deletion impact (SD)	Proband-parent correlation
Current study (22q11.2 deletion)[a]	104 (14)	73 (17)	-2.1	ICC = 0.55 **p = 9.9e-08**	103 (14)	78 (17)	-1.7	ICC = 0.50 **p = 1.9e-06**	105 (13)	71 (16)	-2.3	ICC = 0.50 **p = 1.3e-06**
2014 22q11.2 deletion study[b]	101 (12)	72 (14)	-1.9[d]	* **p < 0.01**	-	-	-	* **p < 0.01**	-	-	-	* **p < 0.01**
2015 16p11.2 deletion study[c]	112 (10)	86 (15)	-1.7	ICC = 0.42 **p = 0.03**	108 (9)	83 (17)	-1.6	ICC = 0.53 **p = 0.003**	114 (10)	88 (17)	-1.7	ICC = 0.20 p = 0.21

	Social (SRS)				Motor (Purdue)			
	Parental scores Mean (SD)	Proband scores Mean (SD)	Deletion impact (SD)	Proband-parent correlation	Parental scores Mean (SD)	Proband scores Mean (SD)	Deletion impact (SD)	Proband-parent correlation
Current study (22q11.2 deletion) [a]	31 (21) [e]	68 (28)	-1.9	ICC = 0.15 p = 0.11	42 (11)	25 (12)	-1.6	ICC = 0.13 p = 0.14
2014 22q11.2 deletion study[b]	-	-	-	-	-	-	-	-
2015 16p11.2 deletion study[c]	30 (18)	75 (33)	-2.2	ICC = 0.52 **p = 0.009**	42 (9)	30 (16)	-1.3	ICC = 0.21 p = 0.22

[a] Current study; Maximum n = 82 probands with a *de novo* 22q11.2 deletion, mean age = 27.2 (9.0) y; see details in text, Table 1 footnote, and eFigure 1 for details of sample sizes for within-family correlation analyses for each phenotype assessed; ICC results include probands with psychotic illness

[b] Olszewski et al., 2014[4]; Maximum n = 69 probands with a *de novo* 22q11.2 deletion, mean age = 18.0 (2.2) y; ICC results include probands with psychotic illness/symptoms. * No biparental data were reported in the Olszewski et al., 2014 study; correlation coefficient R values reported for proband-mother data were: FSIQ R = 0.599, VIQ R = 0.546, PIQ R = 0.437. Note that R is not corrected for within-family effects, hence is not directly comparable to ICC values.

[c] Moreno-De-Luca et al., 2015[5]; Maximum n = 54 probands with a *de novo* 16p11.2 deletion, reported age >2 y; ICC results include probands with autism spectrum disorder

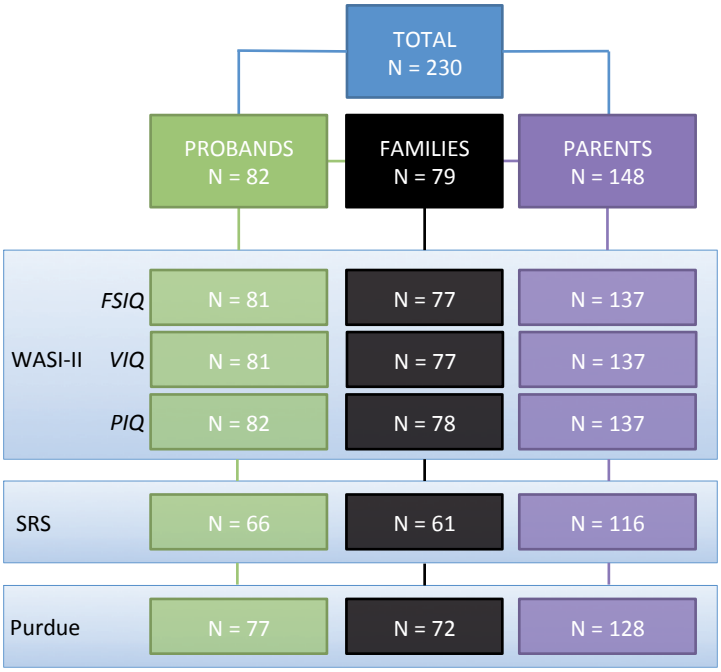
[d] Estimated from data reported in Olszewski et al., 2014

[e] Standardized SRS T-scores: Of the biparental SRS results, 90.2% were within "normal limits" based on population norms; of the proband SRS results, 48.4% were within "normal limits", 46.9% were in the "mild-to-moderate", and 4.7% were in the "severe" range.

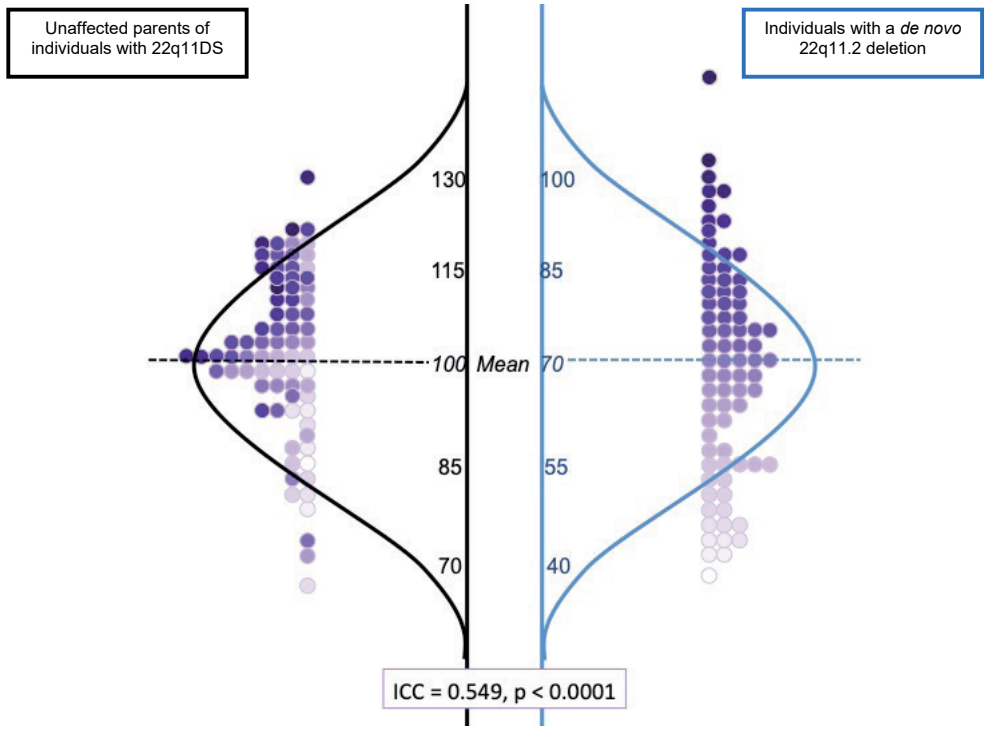
Bold font indicates statistical significance at the $p < 0.05$ level.

NB. General population means for FSIQ, VIQ, and PIQ = 100 (SD = 15); for SRS mean = 30 (SD = 20); and for Purdue mean = 50 (SD = 10).

Supplemental Figure 1. Flowchart of participants in the study overall and per dimensional phenotype.

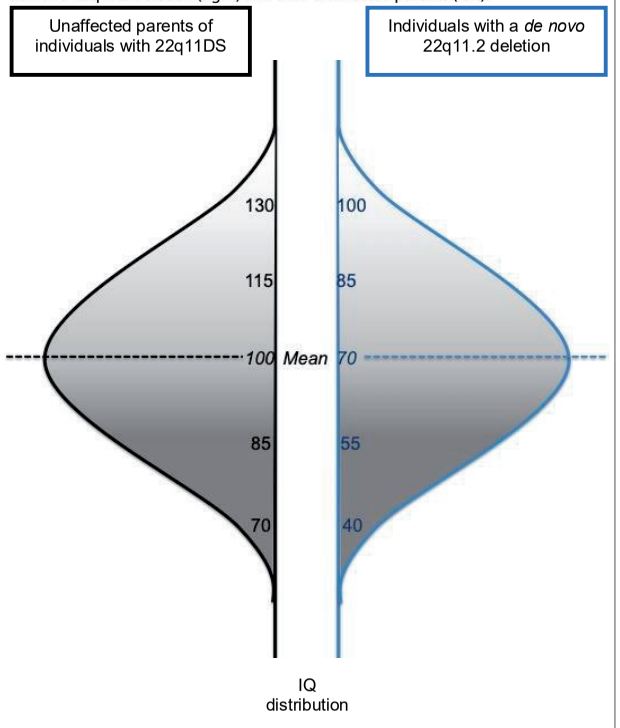


Supplemental Figure 2A. Detailed within-family FSIQ distributions for adult probands with *de novo* 22q11.2 deletions and their unaffected parents data-point color intensity corresponding to proband FSIQ, with idealized distribution curves in mirrored position (see Figure 2 for further details and within-family connector lines).



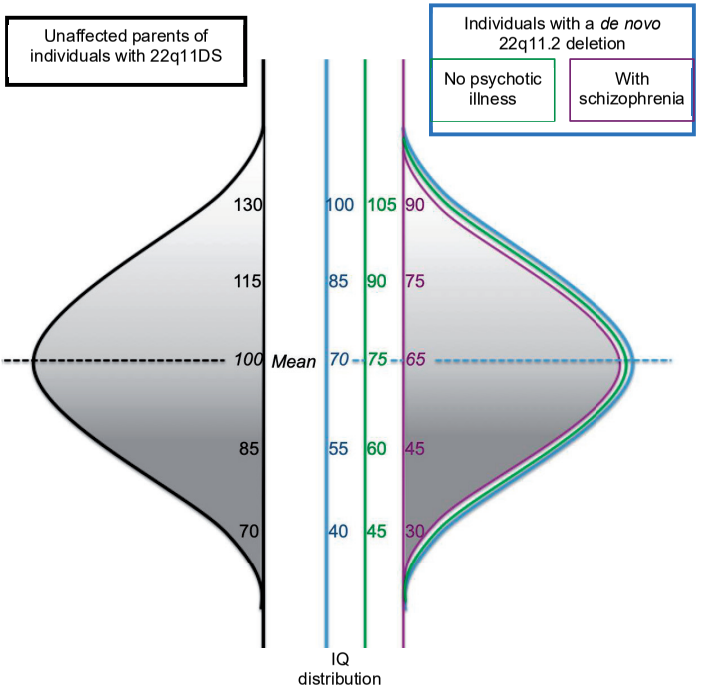
Supplemental Figures 2B and 2C. Idealized schematic representations of FSIQ distributions of probands with *de novo* 22q11.2 deletions and unaffected parents, overall (2B), and probands in no psychotic illness and schizophrenia subgroups (2C).

2B. Idealized schematic depiction of distribution of FSIQ in adult probands with a *de novo* 22q11.2 deletion (right) and their unaffected parents (left).



Despite the shifted IQ-scale in the individuals with a 22q11.2 deletion (average ~30 IQ points), the distribution is not shifted randomly but closely corresponds to the distribution of IQs of their respective parents.

2C. Idealized schematic depiction of distribution of FSIQ in adult probands with a *de novo* 22q11.2 deletion (right), with no psychotic illness, and with schizophrenia, and their unaffected parents (left).



As for the total group of individuals with a 22q11.2 deletion, the significant correlation between parental and proband IQ is preserved, both in those with schizophrenia (mean IQ ≈ 65, SD 15), and in those with no psychotic illness (mean IQ ≈ 75, SD 15).

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CHAPTER 7

7

Using Common Genetic Variation to Examine Phenotypic Expression and Risk Prediction in 22q11.2 Deletion Syndrome

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Abstract

The 22q11.2 deletion syndrome (22q11DS) is associated with a 20 – 25% risk for schizophrenia. In a cohort of 962 individuals with 22q11DS we examined the shared genetic basis between schizophrenia and schizophrenia-related early trajectory phenotypes: subthreshold symptoms of psychosis, low baseline intellectual functioning, and cognitive decline. We studied the association of these phenotypes with two polygenic scores, derived for schizophrenia and intelligence, and evaluated their use for individual risk prediction in 22q11DS. Polygenic scores were not only associated with schizophrenia and baseline IQ, respectively, but schizophrenia polygenic score was also significantly associated with cognitive (verbal IQ) decline and nominally associated with subthreshold psychosis. Further, comparing the tail-end deciles of the schizophrenia and IQ polygenic score distributions, 33% versus 9% of 22q11DS subjects had schizophrenia, and 63% versus 24% had intellectual disability. Collectively, these data show both a shared genetic basis for schizophrenia and schizophrenia-related phenotypes, and highlight the future potential of polygenic scores for risk stratification among individuals with highly, but incompletely, penetrant genetic variants.

Introduction

While schizophrenia (SZ) is typically diagnosed in late adolescence or early adulthood, it is now well established that the first psychotic episode is in fact a manifestation of an advanced stage of this illness.[1] Early behavioral, cognitive and neuroanatomic changes are measurable prior to the first psychotic episode.[2-6] Both lower cognitive ability early in life (the estimated premorbid deficit is 8 IQ points),[7] as well as cognitive decline in early adolescence (estimated IQ-change equal to -1.09 standard deviation),[8] are associated with schizophrenia, with effect sizes in the range of 0.4 to 0.5.[7-13] In addition, subthreshold psychotic symptoms in youth also index increased risk for schizophrenia.[14-16] These observations raise an important question: Do early cognitive phenotypes and subthreshold symptoms of psychosis share a substantial genetic basis with either schizophrenia or intellectual ability?

Early schizophrenia-related phenotypes and trajectories are difficult to study, requiring longitudinal follow-up of large cohorts to capture a sufficient number of schizophrenia cases. At-risk populations facilitate such studies, as fewer individuals need to be followed to obtain the same number of cases. The 22q11.2 deletion syndrome (22q11DS), increasingly identified around birth, provides one such at-risk population,[17] given the associated 20 – 25% risk to develop schizophrenia.[18,19]
Findings from 22q11DS studies reproduce observations related to schizophrenia in the general population, thereby supporting 22q11DS as a genetic model of schizophrenia, including its early trajectory.[20] In 22q11DS, as in the general population, subthreshold psychotic symptoms,[21] low baseline intellectual ability and increasing cognitive deficits over time, particularly in verbal IQ,[22] are all associated with increased risk of subsequent psychotic illness.

A large fraction of the heritability of schizophrenia comes from a polygenic burden of multiple common variants, each of small effect.[23,24] Increasingly, polygenic scores derived from genome-wide association studies (GWAS) have been used to study the genetic relationship between phenotypes.[25] For schizophrenia, a polygenic score using recent GWAS explains up to 7% of the variance on the liability scale. Similarly, polygenic scores for general cognitive function, or proxies thereof, explain 2.5 - 4.3% of its variance.[26,27] Polygenic scores can also be used for phenotype prediction and hence risk stratification.[28] In the general population, they are not yet particularly effective as individual risk predictors[29] given the relatively low population prevalence of phenotypes such as schizophrenia and intellectual disability (ID), and the still modest effect sizes conferred by polygenic scores.[30] However, in high-risk populations such as 22q11DS, the same effect size acts upon a higher baseline prevalence (e.g., 25% for schizophrenia), which may allow for more substantial differences in absolute risk.[31]

The International 22q11.2 Deletion Syndrome Brain Behavior Consortium (IBBC) has assembled the largest genotype-phenotype dataset of individuals with 22q11DS.[20] Previously, the IBBC has reported on genetic associations of both common and rare variants in 520 individuals with 22q11DS, exclusively focusing on schizophrenia.[32] The current study presents several novel analyses, conducted in a substantially larger cohort of individuals with 22q11DS (N = 962) and including longitudinal IQ data. Our main objectives were twofold. First, to study the genetic relationships between schizophrenia and schizophrenia-related phenotypes of low baseline intellectual ability, cognitive decline and subthreshold positive psychotic symptoms. Second, to examine the use of polygenic scores for schizophrenia and IQ for individual risk prediction of schizophrenia and intellectual disability (ID; IQ<70) in individuals with 22q11DS.

Results

Description of dataset

After applying phenotypic classification and performing genotype quality control, data from 962 IBBC cohort members were available for analysis (**Table 1, Methods**). Within this cohort we distinguished those with Schizophrenia Spectrum Disorder (SSD; N = 207),[20] subthreshold psychotic symptoms; N = 158), and those with neither phenotype, grouped into "putative controls" (age <25 ("putative" given the typical age at onset of schizophrenia),[33] N = 382) and "definite controls" (age >= 25, N = 215). Subsequently, we refer to all controls regardless age as "merged controls" (N = 597). Baseline Full Scale IQ (FSIQ) was transformed to z-score as previously described,[22] with an average near 0 (0.03; **Table 1**). VIQ decline, operationalized as exceeding –1 SD (binary), occurred in 5.9% of the cohort.

Table 1. Clinical characteristics of sample cohort.

	SSD	Sub-threshold psychosis	Putative control	Definite control	All	p[c]
N max	207	158	382	215	962	N/A
Sex %M	49	49	54	39	49	0.008
Age at last assessment*	31.6 (12.7) [205], {7,64}	17.9 (5) [158] {8,36}	15.2 (4.6) [382] {5,24}	36.8 (9.9) [215], {25,67}	24 (12.4) [960], {5,67}	$2.0x10^{-167}$
Baseline FSIQ*[a]	-0.34 (0.87) [145], {-2,2}	0.13 (0.96) [127] {-2,3}	0.07 (1.03) [308] {-3,2}	0.24 (0.85) [120], {-2,2}	0.03 (0.97) [700], {-3,3}	$1.8x10^{-6}$
Binary VIQ Decline**[b]	11.9% [59]	5.7% [87]	4.5% [198]	4.7% [43]	5.9% [387]	0.21
Co-morbid mood disorders**	41% [144]	29.2% [154]	16% [363]	38.6% [153]	27.1% [814]	$1.7x10^{-10}$

* quantitative phenotypes are given as mean (SD) [N] {range}, ** binary phenotypes are given as percent true [N], [a] Baseline FSIQ is given as a z-score using previously defined normalization procedure,[22] [b] Binary VIQ decline is operationalized as VIQ decline > -1SD (i.e. 1 z-score), as defined by the reliable change index,[56] [c] p-values are from an ANOVA of phenotype by group and are two-sided and not corrected for multiple comparisons.

Polygenic scores and relationships between schizophrenia, IQ and associated phenotypes

First, we examined known associations. We constructed polygenic scores for schizophrenia[24] (PS_SZ) and intellectual ability[34] (PS_IQ) using standard methods, and performed statistical analyses using either linear or logistic regression as appropriate, adjusting for age, sex and the first five principal components from the imputed genotypes[32] (**Methods**). We observed a significant association between SSD cases versus controls and PS_SZ (N = 802, $p = 4.37x10^{-8}$, marginal Nagelkerke pseudo-$r^2 = 0.053$; p-values reported in the text are nominal), and a similar result when including definite controls only (N = 420, $p = 1.89x10^{-6}$, $r^2 = 0.071$) (**Table 2, Extended Data Figure 1**), corroborating previous reports from the comprehensive IBBC genetic analyses related to schizophrenia in 22q11DS[32]. We also observed a significant association between baseline FSIQ and PS_IQ ($p = 1.08x10^{-7}$), and consistent with the known genetic correlation between schizophrenia and IQ (r2g = -0.234)[26], observed a nominal association between baseline FSIQ and PS_SZ (p = 0.018) as well as a significant association between SSD and PS_IQ ($p = 7.15x10^{-4}$).

Next, we assessed relationships between schizophrenia-related phenotypes and the polygenic scores. We observed a decreasing trend of PS_SZ for phenotypes of SSD (mean = 0.23), subthreshold psychosis (mean = 0.16), putative controls (mean = -0.05), and definite controls (mean = -0.27) (**Figure 1**). PS_SZ was nominally significantly higher in those with subthreshold psychosis compared to the merged control groups (N = 755, p = 0.0247, $r^2 = 0.01$, **Table 2, Figure 1**). Finally, we observed a significant association between VIQ decline and PS_SZ ($p = 5.09x10^{-3}$, **Figure 2**). Neither the association between subthreshold psychosis and PS_IQ (p = 0.056), nor between VIQ decline and PS_IQ (p = 0.658), reached statistical significance.

Investigations into the relationship between subthreshold psychosis and PS_SZ

Post-hoc, we performed three analyses to additionally explore the observed association between subthreshold psychosis and PS_SZ. First, given that some fraction of individuals with subthreshold psychosis will eventually develop SSD, we modelled what proportion would need to develop SSD to be consistent with our findings (**Methods**). Findings showed that observed levels of PS_SZ are consistent with a scenario in which 86% (95% CI 56 - 100%) of individuals with subthreshold psychosis would in fact represent future SSD patients who were not yet identified as such at the time of the assessment. This

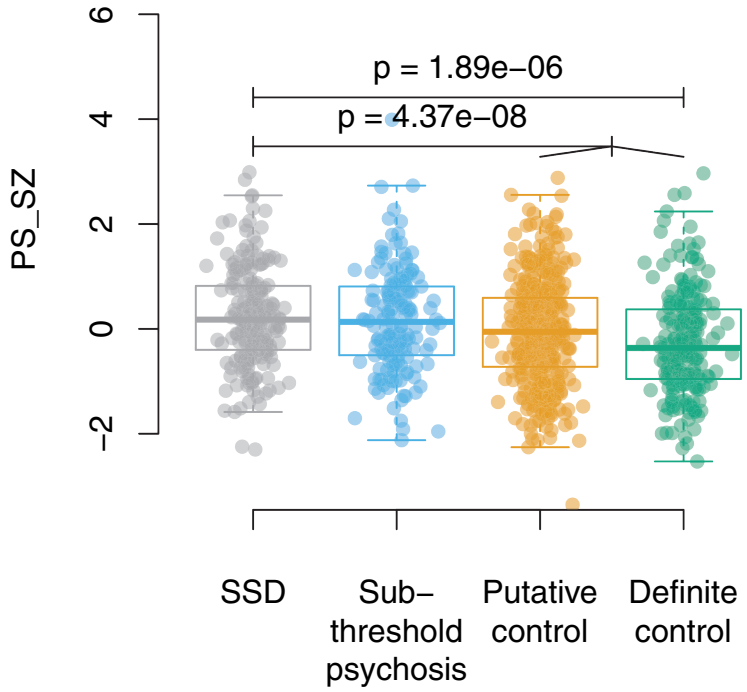
is a proportion inconsistent with known rates of SSD in 22q11DS (see **Extended Data Figure 2**), rendering it unlikely that our result is driven by "future" SSD cases. Second, we examined whether our observation could be due to confounding through psychiatric comorbidity genetically correlated with SSD (**Methods**). In this sample, the rate of comorbid mood disorders in the subthreshold psychosis group was 29.2%, versus 22.7% in the merged controls (**Table 1**). Results from this mediation analysis indicated a lack of attenuation through the mood disorder phenotype (effect size of PS_SZ in model without mood disorder is 0.239, p = 0.025; with mood disorder 0.250, p = 0.021) (**Supplementary Table 1**), indicating that the observed increased PS_SZ in subthreshold psychosis is not readily explained by the higher rate of mood disorders in this group. Third, as a source of additional evidence, we explored the use of residual quantitative variation in the measure of subthreshold psychosis, through a transformed quantitative measure of this phenotype (Structured Interview for Prodromal Syndromes, (SIPS[35])) (**Methods**). When adjusting for the previous binary indicator of subthreshold psychosis versus control, the association between the transformed quantitative SIPS phenotype and PS_SZ was not significant (N = 347, p = 0.77, r^2 = 0.0001; **Supplementary Figure 1**).

Table 2. Key regression results.

Dependent variable	IV	N	beta	r^2	p
SSD	PS_SZ	802	0.56	0.053	4.37×10^{-8} *
Subthreshold psychosis		755	0.24	0.01	0.0247
Baseline FSIQ		720	-0.096	0.0077	0.018
VIQ decline		396	0.66	0.051	0.00509 *
SSD	PS_IQ	802	-0.30	0.020	7.15×10^{-4} *
Subthreshold psychosis		755	-0.18	0.0072	0.056
Baseline FSIQ		720	0.20	0.038	1.08×10^{-7} *
VIQ decline		396	-0.096	0.0013	0.658

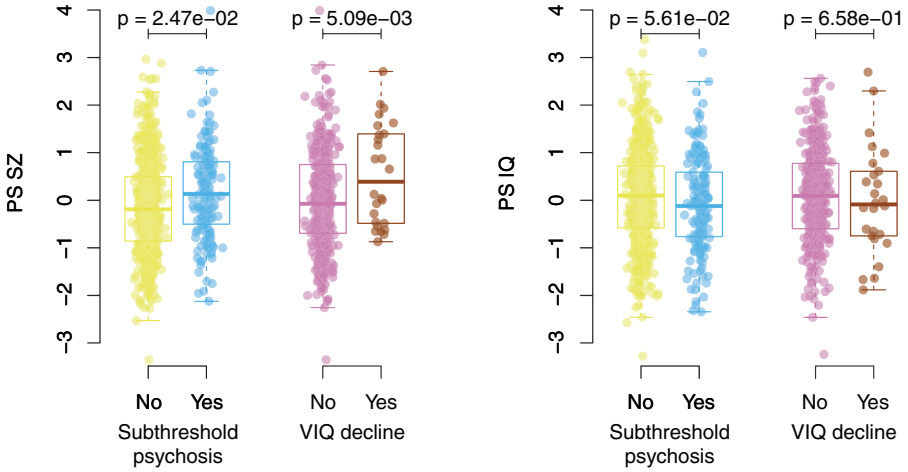
Results are adjusted for standard covariates as described in Methods. Beta is the standard regression effect size estimate. r2 denotes difference between model fit with or without independent variable, using either standard r2 from linear regression or Nagelkerke pseudo-r2. Nominal p-values are reported. Asterisk (*) indicates significant result after Bonferroni correction for 8 independent main analyses in this study (two polygenic scores, four phenotypes). IV = Independent Variable.

Figure 1. Schizophrenia polygenic scores (PS_SZ) among phenotypic subgroups.



Results show per-individual values as well as summaries per group, where minimum and maximum values are directly observable from the plot, the box-plot centre is the median, the boxplot edges represent the 25th and 75th percentiles, and the whiskers represent the lesser of the distance to the minimum or maximum value, or 1.5 times the inter-quartile range. Associations of PS_SZ in 22q11DS with SSD, subthreshold psychosis, putative controls and definite controls. Results for PS_SZ and SSD have been reported previously,32 and are included in this figure for completeness. Total sample sizes for the highlighted associations are N=423 (SSD versus putative controls) and N=802 (SSD versus merged controls). p-values are reported for select comparisons two-sided logistic regression analyses uncorrected for multiple testing using covariates as specified in the Methods.

Figure 2. Relationship between polygenic scores and novel phenotypes



Results show per-individual values as well as summaries per group, where minimum and maximum values are directly observable from the plot, the box-plot centre is the median, the boxplot edges represent the 25th and 75th percentiles, and the whiskers represent the lesser of the distance to the minimum or maximum value, or 1.5 times the inter-quartile range. Results are shown for regressions of subthreshold psychosis versus merged controls (N = 755) and VIQ decline (N = 396) for both PS_SZ (left panel) and PS_IQ (right panel). p-values are reported for two-sided logistic regression analyses uncorrected for multiple testing using covariates as specified in the Methods.

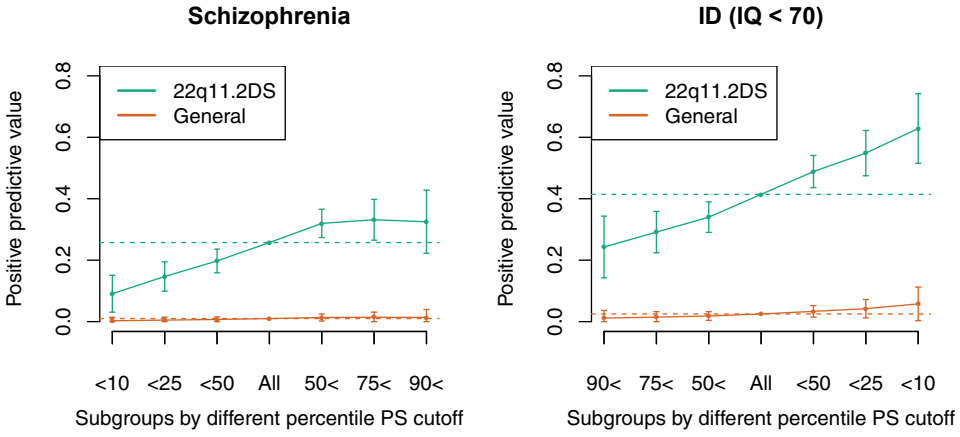
Polygenic score and individual risk prediction

Addressing the second objective of our study, we investigated the extent to which polygenic scores could be used for individualized risk prediction among subjects with 22q11DS. We divided the cohort into quantiles based on polygenic scores, and calculated positive predictive values (PPVs) in each. For SSD, 32% of individuals with scores above the median PS_SZ had SSD (i.e., a PPV of 32%), versus 20% of those with scores below the median (OR = 1.91, 95% CI = [1.38, 2.64], p = 8.4×10^{-5}) (**Figure 3**, **Supplementary Table 2**). Values at the tails showed more extreme differences, with those exceeding the 90th percentile at substantially higher risk (33%) than those in the lowest decile (9.1%). Simulating an effect for the general population, using the observed effect sizes and assuming a general population prevalence of SSD of 1%, generated substantially smaller absolute differences (**Figure 3**).

Similarly, using intellectual ability as a binary outcome (intellectual disability (ID) as IQ < 70), we observed a higher rate of ID among those with a PS_IQ below the median versus above (PPV = 49% vs 34%, OR = 1.85, 95% CI = [1.37, 2.51], p = 7.1×10^{-5}). This effect is accentuated at the tails, with PPVs of 63% for those in the lowest decile for PS_IQ (i.e.

associated with lower IQ in the general population), versus 24% for those in the highest decile of PS_IQ (**Figure 3, Supplementary Table 3**).

Figure 3. Individual risk prediction.



PPVs (y - axis) for SSD (left panel) and ID (right panel) based on various cut-offs of PS_SZ (left panel) or PS_IQ (right panel). Colors differentiate values from the 22q11DS cohort (turquoise) versus values estimated from the general population (orange) given observed prevalences in the population (SSD = 0.01, ID = 0.025; dotted lines) and observed odds ratios. Whiskers represent confidence intervals (+/- 1.96 * standard error) about the central PPV estimate.

Discussion

In this work, we used polygenic scores from large GWAS for schizophrenia and IQ both to better understand the association between schizophrenia and schizophrenia-associated phenotypes, as well as to assess their potential for individual risk prediction. In the first part of the study, we confirmed several results known to occur in the general population, and showed for the first time that known relationships between schizophrenia and IQ extend to individuals with 22q11DS. We observed that a polygenic score for IQ explained ~3.8% of the variance in IQ in 22q11DS, suggesting the previously observed association between parental educational attainment and cognitive outcome in offspring with 22q11DS[36] may be at least partly explained by common variants.

In addition, we identified two novel associations between schizophrenia-related phenotypes and schizophrenia. First, we observed a novel association between subthreshold psychosis and PS_SZ. Given the nominal statistical significance of this observation, we performed several post-hoc investigations to rule out potential confounding sources, showing that neither undiagnosed "future" cases, nor comorbid

mood disorders in our samples, can explain the observed signal. We also examined residual quantitative variation in subthreshold psychosis and found that the association between this transformed quantitative variable and PS_SZ was not significant when adjusting for the previous binary indicator of these phenotypes. However, it is worth noting that a priori power for this analysis was limited and dependent on strong assumptions. Interestingly, studies on genetic correlations between subthreshold psychotic symptoms and PS_SZ in the general population reported to date are conflicting,[37-39] impeding definite evidence in this regard. Regarding subthreshold psychotic symptoms and schizophrenia in 22q11DS, we conclude that our findings tentatively suggest a genetic correlation, but that further studies are required to provide more certainty.

In addition, we observed a novel, significant association between VIQ decline and PS_SZ, but not PS_IQ, suggesting that common risk variants for schizophrenia contribute to cognitive decline, while common variants associated with cognitive ability might not. A possible implication of these results is that cognitive decline prior to the first psychotic episode may not merely be a risk factor for schizophrenia, as reported previously for 22q11DS[22] and idiopathic schizophrenia,[40,41] but also shares its genetic underpinnings. A previous study in a subset of this cohort showed that cognitive decline preceded the onset of the first psychotic episode by several years,[22] making reverse causation – i.e., cognitive decline as a consequence of psychosis – a less likely explanation. The observed cognitive decline in 22q11DS could be caused by the inability of patients to keep up with peers, or alternatively, represent an absolute loss of cognitive abilities, or a combination of both. The current analyses do not distinguish between these, but prior studies in 22q11DS have found evidence in support of both mechanisms.[42,43] We cannot fully exclude the possibility that the observed cognitive decline could be impacted by the negative effect of psychosis on cognitive testing. However, this is an unlikely explanation given that all study sites refrained from assessing subjects when acutely psychotic, as is common clinical policy. Furthermore, in our data the mean age at IQ assessment is below the age at psychosis onset for both baseline (14.8 and 20.6 years respectively) and longitudinal IQ data (18.2 and 20.3 years respectively). In addition, in those without psychosis, 55% show an IQ decline, versus 45% stable or increase ($p = 0.02$), indicating that on average, a modest cognitive decline can be observed in 22q11DS regardless of the occurrence of a psychotic disorder, as previously reported.[22] Taken together, our findings are consistent with the notion that disruption of normal cognitive development is a core component of schizophrenia,[11] and investigation of high-penetrance variants for both phenotypes offers important insights into its mechanism.

In the second part of the study, we examined to what extent polygenic scores could be used for individual risk prediction of SSD and ID among individuals with 22q11DS. Whereas in research the existence of association between test and outcome is most relevant, in clinic

the positive predictive value (PPV) is key, as it enables stratification of individuals into groups with different outcome probabilities that can inform clinical decision-making.[30,44] Previous studies have shown that high-risk copy number variant (CNV) carriers with schizophrenia have increased polygenic scores,[45,46] including specifically 22q11DS,[32,45] but have not looked at stratification within those groups. Importantly, PPV depends not only on the strength of association, but also on the baseline prevalence. Here we examined risk stratification among individuals with 22q11DS, taking advantage of the higher baseline prevalence of schizophrenia and ID compared to the general population (in our sample, 23% and 41%, respectively). Among those in the highest PS_SZ risk decile, 33% had schizophrenia, versus 9% in the lowest decile. Applying the same effect sizes to the general population would yield estimates of 1.5% and 0.3%, respectively. Similarly, 63% of those in the lowest PS_IQ decile had ID, versus 24% in the highest decile.

The observed differences between PPVs in our study are similar to those previously reported for BRCA1 and BRCA2 among females for breast cancer risk[31] and males for prostate cancer risk.[47] The concept of using polygenic background to inform individual risk prediction and clinical decision-making is an area of active investigation,[48] and is being incorporated into clinical trials for common medical conditions (e.g.,[49]). While our findings highlight the potential clinical utility of polygenic scores in the context of a high-penetrance variant like 22q11DS, the PPVs reported here are not yet sufficient to impact clinical decision-making at present. In addition, while risk prediction enables stratification within high-risk populations, it is important to note that the reduction in risk of those in the lowest risk strata within the 22q11DS population does not bring them to population risk levels. At present, compared to the general population, increased risk for certain outcomes remains a clinical reality for all patients with 22q11DS, regardless of PS results. However, as ever-increasing GWAS size improves the strength of PS associations, we suggest that PS may have clinical utility in risk models in the near future,[29] particularly in sub-populations selected for *a priori* increased baseline risk, such as patients with a high-impact mutation like 22q11DS or those with behaviorally defined subthreshold symptoms.[50] Pending more substantial PS effect sizes, as well as robust replication of findings reported here, there are several areas of potential future clinical utility. For example, in the 22q11DS population, elevated PS_SZ could be a reason to further intensify monitoring during adolescence, and PS_IQ may play a role in seeking to prevent misalignment between academic potential and demands.[51] Taken together, our findings highlight the potential clinical utility of polygenic scores in the context of a high-penetrance variant.

Further, estimating risk raises important ethical questions, which require careful consideration. For instance, in the absence of preventative interventions that can alter outcomes such as schizophrenia or ID, it will be essential to examine the balance between benefit and potential harm of exposing caregivers and patients to such information.

Studies are required to examine to what extent early risk knowledge can be used to improve outcomes.[1] Findings like those reported here should prompt a broad societal discussion about the ethical framework in which they can be used.

While this 22q11DS cohort is the largest ever reported, there are limitations to the work shown here. Recruitment into the IBBC cohort is not random so there will be ascertainment biases, which will affect prevalence estimates, but are not expected to substantially impact the interpretation of the genotype-phenotype results reported here. For all analyses, given the current lack of transferability of polygenic score results across genetic backgrounds,[52] and that GWAS for schizophrenia and IQ are only sufficiently large within European populations to be powerful, our results were limited to 22q11DS subjects of European descent. Future large GWAS from diverse backgrounds, and methodological improvements, will allow for analyses in more diverse cohorts. In addition, other uncaptured environmental variables are likely to modulate risk among 22q11DS carriers, and should therefore be included in future studies. Finally, from a multiple testing standpoint, we intentionally restricted the main investigation of schizophrenia and associated phenotypes and polygenic scores to eight tests. Nonetheless, two of the associations, including one of the novel associations, were only nominally significant, necessitating further investigations for more definitive evidence.

In conclusion, common variants associated with schizophrenia risk and IQ variability in the general population modify expression of these phenotypes in 22q11DS. Verbal IQ decline, and subthreshold psychosis at least partly share genetic underpinnings with schizophrenia, highlighting shared causal pathways between these phenotypes. Furthermore, in 22q11DS carriers polygenic scores enable stratification into high and low risk groups substantially in excess of what would be found in a general population setting. We suggest that in populations with high-risk rare pathogenic genetic variants such as 22q11DS, this approach is nearing a level of differentiation required for clinical utility.

Methods

Dataset

All individuals in this study were carriers of the 22q11.2 deletion, confirmed by Multiplex Ligation-dependent Probe Amplification[53] as described previously.[32] All participants were recruited by one of 22 international IBBC sites (total N = 1,789). Local research ethics boards provided appropriate study approval at all sites, and all individuals, as well as parents/guardians where appropriate, provided written informed consent regarding participation in this research.

Psychiatric assessment

Psychiatric assessment was performed using standardized semi-structured interviews,[20] leading to a categorization of each participant in one of the following subgroups: schizophrenia spectrum disorder (SSD), subthreshold psychosis, putative control, and definite control. SSD included schizophrenia, schizoaffective disorder and related psychotic disorders such as delusional disorder or psychotic disorder not otherwise specified, all in accordance with DSM-IV criteria, based on data obtained by semi-structured in-person interviews at each site (see[20] for case consensus procedures). Any individual who had never met criteria for any psychotic disorder diagnosis, but had endorsed clinically significant positive symptoms at any timepoint, was included in the subthreshold psychosis group. Supporting scores from various standardized assessment methods used across sites included symptom scores in the moderate to severe range, i.e., scores of 3-5 on the Structured Interview for Prodromal Syndromes, (SIPS[35]), or above 2 on the Comprehensive Assessment of At-Risk Mental States (CAARMS[54]), or of 2 or higher (probable or definite) on any of the positive symptoms on the Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS[55]). 22q11DS individuals without a lifetime diagnosis of any psychotic disorder and who had never endorsed subthreshold positive psychotic symptoms were considered controls. Given that the risk of developing schizophrenia is most elevated until age 25,[17,33] those younger than 25 years at the most recent assessment were considered "putative controls", while those aged 25 years or older at most recent assessment were classified as "definite controls" (**Supplementary Figure 2,** and demographics in **Supplementary Table 4**).

IQ values and definition of cognitive decline

We previously found baseline Full Scale IQ (FSIQ) to be a significant risk factor for subsequent schizophrenia spectrum disorder in 22q11DS, while the strongest effect size for cognitive decline was observed for verbal IQ (VIQ).[22] To remain consistent with our prior observations, we considered first available FSIQ as a measure of baseline intellectual ability and change in VIQ between the first and last available measurement as an index of cognitive decline. Given the moderate cognitive decline that occurs, on average, in this population,[42] we calculated standardized values (z-scores) derived from the normative chart on which the average IQ trajectory for the 22q11DS population is mapped. Thus, a decline represents a negative deviation from the expected decline in this population.

In seeking to operationalize a cognitive decline as a binary variable, we sought a cut-off between lenient (i.e. requiring less severe decline, but could introduce too much noise), and conservative (more severe decline, but could reduce *a priori* power). This task is further complicated by potential error variance inherent in the data collection across multiple sites, different versions of the Wechsler Intelligence Scale and different age groups.

We initially performed our analysis using a threshold of more than -0.5 SD as the cut-off for verbal IQ decline. Using this cut-off, the observed association with PS_SZ was not statistically significant (N = 396, p = 0.22, r^2= 0.006). Based on the literature on the reliable change index,[56] we subsequently revised our definition of significant change to a more stringent threshold. To minimize the chance that any observed decline was due to chance, we conservatively used the lower boundary of the reliable change index as the cut-off, i.e. defining Verbal IQ decline as a binary variable operationalized as any negative change in z-scores exceeding 1 SD difference.

Genotyping methods and principal components analysis

For a total of 1,789 individuals with a 22q11.2 deletion, phenotypic data were collected in a central consortium database and available DNA samples were genotyped at Albert Einstein College of Medicine, in New York, using Affymetrix Human 6.0 microarrays. We generated imputed genotypes from genotyping microarray data using standard methodological approaches as described elsewhere.[32] After imputation, genotype data for 992 individuals and 6,354,586 autosomal single nucleotide polymorphisms (SNPs) were available for inclusion. We retained 4.0 million (M) SNPs, which had minor allele frequency (MAF) > 10% and were not in the major histocompatibility complex (MHC; chromosome 6, 26-34 Mbp) or in the 22q11.2 region (chromosome 22, 18,820,303 to 21,489,474 bp).

For principal component analysis (PCA), we then intersected this with the available GWAS SNPs described below to yield 3.2M SNPs. We ran PCA on the 992 individuals at the 3.2M SNPs using PLINK version 1.9 release 180612,[57] which revealed between-cohort differences matching geographic ascertainment locations, but no obvious outliers for quality control (QC) or non-European ancestry (**Supplementary Figure 3**). Of the 992 individuals who met criteria for subsequent analysis, 27 did not fall into one of the four pre-specified phenotype groups: 21 individuals who were diagnosed with a mood disorder with psychotic features, but who did not meet criteria for any non-affective psychotic disorder, and 6 individuals with insufficient phenotypic data. We further removed three samples that overlapped with the CLOZUK cohort,[24] which was a component of the PGC schizophrenia GWAS, yielding a total sample for analysis of n=962 (**Supplementary Figure 4**).

Polygenic score construction

We sought out large GWAS that would enable us to generate maximally predictive polygene scores for schizophrenia and IQ. For SSD, we used published summary statistics from a schizophrenia GWAS from the Psychiatric Genomics Consortium (PGC) (max N samples = 77,096).[24] For intellectual ability / IQ, we used results from Davies *et al.*[26] from a GWAS for a general intelligence factor, or "g-factor".[58,59] However, as released GWAS statistics from this work did not contain beta coefficients, which is necessary for polygene score construction, we used summary statistics on the largest available component, i.e.

based on fluid intelligence (max N samples = 108,818) from www.nealelab.is analysis extract of the UK Biobank.[34] In this case, fluid intelligence from the general population should capture any common genetic variants in the same fashion as Full Scale IQ and Verbal IQ, and thus should serve as a suitable proxy.

We built polygene scores using PRSice2 version 2.1.2 beta[60] under default conditions, i.e. using SNPs with an INFO score >0.90, r^2 of 0.10, and distance of 250 kbp, where r^2 was calculated on the target data (i.e. this cohort). We used pre-specified p-value cut-offs for SNPs for inclusion in the polygene score based on the p-value reported in the original GWAS that maximized previously reported prediction ability. For schizophrenia we used a p-value threshold of 0.05 (from Extended Data Figure 5 in reference),[24] and for the UK Biobank Fluid Intelligence / IQ, we used 0.10 (from Supplementary Table 2 in reference, largest explained variance in 2 out of 3 analyses).[26] For the schizophrenia polygene score, there were 80,496 SNPs after clumping, while for the IQ polygene score, there were 80,557 SNPs after clumping.

A priori power analyses and estimation of cohort specific parameter values

We conducted power analyses using simulations under a liability threshold model for our primary investigations using available sample sizes, known heritabilities, genetic correlations, and assumptions regarding the nature of the relationship between schizophrenia and subthreshold psychosis**.** All simulation results assume h2_g SZ = 0.46,[61] h2_g SZ (PRS) = 0.08,[24] h2_g IQ = 0.25,[26] h2_g (PRS) IQ = 0.04,[26] and r_g between SZ and IQ of -0.234.[26] In addition, in the absence of pre-existing literature estimates, we assumed h2_g subthreshold psychosis = 0.46 (based on h2_g SZ), and h2_g VIQ decline = 0.25 (based on h2_g IQ) **(Supplementary Table 5 and 6, and Supplementary Figure 5 and 6)**.

To estimate cohort specific parameters necessary for power analyses, we fit the observed data to a parametric likelihood based model based on the liability threshold model, with parameters as follows: schizophrenia prevalence; subthreshold psychosis prevalence; two shape parameters assuming the age distribution in the population following a beta binomial distribution; mean and SD for age at development of schizophrenia assuming a normal distribution; mean and SD for age at development of subthreshold psychosis assuming a normal distribution.

To explain the model, we considered a generative form, i.e. with population of individuals for study given the parameters above. Subsequently, we first simulated whether an individual would ever develop schizophrenia or subthreshold psychosis, based on the prevalences of the two conditions (i.e. if the prevalence was 20%, then one would simulate

phenotypes under a Bernoulli distribution with probability p=0.20). Next, independently, age was simulated, based on the shape parameters controlling the age distribution. Afterwards, age of diagnosis, conditional on ever developing the phenotype, was simulated, based on the parameters controlling the mean and SD age of development. From these underlying values for each simulated individual of the current age, whether they will ever develop schizophrenia or subthreshold psychosis, and the age at which they develop the phenotype, the present day phenotype of these simulated individuals could be determined.

Using constrained optimization, we obtained the parameters that maximized the likelihood of our real data under the above described model. To obtain a confidence interval for each parameter, we determined the maximum values of that parameter where twice the difference in log likelihood between the maximum likelihood estimation and that point was less than the chi-squared statistic with the appropriate number of degrees of freedom. Before applying the model to real data, we first simulated under the model to verify that we could recover parameter estimates on similar sized datasets, which confirmed the accuracy of the model (results not shown). We next generated parameter estimates on the real data (**Supplementary Table 5**). We used these parameter estimates and other literature derived estimates in the power analyses that were performed.

Regression analysis

We assessed relationships between PS_SZ and PS_IQ and binary phenotypes using logistic regression (SSD, subthreshold psychosis, VIQ decline), and linear regression for quantitative phenotypes (baseline FSIQ), adjusting for age, sex and the first five principal components from the imputed genotypes, with the principal components calculated using PLINK. All statistical tests in this manuscript are two-sided unless otherwise noted. r2 reported from linear regression is standard unadjusted r2 while from logistic regression is Nagelkerke r2.

Effect of future SSD cases as a source of confounding between subthreshold psychosis and PS_SZ

We modelled a scenario whereby the PS_SZ signal would be driven by the presence of individuals with future, as of yet undiagnosed SSD in the subthreshold psychosis group. In essence, we estimated in this scenario what proportion of such future SSD cases would be required to explain the observed PS_SZ in the subthreshold psychosis group

Genetically correlated traits as a source of confounding between subthreshold psychosis and PS_SZ

We examined whether the observed PS_SZ results in the subthreshold psychosis group could originate from increased rates of other psychiatric phenotypes that are genetically correlated with schizophrenia. Available IBBC data allowed us to analyse this possibility for comorbid mood disorders. Underlying assumptions for our mediation analysis were based on extrapolations of the IBBC data and include increased rates of (future) SSD (~40%) and mood disorder (49%) in the subthreshold psychosis group, compared to ~17% rates for both phenotypes in controls.

Quantitative measure of subthreshold psychosis as additional evidence for relationship between subthreshold psychosis and PS_SZ

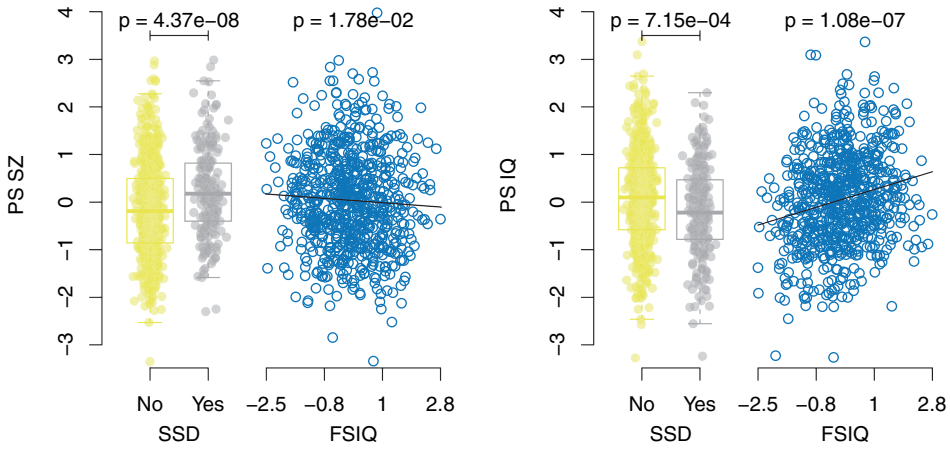
In a subset of 347 of 962 individuals with a well-defined phenotype and imputed genotype data, we were able to obtain an integer-coded measure of subthreshold psychosis from the Structured Interview for Prodromal Syndromes (SIPS).[35] We first generated a transformation from the integer coded, non-normally distributed quantitative SIPS score by fitting an exponential distribution using the least square estimate, yielding a transformation, in R, of "qnorm(pexp(q = x + 0.5, rate = 0.2238))", where x is the original integer coded SIPS score (**Supplementary Figure 7**). This yields a more approximately normally distributed value.

We assessed power to detect an association between the quantitative SIPS based phenotype and PS_SZ using simulations. Using the same assumptions listed before regarding heritabilities and predictive accuracies of polygenic scores, we first simulated an underlying total liability (genetic and environmental) for the quantitative subthreshold psychosis. As before, this total liability becomes binary under a liability threshold model, giving us the binary definition of subthreshold psychosis. In addition, using the continuous total liability, we generated an integer coded value (representing a simulated SIPS score) as "round(qexp(pnorm(Y_sub), rate = 0.2238))". We then re-transformed this to a continuous value using it's inverse "qnorm(pexp(q = x + 0.5, rate = 0.2238))", and from this, could calculate power for detecting an association between the quantitative subthreshold psychosis and PS_SZ, with or without conditioning on the binary phenotype (**Supplementary Figure 8**).

Calculation of Positive Predictive Values (PPV)

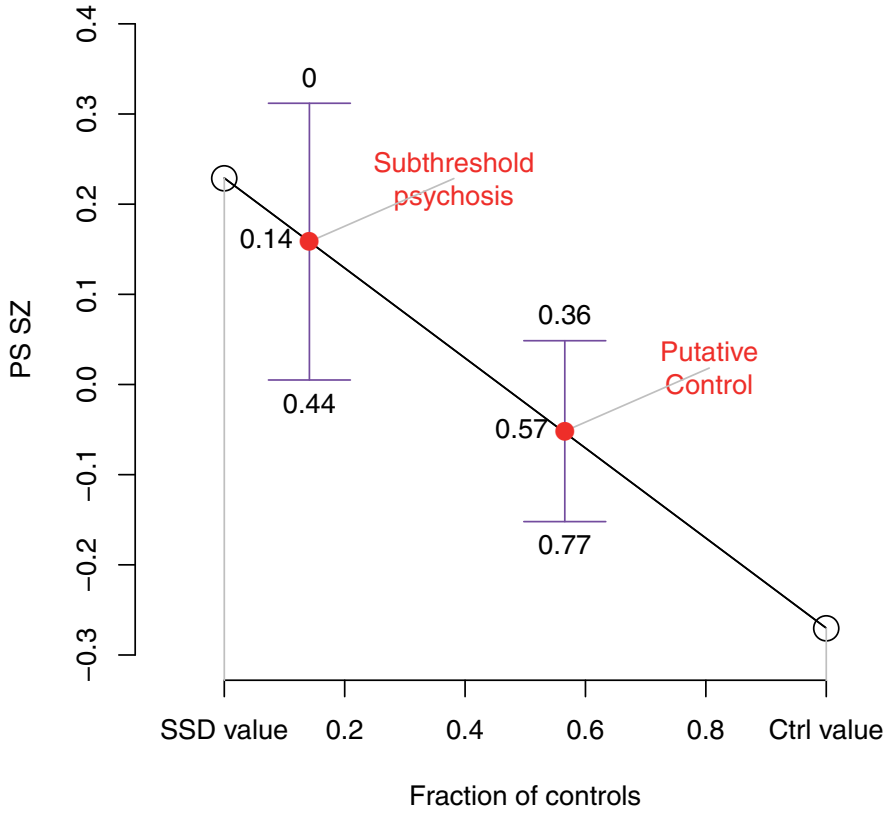
We calculated PPV in the traditional way given binary phenotypes schizophrenia and ID and observed PS_SZ and PS_IQ among 22q11DS samples. We also estimated PPVs for the general population using known estimates of general population prevalence of schizophrenia and ID, as well as sensitivity and specificity values derived from our analysis in this 22q11DS sample.

Extended data figure 1. Relationship between polygenic scores and previously studied phenotypes.



Results for the binary SSD phenotype show per-individual values as well as summaries per group, where minimum and maximum values are directly observable from the plot, the box-plot centre is the median, the boxplot edges represent the 25th and 75th percentiles, and the whiskers represent the lesser of the distance to the minimum or maximum value, or 1.5 times the inter-quartile range. Results are shown for logistic regression of SSD on controls (N = 802) and linear regression for FSIQ (N = 720), for both PS_SZ (left panel) and PS_IQ (right panel). P-values are reported from regression analyses and are two sided and are not corrected for multiple testing.

Extended data figure 2. Inferred contribution of controls and future SSD cases given PS_SZ



Shown on the y-axis are group means of PS_SZ, on the x-axis the fraction of controls. For SDD and controls the fractions of controls were taken as 0 and 1, respectively (open circles). For subthreshold psychosis and putative controls they were inferred through the observed PS-SZ values for each group, using linear interpolation based on fitting a straight line between SSD and control values (red circles). Confidence intervals are shown for the group mean values for subthreshold psychosis and putative controls, as the mean plus or minus 1.96 times the standard error, and above and below these confidence intervals are the inferred fraction of controls this would represent. The observed PS_SZ in the subthreshold group is consistent with a scenario in which 86% (95% CI 56 - 100%) of individuals who had subthreshold psychotic symptoms at the time of the assessment for this study would subsequently transition to SSD, a proportion inconsistent with known rates of SSD in 22q11DS.

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Supplementary Materials

Supplementary Table 1. Mediation analysis between subthreshold psychosis, mood disorders, and PS_SZ. IV = Independent Variable, DV = Dependent Variable.

Purpose	Regression (bold = what in p, effect columns)	N	effect size	p
1 IV and mediator	mediator ~ **IV** + covars	943	-0.405	0.001
1* IV and mediator *(no schizophrenia cases)*	mediator ~ **IV** + covars	725	-0.221	0.202
2 IV and DV *(same as original)*	DV ~ **IV** + covars	755	0.239	0.025
3 mediator and DV	DV ~ **mediator** + covars	725	1.886	0.067
4 IV, DV and mediator	DV ~ **IV** + mediator + covars	725	0.250	0.021

Supplementary Table 2. OR for schizophrenia based on polygenic score cutoffs. Results show for a given binary cutoff based on Polygenic Score percentile, how many 22q11.2DS individuals fall above or below that cutoff, stratified by having SSD, or being a control (regardless of age). ORs and PPVs are given for SSD against merged controls. Prevalence of SSD (observed) is 26% (versus controls).

	Schizophrenia spectrum diagnosis (SSD)	Merged controls (all ages)
90 < PS^{ile}	OR = 1.44 [0.88, 2.37] PPV = 0.325 [0.222, 0.428] N+ = 26, N- = 181	N+ = 54, N- = 543
75 < PS^{ile}	OR = 1.62 [1.14, 2.31] PPV = 0.332 [0.265, 0.398] N+ = 64, N- = 143	N+ = 129, N- = 468
50 < PS^{ile}	OR = 1.91 [1.38, 2.64] PPV = [0.274, 0.366] N+ = 126, N- = 81	N+ = 268, N- = 329
PS^{ile} < 50	OR = 0.52 [0.38, 0.72] PPV = 0.198 [0.159, 0.236] N+ = 81, N- = 126	N+ = 329, N- = 268
PS^{ile} < 25	OR = 0.41 [0.27, 0.62] PPV = 0.147 [0.099, 0.195] N+ = 31, N- = 176	N+ = 180, N- = 417
PS^{ile} < 10	OR = 0.26 [0.12, 0.55] PPV = 0.091 [0.031, 0.151] N+ = 8, N- = 199	N+ = 80, N- = 517

Supplementary Table 3. OR and PPV for ID based on polygenic score cut-offs. Results show for a given binary cut-off based on Polygenic Score percentile, how many 22q11.2DS individuals fall above or below that cut-off, stratified by having ID or not having ID. Odds-ratios and PPVs are shown for each percentile cut-off. ID is defined as IQ < 70. Overall prevalence of ID is 41%.

FSIQ Polygenic Score cutoff (percentile)	22q11.2DS with ID	22q11.2DS without ID
$PS^{ile} < 10$	OR = 2.64 [1.59, 4.4] PPV = 0.629 [0.515, 0.742] N+ = 44, N- = 246	N+ = 26, N- = 384
$PS^{ile} < 25$	OR = 2.07 [1.47, 2.93] PPV = 0.549 [0.475, 0.622] N+ = 96, N- = 194	N+ = 79, N- = 331
$PS^{ile} < 50$	OR = 1.85 [1.37, 2.51] PPV = 0.489, [0.436, 0.541] N+ = 171, N- = 119	N+ = 179, N- = 231
$50 < PS^{ile}$	OR = 0.54 [0.4, 0.73] PPV = 0.34 [0.29, 0.39] N+ = 119, N- = 171	N+ = 231, N- = 179
$75 < PS^{ile}$	OR = 0.49 [0.34, 0.71] PPV = 0.291 [0.224, 0.359] N+ = 51, N- = 239	N+ = 124, N- = 286
$90 < PS^{ile}$	OR = 0.42 [0.24, 0.74] PPV = 0.243, [0.142, 0.343] N+ = 17, N- = 273	N+ = 53, N- = 357

Supplementary Table 4. An overview of demographic differences between included and excluded individuals in the study.

		Mean Age (SD)	Sex (%M)	SSD	Control	Putative ctrl	Sub-threshold	No pheno data
Included	962	24.0 (12.4)	48.6%	207 (21.5%)	215 (22.3%)	382 (39.7%)	158 (16.4%)	0
Excluded	824	18.2 (9.1)	48.5%	123 (14.9%	79 (9.6%)	468 (56.8%)	110 (13.3%)	11 (1.3%)

Note that these differences reflect the history of the IBBC recruitment strategy. In "phase 1" submission of DNA from individuals who were either (schizophrenia spectrum) case or true control (age >25) was encouraged. There are more Affymetrix data available from this "phase 1", because by the time the second wave started, the WGS effort was up and running. This "phase 2" also included individuals who did not directly qualify as either case or definitive control. The main reason for exclusion for the current study was lack of availability of Affymetrix data. Therefore, as a result of the said prioritization of phase 1 (schizophrenia cases and definitive controls), the mean age of subjects with available Affymetrix data is also higher compared to those without Affymetrix data (enriched in phase 2). The age difference occurred because the onset of schizophrenia is generally after age 18 years, and true controls were defined as only those without psychosis and older than 25 years. In addition, given the on average lower age range in the individuals with no available Affymetrix data (hence: not included in this study), it is expected that the proportion of putative controls is higher in the excluded samples.

Supplementary Table 5. Parameter estimates for model that was used to inform power calculations in this study.

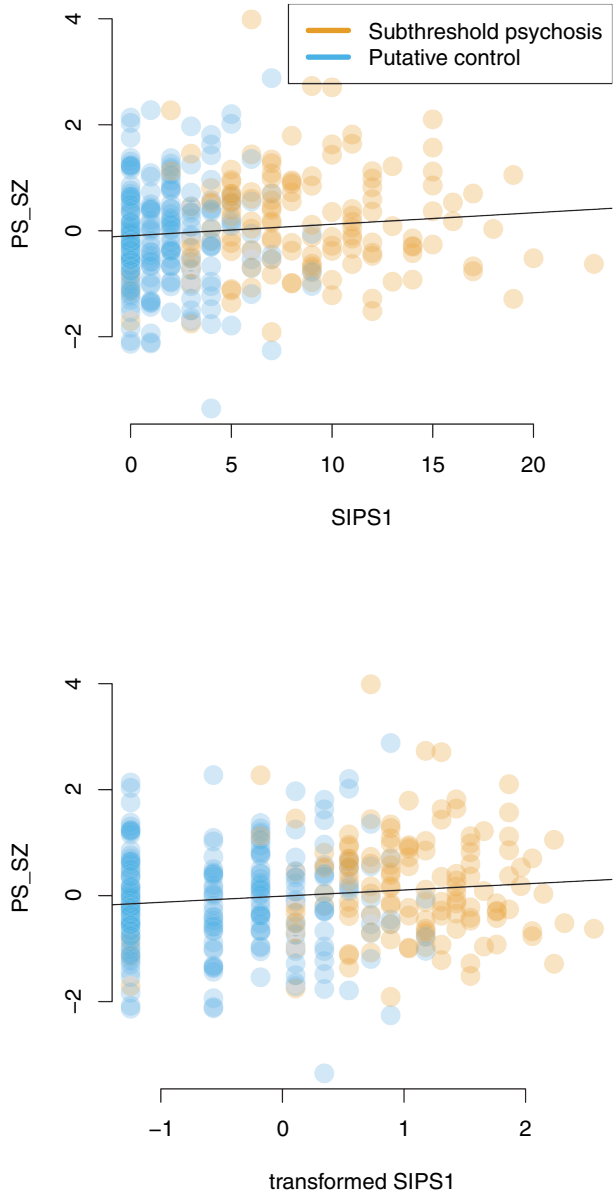
	Estimate	CI Lower Bound	CI Upper Bound
K_SZ	0.45	0.37	0.56
K_subthreshold psychosis	0.32	0.28	0.4
age_shape1	1.63	1.48	1.79
age_shape2	3.58	3.23	3.95
SZ_mean_age	23.05	19.64	27.98
SZ_sd_age	10.33	7.26	15.12
Subthreshold psychosis_mean_age	9.94	8.82	11.89
Subthreshold psychosis _sd_age	1.69	0.76	6.48

Supplementary Table 6. Power analyses for primary analyses regarding genetic relationships between dependent variables (phenotypes) and independent variables (polygenic score).

Dependent variable	IV	Power (alpha = 0.05)*	Relevant Supplementary Figure
SSD	PS_SZ	0.997 [r_g = 1]	5
Subthreshold psychosis		0.062 [r_g = 0], 0.974 [r_g = 0.95]	5
Baseline FSIQ		0.32 [r_g = 1]	6
VIQ decline		0.058 [r_g = 0], 1 [r_g = 0.8]	6
SSD	PS_IQ	0.189 [r_g = 1]	5
Subthreshold psychosis		0.048 [r_g = 0], 0.867 [r_g = 0.95]	5
Baseline FSIQ		1 [r_g = 1]	6
VIQ decline		0.048 [r_g = 0], 0.996 [r_g = 0.8]	6

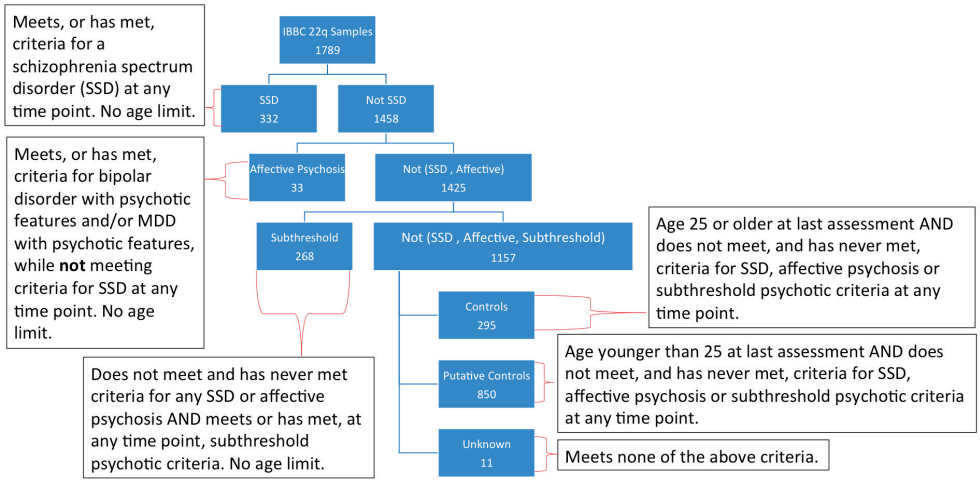
r_g is given between dependent variable and either schizophrenia (first four rows), or IQ (last four rows). Values of r_g between schizophrenia and IQ are fixed at -0.234, while otherwise, conditional on this, we report power for minimum and maximum possible genetic correlation between dependent variable and independent variable. IV = Independent Variable.

Supplementary Figure 1. Correlation plots between PS_SZ and a quantitative measure of subthreshold psychotic symptom severity.

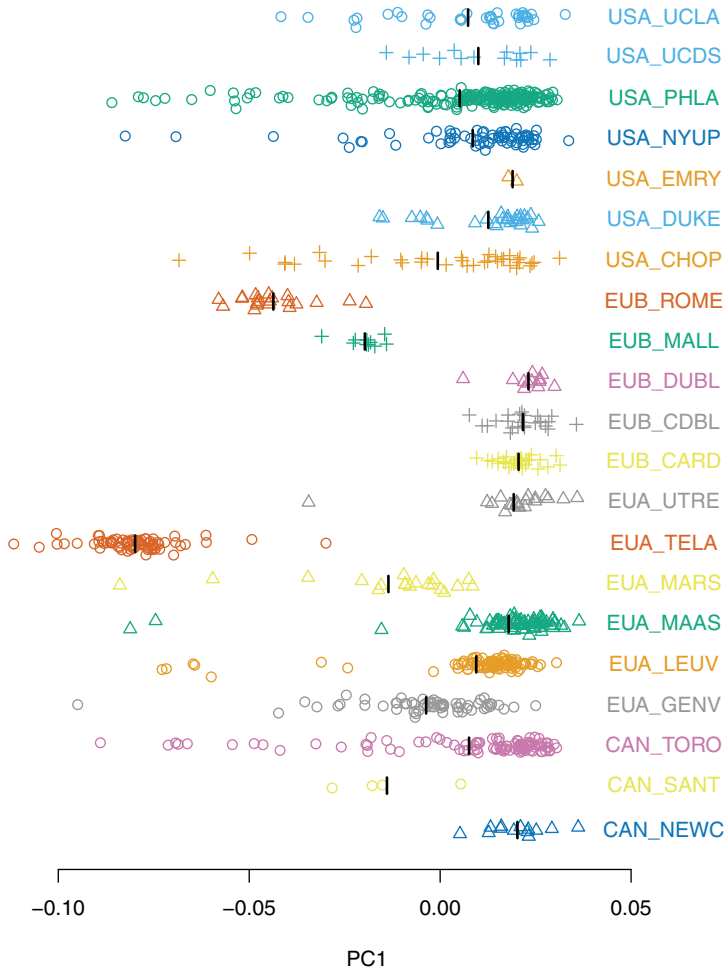


Upper panel shows untransformed SIPS values, lower panel shows transformed SIPS values. When adjusting for the previous binary indicator of subthreshold psychosis versus control, the association between the transformed quantitative SIPS phenotype and PS_SZ was not significant (N = 347, p = 0.77, $r^2 = 0.0001$).

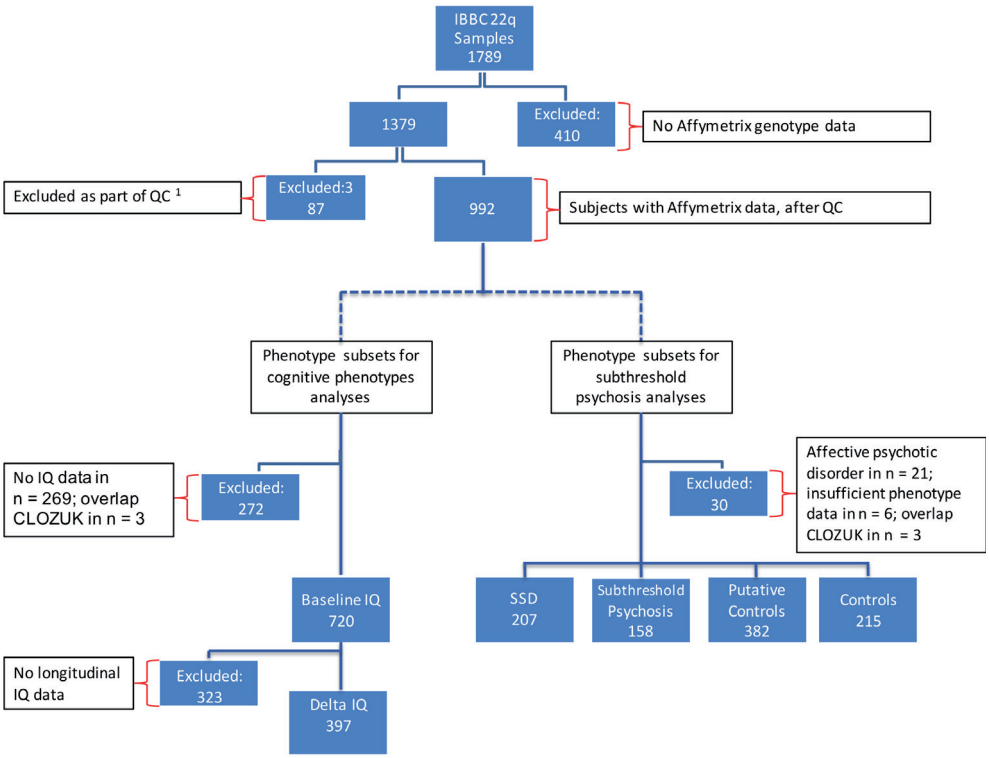
Supplementary Figure 2. Flowchart of IBBC cohort (full cohort) outlining the criteria used to assign IBBC subjects into different diagnostic classes regarding schizophrenia spectrum disorder (SSD) and related phenotypes.



Supplementary Figure 3. Principal component 1 as function of study site. X-axis denotes value per-individual on PC1, while Y-axis is arbitrary to separate study sites plus jitter. Different sites are separated vertically and are grouped together by colour and plot icon. Black vertical bar indicates per study site average.

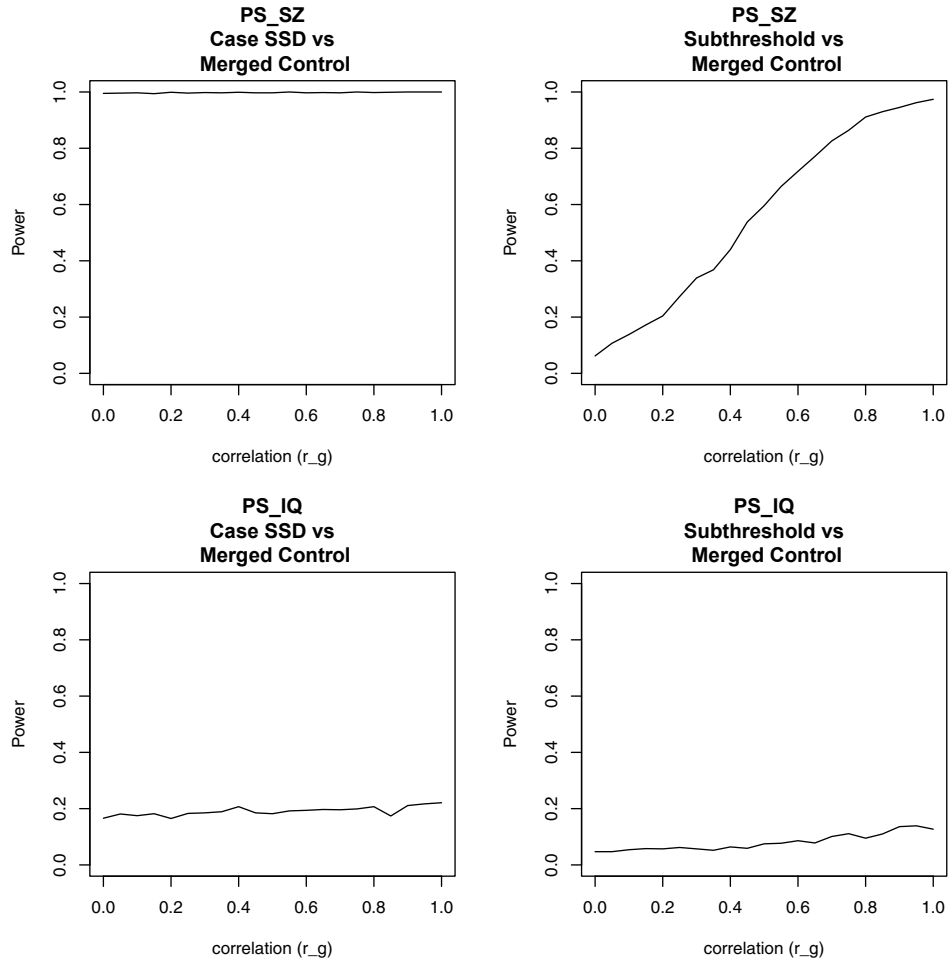


Supplementary Figure 4. Flowchart of subjects of IBBC cohort, outlining the different phenotypic subsets for the current study.



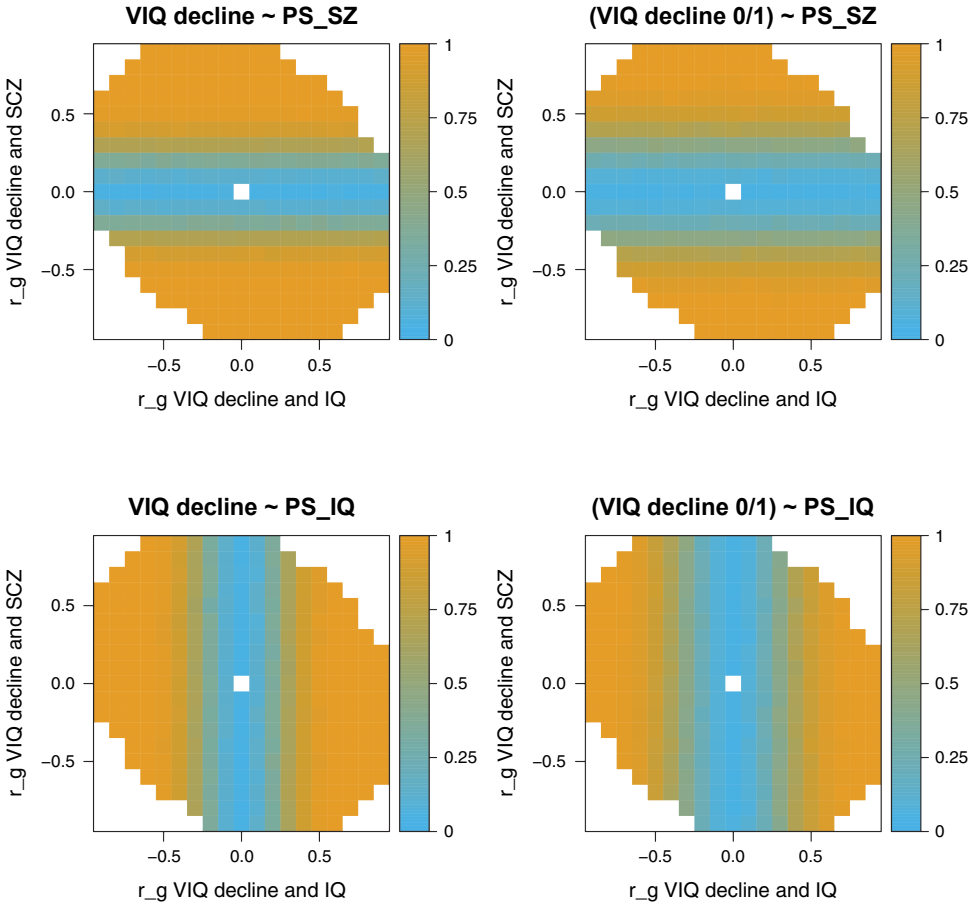
[1.] Arrays excluded for sex coding reasons (5); missingness (84); IBD analysis (174); PCA (124).

Supplementary Figure 5. Power to differentiate SSD status given genetic correlation.



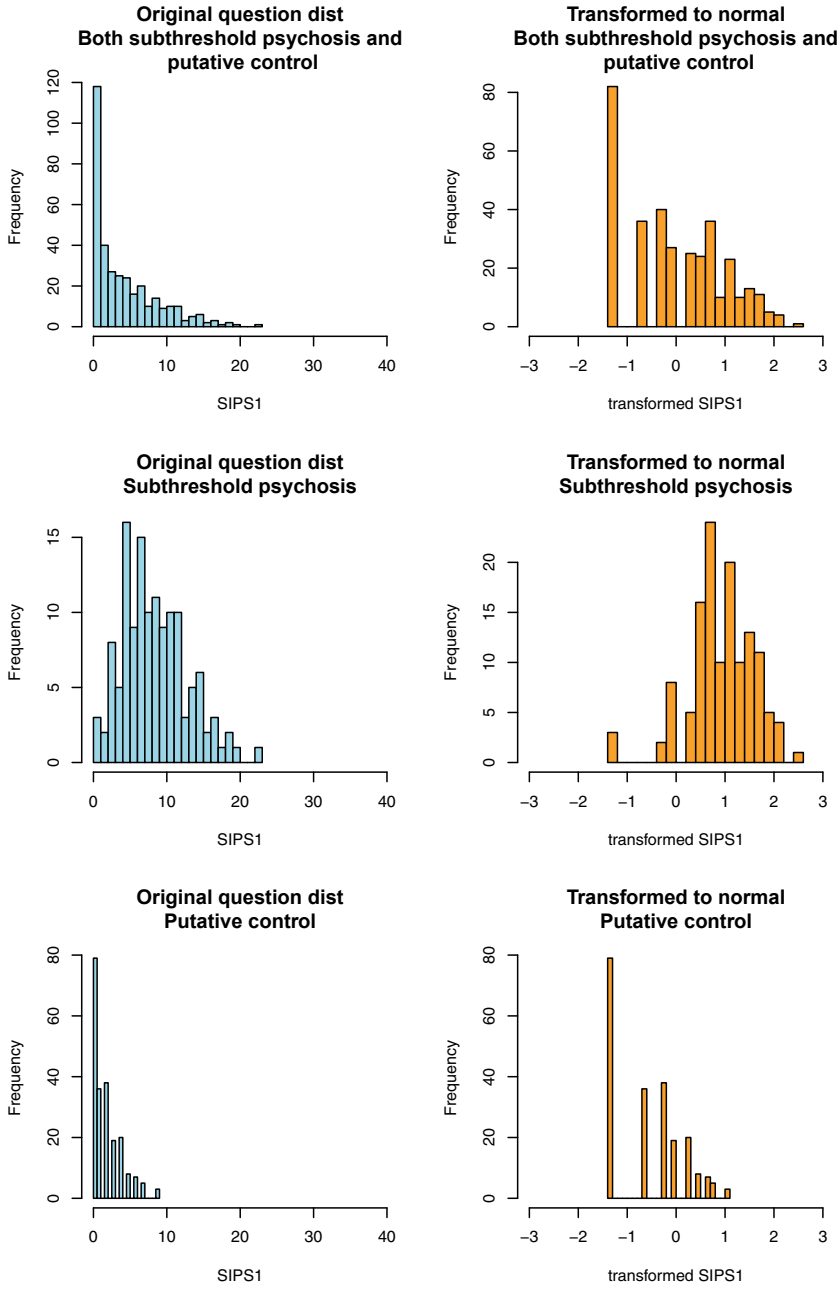
Shown are power at alpha=0.05 when comparing groups as specified in the plot sub-titles for their difference in polygene score as specified in the title, given genetic correlation between subthreshold psychosis and SSD.

Supplementary Figure 6. Power to differentiate VIQ decline given genetic correlations.



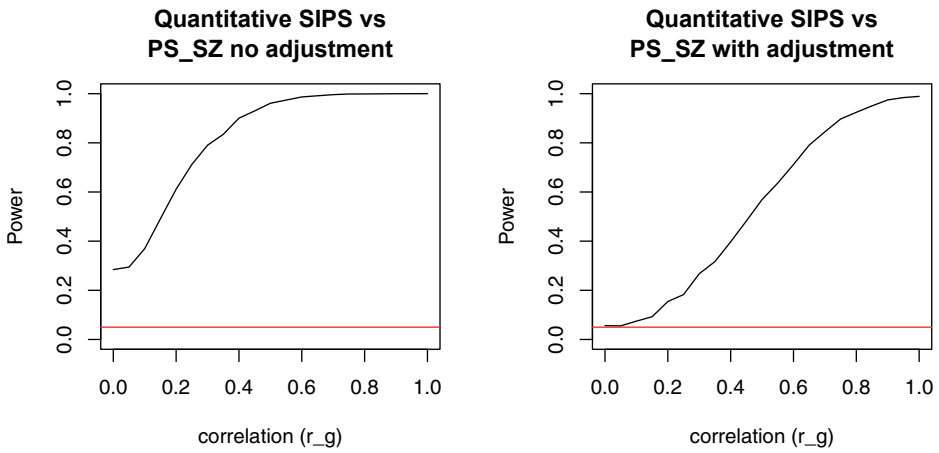
Shown are power at alpha=0.05 when regressing continuous or binary VIQ decline against PS_SZ or PS_IQ, shown as a function of both the genetic correlation between VIQ decline and IQ, as well as between VIQ decline and SSD.

Supplementary Figure 7. Histogram of pre and post transformed SIPS measure.



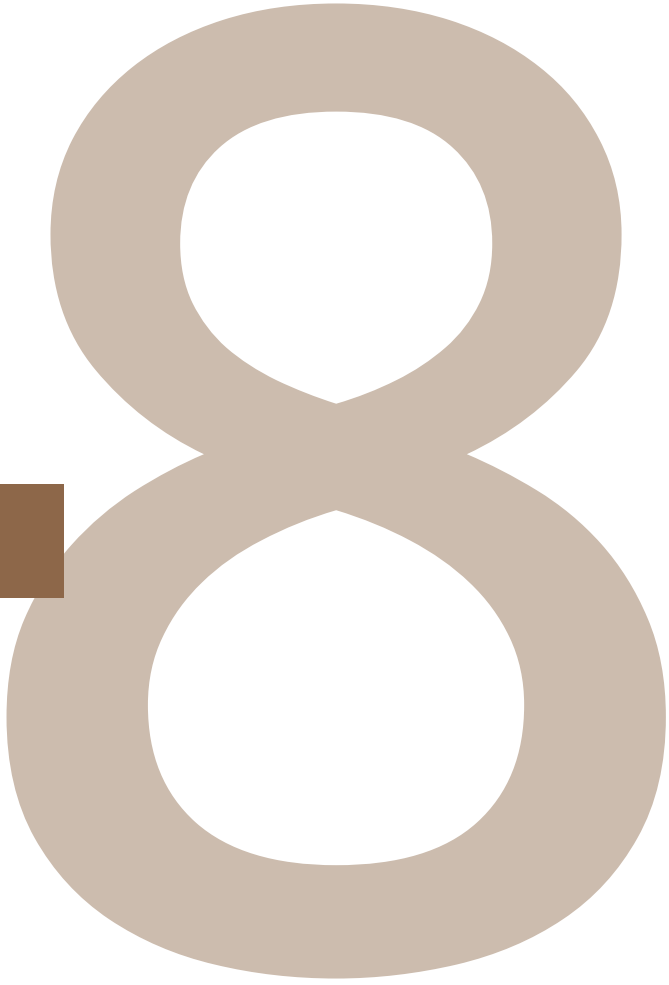
Transformation is defined by "qnorm(pexp(q = x + 0.5, rate = 0.2238))".

Supplementary Figure 8. Power analysis for quantitative subthreshold psychosis measure based on SIPS either without an adjustment for binary subthreshold psychosis (left) or with (right).



Results were generated using simulation including only those simulations where a significant (i.e. alpha < 0.05) observation was made between subthreshold psychosis and PS_SZ. Note that the plot on the right, with the binary conditioning, is unbiased, unlike the plot on the left.

CHAPTER 8



Summary and General Discussion

The work presented in this dissertation collectively aimed to contribute to the understanding of the variable expression of, and mechanisms driving, neuropsychiatric phenotypes in individuals with the 22q11.2 deletion. To this end, we used different, complementary methods and study designs. Our studies included both pediatric and adult cohorts, cross-sectional and longitudinal, and retrospective as well as prospective study designs, allowing the exploration of developmental trajectories of neurobehavioral phenotypes. In this work, we approached these phenotypes both from a categorical perspective, e.g., adhering to the traditional dichotomous classification of psychopathology, while simultaneously exploring the potential of operationalizing neurobehavioral traits in a quantitative manner, e.g., focusing on a dimensional approach to cognitive functioning and other neurobehavioral domains. Some studies were carried out in large samples (IBBC), allowing for the exploration of the role of common genetic variation in the context of a 22q11.2 deletion in major neuropsychiatric outcomes, while others comprised smaller well-characterized samples that allowed for more in-depth phenotyping, in one study including both individuals with 22q11DS and their unaffected parents. While each approach individually contributes to the understanding of one or more aspects of the neuropsychiatric expression of 22q11DS, their synergy may lead to insights into potential mechanisms that shape neuropsychiatric outcomes. Together, the results contribute to illuminating observations and mechanisms that may be specific to 22q11DS and that may be generalizable for populations of individuals with other pathogenic variants and/or idiopathic neuropsychiatric illness.

1. Summary of findings main chapters

Problem 1 = While we know which neuropsychiatric manifestations are associated with 22q11DS, there is no way to predict type and severity of such outcomes for an individual. Among the most prominent of these uncertain outcomes are schizophrenia and level of cognitive functioning.

While one in four individuals with 22q11DS will develop schizophrenia, we are currently unable to differentiate this 25% from the 75% of patients who will not develop schizophrenia at an early developmental stage (*Problem 1*). Some researchers have posited that the social and communicative difficulties and repetitive behaviors, characteristic of an Autism Spectrum Disorder (ASD) and frequently observed in children with 22q11DS, may in fact represent the early stages of schizophrenia [1-3], consistent with conceptualizing schizophrenia as a neurodevelopmental disorder[4]. Indeed, changes in social behavior and deteriorating communicative skills are part of the observed phenotype of schizophrenia and the schizophrenia-prodrome[5,6]. In **Chapter 3**, results from our prospective study of 89 children and adolescents with 22q11DS from the Utrecht cohort demonstrated that

children with ASD are *not* more likely than children without ASD to subsequently develop a psychotic disorder. These results, that replicate those of a previous retrospective study in an independent sample[7], suggest that an early diagnosis of ASD, or symptoms of ASD, cannot be viewed as a clinical marker that indicates increased risk of schizophrenia in individuals with 22q11DS. Rather, ASD and schizophrenia appear to occur independent of one another in the context of a 22q11.2 deletion, indicating that these are two different, pleiotropic[8], consequences of the 22q11.2 deletion. In addition to the traditional operationalization of ASD as a dichotomous variable, we used a quantitative measure of ASD symptomatology, generating the same results with respect to the lack of association with schizophrenia risk. This quantitative approach also highlighted that a large proportion of individuals with 22q11DS have clinically relevant symptoms of ASD, even in the absence of a formal diagnosis[9,10]. The substantial prevalence of such "subthreshold" psychiatric symptoms bears potential implications for clinical care and studies of individuals with 22q11DS (also discussed in **Chapter 2)**, including the importance of considering the early neurodevelopmental expression of 22q11DS in itself; i.e., beyond its potential association with schizophrenia risk.

In **Chapter 4**, we investigated the association between functional (daily life) outcome and domains of neurocognitive functioning, addressing *Problem 1*. Such neurocognitive domains represent more specific abilities than global IQ and have the advantage of being potentially more amenable to interventions. Data from 99 adults with 22q11DS from the Toronto cohort suggest that Executive Performance (representing mental processes that enable us to plan, focus attention, and manage multiple tasks successfully) significantly contributes to the variability in subsequent functional outcome in individuals with 22q11DS, even after accounting for previously identified predictors such as schizophrenia and global cognitive functioning (FSIQ)[11]. In addition, the data in **Chapter 4** revealed a profile of neurocognitive strengths and weaknesses that may be informative for (caregivers of) individuals with 22q11DS (relevant to *Problem 4*), and that bears some similarities to observations in other schizophrenia and schizophrenia high-risk populations[12,13]. In particular, while there are impairments on all domains of neurocognition in individuals with 22q11DS, performance is on average better on tasks related to visual (rather than verbal) memory, and performance is on average worst on motor tasks. In sum, Chapter 4 alludes to the interplay and interdependence of various levels (e.g., global and specific) and domains (e.g., executive performance and motor functioning) of cognitive functioning, schizophrenia (and schizophrenia-risk), and daily life functioning in the context of the 22q11.2 deletion.

Problem 4 = Indices of cognitive functioning and development derived from the general population may not be entirely applicable and sufficiently informative in populations of individuals with pathogenic variants, such as the 22q11.2 deletion.

In **Chapter 5**, we addressed *Problem 4* and studied cognitive data from 1365 individuals with 22q11DS from the IBBC, presenting normative IQ data within this population. We demonstrated the construction of a normative chart for cognitive development for 22q11DS, and that using cognitive norms that are specific to individuals with a certain genetic variant can substantially decrease the sample size necessary in a research context, compared to using standard (general population-based) IQ norms. From a clinical perspective, the variant-specific cognitive norms may provide useful information, including a more accurate and informative interpretation of individual IQ-scores and trajectories, in addition to using (untransformed) population-based IQ norms.

Problem 3: There is a disproportional lack of insight into disease etiology, mechanisms, and early developmental trajectories in the field of neurodevelopmental disorders, including schizophrenia, intellectual disability, and ASD. Important contributing challenges are:

1. The difficulty of (early) identification of individuals at risk for neuropsychiatric conditions.
2. The large etiological heterogeneity of neuropsychiatric conditions.
3. The categorical conceptualization of neuropsychiatric conditions.

In **Chapter 6** we expand our focus from understanding the neurobehavioral phenotypic expressions of 22q11DS, toward including the study of the potential underlying mechanisms, relevant to *Problems 1, 2, and 3*. In a sample of 230 individuals (Toronto cohort), including 82 adults with a *de novo* 22q11.2 deletion and their unaffected parents, we studied expression of three neurobehavioral phenotypes affected by 22q11DS and implicated in major psychiatric disorders. We identified a significant effect of parental functioning on proband functioning for cognitive measures, but not for measures of social or motor functioning. In addition, we showed that, relative to biparental scores, the 22q11.2 deletion confers a negative impact on the phenotypes assessed, with further decrements in those with schizophrenia. Notably, the patterns of influence (i.e., the effect sizes of the deletion, schizophrenia, and parental functioning) differed per phenotype, suggesting different underlying genetic mechanisms. In **Chapter 6**, for the first time, we disentangle the impact of a high-impact variant from the modifying effects of parental background and schizophrenia on important dimensional neurobehavioral phenotypes. This study, demonstrating the benefit of the dimensional assessments of neurobehavioral phenotypes in the context of both a genotype-first approach and a within-family approach, sets the stage for future studies that could aim to further improve individual outcome prediction and compare patterns of influence across different high-impact variants[14,15].

Problem 2: Pathogenic CNVs, such as the 22q11.2 deletion, confer a substantial neuropsychiatric risk at the individual level. However, given their rarity, they hardly have explanatory power at the population level. Common genetic variation, e.g., captured in the polygenic score, on the other hand performs poorly at the level of individual risk prediction, even though it explains a substantial portion of variance at the population level. Our understanding of the role of common genetic variation in the context of a pathogenic structural variant, such as the 22q11.2 deletion, is limited.

In **Chapter 7** we elaborate on the study of genetic mechanisms involved in the phenotypic expression of 22q11DS, and explicitly address *Problem 2*. We directly explore the genetic association of several schizophrenia-associated phenotypes [16-18] with schizophrenia in a sample of 962 individuals with 22q11DS from the IBBC, using polygenic scores derived from the general population. We confirmed previous results that the polygenic score for schizophrenia is associated with schizophrenia in the context of a 22q11.2 deletion[19]. We also demonstrated, for the first time, that the polygenic score for IQ is significantly associated with cognitive functioning in the 22q11DS population. Notably, we found that the polygenic score for schizophrenia is also associated with two schizophrenia-related phenotypes: cognitive decline and subthreshold psychotic symptoms, while the polygenic score for IQ is not associated with those phenotypes. These results suggest that cognitive decline and subthreshold psychotic symptoms may share some of the same genetic underpinnings with schizophrenia, and that these phenotypes may represent earlier stages of the same disease process (addressing *Problem 3*).

In addition, our findings point towards the potential of using polygenic scores to improve risk stratification for key neurobehavioral phenotypes in 22q11DS: schizophrenia and intellectual disability (IQ < 70), addressing *Problem 1*. Comparing the tail-end deciles of the polygenic scores for schizophrenia and IQ respectively, we found that 33% versus 9% had schizophrenia, and 63% versus 24% had intellectual disability. This represents some advance in risk stratification compared to the baseline risk rates of ~25% and ~45% respectively for 22q11DS. These findings are not yet ready for implementation in the clinic (e.g., pending replication). However, they highlight the future potential of using polygenic scores in the context of a population with an *a priori* increased risk, due to a highly, but incompletely penetrant genetic variant, for outcome understanding and prediction[20].

2. Insights and implications

2.i. Overall observations for 22q11DS

The level of global cognitive functioning, captured by overall IQ, in individuals with 22q11DS has consistently been reported to be on average about 30 IQ-points lower than that for the general population (where the mean IQ is 100 with a standard deviation of

15)[21,22]. Our recent studies demonstrate that in addition to this average **leftward shift of -2 SD in IQ** conferred by the 22q11.2 deletion, the characteristics of the IQ-distribution remain notably similar to that in the general population. Specifically, **IQ-scores in the 22q11DS population are normally distributed, with a standard deviation of ~15, but with a mean IQ-score of ~70**, regardless of age or composition of the samples (Chapters 4, 5, and 6). The IQ-data comprising this dissertation also corroborate the previously described discrepancies between the different components of IQ in individuals with 22q11DS. Specifically, **(baseline) VIQ is generally significantly higher than (baseline) PIQ** in individuals with 22q11DS[21,23] (e.g., Chapters 5 and 6).

With respect to the developmental trajectory of global cognitive functioning in individuals with 22q11DS, data demonstrate an, *on average*, **decline in IQ-scores** over time (Chapter 5, [24,25]). Our studies suggest that in this population, a certain decline in IQ points (~-7 IQ-points between the ages of 6 and 12) can be expected. For most individuals with 22q11DS, this "decline" does not necessarily imply an absolute loss of cognitive capacities, but rather, a slower developmental trajectory compared to typically developing peers (i.e., "growing into deficit"[24,26]). Thus, interpreting IQ-scores and IQ-trajectories in individuals with 22q11DS in reference to normative IQ- and IQ-development data tailored for this specific population may complement the traditional approach of using standardized IQ-scored (i.e., adjusted to general population norms) (Chapter 6). The timing of the "decline" in IQ-scores appears to largely coincide with the transition from concrete to more abstract reasoning skills, suggesting that the development of abstract reasoning skills in children with 22q11DS may be relatively more delayed and/or impaired than the development of concrete reasoning abilities. Also, our studies have demonstrated that a negative deviation from the expected IQ-trajectory, i.e., an **IQ-decline (in particular in VIQ) *in excess of* what is expected within this population, is associated with increased risk of schizophrenia** ([25] and Chapter 6).

In individuals with 22q11DS, many of the characteristics of cognitive functioning are associated with psychosis risk, largely converging with findings for idiopathic schizophrenia[16,18,27]. For example, low baseline IQ and IQ-decline are phenotypically associated with schizophrenia; IQ-decline is also genetically correlated with schizophrenia risk; and levels of functioning on certain domains of neurocognitive functioning are, on average, lower in individuals with schizophrenia compared to non-psychotic individuals (Chapters 4, 5, 6, and 7). The data presented in this thesis show no evidence of a cognitive profile that is associated with psychopathology *other* than schizophrenia in individuals with 22q11DS. This is consistent with other reports[28], although a recent study revealed that psychopathology (e.g., ASD and ADHD) was associated with domain-specific cognitive characteristics in an age-specific manner in individuals with 22q11DS[29]. Taken together, such data support the proposition that **the profile of psychopathology in 22q11DS**

cannot be viewed as merely a consequence of overall lower cognitive functioning on average in this population (Chapter 2). While accounting for the large intra- and inter-individual differences observed in this population[30], some aspects of both cognitive functioning and psychopathology, as well as their developmental trajectories, appear to be characteristic of 22q11DS.

2.ii. Potential mechanisms and convergence with results for other populations

Our findings suggest that **shared (genetic) factors play an important role in the variable expressivity of the cognitive phenotype in individuals with 22q11DS**. Chapter 6 demonstrates that the level of cognitive functioning in individuals with a *de novo* 22q11.2 deletion is significantly associated with the level of cognitive functioning of their unaffected parents. In other words, a relatively high parental IQ, e.g., IQ > 120, is *likely* to correspond to a relatively high IQ in the offspring with 22q11DS, e.g., IQ > 80. This may be due to inherited genetic and non-genetic factors that modify the large primary impact of the 22q11.2 deletion. Chapter 7 provides genetic evidence in support of this modifying effect of parental background on offspring IQ. Here, results show that common genetic variants associated with intellectual functioning in the general population (polygenic score for IQ), play a role in shaping the cognitive phenotype in individuals with 22q11DS. While the role of common genetic variation in cognitive outcome in the general population has been well-established[31], this is, to our knowledge, the first time that genetic data suggest the same mechanism in the context of a high-impact genetic variant associated with deviant neurocognitive outcomes. Our studies also suggest that this mechanism may not necessarily be generalizable across different (dimensional) neurobehavioral phenotypes in the context of the 22q11.2 deletion: Chapter 6 revealed no association between parental and offspring functioning on social and motor parameters, in contrast to the cognitive data. Future studies could further address this in GxG studies, while also acknowledging environmental and GxE effects. Such studies may eventually illuminate whether there are differential effects (e.g., of the CNV and of shared genetic and non-genetic factors) across different pathogenic variants that may relate to primary associated neuropsychiatric conditions, as Chapter 6 tentatively suggests[15].

Our studies suggest that cognitive decline is not merely phenotypically associated with subsequent "full-blown" schizophrenia expression, but may represent an early disease stage and likely shares part of its genetic etiology. Common genetic variants for schizophrenia (polygenic score) are enriched in individuals with 22q11DS who demonstrate significant cognitive decline, compared to individuals with 22q11DS but no cognitive decline in excess of the expected trajectory, while the polygenic score for IQ is not significantly different between these two subgroups (Chapter 7). Collectively, these findings suggest that in individuals with 22q11DS, **cognitive decline appears to**

be genetically correlated with schizophrenia rather than with low baseline IQ. Our findings with respect to IQ-decline in 22q11DS converge with findings from the general population. First, there is substantial evidence for the phenotypic manifestation of cognitive decline and its association with subsequent schizophrenia risk[27,32 33-35]. Second, evidence in support of a genetic correlation between schizophrenia and cognitive decline is emerging in the general population[36,37]. A recent study reported that of 540 idiopathic schizophrenia patients studied, those with a significant cognitive decline had the highest schizophrenia polygenic risk score, compared to individuals who remained cognitively stable and/or were already more severely cognitively impaired from an early age onwards[37].

2.iii. 22q11DS as a model?

Over the last two decades, the 22q11.2 deletion has increasingly been identified and recognized as a **valuable genetic model for the study of schizophrenia**. There are several advantages over studies in the general population. Individuals with 22q11DS can be identified very early in life and monitored before the advanced stages of schizophrenia (i.e, active psychosis)[30]; they have a high risk of developing the illness (i.e., reducing required sample size for longitudinal studies)[4,38]; and they represent an etiologically relatively homogeneous population[4,39,40]. Such a *genotype-first* approach to understanding neuropsychiatric phenotypes more broadly requires a certain degree of convergence with observations from idiopathic neuropsychiatric populations. Previous studies have demonstrated a comparable manifestation of schizophrenia spectrum disorders in individuals with 22q11DS to idiopathic schizophrenia[41,42]. Recent findings from the ENIGMA consortium provide a complementary perspective: brain-imaging data from a large international sample of individuals with 22q11DS with and without schizophrenia, both at the cortical, subcortical, and functional level, largely converge with imaging findings for individuals with idiopathic schizophrenia[43-45]. Our findings with respect to the phenotypic expression of schizophrenia, schizophrenia-associated phenotypes and outcomes, and genetic mechanisms underlying these, collectively contribute to the understanding of 22q11DS as a genetic model for schizophrenia, with several findings for 22q11DS that converge with those for idiopathic schizophrenia. The studies in this dissertation are complementary to data from (a subset of) the IBBC that demonstrate that the common genetic variation associated with schizophrenia in the general population is also significantly enriched in individuals with 22q11DS and schizophrenia, compared to those with no psychotic illness, albeit to a lesser degree[19]. One interpretation of this could be that fewer such additional common genetic risk variants are required to result in schizophrenia illness expression in individuals with 22q11DS compared to individuals without 22q11DS; given the *a priori* elevated risk conferred by this CNV.

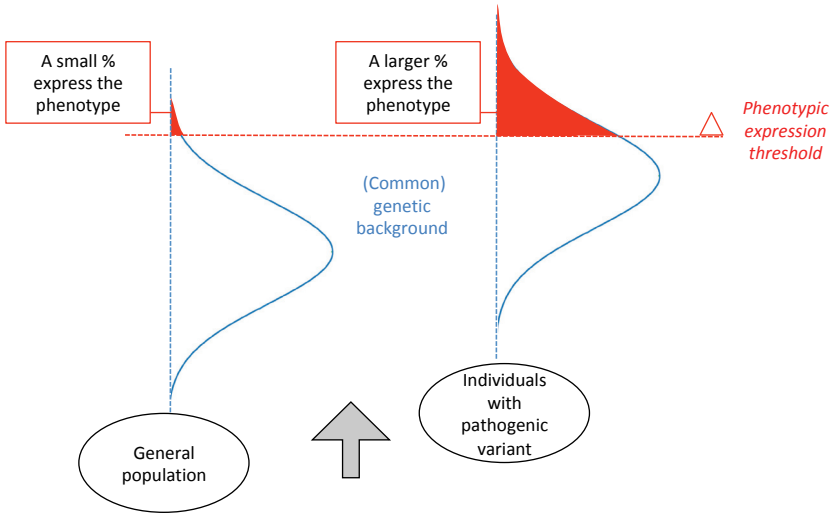
The focus on the elevated risk for schizophrenia conferred by the 22q11.2 deletion is understandable both from a scientific and a mental health care perspective, given the strength of the association; the severity and burden of schizophrenia[46], including long-term outcomes[47] and life expectancy[48]; and the fact that schizophrenia was the first psychiatric phenotype to be reported in individuals with 22q11DS[49-52]. However, a wide scope of associated neurodevelopmental outcomes was progressively revealed by studies including ours. This underlines not only the relevance of studying these phenotypes more extensively in 22q11DS, but also gradually reveals the potential of **22q11DS as a genetic model to study a wide range of neurodevelopmental phenotypes**, including intellectual disability and autism spectrum disorders (Chapter 2). As is the case for schizophrenia, some findings for 22q11DS will likely converge with studies of idiopathic neurodevelopmental populations, while other might not. An example of the latter is that a recent study found that neuroanatomical correlates of ASD symptomatology in individuals with 22q11DS diverge from those in idiopathic ASD[53].

Over the past decades, the list of known pathogenic genetic variants associated with neurodevelopmental outcomes in varying degrees of severity, such as the 22q11.2 deletion, has substantially grown – and continues to do so[54,55]. Among these, typically rare, genetic disorders, the 22q11.2 deletion is relatively prevalent (1/2000-4000), and its genetic description in the early 1980s has preceded by approximately two decades the much more recent discovery of the majority of other rare pathogenic variants associated with aberrant neurodevelopmental outcomes. This has provided studies of 22q11DS with an advance over, thus rendering it a **model for, studies of other pathogenic genetic variants.** Indeed, international consortia comparable to the 22q11DS IBBC have now been established for various pathogenic variants, as well as large collaborative efforts across different genetic variants. In Chapter 5 we propose that the approach to use cognitive norms derived from individuals with 22q11DS for interpreting IQ-scores and trajectories, in addition to general population IQ norms, can be similarly applied to and advantageous for individuals with other pathogenic genetic variants.

Several insights have been obtained from our and other studies of genetic and phenotypic characteristics of the 22q11.2 deletion, and other comparable rare pathogenic genetic disorders. While each of these variants is individually rare, they collectively contribute substantially to human morbidity[39]. A large proportion of our genes are involved in the development and function of our central nervous system. As a result, many of the pathogenic variants may have implications for neurodevelopment. Indeed, neurodevelopmental symptoms are reported in ~74% of individuals diagnosed with a known variant, and pathogenic variants are identified in ~40% of cases of developmental delay and in ~20% of ASD cases[56,57]. Some have seemingly more specific effects; such as the increased risk for schizophrenia of 25-fold, 40-fold, or 18-fold in the 22q11.2 deletion, the 3q29 deletion, and the 15q13.3 deletion respectively, although these prevalence estimates are likely influenced

by ascertainment biases and methods to various degrees[58]. Other rare variants have very large multisystem effect sizes implicated; such as the combination of short stature and significant developmental delay which is a clinical reality for virtually all individuals with trisomy 21[59]. Overall, however, the picture emerging from studies of neuropsychiatric phenomena in individuals with any of a spectrum of rare pathogenic variants consistently includes, to varying degrees, *pleiotropy*, *variable penetrance*, and *variable expressivity*. These phenomena are challenging to study and their underlying mechanisms are difficult to understand. The 22q11.2 deletion, being relatively prevalent and long known among other pathogenic genetic variants, offers a possibility for such investigations. The research chapters in this dissertation contribute to the understanding of these genetic phenomena. Examples include the finding that ASD and schizophrenia are likely pleiotropic consequences of the 22q11.2 deletion (Chapter 3); the examination of factors that potentially contribute to the variable penetrance for schizophrenia and other neurodevelopmental outcomes (Chapters 3, 5, 6 and 7); and the identification and quantification of effects of parental (genetic) background, major psychiatric illness, and the pathogenic 22q11.2 deletion on the variable expressivity of major neurobehavioral traits, including IQ (Chapters 6 and 7). Collectively, our results provide support for a model in which the variable penetrance for neuropsychiatric outcomes in individuals with pathogenic genetic variants is in part explained by the cumulative effect of many (common) genetic variants; i.e., polygenic scores (for example for schizophrenia or IQ) (**Figure 1**).

Figure 1. High-impact genetic variants exert their impact on neuropsychiatric phenotypes in the context of common genetic variation.



In the general population (left), the cumulative burden of common genetic variants that impact neurodevelopment are distributed (blue) such that only a very small proportion of individuals will

be above the phenotype expression threshold (red), and develop a neurodevelopmental disorder. In individuals with a high-impact variant (right), such as the 22q11.2 deletion, the same genetic background factors influence risk for neuropsychiatric outcomes. However, due to the elevated baseline risk conferred by the variant, a much larger proportion will express the phenotype.

Understanding how pathogenic variants contribute to neuropsychiatric expressions may ultimately aid in improving patient care and developing effective treatment strategies for individuals with any of these genetic variants. Specific examples include screening for specific risks, early detection, informed family planning, adequate support and services, and detailed prognosis (and tentative possibilities of treating the underlying pathology of the variant, guided by recent advances in genetically targeted therapies[60,61]). However, being so highly penetrant for certain neuropsychiatric phenotypes, these pathogenic variants also have the potential to provide key insights into developmental trajectories including the underlying biology of idiopathic neuropsychiatric disorders[39]. It is for this reason that the urgency for studies of such genetic variants is increasingly recognized. In this context, the importance of both **using quantitative assessment of neuropsychiatric domains *in addition to* employing a categorical approach to psychopathology** (Chapters 2 – 7), and of **assessing such domains consistently in patients *and* individuals without the pathogenic variant, such as family members** (Chapters 6 and 7) is supported by emerging evidence, including our studies, and progressively emphasized in the field going forward[14,62-64].

The work presented in this dissertation presents various approaches to the genetic and neuropsychiatric characteristics, and potential underlying mechanisms, of the 22q11.2 deletion in *humans*. The study of high-impact variants such as the 22q11.2 deletion also offers the unique possibility of multiple-level, complementary approaches to studying pathophysiological mechanisms of neurodevelopmental outcomes. The well-characterized genetic loci associated with pathogenic variants allow for parallel studies of genomic and phenotypic characteristics in humans and in experimental animal and cellular models[65,66]; a synthesis of approaches that may eventually contribute to the development of new and effective treatments of neuropsychiatric disorders[39]; which is currently hampered by our limited understanding of the mechanisms and pathways that underlie these disorders (*Problem 3*).

Taken together, the study of pathogenic genetic variants is promising in furthering our understanding of the mechanisms underlying complex neuropsychiatric outcomes. While most phenotypic descriptions so far have focused on the individual characterization of each variant; important in and of itself, the combined studying of pathogenic variants, including their commonalities and differences, is increasingly recognized as key[39]. Specifically: **the overlap among neuropsychiatric domains, within and between high-impact variants, provides the opportunity for cross-domain and cross-variant analyses**, which we have begun to explore in Chapter 6. Ultimately, this may assist in unraveling disease mechanisms, aid in the identification of biologically defined

subcategories of neuropsychiatric domains, and pave the way for the translation of scientific discoveries to clinical practice. At the time of writing this dissertation, a new international consortium with precisely this goal has been recently established ("Genes to Mental Health Consortium"; G2MH; https://genes2mentalhealth.com).

3. Limitations and directions for future research

The diversity and complementarity of approaches to the study of individuals with 22q11DS presented in this work have allowed for synergistic findings with respect to the understanding of neuropsychiatric outcomes in this population. These may help pave the way for studies that aim to further improve individualized outcome prediction in individuals with this, and other, high-impact genetic variant(s), and to further elucidate mechanisms underlying expression of neuropsychiatric phenotypes in general. Nonetheless, our studies have *not* taken into account several additional factors that may play a role in this regard. Such factors should be considered in future studies and may include environmental factors (e.g., socio-economic status, and access to and quality of the (mental) health care system (e.g., [67])); additional genetic factors (e.g., *additional* rare (structural and single-nucleotide) variation (e.g.,[19,68])), and pre-, peri-, and post-natal factors (e.g., maternal obesity, and birth complications including hypoxia) (e.g.,[69]).

Improvement of individualized outcome prediction remains a key objective in the field of pathogenic CNVs and neuropsychiatry more broadly. As is the case for the ever-increasing list of pathogenic genetic variants, the variability in (degree of) expressed phenotypes of the 22q11.2 deletion can still only be described in terms of group prevalence rates, causing uncertainty for caregivers with respect to individual needs and daily life expectations[70], and undermining the potential for prevention or early intervention strategies[38]. For example, based on current insights, early prevention strategies for schizophrenia (potentially with adverse side effects) would be applied unnecessarily in three out of four individuals with 22q11DS, given the *a priori* risk of 25%. While the clinical applicability of individualized neuropsychiatric outcome prediction remains, as of yet, hypothetical, several findings of this dissertation represent steps forward in this area and may direct future studies that investigate potential translation to the clinic. These include the variant-specific normative IQ data (Chapter 5); the robust modifying effect of parental cognitive functioning on 22q11DS offspring cognitive functioning (Chapter 6), further corroborated by the association between common variants for IQ (polygenic score) and cognitive functioning in individuals with 22q11DS (Chapter 7); and, more broadly, the potential of using individual common genetic variation data (specifically polygenic scores for IQ and schizophrenia) to improve risk stratification for outcomes of IQ, schizophrenia, and schizophrenia-associated phenotypes (Chapter 7). These findings are not yet ready to

be used as prediction tools in the clinic, but pave the way for further studies to translate to clinical settings, to refine individualized outcome predictions for patients, and possibly to suggest ameliorating strategies.

In addition to the potential of collaborative efforts to study various high-impact pathogenic genetic variants collectively to ultimately contribute to the understanding of mechanisms underlying neuropsychiatric phenomena, future studies may also reveal that **the 22q11.2 deletion and other pathogenic variants may provide a unique potential of bringing scientific advances to clinical implementation.** As is the case other areas of medicine, polygenic risk score findings in neuropsychiatry are not yet ready for use in clinical settings, but are being actively investigated[20,71,72]. However, it is possible that high-impact pathogenic variants will represent the first populations where individual data about common genetic variation will be used in clinical practice to improve understanding and prediction of phenotypic outcomes, and possibly target and guide intervention strategies[20]. In the context of a significantly elevated *a priori* chance of certain neuropsychiatric outcomes in these etiologically relatively homogeneous populations, polygenic risk data may eventually come to add to prediction metrics to a level that is adequate to be used in clinical practice (Chapter 7), pending future studies that will need to demonstrate the utility and feasibility thereof.

Our (e.g., Chapters 6 and 7) and other findings provide impetus to **elaborate on studies elucidating the role of parental background in neurobehavioral phenotypic expression of the 22q11.2 deletion.** Our studies have provided evidence for parent-offspring effects within one phenotypic domain. There may also be other phenotypes in parents (e.g., schizotypy) associated with major phenotypic outcomes in offspring (e.g., schizophrenia)[73]. Evidence for such effects is beginning to emerge. For example, a recent study reported an association between parental anxiety and depression level with offspring psychopathology, which was, notably, stronger in the 22q11DS group compared to a typically developing control group[74]. The study of parental background factors, both genetic and non-genetic, may help in further elucidating mechanisms underlying neurobehavioral phenotypic expression in individuals with 22q11DS, and potentially in identifying factors that play a role in the occurrence of the *de novo* 22q11.2 deletion.

Several observations regarding the domain of PIQ in relation to schizophrenia risk in the 22q11DS population collectively may prompt future research questions, exploring **whether low(er) PIQ is part of the core expression of underlying genetic schizophrenia risk**. A recent study in the general population identified larger genetic overlap between schizophrenia and PIQ, as compared to other domains of IQ[75]. Also, individuals with schizophrenia who have differentially low PIQ (vs. VIQ) are enriched for rare structural genetic variants other than the 22q11.2 deletion[55]. Observations from our studies appear

to be in line with these findings. First, initial IQ assessments in individuals with 22q11DS report on average significantly lower PIQ compared to VIQ (Chapter 5), consistent with other studies[23]. Second, while a decline in PIQ occurs, comparable to what is observed for VIQ (and FSIQ), the association with subsequent development of schizophrenia is strongest for VIQ-decline ([25] and Chapter 5). Third, the impact of the 22q11.2 deletion itself is most pronounced on PIQ, compared to VIQ (and FSIQ, and social and motor functioning, regardless of other background factors and schizophrenia expression (Chapter 6). In addition, the observed parent-proband effect was strongest for PIQ, compared to VIQ and FSIQ, with schizophrenia not even reaching statistical significance in the regression model for offspring PIQ. We also observed a trend-level difference in PIQ between parents of individuals with 22q11DS and schizophrenia versus parents of those with no psychotic illness. Collectively, these findings prompt the question whether there are aspects of the (shared) genetic make-up that lower the threshold for schizophrenia, i.e., account for the increased baseline risk of schizophrenia in individuals with 22q11DS, that may also account for an aberrant development of PIQ abilities. In addition, one could investigate whether this vulnerability-rendering variation (or instability) in the shared genetic make-up derives from the parent-of-origin specifically. A starting point would be to compare PIQ-levels between the parents of origin versus the co-parents of individuals with *de novo* 22q11.2 deletions, and to do so both in parents of 22q11DS with and without schizophrenia.

While a better understanding of mechanisms involved in disease pathways is a vital step towards the development and implementation of effective intervention strategies, studies are also needed to **interrogate the effectiveness of novel as well as existing intervention strategies in individuals with the 22q11.2 deletion**. Recent advances in the field provide guidance in this regard, an example of which are the findings discussed in Chapter 4. Specifically, given its association to subsequent functional outcome, the neurocognitive domain of Executive Performance could be a target for cognitive remediation strategies in individuals with 22q11DS, and more broadly, in individuals with, or at high risk for, schizophrenia[76-78]. A primary research question would be whether cognitive remediation focused on the domain of Executive Performance improves (long-term) functional outcome in individuals with 22q11DS, and a secondary question could be whether such cognitive remediation therapy in early adolescence may abate the risk for subsequent schizophrenia development.

IQ data available up to now in individuals with 22q11DS have resulted in a focus on the constructs of Verbal and Performance IQ, next to the overall Full Scale IQ. However, global cognitive functioning, and the construct of IQ, comprises four main components, that all formally or informally permeate all versions of the Wechsler Scales of Intelligence. Working Memory and Processing Speed are assessed independently from VIQ and PIQ, and reflect key neuropsychological processes. Specific abnormalities in these domains may be

associated with specific psychiatric or neurodevelopmental outcomes[79]. Future studies could **incorporate all core components of IQ, and aim to elucidate Working Memory and Processing Speed data**, to better understand the complete cognitive profile, trajectories, and associations with psychopathology[25,29] in individuals with 22q11DS.
Many individuals with 22q11DS may be hampered in their daily life functioning by one or more significant emotional, behavioral, or social symptom(s), that may or may not be captured in formal psychiatric diagnoses, according to the findings discussed here and elsewhere (e.g., [70,80-82]) and corroborated by clinical observations. Studies could aim to **elucidate the prevalence and character of such "subclinical" symptoms**, motivated by the overall goal to optimize functional outcome in individuals with 22q11DS.

Lastly, the phenotypic association between subthreshold psychotic symptoms and clinically diagnosable schizophrenia could be examined in more detail. Specifically, in Chapter 7 we demonstrated a nominally significant *genetic* association between subthreshold psychosis and schizophrenia in the context of a 22q11.2 deletion. Some previous studies have provided tentative evidence for a phenotypic association as well, both in 22q11DS and in the general population, however, sample sizes were relatively small and the studies were not always designed to investigate this association[18,83,84]. Hence, a large-scale (prospective) study, such as using the IBBC cohort, could provide more definitive evidence with respect to the association between subthreshold psychosis and schizophrenia, and the **potential contribution of subthreshold psychotic symptoms to understanding schizophrenia risk** in the 22q11DS population.

4. Conclusion

The main findings of the research comprising this dissertation can be summarized as follows:

- The early neurodevelopmental phenotypic expression of 22q11DS is complex, variable – both in type and severity of problems-, and important, both for clinical and research purposes, beyond its relevance to schizophrenia risk (Chapter 2).
- Early manifestations of ASD or ASD-like symptomatology cannot be viewed as a clinical marker that precedes schizophrenia onset in individuals with 22q11DS. ASD and schizophrenia are largely independent, different neuropsychiatric outcomes of 22q11DS, indicative of pleiotropy in the context of a high-impact pathogenic genetic variant (Chapter 3).
- Level of functioning on the specific neurocognitive domain Executive Performance is associated with subsequent functional outcome in individuals with 22q11DS, beyond the effects of schizophrenia and global intellectual functioning. There appears to be a profile of neurocognitive strengths and weaknesses that is

characteristic of individuals with 22q11DS, both with and without schizophrenia (Chapter 4).

- On average, global cognitive functioning and development in individuals with 22q11DS substantially differs from observations in the general population, including a modest decline in IQ-scores over the years. Normative IQ- and IQ-development data specific to 22q11DS present a substantial advance in addition to population-based IQ-norms: they reduce required sample size in a research context and allow for clinically more informed interpretation of IQ data and monitoring of patients (Chapter 5).
- Parental IQ is significantly associated with offspring IQ in individuals with *de novo* 22q11.2 deletions, while this effect is not observed for social and motor parameters. Patterns of influence of the pathogenic variant, schizophrenia, and parental functioning vary per phenotype, and may be different across different pathogenic variants, that may relate to primary associated neuropsychiatric conditions that vary across variants (Chapter 6).
- Common genetic variation for IQ modulates cognitive outcome in the context of a 22q11.2 deletion. Common genetic variation for schizophrenia not only modulates schizophrenia risk in this population, but is also associated with (early) schizophrenia-related phenotypes: cognitive decline and subthreshold psychotic symptoms. In the context of a high-impact variant such as the 22q11.2 deletion, common genetic variation, calculated as a PS for schizophrenia and IQ, may significantly improve risk/outcome stratification for these phenotypes (Chapter 7).

Complementary approaches to the study of individuals with 22q11DS at various life stages allowed our studies to collectively improve the understanding of neuropsychiatric outcomes in this population, both at the group level and at the individual level. Given the latter, these insights may contribute to improving clinical care and management of patients. Our studies address complex phenomena that are frequently observed in the context of any pathogenic genetic variant, and important for the understanding of mechanisms underlying neuropsychiatric conditions. The synergy of our genetics-first approach, within-family studies, prospective longitudinal and cross-sectional data, and the complementary analyses of categorical and quantitative neurobehavioral measures may provide key insights for 22q11DS with ramifications for other pathogenic genetic variants. In addition, the findings substantiate the value of the study of individuals with 22q11DS as a genetic model for schizophrenia and other neurodevelopmental disorders, and contribute to the understanding of trajectories and mechanisms underlying such neuropsychiatric conditions.

Appendix: Clinical considerations for 22q11DS

The importance of mental balance

The studies in this dissertation were not specifically designed to shape clinical guidelines for individuals with 22q11DS. Nonetheless, several insights obtained from our studies may be of relevance for clinicians, caregivers and patients. All of the following clinical considerations should be viewed in light of the overall goal of finding and maintaining **mental balance**. Finding and maintaining equilibrium between an individual's individual profile of strengths and weaknesses on the one hand, and environmental demands on the other is important for all humans; regardless genetic background, environmental risk and resilience factors and age. However, given the genetically mediated neuropsychiatric vulnerability of individuals with the 22q11.2 deletion, a healthy mental balance is of particular importance for these individuals. A prolonged mismatch of an individual's capacities and difficulties versus their environmental demands is likely to result in chronic stress. Both in the general population[38] as well as in idiopathic schizophrenia populations[85], stress has been identified as a trigger to the manifestation of (episodes of) psychopathology. In individuals with 22q11DS, high levels of anxiety have been identified as a predictor of transition to psychosis[86], and the role of stress in the transition to psychopathology is becoming increasingly evident. Specifically, evidence is accumulating that stressful life events modulate the risk for psychotic symptoms in adolescents with 22q11DS, and that individuals with 22q11DS may have a differential response, including increased sensitivity, to stress, compared to individuals without 22q11DS[87,88].

Characteristics of the cognitive and psychiatric profile in individuals with 22q11DS over the lifespan add to the complexity of the challenge of finding this mental equilibrium. The following recommendations echo findings from this dissertation, and may be helpful in obtaining optimal insight into an individual's profile, possibly in adjusting environmental demands accordingly, and thereby in finding and maintaining balance.

Regular cognitive assessments

The recommendation of regular evaluation of the cognitive profile of individuals (in particular youth) with 22q11DS[89,90] is substantiated by several observations. First, the overall level of cognitive functioning is significantly below average cognitive functioning in the general population (Chapters 3, 4, 5, and 6).

Second, more often than not, the cognitive profile of relative strengths and weaknesses is complex in individuals with 22q11DS (Chapters 4, 5, and 6). Specifically, a scattered profile with discrepancies between the different domains of cognitive functioning (e.g., a higher VIQ than PIQ) is common. This may easily lead to overestimation in which case a child's relatively high level of functioning in one domain is implicitly taken as an indicator of their global abilities. Frequently, this issue is further exacerbated in individuals with 22q11DS, as the clinical impression is that their initial presentation is often stronger, both

in terms of social and cognitive functioning, than their actual abilities in reality are. As a result, the environmental expectations, e.g., academic or social, may exceed the actual abilities of the individual. While the reverse, i.e., understimulation because a child is only deemed to be able to achieve at the level of their weakest domain, should be similarly avoided, a particular effort should be made to avoid overestimation of individuals with 22q11DS, given their genetic susceptibility for severe psychiatric problems. All in all, one cannot assume an overall level of cognitive functioning based on a relatively well-developed skill that biases initial presentation, particularly in individuals with 22q11DS.

Third, in individuals with 22q11DS, cognitive abilities may not be stable over time (Chapter 5). In contrast to the typical stability of IQ over the lifespan in the general population, individuals with 22q11DS, as a group, show a modest decline in their IQ over time. This observation is at a group-level, meaning that at an individual level, some individuals may remain stable or even show improvement over time, others develop but at a slower pace than expected (resulting in a relative decline in absolute IQ-scores), and yet others may demonstrate an absolute loss of cognitive abilities[25,26]. Taken together, studies provide evidence that some loss of IQ-points can be considered as part of the typical developmental trajectory of cognition in individuals with 22q11DS. This points towards the importance of not only regularly assessing the level of cognitive functioning in this population, but also interpreting the result in light of the normative data for individuals with 22q11DS specifically (Chapter 5), in addition to general population IQ-norms.

Deviations from the 22q11DS-specific cognitive trajectory may be associated with further increased risk for schizophrenia[25] (Chapters 5 and 7), and hence can be viewed as risk markers that require, at the minimum, additional monitoring of psychopathological development.

Regular psychiatric assessments

The psychiatric vulnerability in 22q11DS warrants close monitoring starting early in life. As outlined in the international guidelines for 22q11DS[89,90], it is recommended that all youth with 22q11DS, regardless the presence or absence of behavioral concerns, are regularly screened for any behavioral or emotional difficulties and that their development (cognitive and social) is regularly monitored. This follows directly from several observations. First, any individual with 22q11DS has a higher *a priori* chance of developing psychiatric symptoms[28], as compared to individuals without 22q11DS, with or without intellectual impairment (Chapter 2). Second, different developmental trajectories of psychiatric problems can be observed in individuals with 22q11DS: symptoms and disorders may emerge or intensify with maturation; they may remain constant over time; or they may be outgrown and no longer warrant a formal psychiatric diagnosis[29,86,91].

The variety in type and severity of neuropsychiatric outcomes associated with 22q11DS can provide guidance with respect the nature of these regular psychiatric assessments. First, the broad spectrum of neuropsychiatric phenotypes should be included, i.e.,

beyond a sole focus on schizophrenia spectrum disorders. Second, neuropsychiatric problems require attention even in absence of a formal psychiatric diagnosis (Chapters 2 and 3): "subclinical" symptoms should be considered, and a quantitative assessment of neurobehavioral traits, in addition to the more traditional categorical perspective on psychopathology, may offer insights into an individual's mental health profile.

Recognition of the complexity of the neurobehavioral profile in 22q11DS

In supporting individuals with 22q11DS in their daily living environments and in seeking and providing optimal clinical care, the complexity of the individual's neurobehavioral profile needs to be recognized and, in addition, viewed in the context of potential medical concerns of varying severity. This profile, including cognitive, social, emotional, and behavioral domains, is likely dynamic over time. In addition, both the cognitive and the psychiatric profile of an individual are likely complex, but their interplay further requires an effort to employ an integrative approach. Specifically, without some insight into the cognitive and developmental level of a child, the risk of "over-interpretation" of behavioral symptoms as indicators of psychiatric manifestations is high, while in fact, these "symptoms" may be appropriate for the developmental level of the child. The opposite risk also exists: the tendency for the intellectual disability to "overshadow" any psychiatric disorder. In this scenario, actual psychiatric symptoms are wrongly attributed to the intellectual disability of the individual. Overshadowing may withhold an individual from accessing appropriate treatment, while over-interpretation of symptoms may lead to unnecessary psychiatric treatment[92,93]. Hence, an integrative approach is needed, where cognitive, social, emotional and behavioral functioning are regularly assessed and their interplay is considered in choosing the most optimal management or intervention strategy, including recommendations to help establish and maintain adequate mental balance.

Additional clinical considerations

While early in life, physical health problems are the most likely reason for individuals with 22q11DS to require medical attention, adolescents and emerging adults with 22q11DS are more likely to be brought to medical attention because of mental health concerns[30,89,94,95]. The transition to adult health and social care poses an additional challenge for this age group. Despite increasing awareness of the importance of planned and guided transitions for young people with neurodevelopmental disorders, there is, as of yet, limited insight into and investigation of how to best accomplish this[96]. It would be beneficial if best practice guidelines for young people with 22q11DS would include a planned transition of mental health care, including psychiatric and cognitive (re-) assessments, across different life stages and stressors. It is important to discuss with patients and their families the vulnerability for psychiatric disorders and the importance of maintaining a healthy mental balance throughout different stages of life[90,97].

Having a child with a disability can be stressful for caregivers and siblings[98,99]. Studies in 22q11DS are consistent with this notion[100], and recent findings provide provisional evidence that the level of parental mental wellbeing significantly impacts the level of psychopathology in offspring with 22q11DS[74]. This underlines the importance of considering and assessing the wellbeing of family members in caring for an individual with 22q11DS, and providing adequate support where needed.

While studies into various treatments of psychiatric problems in individuals with 22q11DS are relatively scarce, there is, as of yet, no evidence against general treatment guidelines in this population. Current knowledge about 22q11DS can and should nonetheless be implemented in optimizing treatment, even when following general treatment guidelines. Examples include considering the patient's cognitive profile when choosing the best therapeutic approach (e.g., avoid treatment modalities that rely too heavily on verbal and abstract reasoning), and the recommendations to monitor calcium levels when prescribing anti-psychotic medication[90] and to evaluate the increased sensitivity to develop seizures[89].

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APPENDICES

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Appendices

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SAMENVATTING

Nederlandse samenvatting

Nederlandse samenvatting

1. Achtergrond

Het 22q11.2-deletie-syndroom

Bij mensen met het 22q11.2-deletie-syndroom (22q11DS) ontbreekt een deel (locus 11.2) van het genetisch materiaal (DNA) op de lange arm ("q") van chromosoom 22. In ~90% van de gevallen gaat het om een *de novo* mutatie in het genetisch materiaal; hetgeen betekent dat de deletie niet overgedragen is door één van de ouders, maar spontaan is opgetreden in de zaad- of eicel voorafgaand aan de bevruchting. De 22q11.2-deletie is een *pathogene* zeldzame genetische variant: pathogeen betekent dat het aan ziekte-uitkomsten gerelateerd is, en zeldzaam is gedefinieerd als voorkomend bij minder dan 1 op de 2000 mensen in de bevolking. Echter, vergeleken met andere zeldzame pathogene varianten is 22q11DS relatief veelvoorkomend. Naar schatting wordt 1 op de 2000 - 4000 kinderen geboren met 22q11DS. Ook is 22q11DS al langer bekend dan de meeste andere vergelijkbare genetische varianten.

De uitingen, of fenotypische expressies, van 22q11DS zijn erg variabel: er zijn vele verschillende somatische en neuropsychiatrische aandoeningen met de deletie geassocieerd, die ook nog eens sterk kunnen variëren in ernst (zie Introductie Figuur 1 voor een overzicht). Vrijwel alle mensen met 22q11DS ervaren problemen op een of meerdere gedragsmatige of cognitieve gebieden. Lage cognitieve vermogens (~45% heeft een verstandelijke beperking) en schizofrenie (bij ~20 – 25%) komen bijvoorbeeld vaak voor in deze patiëntengroep. De grote verschillen in neuropsychiatrische beelden, m.a.w. de grote fenotypische variabiliteit, vormen een grote uitdaging voor patiënten, verzorgers, en hulpverleners. De risicocijfers op groepsniveau laten zich niet eenvoudig vertalen naar het individu: ondanks de wetenschap dat ~20-25% van de patiënten met 22q11DS schizofrenie zal ontwikkelen, bijvoorbeeld, kunnen we op geen enkele manier voorspellen welke mensen tot deze groep zullen behoren voordat de ziekte zich grotendeels heeft geopenbaard. Ook in de wetenschappelijke context is dit een belangrijke uitdaging: hoewel we weten dat de 22q11.2-deletie het risico op verschillende neuropsychiatrisch beelden substantieel vergroot, hebben we nog weinig inzicht in de factoren die de uitkomsten voor een individu helpen bepalen, alsook in het vroege beloop en de onderliggende mechanismen van deze neuropsychiatrische uitkomsten.

Probleem 1: Hoewel we weten welke neuropsychiatrische manifestaties geassocieerd zijn met 22q11DS, zijn we niet in staat het type en de ernst van zulke uitkomsten te voorspellen voor een individu. Schizofrenie en niveau van cognitief functioneren zijn twee van de meest prominente onzekere uitkomsten.

Genetica

Ons genetisch materiaal kan gezien worden als een handleiding voor het vormen en functioneren van het menselijk lichaam, inclusief het brein. Het menselijk genoom (d.w.z. het totale DNA) bevat ~20.000 genen. Een gen is een sequentie van DNA-bouwstenen die 'baseparen' heten; hiervan hebben mensen er ~3 miljard. Genen coderen voor specifieke proteïnen, met elk een bepaalde functie in het lichaam. Ieder individu heeft twee kopieën van elk van de ~20.0000 genen (de genen op de geslachtschromosomen van de man uitgezonderd); een overgedragen door de moeder en een door de vader. Het DNA is verdeeld over 46 chromosomen, gerangschikt in 23 paren, waaronder 22 autosomen die genummerd zijn (1-22), en één paar geslachtschromosomen (XX voor vrouwen; XY voor mannen).

De technische mogelijkheden om inzichten in het genoom te verkrijgen zijn in de laatste jaren enorm vooruitgegaan. Zo is het nu bijvoorbeeld duidelijk dat genen een belangrijke rol spelen bij het vormen van uitkomsten in brede zin, inclusief neuropsychiatrische beelden. De variatie in het menselijk genoom is substantieel en bestaat op verschillende niveaus:

- Genetische variatie kan overgeërfd zijn (van moeder of vader), of '*de novo*' optreden.
- Genetische variatie kan op structureel niveau plaatsvinden, of op het niveau van enkelvoudige baseparen. De meest voorkomende structurele varianten hebben betrekking op het aantal kopieën van een deel van een chromosoom: 'Copy Number Variant' (CNV). CNVs kunnen inhouden dat er een deel van een chromosoom *ontbreekt* (een 'deletie' zoals bij de 22q11.2- deletie), of dat er een deel van een chromosoom *extra* is (een 'duplicatie' zoals bij de 22q11.2-duplicatie). Op het niveau van hele chromosomen kan er bijvoorbeeld een extra chromosoom zijn; zoals bij Trisomie 21, oftewel het downsyndroom. Variaties op het niveau van de basenparen worden 'Single Nucleotide Variants' (SNVs) genoemd en hebben dus betrekking op een verandering in één nucleotide.
- Genetische variatie kan zeldzaam zijn of veelvoorkomend; d.w.z. in >1% van de populatie. Zowel structurele varianten als varianten op het niveau van basenparen kunnen zeldzaam ofwel veelvoorkomend zijn, en worden in het laatste geval aangeduid als 'Single Nucleotide Polymorphisms' (SNPs).

Het is inmiddels duidelijk dat genen een rol spelen in observeerbare uitkomsten, waaronder neuropsychiatrische beelden: *genotype* houdt dus verband met *fenotype*. Een methode die heeft bijgedragen aan het begrijpen van genotype-fenotype-relaties is de 'Genome Wide Association Study' (GWAS). Deze methode maakt het mogelijk om veelvoorkomende genetische varianten op het niveau van de basenparen te identificeren, welke vaker voorkomen bij mensen met een bepaalde aandoening (bijvoorbeeld schizofrenie) dan bij mensen zonder deze aandoening. Vervolgens werd het mogelijk

om het risico voor een bepaalde aandoening te berekenen op basis van de met GWAS geïdentificeerde risicovarianten *gezamenlijk*. Deze 'som score' van de totaliteit van risicovarianten van een individu wordt aangeduid als de 'polygene (risico)score' (PS). De PS geeft een idee van de collectieve bijdrage van veel voorkomende varianten (SNPs) aan de variatie in neuropsychiatrische beelden op bevolkingsniveau. Bijvoorbeeld: tot 13% van het risico op schizofrenie en ~4% van de variatie in IQ kan in de algemene bevolking worden verklaard door respectievelijk de PS voor schizofrenie en IQ. Echter, ondanks de toenemende verklaarde variantie op bevolkingsniveau (met grotere steekproeven neemt ook de verklaarde variantie toe), kan de PS niet worden vertaald naar een betekenisvolle voorspeller van risico op individueel niveau. Dit is ten dele te verklaren door de lage prevalentie van de neuropsychiatrische aandoeningen (bijv. schizofrenie) in de algemene populatie.

Studies van zeldzame structurele genetische varianten tonen daarentegen een ander perspectief. Wanneer iemand een dergelijke CNV heeft, is dit doorgaans geassocieerd met een substantieel risico voor bepaalde neuropsychiatrische fenotypes. Derhalve worden zulke CNVs ook als 'pathogeen' aangeduid. De 22q11.2-deletie is hier een voorbeeld van (met bijv. ~20-25% risico op schizofrenie). Echter, doordat deze CNVs zeldzaam zijn, verklaren ze een zeer bescheiden deel van de variantie in neuropsychiatrische uitkomsten op bevolkingsniveau (zie ook Introductie Figuur 2). Daarnaast geldt ook voor deze zeldzame hoog-risico varianten dat ondanks de relatief hoge verklaarde variantie op individueel niveau, we op dit moment niet in staat zijn om betere individuele voorspellingen betreffende neuropsychiatrische uitkomsten te doen dan de basisrisicostatistieken (zie ook Probleem 1). Fenomenen welke consequent worden gezien in de context van pathogene CNVs en die bijdragen aan de uitdaging betreffende onzekerheid over neuropsychiatrische uitkomsten zijn:

- de observatie dat verschillende neuropsychiatrische beelden geassocieerd kunnen zijn met dezelfde variant. Dit wordt aangeduid als *pleiotropie:* bijv. sommige mensen met 22q11DS hebben schizofrenie, anderen hebben een verstandelijke beperking, en weer anderen (maar niet de meesten) hebben beide.
- De observatie dat niet ieder individu met dezelfde genetische variant een bepaald fenotype heeft: *variabele penetrantie*. Bijvoorbeeld: ~25% van de mensen met 22q11DS ontwikkelt schizofrenie, maar ~75% dus niet.
- De observatie dat er grote variatie is in de ernst van neuropsychiatrische beelden: *variabele expressiviteit*. Bijvoorbeeld: hoewel de meeste mensen met 22q11DS een zekere ontwikkelingsachterstand hebben, varieert het cognitief functioneren bij mensen met 22q11DS van zeer laag tot (boven)gemiddeld.

In werkelijkheid bestaan zowel veelvoorkomende SNVs alsook zeldzame CNVs niet in een isolement, maar dragen ze gezamenlijk bij aan het vormen van bepaalde uitkomsten.

De kennis over hoe veelvoorkomende genetische varianten interacteren met grotere structurele zeldzame varianten is vooralsnog zeer beperkt.

Probleem 2: Pathogene CNVs, zoals de 22q11.2-deletie, gaan gepaard met een substantieel verhoogd risico op neuropsychiatrische problemen voor een individu. Echter, vanwege hun zeldzame aard verklaren deze varianten vrijwel geen variatie in neuropsychiatrische uitkomsten op bevolkingsniveau. Veelvoorkomende genetische varianten verklaren anderzijds nauwelijks variatie op het niveau van het individu, hoewel deze wel, zoals gevat in een polygenetische score, een substantieel van de variatie verklaren op bevolkingsniveau. Er is nog te weinig kennis over de rol van veelvoorkomende genetische varianten in de context van een pathogene structurele genetische variant, zoals de 22q11.2-deletie.

Psychopathologie

Wanneer het gedrag, het denken, de emoties, en/of de ervaringen van een persoon substantieel afwijkend zijn en lijden, disfunctioneren, en soms gevaar in het dagelijks leven veroorzaken, kan men spreken van een psychische stoornis of psychopathologie. Schizofrenie is een van de meest ernstige en chronische psychiatrische aandoeningen, waarbij het welzijn maar ook langeretermijnuitkomsten inclusief levensverwachting, substantieel negatief worden beïnvloed. Deze ziekte wordt gekenmerkt door verstoringen in gedachten (bijv. wanen, waarbij iemand vaste overtuigingen heeft welke niet overeenkomen met de werkelijkheid), waarnemingen (bijv. hallucinaties, waarbij iemand stemmen kan horen zonder dat er een waarneembare stimulus is), en gedrag (bijv. sterk onvoorspelbare of ongepaste reacties). Inmiddels is duidelijk dat cognitief disfunctioneren tevens wezenlijk onderdeel uitmaakt van het ziekteproces. Daarbij is schizofrenie illustratief voor de complexiteit van het vroeg herkennen van de mensen die een verhoogd risico hebben op het ontwikkelen van een psychiatrische stoornis: vaak manifesteert de ziekte zich pas volledig rond de leeftijd van 20-25 jaar, terwijl er aanwijzingen zijn dat het ziekteproces reeds veel eerder in gang is gezet. Schizofrenie wordt dan ook steeds vaker geconceptualiseerd als 'ontwikkelingsstoornis'. Andere ontwikkelingsstoornissen worden doorgaans gekenmerkt door eerste symptomen die zich al in de jonge kindertijd openbaren. Een verstandelijke beperking uit zich bijvoorbeeld door een reeds op jonge leeftijd zichtbare zwakkere cognitieve ontwikkeling (IQ <70), in combinatie met beperkingen in het dagelijks functioneren. Autismespectrum- stoornissen (ASS) zijn ontwikkelingsstoornissen die zich kenmerken door problemen met het sociaal en communicatief functioneren, als ook door rigide en/of stereotype gedragingen.

Alle neuropsychiatrische aandoeningen worden op dit moment voornamelijk begrepen en gedefinieerd door hun observeerbare manifestatie. Met andere woorden: de wijze waarop psychiatrische stoornissen gecategoriseerd zijn verschaft weinig inzicht in mogelijke oorzaken (etiologie), ontwikkelingspaden, en ziektemechanismen. Bovendien

lijkt de puur categorische benadering van psychopathologie (d.w.z. iemand heeft een diagnose ja/nee) onvoldoende recht te doen aan de werkelijkheid waarin verschillende neuropsychiatrische beelden in zeer uiteenlopende mate van ernst, tezamen of onafhankelijk van elkaar kunnen voorkomen in de bevolking. Tezamen bemoeilijken deze problemen het verbeteren van individuele uitkomstpredictie, maar ook de ontwikkeling van meer gerichte en effectieve behandelmogelijkheden.

Probleem 3: Er is een substantieel gebrek aan inzichten met betrekking tot etiologie, mechanismen en vroege ontwikkelingstrajecten in neuropsychiatrische beelden, waaronder schizofrenie, verstandelijke beperking, en ASS. Uitdagingen die hieraan in belangrijke mate bijdragen zijn:

1. De complexiteit van het (vroeg) identificeren van mensen met een verhoogd risico voor neuropsychiatrische uitkomsten.
2. De grote etiologische heterogeniteit van neuropsychiatrische beelden.
3. De categorische conceptualisatie van neuropsychiatrische beelden.

In de context van bovenstaande uitdagingen kan het bestuderen van mensen met 22q11DS een waardevolle toevoeging vormen aan het begrijpen van neuropsychiatrische aandoeningen: mensen met 22q11DS zijn doorgaans al vroeg als zodanig geïdentificeerd, en dus ook als 'verhoogd risico' op neuropsychiatrische beelden (1); en genetisch gezien is het een relatief homogene groep (d.w.z. alle mensen hebben nagenoeg dezelfde deletie) (2).

Cognitief functioneren

'Cognitief functioneren' refereert aan het menselijk denk- en redeneervermogen en omvat verschillende vaardigheden, waaronder verbale en perceptuele, maar ook concrete en abstracte redeneervermogens. Het niveau van ons globale cognitief functioneren is een belangrijke determinant voor hoe we ons redden in het dagelijks leven. Menselijk cognitief functioneren kan worden gevat in een IQ-score (intelligentiequotiënt) en er bestaan verschillende gestandaardiseerde, gevalideerde en genormeerde instrumenten om het IQ te meten. Het totale niveau van cognitief functioneren wordt opgebouwd uit een aantal cognitieve domeinen. Volgens de Wechsler IQ-schalen, wereldwijd de meest gebruikte instrumenten, zijn er vier kerncomponenten van het totale IQ: het verbale IQ (VIQ; gerelateerd aan talige vaardigheden); het performale IQ (PIQ; gerelateerd aan perceptuele, structurerende en organisatorische vaardigheden); het werkgeheugen (WM; gerelateerd aan de korte-termijn opslag en bewerkingen van enkelvoudige informatie); en de verwerkingssnelheid (PS; gerelateerd aan de snelheid waarmee een stimulus verwerkt kan worden en een gepaste daaropvolgende actie kan worden ingezet). Naast deze kerncomponenten van het IQ bestaan er tevens specifiekere domeinen van neurocognitie, waaronder executief functioneren of complex geheugen. Deze nauwere

domeinen van neurocognitie zijn mogelijk vatbaarder voor verbetering door interventies, zoals bijvoorbeeld wordt onderzocht in studies die 'cognitieve remediatie' toepassen bij mensen met een hoog risico op schizofrenie. Doorgaans hangen de verschillende componenten van het IQ in hoge mate met elkaar samen en is het cognitief functioneren, gevat in IQ, stabiel over de levensloop – met inachtneming van de veranderingen passend bij opgroeien en verouderen. In de algemene bevolking is er natuurlijke variatie betreffende het niveau van cognitief functioneren tussen mensen. De IQ distributie volgt derhalve een normaalverdeling waarbij een IQ van 100 het gemiddelde is, met een standaard deviatie (SD) van 15 IQ punten (zie ook Introductie Figuur 3).

De rol van cognitief functioneren en cognitieve ontwikkelingstrajecten bij psychiatrische beelden wordt in toenemende mate duidelijk. Tevens wordt steeds duidelijker dat genen een rol spelen in het vormen van cognitieve uitkomsten: globaal cognitief functioneren behoort bijvoorbeeld tot de sterkst erfelijke eigenschappen. Ook worden steeds meer pathogene structurele genetische varianten geïdentificeerd welke geassocieerd zijn met afwijkende cognitieve trajecten en niveaus, waaronder hoge prevalenties van een verstandelijke beperking. Echter, gegeven de vaak afwijkende ontwikkeling en het lagere niveau van cognitief functioneren in mensen met pathogene CNVs, zijn normen voor cognitie die gebaseerd zijn op de algemene bevolking mogelijk niet volledig toepasbaar op mensen met dergelijke CNVs. Deze mismatch kan leiden tot bemoeilijking van het verkrijgen van nieuwe inzichten betreffende cognitieve ontwikkeling en uitkomsten in deze populaties.

Probleem 4: Normen van cognitief functioneren en cognitieve ontwikkeling zijn afkomstig van de algemene populatie en derhalve potentieel niet geheel toepasbaar en onvoldoende informatief in groepen mensen met pathogene genetische varianten, zoals de 22q11.2-deletie.

Overkoepelend doel

Het werk gepresenteerd in dit proefschrift adresseert de vier bovenstaande probleemstellingen en heeft als overkoepelend doel een bijdrage te leveren aan het begrijpen van de variabele expressie – en de onderliggende mechanismen - van neuropsychiatrische beelden bij mensen met een 22q11.2-deletie.

De verkregen inzichten kunnen bijdragen aan:

- Het beter begrijpen van mechanismen die een rol spelen bij de totstandkoming van neuropsychiatrische fenotypes bij mensen met deze, en andere hoog-risico genetische varianten.
- Meer inzicht verkrijgen in ontwikkelingstrajecten en mechanismen van neuropsychiatrische fenotypes, zoals schizofrenie en cognitief functioneren, in de algemene populatie.

- Het verbeteren van de klinische zorg en richtlijnen voor mensen met 22q11DS en hun families.

2. Belangrijkste bevindingen hoofdstukken

Hoewel één op de vier personen met 22q11DS schizofrenie zal ontwikkelen, zijn we momenteel niet in staat om deze 25% in een vroeg ontwikkelingsstadium te onderscheiden van de 75% van de patiënten die geen schizofrenie zullen ontwikkelen (*Probleem 1*). Sommige onderzoekers hebben gesteld dat de sociale en communicatieve moeilijkheden en repetitieve gedragingen, kenmerkend voor een autismespectrumstoornis (ASS) en vaak waargenomen bij kinderen met 22q11DS, in feite de vroege stadia van schizofrenie zouden kunnen zijn, hetgeen consistent is met het concept van schizofrenie als een neurologische ontwikkelingsstoornis. Veranderingen in sociaal gedrag en verslechterende communicatieve vaardigheden maken inderdaad deel uit van het waargenomen fenotype van schizofrenie en het schizofrenie-prodroom. In **Hoofdstuk 3** laten de resultaten van onze prospectieve studie van 89 kinderen en adolescenten met 22q11DS uit het Utrechtse cohort zien dat kinderen met ASS niet meer kans hebben om vervolgens een psychotische stoornis te ontwikkelen dan kinderen zonder ASS. Deze resultaten, die die van een eerdere retrospectieve studie in een onafhankelijke steekproef repliceren, suggereren dat een vroege diagnose van ASS, of symptomen van ASS, niet kan worden gezien als een klinische marker die wijst op een verhoogd risico op schizofrenie bij personen met 22q11DS. ASS en schizofrenie lijken eerder onafhankelijk van elkaar voor te komen in de context van een 22q11.2-deletie, wat aangeeft dat dit twee verschillende, pleiotrope gevolgen zijn van de 22q11.2-deletie. Naast de traditionele operationalisering van ASS als een dichotome variabele, gebruikten we in deze studie een kwantitatieve maatstaf voor ASS-symptomatologie, waarbij we dezelfde resultaten verkregen met betrekking tot het ontbreken van associatie met het risico op schizofrenie. Deze kwantitatieve benadering benadrukte ook dat een groot deel van de mensen met 22q11DS klinisch relevante symptomen van ASS heeft, zelfs als er geen formele diagnose is. De substantiële prevalentie van dergelijke 'subklinische' psychiatrische symptomen heeft mogelijke implicaties voor klinische zorg voor en studies van mensen met 22q11DS (ook besproken in **Hoofdstuk 2**), inclusief het belang van het aandacht hebben voor de vroege psychische ontwikkeling van 22q11DS op zich; d.w.z. buiten de mogelijke associatie met het risico op schizofrenie.

In **Hoofdstuk 4** onderzochten we de associatie tussen functionele (dagelijks leven) uitkomsten en domeinen van neurocognitief functioneren; relevant voor *Probleem 1*. Dergelijke neurocognitieve domeinen vertegenwoordigen meer specifieke vaardigheden dan het globale IQ en hebben het voordeel dat ze mogelijk vatbaarder zijn voor interventies. Gegevens van 99 volwassenen met 22q11DS uit het Toronto-cohort suggereren dat

executief functioneren (hetgeen mentale processen vertegenwoordigt die ons in staat stellen om te plannen, de aandacht te richten en meerdere taken succesvol te managen) significant bijdraagt aan de variabiliteit in de (daaropvolgend gemeten) functionele uitkomst bij mensen met 22q11DS, zelfs rekening houdend met eerder geïdentificeerde voorspellers zoals schizofrenie en globaal cognitief functioneren (FSIQ). Bovendien onthulden de gegevens in Hoofdstuk 4 een profiel van sterke en zwakke neurocognitieve aspecten dat informatief kan zijn voor (zorgverleners van) mensen met 22q11DS (relevant voor *Probleem 4*), en dat enige overeenkomsten vertoont met bevindingen bij andere schizofrenie- en schizofrenie-hoog-risico-populaties. In het bijzonder, hoewel er beperkingen zijn op alle domeinen van neurocognitie bij personen met 22q11DS, zijn de prestaties gemiddeld beter bij taken die verband houden met visueel (in plaats van verbaal) geheugen, en zijn de prestaties gemiddeld het slechtst bij motorische taken. Samenvattend duidt hoofdstuk 4 op de wisselwerking en onderlinge afhankelijkheid van verschillende niveaus (bijv. globaal en specifiek) en domeinen (bijv. executief functioneren en motorisch functioneren) van cognitief functioneren, schizofrenie (en schizofrenie-risico), en het dagelijks functioneren in de context van de 22q11.2-deletie.

In **Hoofdstuk 5** hebben we *Probleem 4* behandeld en bestudeerden we cognitieve gegevens van 1365 individuen met 22q11DS uit de IBBC, waarbij we normatieve IQ-gegevens binnen deze populatie presenteerden. We hebben aangetoond dat een normatieve grafiek voor cognitieve ontwikkeling voor 22q11DS gemaakt kan worden en dat het gebruik van cognitieve normen die specifiek zijn voor individuen met een bepaalde genetische variant, de grootte van een steekproef die nodig is in een onderzoek context aanzienlijk kan verminderen, vergeleken met het gebruik van de standaard IQ-normen (welke gebaseerd zijn op de algemene populatie). Vanuit een klinisch perspectief kunnen de variant-specifieke cognitieve normen nuttige informatie opleveren naast het gebruik van (niet-getransformeerde) populatie-gebaseerde IQ-normen, waaronder een meer accurate en informatieve interpretatie van individuele IQ-scores en -trajecten.

In **Hoofdstuk 6** breiden we onze focus uit van het begrijpen van de neuropsychiatrische fenotypische expressies van 22q11DS, naar de studie van de mogelijke onderliggende mechanismen (relevant voor de *Problemen 1, 2 en 3*). In een steekproef van 230 mensen (Toronto-cohort), waaronder 82 volwassenen met een *de-novo*-22q11.2-deletie en hun niet-aangedane ouders, bestudeerden we de expressie van drie neuropsychiatrische fenotypes die worden aangedaan door 22q11DS en tevens samenhangen met ernstige psychiatrische stoornissen. We identificeerden een significante associatie tussen het niveau van ouders en kind wat betreft de cognitieve maten (IQ), maar niet voor maten van sociaal en motorisch functioneren.
Bovendien toonden we aan dat, in vergelijking met de ouderlijke scores, de 22q11.2-deletie een negatieve impact heeft op de gemeten fenotypes, met verder verlaagde

scores bij degenen met schizofrenie. De invloedspatronen (d.w.z. de effectgroottes van de deletie, schizofrenie en ouderlijke scores) verschilden per fenotype, hetgeen suggereert dat de fenotypes verschillende onderliggende genetische mechanismen zouden kunnen hebben. In Hoofdstuk 6 ontrafelen we voor het eerst de impact van een pathogene variant van de modificerende effecten van ouderlijke scores en schizofrenie op belangrijke dimensionale gedragsfenotypes. Deze studie toont de meerwaarde van het dimensioneel meten van gedragsfenotypen, ter aanvulling op een categorische benadering, in de context van een studie die uitgaat van het genotype (d.w.z. de 22q11.2-deletie; 'genotype-first') en van het functioneren in relatie tot scores binnen het primaire gezin. Dergelijk onderzoek zou de basis kunnen vormen voor toekomstige studies die zouden kunnen streven naar het verder verbeteren van uitkomstvoorspellingen op individueel niveau en naar het vergelijken van invloedspatronen tussen verschillende pathogene varianten.

In **Hoofdstuk 7** gaan we dieper in op de studie van genetische mechanismen die betrokken zijn bij de fenotypische expressie van 22q11DS, waarmee we expliciet *Probleem 2* adresseren. We onderzochten de directe genetische associatie van verschillende, met schizofrenie geassocieerde, fenotypes met schizofrenie in een steekproef van 962 mensen met 22q11DS uit het IBBC-cohort, met behulp van polygene scores die zijn afgeleid van de algemene bevolking. Onze resultaten bevestigen eerdere resultaten: dat de polygene score voor schizofrenie, ook in de context van een 22q11.2-deletie, geassocieerd is met schizofrenie. Ook tonen onze resultaten voor het eerst aan dat de polygene score voor IQ significant geassocieerd is met cognitief functioneren in de 22q11DS-populatie. Tevens ontdekten we dat de polygene score voor schizofrenie ook geassocieerd is met twee aan schizofrenie gerelateerde fenotypes: cognitieve achteruitgang en subklinische psychotische symptomen, terwijl de polygene score voor IQ niet geassocieerd is met die fenotypes. Deze resultaten suggereren dat dezelfde varianten die geassocieerd zijn met een verhoogd risico op schizofrenie, in 22q11DS ook verband houden met het risico op cognitieve achteruitgang en subklinische psychotische klachten, en dat deze fenotypes mogelijk vroegere stadia van hetzelfde ziekteproces zijn (relevant voor *Probleem 3*).
Onze bevindingen wijzen bovendien op het potentieel van het gebruik van polygene scores om de risicostratificatie voor belangrijke gedragsfenotypen in 22q11DS te verbeteren: schizofrenie en verstandelijke beperking (IQ <70) (relevant voor *Probleem 1*). Wanneer we de patiënten met de laagste PS (onder 10e percentiel) vergelijken met de patiënten met de hoogste PS (PS boven de 90e percentiel) voor schizofrenie en IQ vergelijken, zien we dat 33% versus 9% schizofrenie heeft en 63% versus 24% een verstandelijke beperking. Deze resultaten markeren een substantiële vooruitgang in risicostratificatie in vergelijking met de basisrisicocijfers van respectievelijk ~ 25% (schizofrenie) en ~ 45% (verstandelijke beperking) voor 22q11DS. Deze bevindingen zijn nog niet geheel klaar voor implementatie in de kliniek (bijvoorbeeld in afwachting van replicatie). Ze benadrukken echter het toekomstige potentieel van het gebruik van

polygene scores voor het begrijpen en voorspellen van neuropsychiatrische uitkomsten, in de context van een populatie met een *a priori* verhoogd risico, vanwege een sterk, maar onvolledig, penetrante genetische variant.

3. Overkoepelende conclusies

Het werk in deze PhD-dissertatie draagt collectief bij aan het beter begrijpen van de variabele expressie en onderliggende mechanismen van neuropsychiatrische fenotypen bij mensen met de 22q11.2-deletie.
Hiertoe hebben we verschillende, complementaire methoden en studieontwerpen gebruikt. Onze studies omvatten zowel pediatrische als volwassen cohorten, cross-sectioneel en longitudinaal onderzoek, en retrospectieve en prospectieve methoden, waardoor de ontwikkelingstrajecten van neuropsychiatrische fenotypes konden worden onderzocht. In dit werk hebben we deze fenotypen zowel vanuit een categorisch perspectief benaderd, bijvoorbeeld door vast te houden aan de traditionele dichotome classificatie van psychopathologie, terwijl we tegelijkertijd de potentie van het operationaliseren van neuropsychiatrische kenmerken op een kwantitatieve manier hebben onderzocht, bijvoorbeeld door ons te concentreren op een dimensionale benadering van cognitief functioneren en andere gedragsdomeinen. Sommige onderzoeken werden uitgevoerd in grote steekproeven (IBBC), waardoor de rol van veelvoorkomende genetische variatie in de context van een 22q11.2-deletie in belangrijke neuropsychiatrische uitkomsten kon worden onderzocht, terwijl andere, kleinere onderzoeken goed gekarakteriseerde steekproeven omvatten met meer gedetailleerde fenotypering (in één onderzoek met zowel individuen met 22q11DS als hun niet-aangedane ouders). Hoewel elke benadering afzonderlijk bijdraagt aan het begrip van een of meer aspecten van de neuropsychiatrische expressie van 22q11DS, kan hun synergie leiden tot inzichten in mogelijke mechanismen die de neuropsychiatrische uitkomsten helpen bepalen. Samen dragen de resultaten bij aan verhelderende observaties en mechanismen die specifiek kunnen zijn voor 22q11DS en die generaliseerbaar kunnen zijn voor populaties van individuen met andere pathogene varianten en/of idiopathische neuropsychiatrische aandoeningen.

Algemene observaties voor 22q11DS
Een aantal observaties over de groep mensen met 22q11DS komt consequent naar voren in verschillende onderzoeken die deel uitmaken van deze dissertatie. Ten eerste, *gemiddeld* ligt het IQ van de groep mensen met 22q11DS circa 30 IQ punten lager dan het gemiddelde van de algemene bevolking. Naast deze -2-SD-verschuiving lijken de karakteristieken van de IQ-distributie van mensen met 22q11DS overeenkomstig met die in de algemene populatie. In het bijzonder: IQ-scores zijn ook bij mensen met22q11DS normaal verdeeld met een SD van ~15, maar dan met een gemiddelde van ~70. Tevens

zien we consequent discrepanties tussen de verschillende componenten van het IQ bij mensen met 22q11DS, waarbij met name het PIQ doorgaans lager ligt dan het VIQ.

Met betrekking tot het ontwikkelingstraject van globaal cognitief functioneren bij personen met 22q11DS laten gegevens een gemiddelde afname in IQ-scores zien met de tijd. Onze studies suggereren dat in deze populatie een zekere daling in IQ-punten (~ -7 IQ-punten tussen de 6 en 12 jaar) kan worden verwacht. Voor de meeste mensen met 22q11DS impliceert deze 'achteruitgang' niet noodzakelijkerwijs een absoluut verlies van cognitieve capaciteiten, maar eerder een langzamer ontwikkelingstraject vergeleken met normaal ontwikkelende leeftijdsgenoten. Het interpreteren van IQ-scores en IQ-trajecten bij personen met 22q11DS in de context van normatieve IQ- en IQ-ontwikkelingsgegevens die zijn afgestemd op deze specifieke populatie, en kan dus een aanvulling zijn op de traditionele benadering van het gebruik van gestandaardiseerde IQ-scores (d.w.z. genormeerd naar de algemene populatie). De timing van de 'afname' in IQ-scores lijkt grotendeels samen te vallen met de overgang van concreet naar meer abstract redeneervermogen, wat suggereert dat de ontwikkeling van abstract redeneervermogen bij kinderen met 22q11DS relatief meer vertraagd en/of belemmerd kan zijn dan de ontwikkeling van concreet redeneervermogen. Onze studies hebben ook aangetoond dat een negatieve afwijking van het verwachte IQ-traject, dat wil zeggen een IQ-daling (in het bijzonder in VIQ) die hoger ligt dan wat verwacht wordt binnen deze populatie, geassocieerd is met een verhoogd risico op schizofrenie.

Onze data ondersteunen de bewering dat het scala aan psychopathologie geassocieerd met 22q11DS niet enkel gezien kan worden als een gevolg van het lagere cognitieve niveau van deze individuen (zie ook Hoofdstuk 2). Hoewel er substantiele intra- en interpersoonlijke verschillen zijn binnen de 22q11DS-populatie, lijken aspecten van zowel het cognitief functioneren als het profiel van psychopathologie kenmerkend te zijn voor de groep mensen met 22q11DS en niet een 'aspecifiek' gevolg van een gemiddeld lager IQ in deze groep.

Potentiele mechanismen

Onze bevindingen suggereren dat gedeelde (genetische) factoren een belangrijke rol spelen in de variabele expressiviteit van het cognitieve fenotype bij individuen met 22q11DS (zie ook Discussie Figuur 1). Hoofdstuk 6 laat zien dat het niveau van cognitief functioneren bij individuen met een *de novo* 22q11.2-deletie significant geassocieerd is met het niveau van cognitief functioneren van hun niet-aangedane ouders. Met andere woorden, een relatief hoog ouderlijk IQ, bijv. IQ> 120, komt waarschijnlijk overeen met een relatief hoog IQ bij de nakomelingen met 22q11DS, bijv. IQ> 80. Dit kan te wijten zijn aan erfelijke genetische en niet-genetische factoren die de grote primaire impact van de 22q11.2-deletie modificeren. Hoofdstuk 7 biedt genetisch bewijs ter ondersteuning van dit modificerende effect van het ouderlijke IQ op het IQ van nakomelingen. Hier laten de resultaten zien dat veelvoorkomende genetische varianten die geassocieerd zijn met

intellectueel functioneren in de algemene populatie (polygene score voor IQ), een rol spelen bij het vormgeven van het cognitieve fenotype bij personen met 22q11DS. Hoewel de rol van veelvoorkomende genetische variatie in cognitieve uitkomsten in de algemene bevolking goed is vastgesteld, is dit, voor zover wij weten, de eerste keer dat genetische gegevens hetzelfde mechanisme suggereren in de context van een pathogene genetische variant die wordt geassocieerd met neuropsychiatrische uitkomsten. Onze studies suggereren ook dat dit mechanisme niet noodzakelijk generaliseerbaar hoeft te zijn over verschillende (dimensionale) gedragsfenotypes in de context van de 22q11.2-deletie: Hoofdstuk 6 onthulde geen verband tussen het niveau van ouders en nakomelingen op domeinen van sociaal en motorisch functioneren, in tegenstelling tot het domein van cognitief functioneren. Toekomstige studies zouden dit verder kunnen onderzoeken. Dergelijke studies kunnen uiteindelijk uitwijzen of er differentiële effecten zijn (bijv. van de CNV en van gedeelde genetische en niet-genetische factoren) tussen verschillende pathogene varianten die verband kunnen houden met de hieraan primair geassocieerde neuropsychiatrische aandoeningen, zoals hoofdstuk 6 tentatief suggereert.

Onze studies suggereren dat cognitieve achteruitgang niet alleen fenotypisch geassocieerd is met de daaropvolgende 'volledige' expressie van schizofrenie, maar mogelijk een vroeg ziektestadium representeert en waarschijnlijk een deel van zijn genetische etiologie deelt. Veelvoorkomende genetische varianten voor schizofrenie (polygene score) komen in verhoogde mate voor bij personen met 22q11DS die een significante cognitieve achteruitgang vertonen, vergeleken met personen met 22q11DS, maar zonder cognitieve achteruitgang (die groter is dan het verwachte traject), terwijl de polygene score voor IQ niet significant verschilt tussen deze twee subgroepen (hoofdstuk 7). Gezamenlijk suggereren deze bevindingen dat cognitieve achteruitgang bij mensen met 22q11DS genetisch gecorreleerd lijkt te zijn met schizofrenie. Onze bevindingen met betrekking tot IQ-achteruitgang in 22q11DS komen overeen met bevindingen in de algemene bevolking. Ten eerste is er substantieel bewijs voor de fenotypische manifestatie van cognitieve achteruitgang en het verband met het daaropvolgende risico op schizofrenie. Ten tweede is er bewijs ter ondersteuning van een genetische correlatie tussen schizofrenie en cognitieve achteruitgang in de algemene bevolking. Een recente studie rapporteerde dat van 540 idiopathische schizofreniepatiënten die werden bestudeerd, degenen met een significante cognitieve achteruitgang de hoogste polygene risicoscore voor schizofrenie hadden, vergeleken met personen die cognitief stabiel bleven en/of al een ernstigere cognitieve beperking hadden vanaf jonge leeftijd.

22q11DS als model

In de afgelopen twee decennia is de 22q11.2-deletie in toenemende mate geïdentificeerd en erkend als een waardevol genetisch model voor het bestuderen van schizofrenie. Er zijn verschillende voordelen ten opzichte van studies in de algemene populatie. Mensen

met 22q11DS kunnen zeer vroeg in hun leven worden geïdentificeerd en gevolgd vóór de gevorderde stadia van schizofrenie (d.w.z. actieve psychose); ze lopen een hoog risico om de ziekte te ontwikkelen (d.w.z. de vereiste steekproefomvang voor longitudinale onderzoeken is dus lager bij studies van 22q11DS ten opzichte van de algemene bevolking); en ze vormen een etiologisch relatief homogene populatie. Een dergelijke 'genotype-first'-benadering om neuropsychiatrische fenotypes breder te begrijpen vereist een zekere mate van convergentie met waarnemingen van idiopathische neuropsychiatrische populaties. Onze en eerdere studies hebben inderdaad aangetoond dat de manifestaties van schizofrenie-spectrumstoornissen bij mensen met 22q11DS vergelijkbaar zijn met die bij mensen met idiopathische schizofrenie. De bevindingen uit dit proefschrift met betrekking tot de fenotypische expressie van schizofrenie, met schizofrenie geassocieerde fenotypes, en de genetische mechanismen die daaraan ten grondslag liggen, dragen gezamenlijk bij aan het begrijpen van 22q11DS als een genetisch model voor schizofrenie.

De focus op het verhoogde risico op schizofrenie veroorzaakt door de 22q11.2-deletie is begrijpelijk, zowel vanuit een wetenschappelijk als vanuit een klinisch perspectief, gezien de sterkte van de associatie; de ernst en lijdensdruk van schizofrenie; en het feit dat schizofrenie het eerste psychiatrische fenotype was dat werd beschreven bij mensen met 22q11DS. Echter, geleidelijk werd een veel breder scala aan aan 22q11DS geassocieerde neuropsychiatrische fenotypes onthuld door studies inclusief de onze. Dit benadrukt niet alleen de relevantie van het uitgebreider bestuderen van deze fenotypes in 22q11DS, maar toont ook de potentie van 22q11DS als een genetisch model om een breed scala aan neuropsychiatrische beelden te bestuderen, inclusief verstandelijke beperking en autismespectrumstoornissen (zie ook Hoofdstuk 2).

In de afgelopen decennia zijn er steeds meer pathogene genetische varianten ontdekt die geassocieerd zijn met neuropsychiatrische beelden in verschillende mate van ernst, zoals de 22q11.2-deletie. In verhouding tot deze, meestal zeldzame, genetische aandoeningen komt de 22q11.2-deletie relatief veel voor (1/2000-4000), en heeft de genetische beschrijving ervan in het begin van de jaren '80 ongeveer twee decennia eerder plaatsgevonden dan de veel recentere ontdekking van de meeste andere zeldzame pathogene genetische varianten. Dit heeft 22q11DS als het ware een voorsprong opgeleverd, waardoor het als model kan fungeren voor het bestuderen van andere pathogene genetische varianten. Inmiddels zijn internationale consortia, vergelijkbaar met de 22q11DS-IBBC, opgericht voor verschillende pathogene varianten, evenals grote samenwerkingsinspanningen tussen verschillende genetische varianten. Een voorbeeld van hoe onze studies van 22q11DS als opstapje kunnen fungeren voor studies van andere vergelijkbare varianten is hoe we het fenomeen *variabele penetrantie* begrijpen: de resultaten van onze studies ondersteunen een theoretisch model waarbij de variabele penetrantie van neuropscyhiatrische fenotypes in de context van een pathogene genetische variant (zoals de 22q11.2-deletie), deels verklaard kan worden

door veelvoorkomende variatie in de rest van het genoom (zie ook Hoofdstuk 8, Discussie, Figuur 1).

Limitaties en richtingen voor vervolgonderzoek

De onderzoeken binnen dit proefschrift hebben een aantal aspecten *niet* meegenomen, welke mogelijk wel van belang zijn bij het volledig begrijpen van de expressie van neuropsychiatrische fenotypes bij mensen met 22q11DS. Het is wenselijk dat toekomstige studies de rol van dergelijke factoren onderzoeken, inclusief omgevingsfactoren (bijv. sociaal-economische status, blootstelling aan stress, en toegang tot en kwaliteit van de gezondheidszorgsystemen), additionele genetische factoren (bijv. andere zeldzame genetische varianten (zowel op structureel als op basenparenniveau), en pre-, peri-, en post-natale factoren (bijv. overgewicht bij moeder en complicaties bij de geboorte).
Verbetering van geïndividualiseerde uitkomstvoorspellingen blijft een hoofddoelstelling van studies naar 22q11DS en op het gebied van pathogene CNVs en neuropsychiatrie in bredere zin. Onze studies, met name in Hoofdstukken 6 en 7, kunnen dienen als basis voor dergelijk vervolgonderzoek. Daarnaast hebben onze studies de potentie laten zien van het bestuderen van mensen met pathogene genetische varianten voor het vertalen van wetenschappelijke bevindingen naar de klinische praktijk (zie bijvoorbeeld Hoofdstuk 7). Andere vraagstellingen die voortvloeien uit onze bevindingen hebben betrekking op de rol van ouders (zowel wat betreft genetische- alsook omgevingsfactoren) in de expressie van neuropsychiatrische beelden bij mensen met 22q11DS; en op de relatie tussen PIQ en schizofrenie, waarbij een primaire vraag zou zijn of een verlaagd PIQ deel uitmaakt van de kernexpressie van onderliggend genetisch schizofrenierisico. Tot slot geven recente studies, waaronder de onze, aanleiding om de effectiviteit van zowel nieuwe als bestaande interventies gericht op het verbeteren van neuropsychiatrische uitkomsten bij mensen met 22q11DS te onderzoeken, en om te streven naar het in kaart brengen van het *volledige* cognitieve profiel bij mensen met 22q11DS over de levensloop.

Conclusie

Door complementaire benaderingen van het bestuderen van mensen met 22q11DS in verschillende levensfasen dragen onze studies gezamenlijk bij aan het beter begrijpen van neuropsychiatrische expressie in deze populatie, zowel op groepsniveau als op individueel niveau. Sommige verkregen inzichten kunnen uiteindelijk bijdragen aan het verbeteren van de klinische zorg voor patiënten (zie ook Appendix van Hoofdstuk 8: 'Clinical considerations for 22q11DS'). Onze studies adresseren complexe fenomenen die vaak voorkomen in de context van vrijwel alle pathogene genetische varianten, en die belangrijk zijn voor het begrijpen van mechanismen die ten grondslag liggen aan neuropsychiatrische aandoeningen. De synergie van onze studies, waarbij we een "genetics-first" methode combineerden met een intrafamiliale benadering; zowel prospectieve longitudinale als ook cross-sectionele data gebruikten; en een categorische benadering

van psychopathologie complementeerden met kwantitatieve maten van gedrag en cognitie, kan belangrijke inzichten opleveren voor 22q11DS met implicaties voor andere pathogene genetische varianten. Bovendien bevestigen de bevindingen de potentie van 22q11DS als een genetisch model voor schizofrenie en andere neuropsychiatrische fenotypes, en dragen ze bij tot het begrijpen van trajecten en mechanismen die ten grondslag liggen aan dergelijke neuropsychiatrische aandoeningen.

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PUBLICATIONS

List of key publications

List of Key Scientific Publications

Publications: first authored

- A.M. Fiksinski, T. Heung, M. Corral, E.J. Breetvelt, G. Costain, C.R. Marshall, R.S. Kahn, J.A.S. Vorstman, A.S. Bassett (2020). Within-family influences on dimensional neurobehavioral traits in a high-risk genetic model. *Psychological Medicine, in press.*
- A.M. Fiksinski*, R.W. Davies*, […], IBBC, C.E. Bearden, J.A.S. Vorstman (2020). Using common genetic variation to examine phenotypic expression and risk prediction in 22q11.2 deletion syndrome. *Nature Medicine, https://doi.org/10.1038/s41591-020-1103-1.*
- A.M. Fiksinski & J.A.S. Vorstman. Psychiatric profile in childhood and youth. Book chapter in The 22q11.2 Chromosome Deletion Syndrome: A Multidisciplinary Approach to Diagnosis and Treatment, ed.: D.M. McDonald-McGinn (2020). *In press.*
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- A.M. Fiksinski, M. Schneider, J.R. Zinkstok, D. Baribeau, S.J.R.A. Chawner, J.A.S. Vorstman. Neurodevelopmental trajectories and psychiatric morbidity: lessons learned from the 22q11.2 deletion syndrome. *Under review (Current Psychiatry Reports, November 2020).*
- A.M. Fiksinski, C.E. Bearden, A.S. Bassett, R.S. Kahn, J.R. Zinkstok, S.R. Hooper, W. Tempelaar, IBBC, J.A.S. Vorstman*, E.J. Breetvelt*. A normative chart for cognitive development in a genetically selected population. *Under review (Neuropsychopharmacology, July 2020).*

Publications: co-authored

- C. Ching, […], A.M. Fiksinski, […], C.E. Bearden (2020). Mapping subcortical brain alterations in 22q11.2 deletion syndrome: effects of deletion size and convergence with idiopathic neuropsychiatric illness. *American Journal of Psychiatry 177 (7): 589-600.*
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BIOGRAPHICAL

Biographical sketch

Biographical sketch

Anna Maria (Ania) Fiksinski was born on June 26th 1990 in Enschede, the Netherlands. After obtaining her VWO-diploma in 2007, she enrolled in professional dance and theatre training. Starting in 2009, she completed her BA. in Psychology and Linguistics at University College Utrecht. In 2013, she obtained her MSc. in Clinical and Health Psychology at the University of Utrecht. Over the course of several years, she combined her academic training with part-time work as a psychiatric social worker at an assisted-living facility for children and young adults from multi-problem families with intellectual disability and comorbid psychiatric disorders. In November 2013, Ania started working as a psychologist at the Children's Hospital in Utrecht (WKZ). Her academic curiosity was further spurred by working together with Dr. Jacob Vorstman at the University Medical Center in Utrecht (UMCU), and resulted in a PhD position under the joint supervision of Prof. Dr René Kahn (UMCU and Icahn School of Medicine at Mount Sinai, New York, USA), Prof. Dr. Anne Bassett and Dr. Jacob Vorstman (both at the University of Toronto, Canada) starting in 2016. During her PhD, Ania coordinated the 22q11DS psychiatry study in Utrecht and helped initiate and execute a novel study including families of individuals with 22q11DS in Toronto, working closely with Dr. Bassett. She spent over a year in Toronto, seeing many adult patients with 22q11DS and their families, while mostly seeing children and youth with 22q11DS in the Netherlands. During her PhD, the focus of which was to better understand (trajectories of) neurobehavioral phenotypes in individuals with 22q11DS, she was awarded several grants and prizes that supported her research and international collaborations. Ania is also actively involved in patient/family organizations for 22q11DS, both on a national and international level. She acts as an Advisor to the International 22q11DS Society. Ania is looking forward to continuing her academic career in combination with continued clinical specialization as a psychologist. She currently lives in Utrecht (NL) with her husband Bruno and their daughter Verena.

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