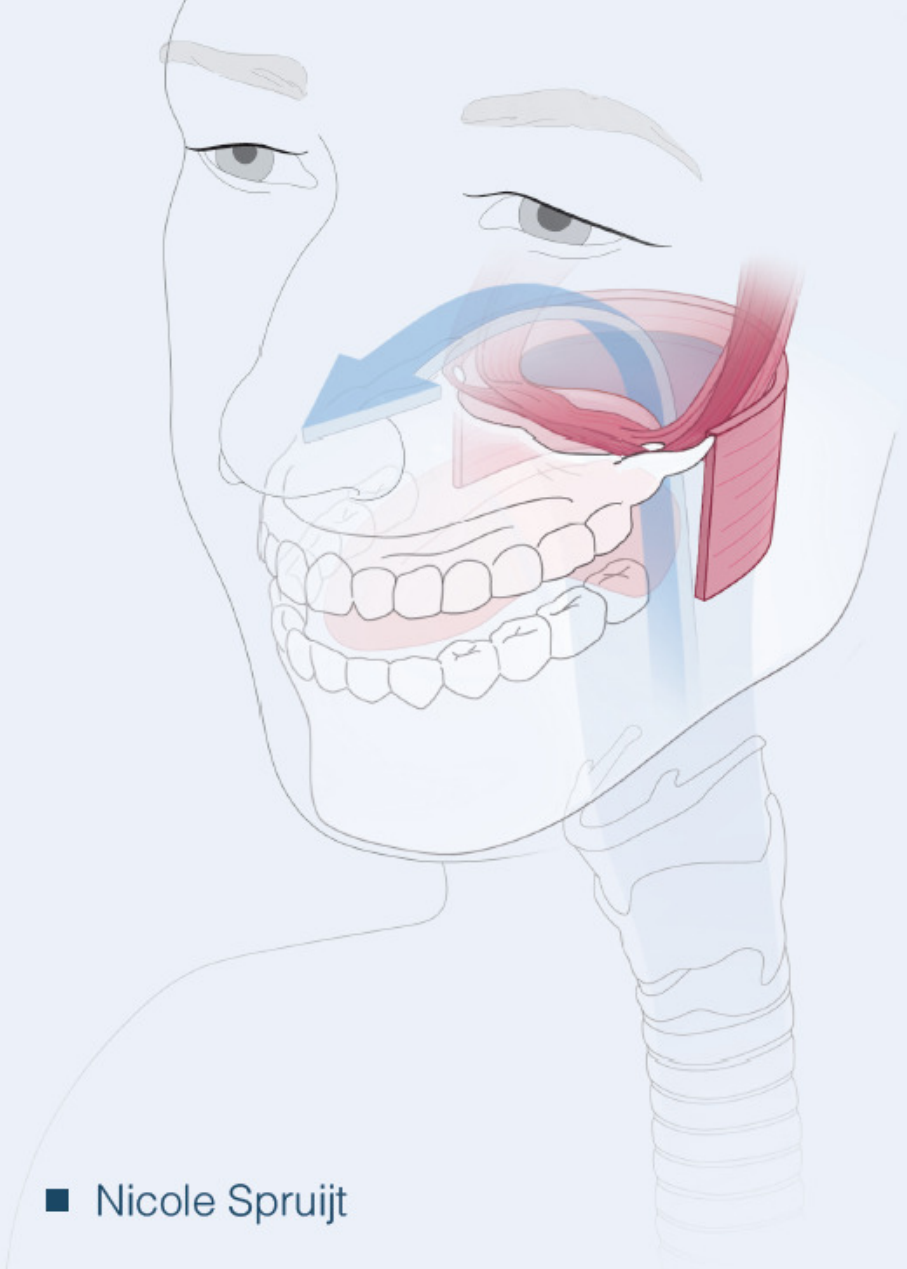
UNDERSTANDING SPEECH PROBLEMS IN 22Q11.2 DELETION SYNDROME FOR OUTCOME PREDICTION



Nicole Spruijt

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Nederlandse Vereniging voor Plastische Chirurgie
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Understanding Speech Problems in 22q11.2 Deletion Syndrome for Outcome Prediction

Spraakproblemen begrijpen in het 22q11.2 deletie syndroom om uitkomsten te voorspellen

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college van promoties in het openbaar te verdedigen op dinsdag 26 augustus 2014 des middags te 4.15 uur

door

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geboren op 3 oktober 1984

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"I was merely thinking God's thoughts after him."

Johannes Kepler (1571 – 1630), German scientist and theologian

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Introduction

The 22q11.2 deletion syndrome (22q11DS) is the most frequent human microdeletion syndrome (Saitta et al 2004), affecting around 1 in 2000-4000 newborns (Devriendt et al 1998; Shprintzen 2008). It results from the deletion of a 3 megabase region on chromosome 22 containing about 45 genes (Bassett et al 2011). The deletion affects the embryology of the pharyngeal arches and pouches (Scambler 2010). Over 180 clinical features, including every organ system, have been associated with the deletion (Robin & Shprintzen 2005). The phenotypes among patients with 22q11DS vary greatly (Bassett et al 2011; Cirillo et al 2014). Subsequently, parents and those who care for children with 22q11DS are left with many questions about the likely manifestations and the course of these problems.

Overall, the estimated severity of the syndrome is gauged to be "likely to require special schooling; may be employable in adulthood, but likely to need support in daily living" (Bishop 2010). This decreased quality of life is compounded by multiple physical, mental, and psychological problems for which children with 22q11DS undergo numerous treatments (Looman et al 2010). An international group of researchers and clinicians have drawn up a guideline for managing the array of issues these patients may face (Bassett et al 2011; Habel et al 2014).

As a member of the multidisciplinary team that cares for patients with 22q11DS, the plastic surgeon is involved in management of cleft lip- and/or palate and velopharyngeal dysfunction (VPD). VPD is the incomplete closure of the velopharyngeal valve which normally separates the oral and nasal cavities (the ring of muscles illustrated on the cover). VPD allows excess air to escape through the nose during speech (this is illustrated with the arrow on the cover). This hypernasality is socially noticeable and can hamper understandability. In addition to having VPD, children with 22q11DS begin speaking at an older age. An impaired ability to communicate with others may result in social withdrawal and poor social skills (Lipson et al 1991; Swillen et al 2000).

All children with VPD receive speech and language therapy. When this insufficiently corrects VPD due to anatomic deficits, the velopharyngeal gap can be decreased in size by obturation with a prosthesis, inserting autologous or synthetic materials, or surgically. Algorithms have been suggested to guide the clinician's treatment choice (Marsh 2003; Mehendale et al 2004; Sie & Chen 2007).

The same surgical techniques are employed to reduce hypernasal speech in non-syndromic patients who have VPD following closure of their cleft palates. In general, the speech outcome after surgery has been reported to be worse in patients with 22q11DS than in patients without the syndrome (D'Antonio et al 2001a; D'Antonio et al 2001b; Losken et al 2003; Losken et al 2006; Nicolas et al 2011; Sie et al 1998; Sie et al 2001; Widdershoven et al 2008b), but some patients with 22q11DS fare as well as their non-syndromic counterparts after surgery (Argamaso et al 1994; Brandao et al 2011; Meek et al 2003; Milczuk et al 2007; Perkins et al 2005; Pryor et al 2006; Rouillon et al 2009).

A possible explanation for the different outcomes after surgery is the underlying cause of the hypernasal speech. If the valve mechanism does not function because there is an unrepaired cleft palate, the cleft can be repaired. If the palate is too short, it can be lengthened. In 22q11DS a series of factors contribute to VPD (Widdershoven et al 2008a) including pronounced hypodynamism of the muscles (Arneja et al 2008) and an abnormally obtuse cranial base angle which deepens the pharynx (Arvystas & Shprintzen 1984). All surgical techniques rely on some intrinsic muscle activity for closure of the remaining velopharyngeal port (McDonald-McGinn & Sullivan 2011).

Given the costs and potential complications associated with surgery, can we identify which patients will benefit before subjecting them to surgery? As suggested by Witt et al. (Witt et al 1995a), suboptimal postoperative functional outcome may represent errors in patient selection rather than errors in operative technique.

Objectives

Naturally, parents are interested to know whether their child will benefit from surgery. However, prognostic factors remain elusive (Losken et al 2003). Since the individual outcome is variable and difficult to predict, there is often disappointment. These studies have been completed to increase the understanding of the etiology of VPD in 22q11DS (Chapters 1, 2, and 3) and find prognostic factors for outcome (Chapters 4, 5, and 6). Subsequently, changes may be made in the management strategy and/or expectations. The ultimate goal is to achieve more predictable and reliable results for each patient with 22q11DS and VPD.

CHAPTER 1:
Histology of the Pharyngeal Constrictor Muscle in 22q11.2 Deletion Syndrome and Non-Syndromic Children with Velopharyngeal Dysfunction

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Abstract

Plastic surgeons aim to correct velopharyngeal dysfunction manifest by hypernasal speech with a velopharyngoplasty. The functional outcome has been reported to be worse in patients with 22q11.2 deletion syndrome than in patients without the syndrome. A possible explanation is the hypotonia that is often present as part of the syndrome. To confirm a myogenic component of the etiology of velopharyngeal dysfunction in children with 22q11.2 deletion syndrome, specimens of the pharyngeal constrictor muscle were taken from children with and without the syndrome. Histologic properties were compared between the groups. Specimens from the two groups did not differ regarding the presence of increased perimysial or endomysial space, fiber grouping by size or type, internalized nuclei, the percentage type I fibers, or the diameters of type I and type II fibers. In conclusion, a myogenic component of the etiology of velopharyngeal dysfunction in children with 22q11.2 deletion syndrome could not be confirmed.

Introduction

The 22q11.2 deletion syndrome (22q11DS) is the most common human microdeletion syndrome (Saitta et al 2004) with an estimated frequency around 1 in 4000 (Devriendt et al 1998) but possibly as high as 1 in 2000 surviving newborns (Shprintzen 2008). It encompasses the phenotypes previously known as DiGeorge syndrome, velocardiofacial syndrome, conotruncal anomaly face syndrome, many cases of the autosomal dominant Opitz G/BBB syndrome, and Cayler cardiofacial syndrome (asymmetric crying facies). Over 180 clinical features including every organ system have been associated with the deletion (Robin & Shprintzen 2005).

One of the presenting features of 22q11DS is velopharyngeal dysfunction (VPD). Velopharyngeal dysfunction is the failure of the soft palate to reach the posterior pharyngeal wall to close the opening between the oral and nasal cavities, resulting in hypernasal speech. Incomplete velopharyngeal closure is most frequently related to structural abnormalities such as cleft palate or submucous cleft, but may also be the corollary of neuromuscular impairment (Dworkin et al 2004). Both seem to be factors in the etiology of VPD in patients with 22q11.2 deletion syndrome where palatal defects, adenoid hypoplasia, and platybasia enlarge the pharyngeal gap (Widdershoven et al 2008a), and the hypodynamic pharynx as viewed by nasendoscopy has been described as a “black hole” (Arneja et al 2008).

Surgical repair of palatal clefts does not sufficiently correct VPD in 10-31.8% of all patients with VPD not restricted to those with 22q11DS (Farzaneh et al 2008; Inman et al 2005; Phua & de Chalain 2008; Sell et al 2001; Van Lierde et al 2004), possibly due to stiffness or shrinkage of the velum due to scarring (Dworkin et al 2004). Secondary velopharyngoplasty to correct the VPD may then follow. The functional outcome has been reported to be worse in patients with 22q11DS than in patients without the syndrome (D'Antonio et al 2001b; Losken et al 2003; Losken et al 2006; Sie et al 1998; Sie et al 2001; Widdershoven et al 2008b). A possible explanation is the hypotonia that is often present as part of the syndrome and which cannot be corrected by surgery.

Velopharyngeal closure is achieved by the concert action of multiple muscles, including palatal lift by the levator veli palatini and circular pharyngeal closure by the pharyngeal constrictor muscle (PCM) (Adachi et al 1997; Kogo et al 1996). A previous study of the PCM, shows that patients with 22q11DS have proportionally more type I fibers and the diameter of these fibers is smaller than those in people without the syndrome (Zim et al 2003). In that study, muscle biopsies from children were compared with specimens from elderly cadavers. Muscle fiber hypoplasia or atrophy with subsequent pharynx hypotonia may be primarily myogenic or neurogenic.

Muscular and neurologic problems have been associated with 22q11DS both clinically and genetically. Specific myopathies are rare (Bolland et al 2000; Mongini et al 2001; Okiyama et al 2005), but neurologic disorders including delayed motor and mental development (Gerdes et al 2001; Kobrynski & Sullivan 2007; Van Aken et al 2007) and dysfunction of cranial nerves III, VII, VIII, IX, X, and XII (Hultman et al 2000) affect at least 33% of patients (Oskarsdottir et al 2005a; Roubertie et al 2001). General hypotonia, which affects 23-76% of patients with 22q11DS (Gerdes et al 1999; Kitsiou-Tzeli et al 2004; Oskarsdottir et al 2005a), was found to be universally prevalent among children with 22q11DS and VPD (Havkin et al 2000).

About 40 genes (Shprintzen 2008), including TBX1, are located in the 3.0 megabase region deleted in 22q11DS (Saitta et al 2004), affecting countless downstream signaling pathways. The central roles of the TBX1 and CRKL genes in the anomalous developmental of pharyngeal structures in 22q11DS have recently been reviewed (Wurdak et al 2006). The murine *Tbx1*$^{-/-}$ model for 22q11DS has hypoplastic branchiomeric muscles (Kelly et al 2004; Xu et al 2005), but the sporadic muscles that develop have a normal distribution of muscle fibers types (Grifone et al 2008). In patients with 22q11DS, decreased PCM muscle thickness on MRI (Zim et al 2003) suggests hypoplasia. The temporal *Tbx1* gradient follows the cranial-caudal development of pharyngeal structures (Xu et al 2005), causing structures that are derived from more cranially located pharyngeal arches, such as the levator palatini muscles, to be less affected by the mutation than structures

derived from more caudally located pharyngeal arches, such as the PCM muscle (Vitelli et al 2002b; Walker & Trainor 2006). Although *Tbx1* is not expressed in primary neural crest cells (Garg et al 2001), *Tbx1* mutants have aberrant structures derived from neural crest cells including cranial nerves (Vitelli et al 2002b) since defective *Tbx1* expression in the pharyngeal endoderm affects the downstream expression *Fgf8* and *Fgf10* which are necessary for neural crest cell migration (Abu-Issa et al 2002; Arnold et al 2006; Vitelli et al 2002b). As suggested by studies on the deleted *TBX1* gene (Grifone et al 2008; Kelly et al 2004; Vitelli et al 2002b), primary aberrant myogenesis leads to aberrant neurogenesis.

In summary, the poorer functional outcome after velopharyngoplasty in patients with 22q11DS may be attributed to pharyngeal hypotonia. Anomalous myogenesis and neurogenesis which may underlie the hypotonia have been reported in a murine model for 22q11DS. In this study we aimed to confirm a myogenic component of the etiology of VPD in children with 22q11DS by analyzing the histology of the PCM muscle. Our clinical experience is that the PCM seems thicker in children with 22q11DS. We expect to find fiber hypertrophy as a corollary of the muscle hypoplasia (Kelly et al 2004; Xu et al 2005) necessitating the few fibers present to take on a heavier workload.

Methods

Ethics Statement

This study was approved by the institutional medical ethics review board (Utrecht University Medical Center Ethics Review Board) and the patients' parents gave written informed consent to participate.

Patients

The University Medical Centre in Utrecht is the Dutch national centre for children with 22q11DS. Children undergoing velopharyngoplasty for VPD with and without the 22q11DS were included in the study. Children with contra-indications

for velopharyngoplasty (including bleeding disorders or extensive comorbidity such as cardiac problems) and known neurological disorders were excluded.

Sample size calculation

Using the results of the only previous study on PCM histology in 22q11DS (Zim et al 2003) which found a difference of mean diameter of type I fibers of 5.0 μm between patients with and without 22q11DS, with a standard deviation of 2.0 μm, an alpha of 0.05, and a power of 0.80 in the two-tailed two-sample *t*-test sample size formula yields a sample size of 4 subjects in each group. This number was arbitrarily doubled as the difference between two groups of children is likely smaller than the difference between children and adults in the previous study.

Muscle specimens

During velopharyngoplasty, a cranially attached pharyngeal flap (measuring around 10 x 40-50 mm) is mobilized from the dorsal pharyngeal wall and attached to the velum. This flap is comprised of part of the PCM muscle and the overlying mucosa. Muscle at the caudal end of the flap is trimmed (measuring around 10 x 3 mm) and delivered fresh to the pathologist in a damp gauze for histological evaluation.

Outcome parameters

Histological evaluation of the muscle specimens included qualitative analysis and quantitative measurements. The analysts were blinded for age, gender and presence of the syndrome. The specimens were qualitatively evaluated for the presence of increased perimysial and endomysial space, muscle fiber grouping by size or type, and presence of internalized nuclei. After staining with ATPase at pH 4.3, representative areas from each specimen were photographed. For quantitative analysis, muscle fibers were counted and the percentage of type I muscle fiber was calculated per patient. The diameters of up to 100 fibers of each type were measured for each patient. For each muscle fiber type, the mean fiber diameter and variance ((SD x 1000)/mean diameter) were calculated per group (males, females, and children with and without 22q11DS).

Statistical analysis

The genders of children with and without 22q11DS were compared using the Chi-square test. Age at surgery of males and females and children with and without 22q11DS were compared using the independent samples *t*-test. The presence of increased perimysial and endomysial space, muscle fiber grouping by size and type, and internalized nuclei was compared between the two groups using Fisher's exact test. The relationship between age at surgery and fiber diameters was examined using the Spearman correlation. The independent samples *t*-test was used to compare the mean percentage of type I fibers and muscle fiber diameters between males and females and between children with and without 22q11DS.

Results

Patients

Muscle specimens were available for 16 children, eight with 22q11DS and eight without 22q11DS. The groups did not differ regarding gender (5/8=63% and 4/8=50% female, respectively, p=0.63) or age at surgery (6.5 and 7.0 years, respectively, p=0.68) (Figure 1.1). Males and females did not differ regarding age at surgery (7.4 and 6.2 years, respectively, p=0.39).

Figure 1.1. Group demographics.

O: males, X: females.

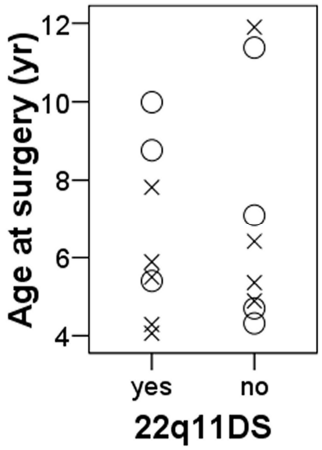
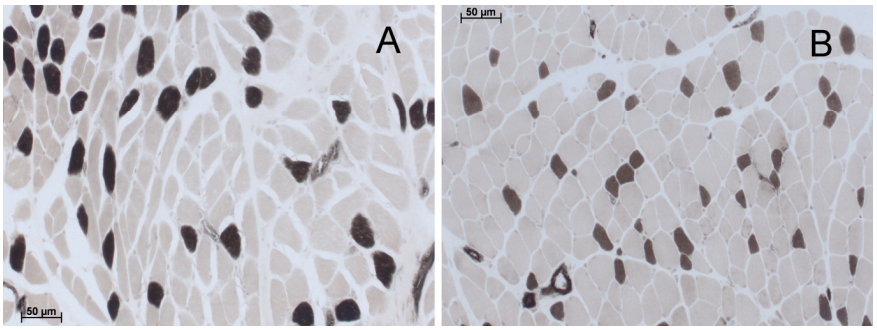


Figure 1.2: Histological specimens with ATPase stain at pH 4.3. A) a 5-year-old female without 22q11DS but with increased perimysial and endomysial space. B) a 10-year-old male with 22q11DS and without increased perimysial and endomysial space. Bars 50 µm.



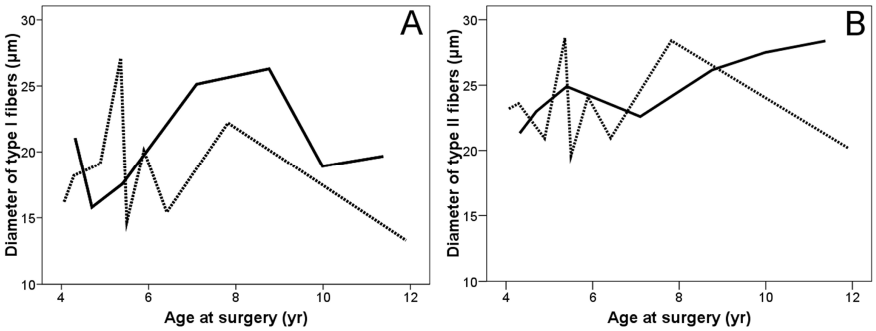
Qualitative analysis

No structural differences were seen between histological specimens from children with and without 22q11DS (Table 1.1, Figure 1.2). Increased perimysial and endomysial space was seen equally in both groups. No grouping by muscle fiber type was seen in any patient. One non-syndromic patient had localized grouping of smaller fibers, but these were round fibers without nuclear clumping which do not suggest neurogenic atrophy or other signs of fiber degeneration and regeneration. One patient with 22q11DS had an increased percentage of internalized nuclei.

Table 1.1: Qualitative analyses.

Parameter	22q11DS (n=8)	No 22q11DS (n=8)	p-value
Increased perimysial space, No. (%)	5 (63)	5 (63)	1
Increased endomysial space, No. (%)	4 (50)	6 (75)	0.61
Grouping by size, No. (%)	0 (0)	1 (13)	1
Grouping by fiber type, No. (%)	0 (0)	0 (0)	1
Internalized nuclei, No. (%)	1 (13)	0 (0)	1

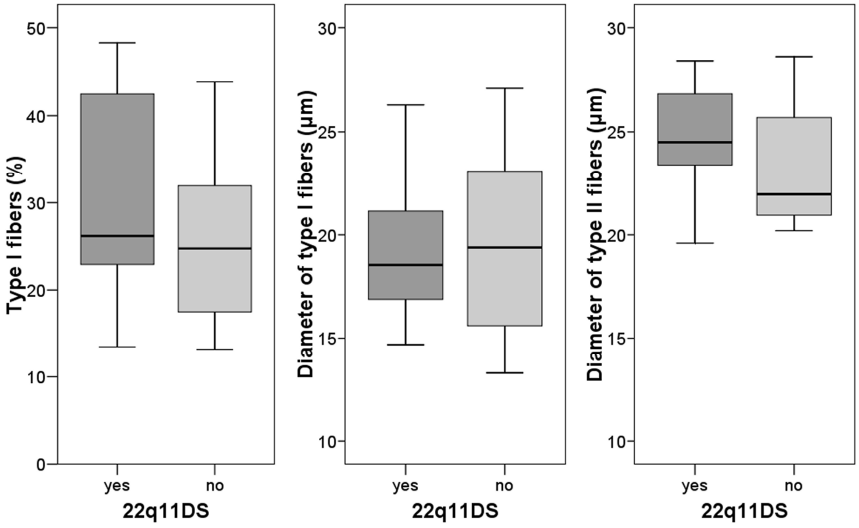
Figure 1.3: Mean diameters of type I (A) and type II (B) muscle fibers and age at surgery. Solid lines: males, dashed lines: females.



Quantitative measurements

There was no correlation between muscle fiber diameter and age at surgery (p=0.78 for type I fibers and p=0.48 for type II fibers, Figure 1.3). Neither the percentage of type I fibers nor the diameters of the fiber types differed significantly between males and females or between children with and without 22q11DS (Table

Figure 1.4: Muscle fiber type measurements for children with and without 22q11DS. Bands, means. Boxes, 25th-75th percentiles. Whiskers, 95% confidence intervals.



1.2, Figure 1.4). All calculated fiber diameter variances were less than 250 (Table 1.2). For all groups, the mean diameters of type I fibers were more than 12% smaller than the mean diameters of the larger type II fibers.

Table 1.2: Quantitative analyses.

Parameter	Male	Female	Mean difference (95% CI)	p-value	22q 11DS	No 22q 11DS	Mean difference (95% CI)	p-value
Type I fibers, % (SD)	24.8 (10.3)	30.7 (11.9)	-6 (-18, 6)	0.43	30.6 (12.3)	25.7 (10.5)	4.9 (-7, 17)	0.46
Type I fiber diameter, μm (SD), variance	20.6 (3.9), 189	18.5 (4.3), 232	2 (-2, 7)	0.32	19.3 (3.7), 192	19.6 (4.8), 245	-0.3 (-5, 4)	0.92
Type II fiber diameter, μm (SD), variance	24.8 (2.6), 105	23.3 (3.3), 142	2 (-2, 5)	0.37	24.7 (2.8), 113	23.3 (3.4), 146	1.4 (-2, 5)	0.25

Discussion

Few studies have looked at the histology of the PCM. With the exception of specimens obtained from patients undergoing pharyngoplasty (Zim et al 2003) or laryngectomy (Sundman et al 2004), most only study specimens from cadavers.

Morphology

Our qualitative analysis revealed no morphologic differences between PCM muscles in children with and without 22q11DS (Table 1.1). We found increased perimysial and endomysial space in both groups. While increased space is associated with chronic muscle damage, it is unclear whether this is also true for pharyngeal constrictors. Since it affects both groups equally, it is unlikely to be a factor in the poorer speech in children with 22q11DS. Zim et al. (Zim et al 2003) found increased endomysial space in children with 22q11DS relative to adults without the syndrome, but did not test the difference for significance. Like Zim et al. (Zim et al 2003), we

did not find any grouping by muscle fiber type, indicating the absence of innervation distubances.

Fiber type

We found 30.6% (SD 12.3) and 25.7% (SD 10.5) type I muscle fibers, respectively, in children with and without 22q11DS. Zim et al. (Zim et al 2003) found 27.7% (SD 2.01) and 17.9% (SD 2.15) type I muscle fibers, respectively, in children with and adults without 22q11DS. The significant difference between the groups in the study by Zim et al. may not necessarily be attributed to the presence of the syndrome, but may be distorted by the unusually small percentage of type I fibers found in the adult controls (81-86 years, cadavers). Other studies on pharyngeal constrictor specimens in adults found 35% (43-77 years, live) (Sundman et al 2004), 49% (SD 9.2) (38-61 years, cadavers) (Smirne et al 1991), and 33.7% (SD 12.0) (over 50 years, cadavers) (Leese & Hopwood 1986) type I fibers. Leese and Hopwood (Leese & Hopwood 1986) report 20.4% (SD 8.7) type I fibers in infants (0-3 years) and 30.2% (SD 15.3) type I fibers in young adults (12-49 years). While they report no significant change with respect to age, they also report that infant muscle fibers exhibit a significantly lesser percentage of type I fibers.

Fiber diameter

Previous reports on the mean diameter of type I muscle fibers in pharyngeal constrictor muscles in adults without 22q11DS range from 26.6 to 29 μm (Smirne et al 1991; Zim et al 2003). In children without 22q11DS we found a mean diameter of 19.6 μm (SD 4.8). In children with 22q11DS, Zim et al. (Zim et al 2003) found a mean diameter of 21.6 μm (SD 2.09) and we found a mean diameter of 19.3 μm (SD 3.7). It is tempting to conclude that, as with limb muscles, mean fiber diameter is related to age (Brooke & Engel 1969). However, we did not find a correlation between age and diameter among children of different ages (Figure 1.3) and Leese and Hopwood (Leese & Hopwood 1986) failed to find a relationship among adults of different ages. They did find a significant difference between fiber diameters in infants (0-3 years) and adults (over 12 years). Like Leese and Hopwood (Leese &

Hopwood 1986), we found no difference in fiber diameter between males and females, reflecting similar usage of the muscles by both genders.

The similar diameters of both type I and II muscle fibers in children with and without 22q11DS found in this study reflect similar strain put on this muscle by all children with VPD. Unfortunately, we did not have a control group of PCM specimens from children without VPD. Presumably, children without structural abnormalities that lead to VPD will have smaller muscle fiber diameters as they have do not have to employ the pharyngeal muscles as vigorously to close the oropharynx off from the nasopharynx.

Fiber type disproportion, reflected in a difference between the mean fiber type diameters of more than 12% of the mean diameter of the larger fiber type, is characteristic of congenital myopathies (Brooke & Engel 1969). In this study, the type II fibers were more than 12% larger than the type I fibers in both children with and without 22q11DS. In the study by Zim et al. (Zim et al 2003), the diameters of the type II fibers were also more than 12% larger than the type I fibers in children with 22q11DS, while the muscle fiber types had similar diameters in adults without 22q11DS. The disproportion is likely a result of selective type II hypertrophy rather than type I atrophy as children with VPD place extra strain on the fast type II fibers while attempting to articulate properly and preventing nasal regurgitation while swallowing.

We found greater variance in muscle fiber diameter (192 and 113) than Zim et al. (Zim et al 2003) (97 and 77, respectively, for type I and II fibers in children with 22q11DS). Our measurements are based on more fibers per patient (171 to 200) than the study by Zim et al. (Zim et al 2003) (64 to 113 fibers per patient). We found greater variance among children without 22q11DS (245 and 146, respectively, for type I and II fibers), but no groups had variances greater than 250, which is considered pathologic in limb muscles, but has been found in healthy palatal muscles (Stal & Lindman 2000).

Conclusion

Therefore, we conclude that there is no evidence of innervation or myogenic disturbances in the histologic specimens of the PCM in children with 22q11DS relative to non-syndromic counterparts. The absence of histologic deficits in the PCM muscle of patients with 22q11DS does not preclude the functional deficits manifest in the hypodynamic pharynx seen on nasendoscopy and poorer functional outcome after velopharyngoplasty. Future studies to elucidate the etiology of the pharyngeal hypotonia in 22q11DS should investigate the role of the central nervous system, such as by comparing fMRI images taken during speech. Meanwhile, unanswered etiologic and clinical questions hamper adequate management of the compromised speech understandability in patients with 22q11DS, contributing to poor social functioning and quality of life.

Acknowledgements

The authors would like to thank Paul Westers for giving expert advice regarding statistical calculations.

CHAPTER 2:
Exploring a Neurogenic Basis of Velopharyngeal Dysfunction in *Tbx1* Mutant Mice: No Difference in Volumes of the Nucleus Ambiguus

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Abstract

Objective: Velopharyngeal hypotonia seems to be an important factor in velopharyngeal dysfunction in 22q11.2 deletion syndrome, but the etiology is not understood. Because *TBX1* maps within the typical 22q11.2 deletion and *Tbx1*-deficient mice phenocopy many findings in patients with the 22q11.2 deletion syndrome, *TBX1* is considered the major candidate gene in the etiology of these defects. *Tbx1* heterozygosity in mice results in abnormal vocalization 7 days postnatally, suggestive of velopharyngeal dysfunction. Previous case-control studies on muscle specimens from patients and mice revealed no evidence for a myogenic cause of velopharyngeal dysfunction. Velopharyngeal muscles are innervated by cranial nerves that receive signals from the nucleus ambiguus in the brainstem. In this study, a possible neurogenic cause underlying velopharyngeal dysfunction in *Tbx1* heterozygous mice was explored by determining the size of the nucleus ambiguus in *Tbx1* heterozygous and wild type mice.

Methods: The cranial motor nuclei in the brainstems of postnatal day 7 wild type (n=4) and *Tbx1* heterozygous (n=4) mice were visualized by *in situ* hybridization on transverse sections to detect *Islet-1* mRNA, a transcription factor known to be expressed in motor neurons. The volumes of the nucleus ambiguus were calculated.

Results: No substantial histological differences were noted between the nucleus ambiguus of the two groups. *Tbx1* mutant mice had mean nucleus ambiguus volumes of 4.6 million μm^3 (standard error of the mean 0.9 million μm^3) and wild type mice had mean volumes of 3.4 million μm^3 (standard error of the mean 0.6 million μm^3). Neither the difference nor the variance between the means were statistically significant (*t*-test p=0.30, Levene's test p=0.47, respectively).

Conclusions: Based on the histology, there is no difference or variability between the volumes of the nucleus ambiguus of *Tbx1* heterozygous and wild type mice. The etiology of velopharyngeal hypotonia and variable speech in children with 22q11.2 deletion syndrome warrants further investigation.

Introduction

The 22q11.2 deletion syndrome (22q11DS) is the most frequent survivable human syndrome that is caused by a hemizygous microdeletion within a chromosome (Scambler 2010). In approximately 85% of all 22q11DS patients, a 3 megabase (Mb) region on chromosome 22 is deleted (Saitta et al 2004) containing about 45 genes (Bassett et al 2011). One of the genes that maps within the deleted region is *Tbx1*, which is expressed in pharyngeal endodermal pouches, in pharyngeal mesoderm including the mesodermal cores of the pharyngeal arches, and in head mesenchyme during embryonic development (Scambler 2010) and in the brain after birth (Paylor et al 2006). Major phenotypes of 22q11DS can be related to aberrant development of the pharyngeal arches and pouches 3, 4, and 6, including facial dysmorphism, feeding and speech problems due to velopharyngeal dysfunction (VPD), hypocalcaemia due to parathyroid dysfunction, immune disorders due to thymus dysfunction, and congenital heart disease.

VPD occurs when the valve mechanism of the soft palate and the lateral and posterior pharyngeal walls fail to close the port between the oral and nasal cavities, resulting in hypernasal speech. Some children with VPD undergo surgery to decrease the size of the velopharyngeal port. In general, postoperative residual VPD is more prevalent among children with 22q11DS than in children without the syndrome (D'Antonio et al 2001a; D'Antonio et al 2001b; Losken et al 2003; Losken et al 2006; Sie et al 1998; Sie et al 2001; Widdershoven et al 2008b), but some patients with 22q11DS fare as well as their non-syndromic counterparts (Argamaso et al 1994; Meek et al 2003; Milczuk et al 2007; Perkins et al 2005; Pryor et al 2006; Rouillon et al 2009). It is not clear why some children with 22q11DS benefit more from surgery than others (Losken et al 2006; Spruijt et al 2011). Phenotype variability of VPD in 22q11DS has been one of the research foci of the 22q11DS team at our tertiary hospital.

All surgical techniques rely on some intrinsic muscle activity for closure of the remaining velopharyngeal port (McDonald-McGinn & Sullivan 2011). A possible explanation for the different postoperative outcomes is a neuromuscular

component of VPD in 22q11DS as seen on nasendoscopic views of attempted velopharyngeal closure (Witt et al 1995b). On magnetic resonance imaging, the pharyngeal constrictor muscle in patients with 22q11DS was found to be hypotrophic compared to controls (Zim et al 2003), which may be the result of abnormal development of the muscle or its innervation. The etiology of velopharyngeal hypotonia is uncertain, but may primarily result from myogenic or neurogenic abnormalities. Superior constrictor muscle biopsies taken from children with and without 22q11DS revealed no clear histological differences, suggesting a nonmyogenic origin of velopharyngeal hypotonia in patients with 22q11DS (Widdershoven et al 2011a). Whether a neurogenic cause underlies VPD in patients with 22q11DS is unclear.

Neurogenic pharyngeal weakness is seen in amyotrophic lateral sclerosis, a neurodegenerative disease accompanied by a decreased number of cells in the brainstem nucleus ambiguus (nA) (Ferrucci et al 2009; Zang et al 2004). The nA transmits signals from the cerebral cortex to the vagal (n.X) and accessory (n.XI) cranial nerves which innervate the pharyngeal muscles (German & Palmer 2006; Keller et al 1984; Standring 2005). Additionally, some patients with Möbius syndrome, which is characterized by congenital weakness or paralysis of the muscles innervated by the facial nerve (n.VII), have hypoplastic brainstem facial cranial nerve nuclei with fewer neurons than controls (Richter 1960; Towfighi et al 1979; Verzijl et al 2005). Similarly, congenital VPD in 22q11DS could be caused by hypoplastic development of the nA. Unfortunately, noninvasive imaging does not permit an accurate estimation of the size the brainstem nuclei (Komisaruk et al 2002), necessitating a histological analysis of brainstem tissue.

Postmortem human brainstem material is difficult to obtain, therefore we resorted to studying an animal model of 22q11DS. Among vertebrate model organisms, the neuronal architecture of the mouse is the most similar to that of humans (Cordes 2001). Mouse models for 22q11DS have been generated by deleting a 1 Mb homologous region on mouse chromosome 16 (*Df (16)1, LgDel*) including *Tbx1*, or specifically disrupting the *Tbx1* gene (Lindsay et al 2001; Paylor & Lindsay 2006). The phenotype of *Tbx1* heterozygous mutant mice ($Tbx1^{+/-}$) is less

penetrant and does not phenocopy the entire phenotypic spectrum of patients with 22q11DS. However, recent findings demonstrated that seven to eight-day-old *Tbx1*$^{+/-}$ mouse pups (P7-8) may have VPD since they vocalize at a lower frequency and for a shorter duration compared to wild type littermates (Hiramoto et al 2011). Interestingly, a loss-of-function point-mutation of *TBX1* in patients without the typical 22q11.2 deletion, results in phenotypes similar to those found in patients with 22q11DS, including VPD (Yagi et al 2003). Therefore, *Tbx1*$^{+/-}$ mice can be used as an adequate model to study the VPD phenotype found in 22q11DS.

Moreover, as in patients with 22q11DS, phenotypic variance is seen in the *Tbx1*$^{+/-}$ mouse model (Vitelli et al 2002a): all *Tbx1*$^{+/-}$ embryos have fourth pharyngeal arch artery hypoplasia at E10.5, but at term only 30-50% have fourth pharyngeal arch artery-derived cardiovascular defects (Lindsay et al 2001). The differences in phenotypic penetrance depends on the genetic background of the mouse strains (Taddei et al 2001; Vitelli et al 2002a; Zhang et al 2005), and on genetic modifiers including *Vegfa, Nrp1, Spry*, and retinoic acid (Ryckebusch et al 2010; Simrick et al 2012; Stalmans et al 2003; Vermot et al 2003; Zhou et al 2012).

The presence of velopharyngeal hypotonia as underlying cause for the VPD was not specifically mentioned in the study with mouse pups (Hiramoto et al) nor in the study with patients with the *TBX1* point-mutation (Yagi et al 2003). The requirement of *Tbx1* during development of velopharyngeal muscles and nerves has been shown in *Tbx1*-deficient (*Tbx1*$^{-/-}$) mice which die during fetal and neonatal stages: *Tbx1*$^{-/-}$ mice have hypoplastic branchiomeric head and neck muscles (Grifone et al 2008; Kelly et al 2004) and abnormally fused ganglia of the glossopharyngeal (n.IX) and n.X nerves (Calmont et al 2010; Vitelli et al 2002b). Thus, although *Tbx1* is not expressed in primary neural crest cells (Garg et al 2001), the neural crest-derived ganglia are aberrantly formed in the absence of *Tbx1* (Angeles Fernandez-Gil et al 2010).

The objective of this study was to explore the possibility that a neurogenic defect causes velopharyngeal hypotonia in 22q11DS by comparing the gross histology of the nA in the *Tbx1*$^{+/-}$ mouse model for 22q11DS to that of wild type mice. Diminished or absent activity of *Tbx1* gene may indirectly effect the brainstem

as it does the cranial nerves (Calmont et al 2010; Vitelli et al 2002b). Our results indicate that the volume of the nA is not significantly affected by *Tbx1* haplosufficiency.

Materials and Methods

Mice

Tbx1$^{+/lacZ}$ mice (Lindsay et al 2001) were intercrossed to generate wild type and heterozygous mutant pups. Genotypes were confirmed by PCR using primers specific for the *lacZ* gene (Lindsay et al 2001). All mice were maintained on an FVB background. Animal care was in accordance with national and institutional guidelines. The experimental procedure was approved by the animal ethics committee of the Academic Medical Center in Amsterdam, the Netherlands. On postnatal day 7 (P7) the pups (n=4 of each genotype) were brought into a hypercapnic coma in a sealed cage and sacrificed for tissue isolation. The brainstems were isolated in ice-cold phosphate-buffered saline (PBS 1x), fixed by overnight immersion in 4% paraformaldehyde (PFA), and embedded in paraplast for further processing.

In situ hybridization

Embedded brainstem tissue was cut into 10 µm thick transverse sections with a Leica RM 2165 rotation microtome, mounted on Starfrost slides, and processed for non-radioactive *in situ* hybridization (ISH) as described (Moorman et al 2001). The brainstem motor nuclei were visualized by ISH with a DIG-labeled *Islet-1* (*Isl1)* (deLapeyriere & Henderson 1997; Pfaff et al 1996) mRNA probe (Moorman et al 2001). The sections were photographed using a camera connected to a Zeiss Axiophot microscope.

Outcome

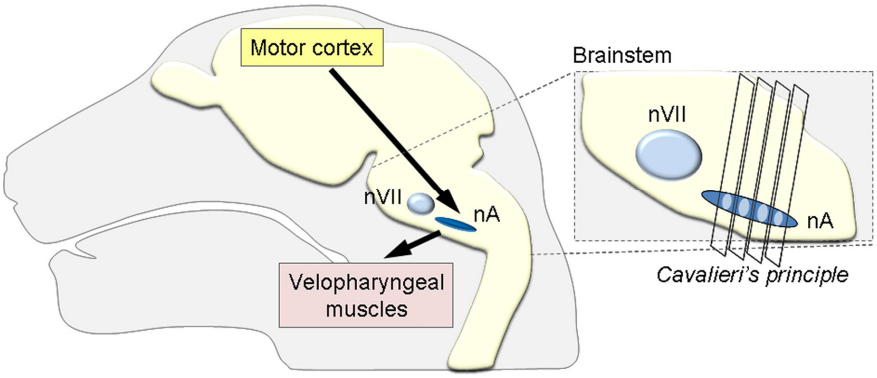
Morphometric analyses were performed blinded using imaging software (Amira 5.4, Visage Imaging, San Diego, CA, USA). The nA of the mutant and the

wild type pups were compared qualitatively by describing the appearance, and quantitatively by calculating the volume marked by *Isl1*. Rather than measuring every section that contained the nA, the surface area of the nA on a minimum of 10 equally spaced sections encompassing the nA were measured. Using Cavalieri's principle, the sum of the measured areas was multiplied by the distance between the selected sections (Figure 2.1). This approximation of the volume is accurate to within 5% of the true volume (Howard & Reed 1998). The volumes of the nA of *Tbx1*$^{+/-}$ and wild type mice were compared using a two-tailed *t*-test. The variance was measured with Levene's test. Statistical calculations were performed using IBM SPSS Statistics for Windows (Version 20.0. Armonk, NY, USA).

Sample size calculation

The number of pups needed to obtain statistically significant results, was determined based on a study in which n.X innervation of the stomach was compared between wild type and *Tbx1*$^{+/-}$ mice (Calmont et al 2010). At embryonic day 16.5 (E16.5), significantly less n.X fibers intersected in the stomachs of *Tbx1*$^{+/-}$ mice (n=9) than in wild type mice (n=9) (14.6±1.6 versus 20.4±1.3, $p<0.05$). With these

Figure 2.1: Methods. Sagittal view of a mouse brain and brainstem showing the locations of the facial nucleus (nVII) and nucleus ambiguus (nA). Signals from the cerebral motor cortex are relayed to the velopharyngeal muscles via the nA. Inset showing magnification and Cavalieri's principle of calculating the volume of a structure based on equally-spaced transverse sections.

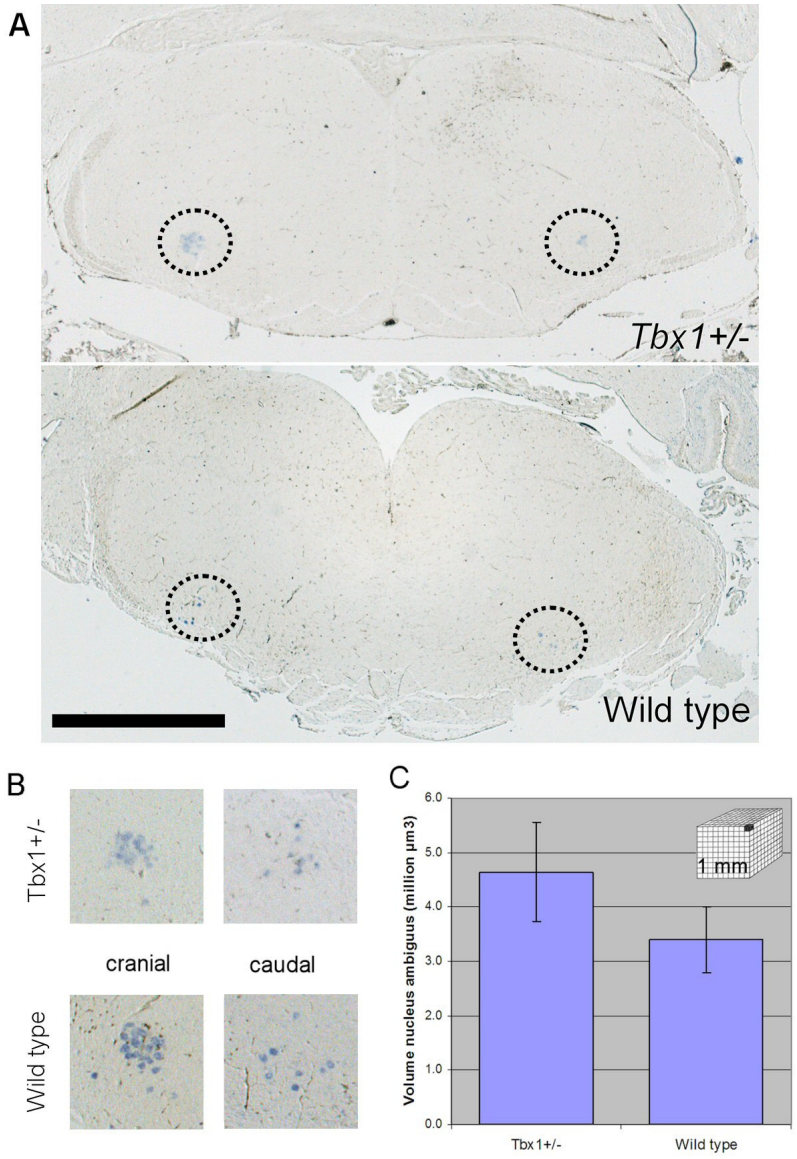


numbers, the required sample size to find a similarly significant difference in nA volumes between the genotypes, with an alpha of 0.05, and a power of 0.80 is only n=2 pups per genotype. Since this calculation is based on the n.X and not the nA, this number was empirically doubled so n=4 pups per genotype were used in this pilot study.

Results

To test whether a neurogenic abnormality underlies velopharyngeal hypotonia in patients with 22q11DS, we harvested and analyzed brainstems from *Tbx1*$^{+/-}$ (n=4) and wild type (n=4) pups from two litters. No macroscopic qualitative differences were noted between both genotypes. As previously described (Bieger & Hopkins 1987; Brown 1990; Kitamura et al 1993), the spindle-shaped nA extends cranio-caudally from the facial nucleus (nVII) to the pyramidal decussation (Friedland et al 1995). To identify and localize the nA (Figure 2.2A), we used a riboprobe directed against *Isl1* mRNA, encoding a LIM domain-containing transcription factor. *Isl1* is expressed in motor neurons of all cranial nerves, including the oculomotor, trochlear, trigeminal, abducens, facial, ambiguus, and hypoglossal nuclei in the brainstem (Jurata et al 1996; Pfaff et al 1996; Varela-Echavarria et al 1996). This approach furthermore allowed the identification of the facial nucleus directly cranial to the nA and the hypoglossal nucleus dorsal to the nA (VanderHorst & Ulfhake 2006), facilitating the localization of the nA. We found that within the nA, the cranially located neurons are packed more compactly and the caudally located neurons are more loosely arranged (Figure 2.2B). The nA in the two genotype groups did not differ in shape or cell density. Quantitatively, *Tbx1*$^{+/-}$ mutant pups had mean nucleus ambiguus volumes of 4.6 million μm^3 (standard error of the mean (SEM) 0.9 million μm^3) and wild type mice had mean volumes of 3.4 million μm^3 (SEM 0.6 million μm^3) (Figure 2.2C). The difference between the means was not statistically significant (*t*-test p=0.30), nor was the variance (Levene's test p=0.47).

Figure 2.2: Nucleus ambiguus of *Tbx1*$^{+/-}$ and wild type P7 pups. A) *Isl1 in situ* hybridization-labeled transverse brainstem sections. Scale: bar = 1mm. B) Magnification of nucleus ambiguus, showing cranial compact and caudal loosely spread cells. C) Mean volumes of the nucleus ambiguus. Error bars = standard error of the mean. Scale: small dark cube = 1 million µm^3.



Discussion

Velopharyngeal hypotonia is a common cause of VPD in patients with 22q11DS. However, the etiology and subsequent speech problems in children with 22q11DS is still poorly understood. We previously demonstrated that myogenic disturbances did not seem to underlie VPD in 22q11DS children (Widdershoven et al 2011a). In this study, we aimed to determine whether a neurogenic cause underlies velopharyngeal hypotonia. The size of the nA is decreased in other diseases with velopharyngeal hypotonia (Ferrucci et al 2009; Richter 1960; Towfighi et al 1979; Verzijl et al 2005; Zang et al 2004). We measured the volume of the nA in mice heterozygous for *Tbx1*, the major candidate gene in the etiology of 22q11DS, and did not observe a clear difference or variability in the volumes of the nA compared with wild type mice.

Evidence of neurologic deficits in *Tbx1* mouse mutants and 22q11DS human patients

The lack of difference in nA volumes between the genotypes does not disprove a neurologic etiology of the supposed VPD in $Tbx1^{+/-}$ mice. Cerebral deficits are apparent in $Tbx1^{+/-}$ mice: relative to wild type mice, they have reduced prepulse inhibition (Paylor et al 2006), lower grip strength, and delayed movement initiation (Long et al 2006). The defects seem to be subtle: even in the *LgDel* model, adult mouse brains show no significant changes in weight or gross morphological appearance (Meechan et al 2010). *Tbx1* expression is limited to the brain vasculature, suggesting that microvascular abnormalities contribute to the phenotypes found in these mutants (Paylor et al 2006). Distal to the nA, the morphology and volumes of the n.X ganglia do not differ between $Tbx1^{+/-}$ and wild type mouse embryos, but a significant decrease in the number of n.X fibers that intersect the stomach was observed in $Tbx1^{+/-}$ mutants compared to wild type mouse embryos, suggesting defective n.X projections (Calmont et al 2010). Pharyngeal projections have not been studied beyond embryonic day 10 thus far (Vitelli et al 2002b).

Clinically, impaired n.X function as evidenced by velar paresis (n=10/13) (Hultman et al 2000), as well as velar and pharyngeal motion are negatively affected in 22q11DS patients compared to nonsyndromic patients with a repaired cleft palate (Widdershoven et al 2011b; Ysunza et al 2011). Moreover, dysfunction of the muscles normally innervated by the nA in 22q11DS are suggested to cause polyhydramnios (Heuschkel et al 2003; Vantrappen et al 1999) due to swallowing disorders (Eicher et al 2000), hoarse voice (D'Antonio et al 2001b; Gerdes et al 1999; Goldmuntz 2005; Kobrynski & Sullivan 2007; Rommel et al 1999; Solot et al 2001; Solot et al 2000) presumably due to laryngeal muscle hypotonia (Dyce et al 2002; Walker 1990), and case reports of aspiration (Fryburg et al 1996; Heuschkel et al 2003; Lee & Han 2011). These phenotypic characteristics could potentially result from neurogenic, myogenic or neuromuscular abnormalities.

The neurogenic component of the syndrome needs attention. In some patients with 22q11DS the velopharyngeal valve mechanism is sufficient during swallowing, but not during speech. The nA relays motor signals to the velopharynx as well as to the instrinsic muscles of the larynx and the upper esophagus. The nA is active during vocalization, respiration, sneezing, coughing, swallowing, and the gag reflex (Standring 2005). In all motor neurons that are related to both swallowing and vocalization, higher electromyographic (EMG) activity levels were achieved during swallowing, reflecting that more forceful adduction of the vocal folds is needed for glottal closure during swallowing to protect the airway against aspiration (Yajima & Larson 1993). If the muscles can be sufficiently forcefully activated during reflexive swallowing in 22q11DS, hypotonia during speech may indicate impairment of the cerebral voluntary component of vocalization.

Limitations of this study

The *Tbx1* mouse background used in this study (FVB) differs from that used in the vocalization study (C57BL/6J) (Hiramoto et al 2011), which could affect the penetrance of defects (Taddei et al 2001; Vitelli et al 2002a), because an FVB genetic background offers a protective effect with regard to fourth pharyngeal arch artery development (Zhang et al 2005). Therefore, an FVB background could also be

protective with regard to nA development and VPD. Additionally, the phenotypic variability of 22q11DS may reflect the array of genetic variability in humans which could not be recapitulated in experimental animals with a homogeneous background.

Another important limitation of this study is that only the histology of the nA has been studied by labeling *Isl1*. No evidence has been presented that could prove normal function. During embryology and postnatal growth, morphology and physiological function are not necessarily temporally coincident (Sato et al 1998); abnormalities may be apparent on electrophysiological examination without histopathological abnormalities (Cordes 2001).

Finally, in 22q11DS typically around 45 genes are deleted. Although the lower frequency and decreased duration of vocalization in *Tbx1*$^{+/-}$ mutants suggests that this gene may primarily contribute to VPD, other genes could function in parallel or in the same genetic pathway (Grifone et al 2008). Other candidate genes in the deleted region include CLTD which is expressed in skeletal muscle, its deletion may contribute to hypotonia (Sirotkin et al 1996); CRKL which is expressed in migrating neural crest cells; its deletion results in hypoplastic n.IX and n.X (Guris et al 2001); and Cdcrel-1 (Pnut1) which is expressed in the n.IX and n.X (Maldonado-Saldivia et al 2000). Haplosufficiency of six other genes (*Slc25a1*, *Prodh*, *Mrpl40*, *αZdhhc8*, *Txnrd2*, and *T10*) deleted in the syndrome might negatively affect synaptogenesis which peaks at P0 (Meechan et al 2010). Haplosufficiency of three other genes (DGCR6 (L) and PRODH) may contribute to neurochemical imbalance in the excitatory and inhibitory neurotransmitters GABA and glutamate (Sobin et al 2005; Zunner et al 2009).

Future studies

To further differentiate between a neurogenic and myogenic etiology of velopharyngeal hypotonia in 22q11DS, an invasive neuromuscular conduction study (EMG) of the velopharyngeal muscles could be performed (Velepic et al 1999). Electrophysiological analysis in distinct regions of the brain in wild type and *Tbx1* heterozygous mice should shed light on the contribution of the cerebral cortex to velopharyngeal closure (Meechan et al 2010).

Conclusions

This study is a step in the process of unraveling the hitherto inadequately explained variation between genotype and phenotype in 22q11DS (Vitelli et al 2002a). The phenotypes among patients with 22q11DS vary greatly (Bassett et al 2011; Vitelli et al 2002a). Parents and caretakers of children with 22q11DS are left with many questions about the likely manifestations and the course of these problems in time. Mice deficient for *Tbx1* are known to display a variety of abnormalities similar to those described in 22q11DS, but it does not seem to affect the morphology or volume of the nA since there were no differences between *Tbx1* heterozygous and wild type mice. The volumes did not vary more within the mutant group than in the wild type group. The underlying cause of velopharyngeal hypotonia and subsequent speech problems in children with 22q11DS continues to be unknown. It is likely that genetic modifiers beyond the 22q11DS genes play a key role in determining 22q11DS phenotypic severity (Meechan et al 2010). With increasing knowledge, the parents and caretakers of children with 22q11DS can be better informed about the expected outcome after velopharyngeal surgery for VPD. Finally, improving our understanding the underlying mechanisms that cause VPD in 22q11DS may lead to novel therapeutic and/or diagnostic methods.

CHAPTER 3:
Platybasia in 22q11.2 Deletion Syndrome is not Correlated with the Speech Resonance

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Abstract

Background: An abnormally obtuse cranial base angle, also known as platybasia, is a common finding in patients with 22q11.2 deletion syndrome (22q11DS). Platybasia increases the depth of the velopharynx and is therefore postulated to contribute to velopharyngeal dysfunction. Our objective was to determine the clinical significance of platybasia in 22q11DS by exploring the relationship between cranial base angles and speech resonance.

Methods: In this retrospective chart review at a tertiary hospital, 24 children (age 4.0-13.1 years) with 22q11DS had speech assessments and lateral cephalograms which allowed measurement of the cranial base angles.

Results: One patient (4%) had hyponasal resonance, 8 (33%) had normal resonance, 10 (42%) had hypernasal resonance on vowels only, and 5 (21%) had hypernasal resonance on vowels and consonants. The mean cranial base angle was 136.5° (SD 5.3°, range 122.3 to 144.8°). The Kruskal-Wallis test showed no significant relationship between the resonance ratings and cranial base angles (p=0.242). Cranial base angles and speech ratings were not correlated (Spearman correlation =0.321, p=0.126). The group with hypernasal resonance had a significantly more obtuse mean cranial base angle (138° vs 134°, p=0.049) but did not have a greater prevalence of platybasia (73% vs 56%, p=0.412).

Conclusions: In this retrospective chart review of patients with 22q11DS, cranial base angles were not correlated with the speech resonance. The clinical significance of platybasia remains unknown.

Introduction

The hemizygous deletion of a region on the long arm of the 22nd chromosome results in a series of physical and mental ailments collectively known as the 22q11.2 deletion syndrome (22q11DS, OMIM #192430/188400) (Shprintzen 2008). Genes in this region contribute, amongst others, to the embryonic development of pharyngeal arches 3, 4 and 6. Therefore, deletion of this these genes often leads to dysmorphism and/or dysfunction of structures that are derived from those pharyngeal arches including the face, velum, parathyroid, thymus, and heart.

The phenotype varies greatly among patients, but often includes hypernasal speech due to velopharyngeal dysfunction (VPD). Many factors may contribute to the etiology of the VPD in 22q11DS (Widdershoven et al 2008a). An abnormally obtuse cranial base angle, also known as platybasia, is a common finding in 22q11DS (Heliovaara & Hurmerinta 2006; Ricchetti et al 2004; Ruotolo et al 2006). Platybasia increases the depth of the velopharynx and is therefore postulated to contribute to VPD (Arvystas & Shprintzen 1984).

In the sagittal midline of the skull, the frontal, ethmoid, sphenoid, and occipital bones form the cranial base angle. During embryology, neural crest cells migrating from the region of the hindbrain to pharyngeal arches 3, 4, and 6, which are known to be affected in 22q11DS, pass through the region which becomes the skeletal cranial base (Molsted et al 2010). In the general population, between the ages of 6 and 21 years, the cranial base angle remains stable in females and decreases only slightly in males (Axelsson et al 2003).

Mechanical forces increase chondrocyte proliferation and cranial base growth (Cohen 2002; Persing et al 1991; Wang & Mao 2002). Hypothetically, since pharyngeal muscles influence the size and shape of the cranial base, weakness of the muscles may cause a tendency towards platybasia (Arvystas & Shprintzen 1984). Continuing in this line of thought, surgical treatment of VPD, which involves rotating velopharyngeal muscles, could potentially decrease the cranial base angle by tethering the posterior pharyngeal wall to the velum (as in a pharyngeal flap procedure) or constriction (as in a sphincter pharyngoplasty).

Velopharyngeal muscle hypotonia and surgery for VPD may both affect the cranial base angle in patients with 22q11DS. However, to date the clinical significance of platybasia has not been shown (Ricchetti et al 2004). Studies in which the cranial base angle was discussed in the context of speech problems only assessed cohorts of patients with hypernasal speech (Havkin et al 2000; Leveau-Geffroy et al 2011; Nachmani et al 2012; Wang et al 2009b) or those requiring surgery for VPD (Veerapandiyan et al 2011). The objective of this study was to explore the relationship between cranial base angles in patients with 22q11DS and speech resonance or previous palato- and/or pharyngoplasty. We hypothesized that patients with hypernasal speech would have more obtuse cranial base angles. In addition, patients with previous palato- or pharyngoplasties were expected to have more acute cranial base angles.

Materials and Methods

Patients

The cranial base angle can be measured on lateral cephalograms. Lateral cephalograms are not routinely made for all patients with 22q11DS who attend the multidisciplinary outpatient clinic at our tertiary referral center. At the discretion of the orthodontist in the multidisciplinary team, many patients have had lateral cephalograms made in conjunction with dental panoramic radiographs. The hospital's electronic patient database allowed access to lists of patients who attended the clinic in the past 21 months (March 2012 – November 2013). A search in this electronic database yielded 24 patients with 22q11DS who had a lateral cephalogram and whose speech was assessed by a speech-language pathologist in the team. In accordance with the Health Code of 2005 based on the Code of Good Conduct 1995, our institutional review board grants a universal waiver for retrospective chart reviews, such as this study, in which patient data is completely anonymous and not identifiable.

Power calculation

Using an alpha of 0.05 and a power of 0.80, the sample size of 24 patients was sufficient to find a correlation coefficient rho of 0.5.

Speech evaluation

The speech-language pathologists in our multidisciplinary team are specialized in assessing cleft speech. They rated the perceptual resonance of patients' speech using the three-point scale used by the Dutch Association for Cleft and Craniofacial Anomalies (Meijer 2003): A score of 1 denotes normal resonance is heard on vowels. A score of 2 denotes hypernasal resonance is heard on vowels only. A score of 3 denotes hypernasal resonance is heard on vowels and weak consonants (for example, [b, d, k] are heard as [m, n, ng]). In this study a score of 0 was added for resonance that was deemed hyponasal.

Cranial base angle

On the lateral cephalogram, the angle (°) was measured between the nasion-sella turcica-basion (Axelsson et al 2003) using ImageJ software (version 1.46r, National Institutes of Health USA) (Figure 3.1). The assessor was blinded to the participant's age, gender, surgical history, and resonance rating. Accuracy of the measurements was tested by repeating measurements on all lateral cephalograms on two separate occasions at least three weeks apart. The intraclass correlation coefficient of duplicate

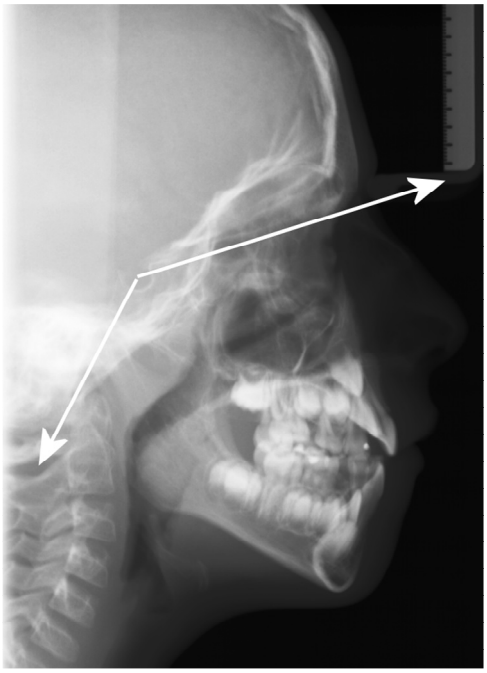

Figure 3.1: Lateral cephalogram. The arrows indicate where the cranial base angle was measured.

measurements was 0.94, denoting excellent intra-observer reliability.

There is no agreed threshold cranial base angle to define platybasia. Some refer to the normal range of 128.9-131.6° with a standard deviation (SD) of approximately 5° (Nachmani et al 2012). By defining platybasia as a cranial base angle that is 1 SD more obtuse that the mean cranial base angle, a cranial base angle >136° was considered platybasia in this study (Ricchetti et al 2004).

Outcomes

Two-tailed Spearman correlations were tested between 1) cranial base angles and resonance ratings, 2) cranial base angles and ages, and 3) ages and resonance ratings. To further assess the relationship between speech resonance and cranial base angle, the Kruskal-Wallis test was performed. Demographics and cranial base angles were compared between the groups of patients with normal resonance (rating ≤1) and those with hypernasal resonance (rating >1) using the Mann-Whitney U, Chi-square, or Fisher's exact tests where appropriate. Additionally, demographics and resonance ratings were compared between the group of patients with normal cranial base angles and those with platybasia using Mann-Whitney U, Chi-square, or Fisher's exact tests where appropriate. Statistical calculations were performed using IBM SPSS Statistics for Windows (Version 20.0. Armonk, NY, USA), where $p<0.05$ was considered a significant finding.

Results

The 24 patients who had lateral cephalograms and speech assessments had a mean age of 8.3 years (SD 2.2 years, range 4.0 to 13.1 years). Thirteen (54%) were female. One (4%) had hyponasal resonance, 8 (33%) had normal resonance, 10 (42%) had hypernasal resonance on vowels only, and 5 (21%) had hypernasal resonance on vowels and consonants. Cleft palate and surgical history are listed in Table 3.1. Patients who were found to have a cleft palate but had not had a palatoplasty (all had submucous cleft palates) were on the waiting list for a modified Honig pharyngoplasty which includes a velar pushback. The mean cranial base

angle was 136.5° (SD 5.3°, range 122.3 to 144.8°). The Kruskal-Wallis test showed no significant relationship between the resonance ratings and cranial base angles (p=0.242).

Cranial base angles and resonance ratings were not correlated (Spearman correlation =0.321, p=0.126), nor were age and cranial base angles (Spearman correlation = -0.264, p=0.212). Age and resonance ratings were correlated: older patients had more normal resonance (Spearman correlation = -0.419, p=0.042, Figure 3.2).

Comparing participants with normal resonance (rating ≤1, n=9) to those with hypernasal resonance (rating >1, n=15) showed significant demographic differences (Table 3.1). Patients with normal resonance were significantly older (p=0.009), had no cleft palates or palatoplasties (p=0.027), and were not on the waiting list for a pharyngoplasty (p=0.015). The group with hypernasal resonance had a significantly more obtuse mean cranial base angle (138° vs 134°, p=0.049) but did not have a greater prevalence of platybasia (73% vs 56%, p=0.412).

Comparing participants with normal cranial base angles (≤136°, n=8) to those with platybasia (>136°, n=16) showed no significant demographic differences nor differences in resonance (p=0.397) (Table 3.1).

Figure 3.2: Scatterplots with cranial base angles and resonance. There was no correlation between A) cranial base angles and resonance ratings, nor B) cranial base angle and age. C) Age and resonance rating were correlated; older patients had more normal resonance. Each O, X, and Δ represents one participant. IVP, intravelar veloplasty.

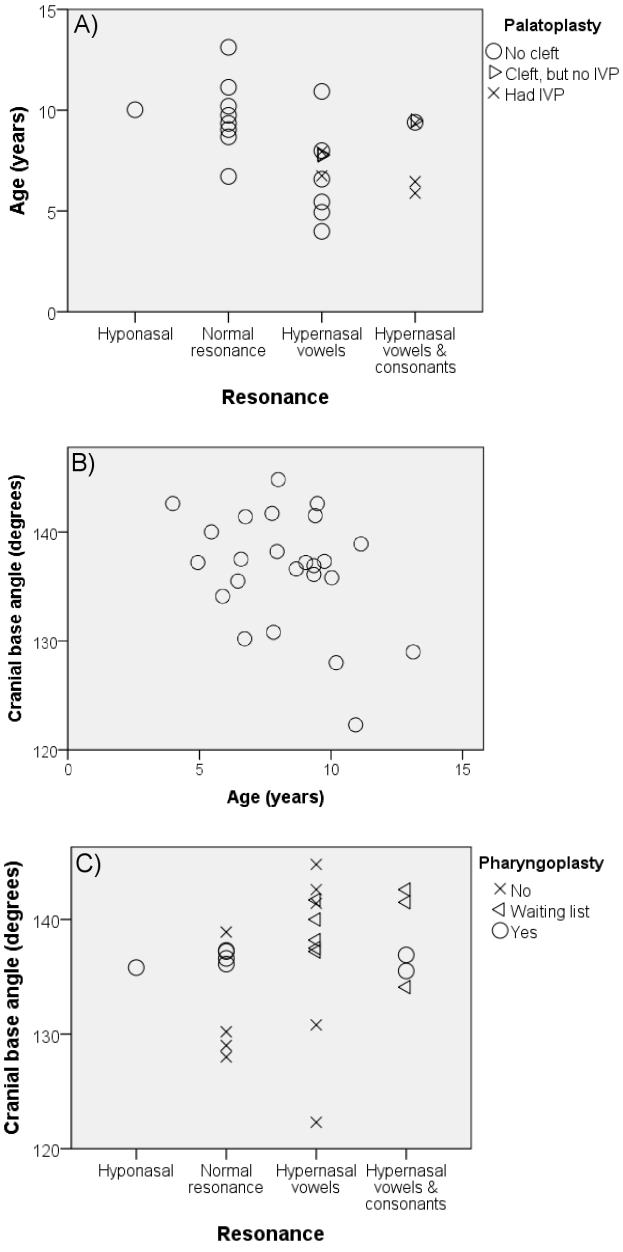

Table 3.1: Group demographics and comparisons between those with 1) normal and hypernasal resonance, and those with 2) normal cranial base angles and platybasia.

Factor	**All patients n=24**	**Resonance**			**Cranial base angle**		
		Normal, n=9	**Hyper-nasal, n=15**	**p-value**	**Normal, n=8**	**Platy-basia, n=16**	**p-value**
Age (mean years ± SD, range)	8.3±2.2 (4.0-13.1)	9.8±1.8 (6.7-13.1)	7.4±1.9 (4.0-10.9)	0.009 [a)]	8.9±2.6 (5.9-13.1)	8.0±2.0 (4.0-11.1)	0.426 [a)]
Female	13 (54)	4 (44)	9 (60)	0.675 [c)]	4 (50)	9 (56)	1.000 [c)]
Cleft palate							
• *No*	16 (67)	9 (100)	7 (47)	0.027 [b)]	5 (63)	11 (69)	0.665 [b)]
• *Submucous cleft palate*	7 (29)	0 (0)	7 (47)		3 (37)	4 (25)	
• *Cleft lip and palate*	1 (4)	0 (0)	1 (6)		0 (0)	1 (6)	
Palatoplasty							
• *No cleft*	16 (67)	9 (100)	7 (47)	0.027 [b)]	5 (63)	11 (69)	0.936 [b)]
• *Cleft, but no palatoplasty*	3 (12)	0 (0)	3 (20)		1 (12)	2 (12)	
• *Palatoplasty*	5 (21)	0 (0)	5 (33)		2 (25)	3 (19)	
Pharyngoplasty							
• *No*	9 (38)	4 (44)	5 (33)	0.015 [b)]	5 (62)	4 (25)	0.163 [b)]
• *Waiting list*	8 (33)	0 (0)	8 (53)		1 (13)	7 (44)	
• *Yes*	7 (29)	5 (56)	2 (13)		2 (25)	5 (31)	
Resonance							
• *Hyponasal*	1 (4)	1 (11)	0 (0)	0.000 [b)]	1 (12)	0 (0)	0.397 [b)]
• *Normal*	8 (33)	8 (89)	0 (0)		3 (38)	5 (31)	
• *Hypernasal vowels only*	10 (42)	0 (0)	10 (67)		2 (25)	8 (50)	
• *Hypernasal vowels and consonants*	5 (21)	0 (0)	5 (33)		2 (25)	3 (19)	
Cranial base angle (mean ± SD, range)	136±5 (122-145)	134±4 (128-139)	138±6 (122-145)	0.049 [a)]	131±5 (122-136)	139±3 (137-145)	0.000 [a)]
• *Platybasia*	16 (67)	5 (56)	11 (73)	0.412 [c)]	0 (0)	16 (100)	0.000 [c)]

Values are presented as n (%). SD, standard deviation.

a) Mann-Whitney U test.

b) Chi-square test.

c) Fisher's exact test.

Discussion

The role of platybasia in the etiology of VPD was suggested over a half century ago (Ricketts 1954). Yet, to date the clinical significance of the platybasia in patients with 22q11DS had not been shown (Ricchetti et al 2004). In this retrospective chart review of patients with 22q11DS, cranial base angles were not correlated with the speech resonance. We did find a trend that the mean cranial base angle was more obtuse in the group of patients with hypernasal speech.

While the meaning of platybasia is clear (literally, "flat skull"), there is no standardized definition. Some have reported platybasia in 22q11DS relative to a control group (Arvystas & Shprintzen 1984; Dalben Gda et al 2010; Glander & Cisneros 1992; Heliovaara & Hurmerinta 2006; Molsted et al 2010; Nachmani et al 2012). Others use cut-off values, defining platybasia as 7° (Oberoi & Vargervik 2005) or 10° (Veerapandiyan et al 2011) above the normal mean without specifying SDs. Yet others define platybasia as being 1 SD (Havkin et al 2000; Nachmani et al 2012; Ricchetti et al 2004), 1.5 SD (Ruotolo et al 2006), or 2 SD above the normal mean. The choice to define platybasia as 1 SD above the normal mean was somewhat arbitrary. The lack of correlation of cranial base angle with resonance rating (Figure 3.2) provides more insight into the, thus far, clinical insignificance of measuring the cranial base angle.

The finding that speech problems are not correlated with platybasia is echoed by another syndrome that is characterized by a high prevalence of platybasia: Hajdu-Cheney Syndrome. Patients with this syndrome have platybasia but do not have VPD; the symptoms that are attributed to the platybasia include headaches, hydrocephalus, poor balance, dizziness, muscle weakness, decreased sensitivity to pain and temperature, and visual loss (Brennan & Pauli 2001). In our current study we did not assess these symptoms in patients with 22q11DS.

Potential factors affecting the cranial base angles in 22q11DS are the presence of a cleft palate and/or a history of palato- and/or pharyngeal surgery. A recent large study showed that the prevalence of platybasia decreases successively from patients with occult submucous cleft palate to submucous cleft palate to cleft

palate to cleft lip and palate (Nachmani et al 2012), perhaps reflecting the effect of differing degrees of surgical correction. Yet, young adults with repaired unilateral cleft lip and palate still have more obtuse cranial base angles than controls (Dogan et al 2006), suggesting any effect of surgical correction does not overcome the congenitally obtuse angle. We did not find a significant relationship between cleft palate and platybasia, nor palatoplasty and platybasia.

The lack of correlation of the cranial base angle with palato- and/or pharyngoplasties found in this study is confirmed by others who studied patients without the 22q11DS. Although not a longitudinal study, the cranial base angles of males without a cleft palate were compared to those of boys with an unrepaired cleft palate, and those of boys whose cleft palates were repaired with a pushback and pharyngeal flap. The cranial base angles did not differ significantly between the groups (Smahel & Mullerova 1992). In another study, the cranial base angles did not differ between children with unilateral cleft lip, cleft palate, and controls at ages 4 months and 2, 4, and 8 years, suggesting the growth of the cranial base is not much influenced by surgical intervention (Han et al 1995).

In conclusion, in this study among patients with 22q11DS, while we found that patients with hypernasal speech had a significantly more obtuse mean cranial base angle, the prevalence of platybasia among those patients was not significantly greater, and there was no correlation between resonance and the cranial base angle. The clinical significance of platybasia remains unknown. The etiology of VPD in 22q11DS is multifaceted (Widdershoven et al 2008a), and is likely a sum of many factors. Our findings indicate that platybasia does not play a prominent role in VPD.

CHAPTER 4:
Velopharyngeal Dysfunction and 22q11.2 Deletion Syndrome: A Longitudinal Study of Functional Outcome and Preoperative Prognostic Factors

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Abstract

Objective: To describe the effect of time after velopharyngoplasty on outcome and search for preoperative prognostic factors for residual hypernasality in patients with 22q11.2 deletion syndrome (22q11DS).

Design: Retrospective chart review.

Setting: Tertiary hospital.

Patients: Patients with 22q11DS and velopharyngeal dysfunction (VPD) who underwent a primary (modified) Honig velopharyngoplasty between 1989 and 2009.

Main outcome measures: Clinically obtained perceptual and instrumental measurements of resonance, nasalance, and understandability before and after velopharyngoplasty.

Results: Data was available for 44 of 54 patients (81% follow-up), with a mean follow-up time of 7.0 years (range 1.0-19.4 years). During follow-up, 24 (55%) patients attained normal resonance and 20 (45%) had residual hypernasality or underwent revision surgery. Mean postoperative nasalance and understandability scores were closer to normal values than mean preoperative scores (2.0 vs 5.5 SD for the normal passage, 1.3 vs 8.1 SD for the non-nasal passage, and 2.3 vs 4.1 understandability). Serial measurements revealed that hypernasality only resolved on average five years after surgery, and three patients' whose resonance initially normalized later relapsed to hypernasality. Gender, age at surgery, lateral pharyngeal wall adduction, velar elevation, presence of a palatal defect, previous intravelar veloplasty, nasalance, understandability, adenoidectomy, hearing loss, and IQ were not able to predict poor outcome following primary velopharyngoplasty (all $p>0.05$).

Conclusions: In this chart review of patients with 22q11DS and VPD, residual hypernasality persisted in many patients after velopharyngoplasty. None of the preoperative factors that were studied had prognostic value for the outcome.

Introduction

The 22q11.2 deletion syndrome (22q11DS) is the most frequent human microdeletion syndrome (Saitta et al 2004). The frequency is estimated around 1 in 4000 (Devriendt et al 1998) but may be as high as 1 in 2000 surviving newborns (Shprintzen 2008). Over 180 clinical features, including every organ system, have been associated with the deletion (Robin & Shprintzen 2005).

One of the most common clinical features is velopharyngeal dysfunction (VPD), affecting 27-92% of children with 22q11DS (Kobrynski & Sullivan 2007). The 22q11DS is the most common diagnosis in patients with VPD of unknown cause (Zori et al 1998). VPD is the incomplete closure of the velopharyngeal valve which normally separates the oral and nasal cavities, resulting in nasal regurgitation during feeding, frequent otitis media, and hypernasal speech (McDonald-McGinn et al 1999). In 22q11DS, the etiology is related to structural abnormalities such as palatal anomalies in 34% of patients (McDonald-McGinn et al 1999), but may also be the corollary of cranial nerve dysfunction (Hultman et al 2000). Surgeons aim to correct VPD by improving the velopharyngeal closure. This can be done by lengthening the palate, mobilising a pharyngeal flap that spans the center of the gap but retains lateral ports, or rotating lateral flaps to augment the sphincter (Sie & Chen 2007). In general, the speech outcome after surgery has been reported to be worse in patients with 22q11DS than in patients without the syndrome (D'Antonio et al 2001a; D'Antonio et al 2001b; Losken et al 2003; Losken et al 2006; Sie et al 1998; Sie et al 2001; Widdershoven et al 2008b), but some patients with 22q11DS fare as well as their non-syndromic counterparts after surgery (Argamaso et al 1994; Meek et al 2003; Milczuk et al 2007; Perkins et al 2005; Pryor et al 2006; Rouillon et al 2009). Naturally, parents are interested to know whether their child will benefit from surgery. However, prognostic factors remain elusive (Losken et al 2003).

All postoperative outcome studies to date have mean follow-up periods of less than 5 years. This report includes an analysis of the functional outcome after a follow-up up to 19 years after primary velopharyngoplasty in patients with 22q11DS. To do this, a group of patients previously reported on (Widdershoven et al

2008b) was augmented with more recent patients. The purpose of this study was to describe the effect of time on functional outcome and search for preoperative prognostic factors for residual perceptual hypernasality or the need for surgical revision following velopharyngoplasty.

Methods

Patients

Postoperative functional outcome was inventoried from the medical records of patients with FISH-confirmed 22q11DS who underwent a primary (modified) Honig velopharyngoplasty for VPD between 1989 and 2009 in our tertiary hospital. These surgeries include both palatal lengthening by pushback and raising a superiorly based pharyngeal flap from the posterior pharyngeal wall. The lateral edges of the flap curl under causing it to tube. While the conventional Honig velopharyngoplasty uses full thickness mucoperiosteal flaps for the oral lining of the defect, the modified technique uses only mucosal flaps (Mink van der Molen et al 2008). Only patients for whom resonance was measured preoperatively and at least one year postoperatively was available were included since resonance takes at least a year to stabilize after surgery (Arneja et al 2008; Conley et al 1997; Rouillon et al 2009; Tatum et al 2002; Widdershoven et al 2008b). The outcome of a subgroup of 25 patients was previously reported after a mean follow-up time of five years (Widdershoven et al 2008b). These patients were invited to return for long term follow-up assessment at the outpatient clinic.

Between 1989 and 2009, 54 patients with 22q11DS underwent a primary (modified) Honig velopharyngoplasty at our institution. All patients had intensive speech therapy before and after surgery. Assessments of resonance both preoperatively and at least one year after primary velopharyngoplasty were available for 44 of these patients (81% follow-up). One patient was excluded because she did not speak preoperatively, precluding preoperative resonance assessment. The other nine patients were excluded because they only returned for follow-up assessments within one year after primary velopharyngoplasty. No reasons were recorded for

discontinued follow-up. Patient demographics are listed in Table 4.1. As indicated by the inclusion criteria, the minimum postoperative follow-up time to speech assessment was one year. The maximum follow-up time was 19.4 years after primary velopharyngoplasty with a mean of 7.0 years. Intravelar veloplasty constitutes the anatomic dissection and repositioning of the velar muscles. On all but one occasion when revision surgery was performed, this was for residual hypernasal speech. The exception was one patient without residual hypernasality whose speech continued to be perceptually bothersome after over eight years of speech therapy after primary pharyngeal flap surgery. The first and second revisions were performed an average of 6.2 years (range 1.5-11.0 years) and 8.6 years (range 5.1-12.1 years) after primary velopharyngoplasty, respectively. Based on patient histories, no patients suffered from obstructive sleep apnea postoperatively.

Table 4.1: Patient demographics.

Characteristic	**Prevalence**
Female (n, %)	30/44 (68%)
Age at primary velopharyngoplasty (mean, range)	6.0 years (range 3.4-13.9 years)
Maximum follow-up time (mean, range)	7.0 years (range 1.0-19.4 years)
Age at maximum follow-up (mean, range)	13.0 years (range 5.2-27.2 years)
Palatal anomaly (n, %)	23/40 (58%)
• Cleft lip and palate	1/40 (2%)
• Overt cleft palate	2/40 (5%)
• Submucous cleft palate	15/40 (34%)
• Bifid uvula	5/40 (13%)
• Unknown	4/44 (9%)
Intravelar veloplasty (n, %)	15/38 (38%)
• During previous cleft palate repair	6/38 (16%)
• During velopharyngoplasty	9/38 (24%)
• Unknown	6/44 (14%)
Revision surgery (n, %)	8/44 (18%)
• Two revision surgeries	2/44 (5%)
• Sphincter pharyngoplasty	7/10 revisions (70%)
• (Modified) Honig velopharyngoplasty	3/10 revisions (30%)
Obstructive sleep apnea (n, %)	0/44 (0%)

Outcome measures

By surgically creating an autologous obturator between the oro- and nasopharynx, treatment for VPD most directly aims to ameliorate hypernasality. Resonance was tested during live assessment preoperatively and postoperatively at varying follow-up times using standardized passages. The 'normal' passage has a proportion of nasal sounds representative for Dutch language similar to the Rainbow passage. The 'non-nasal' passage is similar to the Zoo passage in that it has no nasal sounds (van der Weijer & Slis 1991).

Perception is the gold standard of speech assessment (Kuehn & Moller 2000). Speech pathologists graded hypernasality using the three-point-scale used by the Dutch Association for Cleft and Craniofacial Anomalies (Meijer 2003; Sullivan et al 2009). A score of 1 denotes normal resonance on vowels, a score of 2 denotes hypernasality on vowels, and a score of 3 denotes hypernasality on vowels and approximants. Documentation in patient charts, however, was inconsistent, often only stating whether resonance was normal or hypernasal. Therefore, only normal or hypernasal resonance was inventoried for this chart review.

While the perceptual speech test used by the Dutch Cleft Palate Association has not officially been tested for validity, some believe there is poor inter- and intrarater reliability for the perceptual assessment of hypernasality. Therefore, the speech pathologists at our center frequently measured nasalance instrumentally with the Nasometer 6200 (Kay Elemetrics) until 1999, and the NasalView (Tiger DRS Electronics) from 2000 onwards. These measurements were inventoried as secondary outcome measures. As these machines have different calibrations (Hogen Esch & Dejonckere 2004; van der Weijer & Slis 1991), the nasalance percentage scores could not be compared directly. Instead, the standard deviations were calculated for the percentage scores. Values within two standard deviations (SDs) greater than or less than the normal score were considered to be within the normal range (Hogen Esch & Dejonckere 2004; van der Weijer & Slis 1991). Occasionally, when perceptual resonance was normal, speech pathologists forewent instrumental measurements.

Speech understandability is less directly influenced by surgery as it a sum of many speech components besides resonance, including articulation and voice quality. It was inventoried for this study as it is socially important (Lipson et al 1991). Based on live conversational speech, a speech pathologist graded the understandability together with the patients and their parents using the five-point-scale used by the Dutch Association for Cleft and Craniofacial Anomalies (Meijer 2003; Sullivan et al 2009) (Table 4.2). A score of 1 indicated normal speech and a score of 4 or 5 indicated poor speech understandability.

Table 4.2: Understandability scale (Dutch Association for Cleft and Craniofacial Anomalies).

Rating	***Description***
1	The speech is understandable and normal.
2	The speech differs from others. This does not lead to comments and the speech is understandable.
3	The speech differs from others. This does lead to comments and the speech is understandable.
4	The speech is understandable with some difficulty.
5	The speech is not understandable.

As aforementioned, resonance takes at least a year to stabilize after surgery (Arneja et al 2008; Conley et al 1997; Rouillon et al 2009; Tatum et al 2002; Widdershoven et al 2008b). Lipson et al. (Lipson et al 1991) found improvement occurred up to four years after surgery. To examine whether speech continues to change, serial assessments in patients with multiple assessments were compared. As this was a retrospective study, patients had not been invited for measurements at regular intervals. Limited data precluded statistical analysis; descriptive analyses are presented.

Poor outcome after primary pharyngeal flap surgery was defined as residual perceptual hypernasality or the need for surgical revision. Based on studies including both syndromic and non-syndromic patients with VPD, the following potential preoperative prognostic factors for poor postoperative outcome were analyzed in the 22q11DS population: male gender (Kasten et al 1997), age >7 years

at surgery (Riski 1979), poor or moderate lateral pharyngeal wall adduction (Argamaso et al 1980; Armour et al 2005; Lam et al 2007; Schmelzeisen et al 1992), poor or moderate velar elevation, presence of a palatal defect (de Buys Roessingh et al 2006), previous intravelar veloplasty, adenoidectomy, hearing loss of at least 40dB in both ears, IQ <70 (Moll et al 1963), poor understandability, and high nasalance scores (Losken et al 2006). Lateral pharyngeal wall adduction and velar elevation had been assessed during nasal endoscopy with a Pentax 2.3-mm flexible endoscope. Motion had been categorized as either poor, moderate, or good. IQ was measured using the age-appropriate WPPSI-R, SON-R, or WISC-III scales.

Statistical analysis

The independent *t*-test was used to compare the mean follow-up time to resolution of hypernasality, mean age at primary velopharyngoplasty, mean preoperative nasalance, and mean preoperative understandability between the group that attained normal resonance and the group that had residual hypernasality or underwent revision surgery. A two-tailed Spearman correlation was used to quantify the correlation between resonance and nasalance, and between resonance and understandability. The values of the potential prognostic factors for predicting poor outcome were tested using the Fisher exact test. Only complete data pairs were used.

Results

Pre- and postoperative speech assessments

Hypernasal resonance is the indication for velopharyngoplasty. After primary velopharyngoplasty, 24 (55%) patients attained normal resonance and 20 (45%) had residual hypernasality or underwent revision surgery (Figure 4.1). The follow-up time to either outcome did not differ significantly between the groups (5.2 years (range 1.1-17.4) vs 4.9 years (range 1.0-15.5), p=0.80), neither did the age at primary velopharyngoplasty (5.9 years (range 3.4-10.0) vs 6.0 years (range 3.4-13.9), p=0.88). Normal resonance was not limited to those who had been followed for at least 5 years. Three of the patients who attained normal resonance relapsed to

hypernasality on average 2.0 years after achieving normal resonance. Of the eight who underwent revision surgery, two went on to attain normal resonance. At maximum follow-up, 21 (48%) had residual hypernasality, including six who remained hypernasal after revision surgery.

Given the retrospective nature of this cohort study, although resonance assessments were available for all patients based on the inclusion criteria, nasalance and understandability scores were not. The mean nasalance and understandability scores at maximum follow-up showed an improvement relative to the mean preoperative scores (Table 4.3). Resonance was more highly correlated to the nasalance scores (0.67 for the normal passage, and 0.71 for the non-nasal passage) than to understandability (0.48) (Figure 4.2).

Figure 4.1: Resonance at maximum follow-up, including the total number of patients with each outcome and stratification by follow-up time.

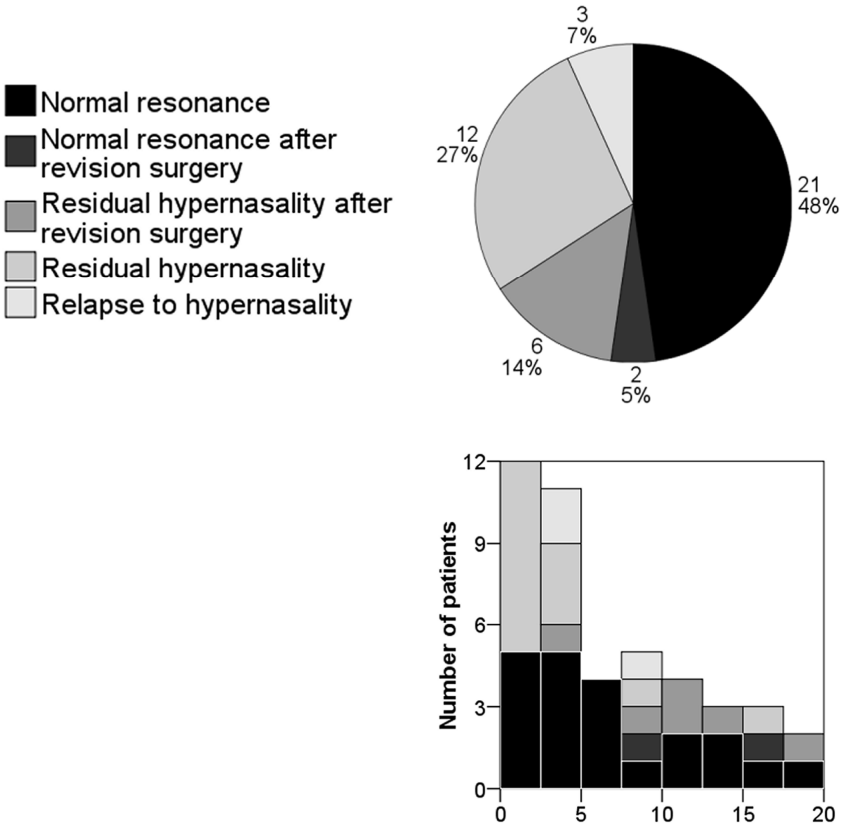
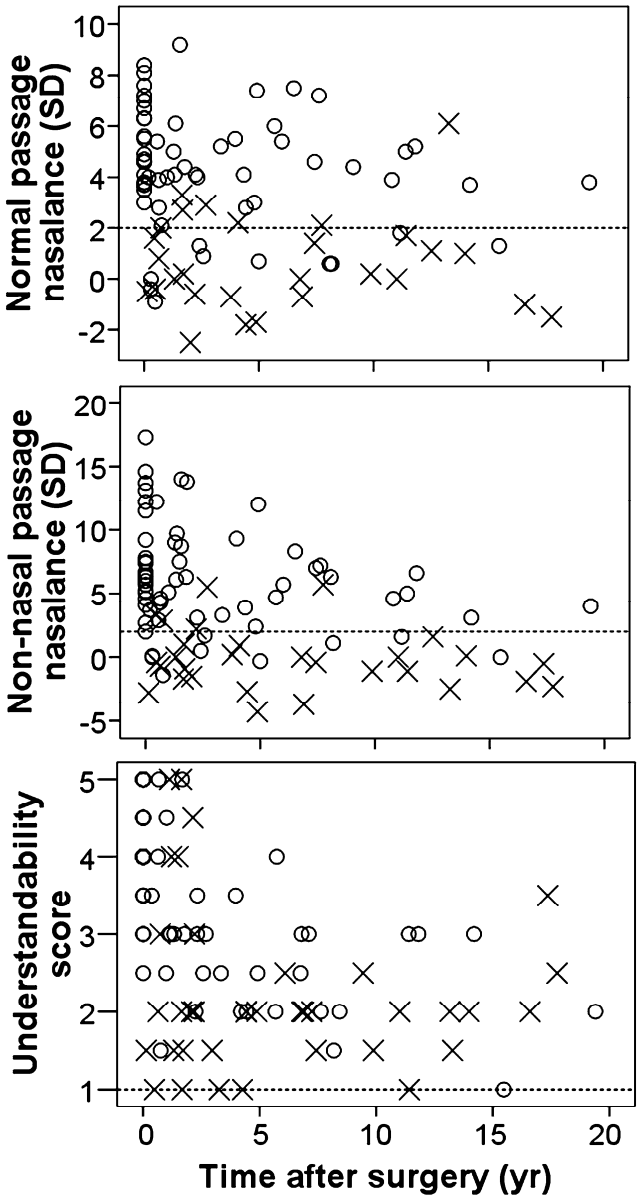


Table 4.3: Nasalance and understandability scores measured preoperatively and at maximum follow-up.

	Time of measurement	*Preoperative*	*Maximum follow-up*
Normal passage nasalance (SD)	Mean (SD)	5.5 (1.6)	2.0 (2.5)
	Range	3.0-8.4	-1.8-7.2
	n	20	28
	Normal (-2; 2)	0 (0%)	16 (57%)
Non-nasal passage nasalance (SD)	Mean (SD)	8.1 (4.1)	1.3 (3.4)
	Range	2.0-17.3	-3.7-8.7
	n	21	27
	Normal (-2; 2)	1 (5%)	14 (52%)
	Hyponasal (<-2)	0 (0%)	4 (15%)
Understandability score	Mean (SD)	4.1 (0.7)	2.3 (0.9)
	Range	2.5-5.0	1.0-5.0
	n	28	36
	Understandable (<4)	7 (25%)	34 (94%)

Figure 4.2: Nasalance, understandability, and resonance measured preoperatively (time after surgery 0) and at varying times after velopharyngoplasty. Dashed lines: upper limits of normal nasalance and understandability scores. X, normal resonance; O, hypernasal.



Prognostic factors

Again, as this was a retrospective study, not all of the potential preoperative prognostic factors had been measured or recorded for all patients. None of the factors tested were prognostic for poor outcome following primary velopharyngoplasty (Table 4.4). The group of patients with poor outcome did not differ from the group that attained normal resonance regarding the preoperative nasalance while reading or repeating the normal passage (5.9 vs 5.0 SD. p=0.24) or the non-nasal passage (9.1 vs 6.9 SD, p=0.23), nor understandability (4.0 vs 4.1, p=0.55).

Table 4.4: Potential preoperative prognostic factors for residual hypernasality or surgical revision following primary velopharyngoplasty.

Potential preoperative prognostic factors	*Among those with poor outcome n (%)*	*Among those with normalized resonance n (%)*	*p-value (2-sided)*
Male gender	5/20 (25)	9/24 (38)	0.52
Age >7 years at surgery	14/20 (70)	19/24 (79)	0.51
Poor or moderate lateral pharyngeal wall adduction	3/6 (50)	4/10 (40)	1.00
Poor or moderate velar elevation	13/15 (87)	14/19 (74)	0.43
Palatal defect	9/18 (50)	14/22 (64)	0.52
Previous intravelar veloplasty	19/20 (95)	19/24 (79)	0.20
Adenoidectomy	9/14 (64)	15/22 (68)	1.00
Hearing loss	2/7 (29)	3/14 (23)	1.00
IQ <70	2/6 (33)	1/12 (8)	0.25

Discussion

Speech generation and perception are complex, involving cognition, language, and voice. Understanding and managing speech problems in patients with 22q11DS is especially challenging because of the gamut of clinical findings associated with the syndrome and the heterogeneous presentation among patients (Widdershoven et al 2008a). Anatomically, palatal defects, adenoid hypoplasia

(Havkin et al 2000), and platybasia (Ricchetti et al 2004) enlarge the pharyngeal gap, and medially placed internal carotid arteries call for extra caution during surgery (Mehendale & Sommerlad 2004). Comorbidities such as cardiac anomalies and immune deficiencies may delay or preclude surgery. Additionally, muscle hypotonia (Gerdes et al 1999; Kitsiou-Tzeli et al 2004; Oskarsdottir et al 2005a), asymmetric palatal elevation (Chegar et al 2006; Cheng et al 2006; Solot et al 2000; Tatum et al 2002), hearing disorders (Solot et al 2000), schizophrenia (Debbane et al 2009), and learning disabilities (Gerdes et al 2001; Kobrynski & Sullivan 2007; Van Aken et al 2007) may hamper speech therapy.

The effect of time on functional outcome

Rouillon et al. (Rouillon et al 2009) showed that postoperative speech in patients with 22q11DS was inferior to that of patients without 22q11DS at 9 months, but equal at 24 months. The authors postulated that this delayed improvement can be attributed to psychomotor retardation or acquisition difficulties which are common in 22q11DS (Gerdes et al 2001; Kobrynski & Sullivan 2007; Van Aken et al 2007). As was described by Widdershoven et al. (Widdershoven et al 2008b), our experience confirms that the adjustment to the new anatomic situation after surgery is markedly slower in patients with 22q11DS than in patients without 22q11DS. Witt et al. (Witt et al 1999b) found that perceptual speech scores at ages 6 and 12 years after cleft palate repair are stable in children without 22q11DS. Following pharyngeal flap surgery in children without 22q11DS, Riski et al. (Riski 1979) report that the percentage of patients with acceptable resonance remained consistent 2 and 5 years after, and Cable et al. (Cable et al 2004) found that overall resonance continued to be adequate up to 14 years after pharyngeal flap surgery. Given the complex nature of the speech problems in 22q11DS, we were curious whether postoperative speech outcome changes over time in this population. All postoperative outcome studies in patients with 22q11DS to date have mean follow-up periods of less than 5 years. In this report, we present the functional outcome up to 19 years after primary pharyngeal flap velopharyngoplasty.

Previous studies only note that resonance takes at least a year to stabilize after surgery (Conley et al 1997; Tatum et al 2002; Widdershoven et al 2008b). In this study we show that speech continues to evolve as patients age. In patients whose resonance normalized, this only occurred an average of 5 years after primary velopharyngoplasty. Perhaps the patients with residual hypernasality who were followed for less than 5 years will eventually attain normal resonance and were therefore erroneously categorized as having a poor outcome. Once normal resonance was attained, some patients relapsed to hypernasal speech. These continuing changes in resonance make conclusions about postoperative outcome questionable.

The upper limits of the ranges of follow-up times around each of the calculated means may illustrate the marked phenotypic heterogeneity in 22q11DS, or be artifacts of this retrospective study in which patients had not been assessed at regular intervals after surgery. For example, the patient who only attained normal resonance 17.4 years after primary velopharyngoplasty had been dismissed from clinical follow-up 5.7 years after surgery, at which point his resonance was still hypernasal. When he was invited to return for re-assessment, his resonance was normal. It is unclear when his resonance normalized. Patients with poorer speech return more frequently for follow-up consultations and measurements, introducing a selection bias. A prospective study where all patients are measured at regular intervals should avoid selection bias and ensure sufficient data pairs for statistical analysis.

Outcome measures

While perceptual speech is the gold standard for assessing the success of a velopharyngoplasty (Kuehn & Moller 2000), there is no standardized reporting system. Henningsson et al. (Henningsson et al 2008) have suggested a system that including the parameters hypernasality, hyponasality, audible nasal air emission and/or nasal turbulence, voice disorder, consonant production errors, understandability, and acceptance. They suggest continued usage of local measures with mapping to a universal scale to allow comparison of outcomes between centers. The speech test developed and used by the Dutch Association for Cleft and

Craniofacial Anomalies (Meijer 2003) measures all parameters except voice disorder, but has not officially been tested for validity nor reliability. The speech test uses a three-point scale to rate resonance differentiating between hypernasality on vowels or consonants. However, due to inconsistent reporting in the charts, in this study resonance was recorded as either normal or hypernasal. Dichotomous scales generally yield higher agreement and reliability, but this made it impossible to grade improvements in resonance other than complete normalization, underestimating the effect of velopharyngoplasty in partially correcting hypernasal resonance.

Along with perceptual speech rated by a speech pathologist, various surrogate outcome measures are used to assess speech and the success of a velopharyngoplasty. For example, revision rates are easy to measure. However, they are not always indicative of success (Losken et al 2006): sometimes the surgeon gauges that further surgery will not be beneficial, and sometimes patients are satisfied with improved speech and therefore do not opt for further surgery to optimize speech. Patient satisfaction, another outcome measure, is more subjective than perceptual speech assessed by a speech pathologist. Some find that hyponasality causes less social stigmatization than hypernasality (Witt et al 1999a). Nasendoscopic velopharyngeal closure is difficult to assess objectively (Paal et al 2005; Pigott 2002; Sie et al 2008). Objective measures such as and nasalance measured with the Nasometer or NasalView are often reported, however the correlation with perceptual resonance varies from 0.31 to 0.74, limiting its use to measuring the degree of hypernasality once hypernasality has been diagnosed perceptually (Keuning et al 2002; Sweeney & Sell 2008) (Figure 4.2).

Understandability, which is perhaps the most important outcome measure for social interaction, is only partially affected by resonance, as is illustrated by the poor correlation (0.48, Figure 4.2). Normalized resonance may not lead to improved understandability if articulation does not improve (Meek et al 2003). Compensatory articulation is common among patients with 22q11DS.

Prognostic factors

Nearly half of our patients had residual hypernasality following velopharyngoplasty. Given the costs and potential complications associated with this procedure, can we identify this subset before subjecting them to surgery? As suggested by Witt et al. (Witt et al 1995a), suboptimal postoperative functional outcome may represent errors in patient selection rather than errors in operative technique. At our center, all children with 22q11DS and residual speech problems following intensive speech therapy undergo velopharyngoplasty. Preoperative prognostic factors have been sought to determine whether it can be predicted which patients are less likely to benefit from surgery. Studies in the larger VPD population including those with 22q11DS and non-syndromic cleft palate present conflicting results regarding the predictive value of the preoperative factors we tested.

Like Losken et al. (Losken et al 2006), who are the only previous group to report on prognostic factors for postoperative outcome in patients with 22q11DS, we did not find gender to be a predictive factor. In the larger VPD population including non-syndromic patients, Kasten et al. (Kasten et al 1997) found that males had worse postoperative speech scores than females, Sie et al. (Sie et al 2001) found that females had worse scores, while four larger studies showed that gender was not a predictor for outcome (Losken et al 2003; Perkins et al 2005; Pryor et al 2006; Riski et al 1992; Sie et al 2001).

One may postulate that those undergoing surgery at an older age may be disadvantaged since compensations are more ingrained and their brains have less plasticity to relearn speaking techniques. However, neither we nor Losken et al. (Losken et al 2006) found age to be an outcome predictor. In the larger VPD population including non-syndromic patients, some studies found that an older age at surgery led to worse postoperative results (Meek et al 2003; Moll et al 1963; Peat et al 1994; Riski et al 1992; Schmelzeisen et al 1992), while others found that older patients did not have a poorer outcome (Albery et al 1982; Armour et al 2005; Becker et al 2004; Losken et al 2003; Perkins et al 2005; Pryor et al 2006; Seyfer et al 1988; Sie et al 1998; Sie et al 2001; Van Demark & Hardin 1985) but in fact had a better outcome (Kasten et al 1997). It is impossible to draw a general conclusion

because these studies use different methods: some studies compare the mean ages of patients with successful outcome to those without, while others, like ours, test the success rate above and below a below a cut off age.

The murine model for 22q11DS has hypoplastic branchiomeric muscles (Kelly et al 2004; Xu et al 2005) and aberrant cranial nerves (Vitelli et al 2002b). The clinically hypodynamic pharynx in patients with 22q11DS (Milczuk et al 2007; Rouillon et al 2009; Witt et al 1999a) echoes a neuromuscular component in the etiology of VPD. We expected to find that patients without good lateral pharyngeal wall adduction would be less likely to attain normal resonance following a (modified) Honig velopharyngoplasty, but failed to find a significant relationship. This may be explained by the presence of a pharyngeal flap changing postoperative lateral pharyngeal wall adduction (Karling et al 1999a). In the larger VPD population including non-syndromic patients, Sie et al. (Sie et al 1998) did not find worse postoperative outcomes among patients with less lateral wall movement, but larger studies did (Argamaso et al 1980; Armour et al 2005; Lam et al 2007; Schmelzeisen et al 1992). Likewise, in our study, patients with poor or moderate velar elevation were not more likely to remain hypernasal than their counterparts with good velar elevation. Witt et al. (Witt et al 1995a) also did not find a correlation between velar activity and postoperative speech outcome in a VPD population including non-syndromic patients.

We did not find preoperative nasalance or understandability to be predictive for residual hypernasality after surgery. In the larger VPD population including non-syndromic patients, preoperative speech scores have been predictive for postoperative speech scores (Losken et al 2003; Riski et al 1992; Schmelzeisen et al 1992). In patients with 22q11DS, Losken et al. (Losken et al 2006) found that lower preoperative nasalance scores correlated with a decreased need for surgical revision.

In our study population, patients with a palatal defect were not more likely to remain hypernasal. Likewise, those who underwent intravelar veloplasty prior to pharyngeal flap surgery did not fare worse than those who did not. This may affirm the adequacy of the (modified) Honig velopharyngoplasty technique for correcting

anatomical aberrations. In the larger VPD population including non-syndromic patients, only de Buys Roessingh et al. (de Buys Roessingh et al 2006) found that patients with palatal defects who underwent velopharyngoplasty had worse postoperative speech outcomes, while all other studies found no predictive value (Perkins et al 2005; Pryor et al 2006; Riski et al 1992; Schmelzeisen et al 1992; Seyfer et al 1988; Sie et al 2001).

While adenoidectomy predisposes to VPD (Conley et al 1997; Ford et al 2000; Saunders et al 2004), in our study prior adenoidectomy was not predictive for outcome. Likewise, Witt et al. (Witt et al 1995b) found no overt correlation between removal of lymphoid tissue and outcome in the larger VPD population including non-syndromic patients. Since the adenoids are often hypoplastic in 22q11DS (Havkin et al 2000; Mehendale et al 2004; Williams et al 1987) there may not be a real difference between the groups who did and did not undergo adenoidectomy.

Hearing loss in 22q11DS can be sensorineuronal (Reyes et al 1999) or conductive following recurrent otitis media (Solot et al 2000). As postulated by Willging (Willging 1999), hearing loss hampers VPD resolution since it reduces the patient's ability to self-correct the problem. Albery et al. (Albery et al 1982) did not find hearing to be prognostic in the larger VPD population including non-syndromic patients and we did not find a correlation between preoperative hearing loss and postoperative resolution of hypernasal resonance in patients with 22q11DS.

Those with a higher IQ may more readily learn to employ the new anatomical situation after surgery for understandable speech. In the larger VPD population including non-syndromic patients, Moll et al. (Moll et al 1963) found that IQ is prognostic for postoperative speech outcome while Albery et al. (Albery et al 1982) did not find intelligence to be prognostic. We did not find IQ to be prognostic in our 22q11DS population. All our patients had speech therapy before and after surgery, however the amount was not documented in the charts and therefore unavailable for testing as a prognostic factor. Speech therapy is essential in learning to speak understandably (Wang et al 2009a).

Major weaknesses of this study include: that speech had not been evaluated by speech therapists who were blinded to the study, that previous live assessments

by speech therapists preclude controlling the data, and that much data is missing. Admittedly, being able to review recordings and having more complete data would be preferred, but these ideals are impossible to realize in a retrospective study. Yet, a retrospective design was necessary to yield the largest number of patients.

Although our center is a referral center, and we inventoried data from the past 20 years, we did not have a large enough population to definitively refute the null-hypothesis. Performing a sample size calculation with our ratio of patients with poor outcome to those who attained normal resonance (1:1), to find a prognostic factor that is deemed clinically significant when 70% of the patients with poor outcome have the factor while only 40% of patients with normalized resonance do, using an alpha of 0.05, a power of 0.8, and a continuity correction, yields a necessary sample size of 96 patients (48 per group). With those assumptions, even with complete data for all the 22q11DS patients treated at our center in the past 20 years we would not have sufficient patients. A multicenter cohort study will therefore be necessary to get sufficient numbers to find prognostic factors for postoperative resonance in 22q11DS and inform parents whether their child is expected to benefit from surgery.

Conclusion

In patients with 22q11DS and VPD, this chart review shows that residual hypernasality persisted in many patients after primary velopharyngoplasty. Resonance continued to change years after surgery, making conclusions about postoperative outcome questionable. No preoperative prognostic factors were found for residual hypernasality and/or undergoing revision surgery. A prospective study or a meta-analysis of current data from multiple centers is needed to elucidate prognostic factors.

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CHAPTER 5:
In Search of the Optimal Surgical Treatment for Velopharyngeal Dysfunction in 22q11.2 Deletion Syndrome: A Systematic Review

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Abstract

Background: Patients with the 22q11.2 deletion syndrome (22q11DS) and velopharyngeal dysfunction (VPD) tend to have residual VPD following surgery. This systematic review seeks to determine whether a particular surgical procedure results in superior speech outcome or less morbidity.

Methodology and Principal Findings: A combined computerized and hand-search yielded 70 studies, of which 27 were deemed relevant for this review, reporting on a total of 525 patients with 22q11DS and VPD undergoing surgery for VPD. All studies were levels 2c or 4 evidence. The methodological quality of these studies was assessed using criteria based on the Cochrane Collaboration's tool for assessing risk of bias. Heterogeneous groups of patients were reported on in the studies. The surgical procedure was often tailored to findings on preoperative imaging. Overall, 50% of patients attained normal resonance, 48% attained normal nasal emissions scores, and 83% had understandable speech postoperatively. However, 5% became hyponasal, 1% had obstructive sleep apnea (OSA), and 17% required further surgery. There were no significant differences in speech outcome between patients who underwent a fat injection, Furlow or intravelar veloplasty, pharyngeal flap pharyngoplasty, Honig pharyngoplasty, or sphincter pharyngoplasty or Hynes procedures. There was a trend that a lower percentage of patients attained normal resonance after a fat injection or palatoplasty than after the more obstructive pharyngoplasties (11-18% versus 44-62%, p=0.08). Only patients who underwent pharyngeal flaps or sphincter pharyngoplasties incurred OSA, yet this was not statistically significantly more often than after other procedures (p=0.25). More patients who underwent a palatoplasty needed further surgery than those who underwent a pharyngoplasty (50% versus 7-13%, p=0.03).

Conclusions: In the heterogeneous group of patients with 22q11DS and VPD, a grade C recommendation can be made to minimize the morbidity of further surgery by choosing to perform a pharyngoplasty directly instead of only a palatoplasty.

Introduction

The 22q11.2 deletion syndrome (22q11DS) is the most frequent human microdeletion syndrome (Saitta et al 2004), with a frequency estimated around 1 in 4000 (Devriendt et al 1998). There is marked phenotypic heterogeneity among patients. The most common concerns during infancy include congenital heart disease, immune disorders, feeding problems, and hypocalcaemia. In toddlers and school age children, developmental delay and speech problems surface. In adolescents and adults psychiatric issues arise (Kobrynski & Sullivan 2007; McDonald-McGinn & Sullivan 2011; Shprintzen 2008).

The speech problems are mostly attributed to velopharyngeal dysfunction (VPD). The velopharyngeal valve which normally separates the oral and nasal cavities shows incomplete closure resulting in feeding difficulties and hypernasal crying in infants and hypernasal speech in older children. Hypernasality can impair speech intelligibility with subsequent frustration and social withdrawal (Lipson et al 1991). Language acquisition is often delayed (Dyce et al 2002; Eliez et al 2000; Gerdes et al 1999; Persson et al 2006; Scherer et al 1999; Van Lierde et al 2007). The etiology of VPD in patients with 22q11DS includes palatal defects, adenoid hypoplasia, and platybasia which enlarge the pharyngeal gap (Widdershoven et al 2008a). Furthermore, nasendoscopic views of attempted velopharyngeal closure show pharyngeal hypotonia (Ysunza et al 2011).

Patients with hypernasal speech which is resistant to speech therapy or patients with VPD based on anatomic deficits are candidates for velopharyngeal surgery. Surgeons aim to correct VPD by decreasing the size of the velopharyngeal gap by injecting fat in the posterior pharyngeal wall, lengthening the palate, mobilizing a pharyngeal flap (PF) that spans the center of the velopharyngeal gap but retains lateral ports, and/or rotating lateral flaps to reduce the velopharyngeal port diameter (Sie & Chen 2007). There is little evidence guiding the choice between these procedures.

Theoretically, PFs are only appropriate for patients with good lateral wall motion (Abyholm et al 2005; Argamaso et al 1980; Armour et al 2005; Lam et al

2007; Schmelzeisen et al 1992). When there is good velar elevation and poor lateral wall motion, a sphincter pharyngoplasty (SP) or Hynes pharyngoplasty can be used, provided the level of the flap inset is high enough to provide velopharyngeal competence (Arneja et al 2008) and low enough to avoid hyponasality (Huang et al 1998).

VPD treatment algorithms based on these theories state that surgical procedures should be tailored to preoperative findings such as velopharyngeal gap size and gap shape (Marsh 2003; Mehendale et al 2004; Sie & Chen 2007). Patients with coronal closure patterns are predicted to benefit from SPs (Sie et al 1998) while patients with sagittal closure patterns are predicted to benefit from PFs (Armour et al 2005; Marsh 2003). However, these recommendations are not based on clinical trials or systematic reviews.

Given both the costs and potential complications associated with surgery, it is important to help surgeons chose which patients to operate on and which procedure to employ (Goldberg et al 2005). Clinical trials comparing PFs to sphincter pharyngoplasties in nonsyndromic patients show no difference in outcome when treatment allocation is randomized (Abyholm et al 2005; Ysunza et al 2002; Ysunza et al 2004) or tailored to lateral pharyngeal wall and velar motion (Lam et al 2007; Peat et al 1994; Ysunza et al 2001). Patients with 22q11DS were excluded from these clinical trials, therefore the question whether creating a PF is more effective than an SP in resolving VPD remains unanswered for this population.

This study aims to determine whether in patients with 22q11DS and VPD a particular surgical procedure results in a greater percentage of postoperative normal resonance by systematically reviewing the available literature. Sub-questions include which procedure results in less morbidity and whether tailoring the procedure to preoperative patient characteristics results in superior outcome. Ideally, these questions should be answered in a clinical trial. However, patient acquisition rates necessitate multi-center collaboration (Marsh et al 1989), and surgeon preferences for certain procedures limit participation (Abyholm et al 2005). This retrospective study contributes to further insight in the outcome of different surgical procedures.

Methods

Ethics

No ethical approval was required to conduct a systematic review of the literature. Approval was granted by the Institutional Review Board at the University Hospital in Leuven, Belgium to include unpublished data from a chart review.

Searching

No protocol exists for this systematic review, nor was such a protocol prospectively registered in the Cochrane database. Studies were found via computerized searches of the MEDLINE and EMBASE databases and the Cochrane CENTRAL Register of Controlled Trials on 11-11-11. The search syntax included synonyms of 22q11DS (Di-George OR DiGeorge OR "Di George" OR velocardiofacial OR 22q11* OR del22q11* OR "velo-cardio-facial" OR Shprintzen OR "catch 22") and surgery for VPD ((fat AND inject*) OR palatoplast* OR Furlow pharyngoplast* OR velopharyngo* OR "pharyngeal flap" OR Honig OR Hynes). No limits were imposed on publication type, date, or language. Additionally, references of the relevant studies were hand-checked to confirm that no relevant publications were missed by the electronic search. Finally, data from personal unpublished files was included.

Selection

The search hits were scanned for relevance using the inclusion criteria: (1) report outcome after surgery for VPD, and (2) report separate results for patients with 22q11DS. Where relevance could not be determined based on title and abstract, the full-text was assessed.

Validity assessment

The methodological quality of each study was appraised using criteria based on the Cochrane Collaboration's tool for assessing risk of bias in therapeutic studies (2011). One point was accredited for each positive criterion: (1) genetic confirmation of 22q11DS, (2) inclusion of all patients with 22q11DS and VPD who

underwent surgery at the center, (3) the choice for the specific surgical procedure was randomized, (4) speech outcome was assessed at least one year postoperatively for all patients, (5) speech assessors were blinded to the surgical procedure, (6) the speech test was validated, and (7) results included the number of patients with postoperative normalized resonance. Patient inclusion criteria were collected to determine whether the study results could be generalized to all patients with 22q11DS with VPD requiring surgery. Outcome assessment at least one year postoperatively was considered important since resonance takes at least a year to stabilize after surgery (Arneja et al 2008; Conley et al 1997; Rouillon et al 2009; Tatum et al 2002; Widdershoven et al 2008b).

Data abstraction

Data abstraction was completed independently. When patients with isolated VPD or other syndromes with VPD were included in studies, only data from patients with 22q11DS and VPD were included in this review. Data was collected from the studies including patient age at surgery, prevalence of palatal anomalies, details of the preoperative imaging and whether this was used to tailor the procedure, specifics on the surgery, the length of postoperative follow-up time until speech was assessed, and speech outcome variables. The surgery was considered tailored when preoperative imaging studies affected the surgeon's choice for a particular surgical technique. For example, only patients with good pharyngeal lateral wall adduction received PFs, or the amount of pharyngeal lateral wall adduction determined the PF width.

Surgical procedures were categorized as either fat injection, Furlow, intravelar veloplasty (IVP), PF, Honig, SP, or Hynes. In a Honig procedure a velar retropositioning is combined with the creation of a PF. The pedicle of the flap tubes postoperatively, minimizing the obstruction (Mink van der Molen et al 2008l). A Hynes procedure is derived from an SP with high inset of the lateral flaps implying splitting of the palate (Mehendale et al 2004).

Non-standardized reporting of speech scores impeded comparison of preoperative baseline characteristics and postoperative outcome, and different

definitions were used to indicate 'improved' speech. Therefore, it was not possible to inventory the degree of preoperative VPD. Yet, where possible, the numbers of patients with postoperative normal perceptual resonance, nasal emission, and understandable speech were distilled from the studies. The definition of normal scores differed per study, introducing a bias that may affect the cumulative evidence.

Quantitative data synthesis

To compare the outcome of the various procedures, the percentage of patients who attained normal perceptual resonance, normal nasal emissions, understandable speech, hyponasal speech, obstructive sleep apnea (OSA), and those requiring further surgery were included in a weighted ANOVA with weights based on the number of patients each outcome was measured in. Where there were significant differences, these were further tested using contrasts with a Bonferroni correction. The following pairs were compared: 1) fat injection versus Furlow or IVP since these less obstructive procedures tend to be performed on patients with some velopharyngeal movement, 2) fat injection versus SP or Hynes since both augment the posterior pharyngeal wall, 3) fat injection, Furlow, or IVP versus PF, Honig, SP, or Hynes since the previous tend to be less obstructive than the latter, 4) Furlow, or IVP versus PF, Honig, SP, or Hynes to compare palatoplasties to pharyngoplasties, 5) PF versus Honig to compare the effect of differing flap width to creating a narrow PF combined with palatal retropositioning, and 6) PF versus SP or Hynes, and 7) Honig versus SP or Hynes to compare the different types of pharyngoplasties. No assessment of publication bias was done.

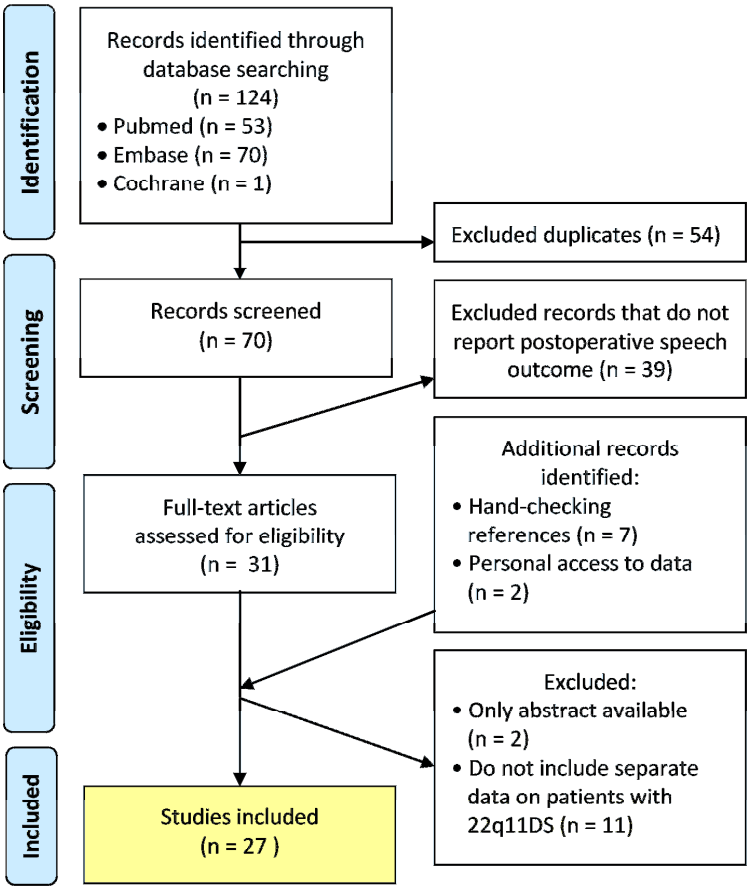
Results

Search and selection

After filtering for duplicates, this electronic search strategy yielded 70 studies (Figure 5.1). Thirty-nine studies were excluded that did not report postoperative speech outcome. Hand-checking references yielded seven additional studies that report postoperative outcome but were missed by the electronic search

because a synonym of 22q11DS was not mentioned in the title or abstract, but only in the body text (Husein et al 2004; Perkins et al 2005; Pryor et al 2006; Seagle et al 2002) or a table (Baylis et al 2008; de Buys Roessingh et al 2008; de Serres et al 1999). For two of the relevant studies, only the abstracts have been published, hampering data extraction (Ghanem et al 2011; Solot et al 2011). Eleven studies were excluded that did not report separate results for patients with 22q11DS. The authors have personal access to data from another relevant article which has been published in a dissertation (Widdershoven et al 2011b) and to data from the University Hospital in Leuven, Belgium (Hens and Vander Poorten, co-authors). The combined electronic and hand-search strategy yielded 27 relevant studies for which data was accessible for analysis.

Figure 5.1: Study selection. Computerized search conducted on 11-11-11.



Validity assessment

None of the studies met all the criteria indicating good methodological quality (Table 5.S1). Genetic confirmation of the deletion was not always performed or reported. In most studies only a specific subgroup of patients who underwent surgery for VPD was reported. The choice for a particular surgical procedure was not randomized in any study. In only eight studies was the follow-up time at least one year for all patients. When loss to follow-up was reported, it ranged from 0-34%. In ten studies the outcomes of multiple surgical procedures were reported. In three of these studies the speech assessor was blinded to the surgical procedure (Baylis et al 2008; Mehendale et al 2004; Milczuk et al 2007).

In five studies postoperative speech was only reported in terms of improvement without data on the number of patients with normalization of resonance (Losken et al 2006; Milczuk et al 2007; Perkins et al 2005; Rottgers et al 2011; Rouillon et al 2009). In three studies resonance was not one of the postoperative outcome measures (D'Antonio et al 2001a; Wang et al 2009a; Witt et al 1998a). These eight studies could not be used to answer the main question of this review, namely, whether a particular surgical technique leads to a higher percentage of patients with normal resonance postoperatively. However, these studies were still included in the analyses since they reported on the morbidity of the procedures.

In a handful of studies the inter- or intrarater reliability for the speech test were reported, indicating the validity of the test (Arneja et al 2008; Baylis et al 2008; Brandao et al 2011; Wang et al 2009a; Ysunza et al 2009). Others used the previously validated Cleft Audit Protocol for Speech (Mehendale et al 2004) or Pittsburgh Weighted Speech Score (Rottgers et al 2011; Swanson et al 2011; Widdershoven et al 2011b). No formal validation has been done for the Borel-Maisonny scale (Leuchter et al 2009; Nicolas et al 2011; Rouillon et al 2009), the SISL (Screening Instrument for Cleft Palate Speech in Leuven) (Hens and Vander Poorten, co-authors), or the test developed by the Dutch Association for Cleft and Craniofacial Anomalies (Spruijt et al 2011).

Most study designs were outcomes research evaluating the speech of patients with 22q11DS and VPD after undergoing surgery which is considered level

2c evidence (Phillips 1998). Three studies were cohort studies in which patients with 22q11DS and VPD who underwent surgery and those who did not were followed up. However, these studies were of poor quality deeming them level 4 evidence (Phillips 1998) due to ascertainment bias in recruiting patients to participate in the study (Baylis et al 2008), loss to follow-up >20% (Lipson et al 1991), or because follow-up times were not reported (MacKenzie-Stepner et al 1987).

Data from all studies were used to determine which surgical procedure was most effective for resolving VPD in patients with 22q11DS.

Study characteristics

A comparison of the characteristics of the 27 studies, further subdivided by the groups of patients undergoing different procedures, revealed marked heterogeneity regarding which patients underwent surgery, the preoperative imaging, and the postoperative outcome measurements (Table 5.1, Table 5.S2). Study sizes ranged from 1 to 44 patients. Many patients had palatal anomalies (57%, n=282/494). Some had previous surgery on the palate or pharynx (n=25) (Leuchter et al 2009; Nicolas et al 2011; Spruijt et al 2011; Swanson et al 2011; Tatum et al 2002; Widdershoven et al 2011b; Ysunza et al 2009) or adenoid or tonsils removed (n=69) (Hens and Vander Poorten, co-authors) (Goorhuis-Brouwer et al 2003; Lipson et al 1991; MacKenzie-Stepner et al 1987; Rottgers et al 2011; Spruijt et al 2011; Swanson et al 2011; Tatum et al 2002; Widdershoven et al 2011b; Ysunza et al 2009). The patients included in the study by Argamaso et al (Argamaso et al 1994) underwent surgery when they were on average twice as old as the patients in the other studies.

Pre-operative imaging included nasendoscopy and/or X-ray cephalograms or (video)fluoroscopy to confirm VPD or assess pharyngeal movement, including pharyngeal lateral wall motion, velar movement, gap size on attempted closure, and the closure pattern. Patients who underwent fat injections or palatoplasties tended to have better movement and smaller gap sizes than patients who underwent pharyngoplasties (Table 5.S2).

The course of the carotid arteries was noted during nasendoscopy, using magnetic resonance imaging (Arneja et al 2008; Nicolas et al 2011; Rouillon et al 2009; Swanson et al 2011; Tatum et al 2002; Widdershoven et al 2011b), or intra-operatively. When an aberrant medialized course was found, some considered this a contraindication for surgery (Rouillon et al 2009), other created a narrow PF (MacKenzie-Stepner et al 1987), others suggested a palatoplasty would be safer than a pharyngoplasty (Mehendale et al 2004), and others stated it had no consequence for the subsequent therapy (Widdershoven et al 2011b).

Table 5.1: Study characteristics and outcome, sorted by surgical procedure.

First author, publication year	Procedure	n	Palatal anomaly (%)	Age at surgery (mean years, range)	Follow-up (mean years, range)	Normal resonance (%)	Normal nasal emission (%)	Understandable (%)	Hyponasal (%)	OSA (%)	Needing further surgery (%)
Leuchter 2009	fat injection	3	3/3 (100)	13.7 (11-17)	0.75 (0.4-1)	0/3 (0)	3/3 (100)	3/3 (100)	0/3 (0)	0/3 (0)	1/3 (33)
Nicolas 2011	fat injection	6	2/6 (33)	11.0 (7.7-17.9)	1.6 (1.0-2.6)	1/6 (17)	0/6 (0)	3/6 (50)	-	0/6 (0)	2/6 (33)
Milczuk 2007	Furlow	2	2/2 (100)	8.6 (6.4-10.8)	4.6 (3.2-6.1)	I	I	I	-	0/2 (0)	0/2 (0)
d'Antonio 2001	Furlow	4	4/4 (100)	-	-	-	-	-	-	-	4/4 (100)
Rottgers 2011	Furlow	13	13/13 (100)	(5.2)	2	I	I	-	-	0/13 (0)	2/13 (15)
Perkins 2005	Furlow	16	16/16 (100)	-	0.25	I	-	-	-	0/16 (0)	-
Mehendale 2004	IVP	14	14/14 (100)	5.7	(4 (0.6-9.17))	2/14 (14)	5/14 (36)	-	1/14 (7)	0/14 (0)	0/14 (0)
Brandao 2011	IVP	19	19/19 (100)	(9 (5-27))	1.4	4/19 (21)	-	-	-	-	-
MacKenzie 1987	PF	1	0/1 (0)	>3	-	0/1 (0)	0/1 (0)	-	0/1 (0)	0/1 (0)	1/1 (100)
Argamaso 1994	PF	2	-	25 (19, 31)	7 (5-9)	2/2 (100)	-	-	0/2 (0)	0/2 (0)	0/2 (0)
Baylis 2008	PF	3	2/3 (67)	<6.9 (<6.2-7.5)	-	0/3 (0)	-	1/3 (33)	1/3 (33)	-	-
Rottgers 2011	PF	4	4/4 (100)	(5.2)	0.8	I	I	-	-	0/4 (0)	0/4 (0)
Witt 1998	PF	5	5/5 (100)	6.4 (4-9)	-	-	-	-	-	-	4/5 (80)
Brandao 2011	PF	6	1/6 (17)	(9 (5-27))	1.4	3/6 (50)	-	-	-	-	-
Arneja 2008	PF	8	1/8 (13)	7.25 (4-11)	1	2/8 (25)	-	-	-	0/8 (0)	0/8 (0)
Rouillon 2009	PF	11	0/11 (0)	7.7	2	(10/11 (91)*)	-	-	-	0/11 (0)	0/11 (0)
Goorhuis 2003	PF	18	≥4/18 (22)	(4-6)	-	14/18 (78)	-	-	-	-	0/18 (0)
Tatum 2002	PF	20	19/20 (95)	6.2 (4-17)	0.5-2	18/20 (90)	18/20 (90)	-	0/20 (0)	0/20 (0)	0/20 (0)

First author, publication year	Procedure	n	Palatal anomaly (%)	Age at surgery (mean years, range)	Follow-up (mean years, range)	Normal resonance (%)	Normal nasal emission (%)	Understandable (%)	Hyponasal (%)	OSA (%)	Needing further surgery (%)
Ysunza 2009	PF	20	20/20 (100)	(8 (5-25))	>0.5	12/20 (60)	-	-	0/20 (0)	0/20 (0)	-
Swanson 2011	PF	33	33/33 (100)	6.4 (4.4-19)	0.3 (0.1-8.8)	15/33 (45)	8/33 (24)	-	5/33 (15)	1/33 (3)	1/33 (3)
Wang 2009	PF	33	0/33 (0)	7.2 (4-17)	0.7 (0.2-2.1)	-	-	I	-	-	-
Lipson 1991	(likely PF)	32	19/32 (60)	<15.2)	>0.5	15/24 (63)	15/24 (63)	23/24 (96)	0/24 (0)	0/24 (0)	0/24 (0)
Widdershoven 2011	PF (33), SP (7)	40	13/40 (33)	7.5 (3.9-16.3)	2.4 (1-6.3)	28/40 (70)	18/40 (45)	-	0/40 (0)	1/40 (3)	4/40 (10)
Rottgers 2011	PF + Furlow	10	10/10 (100)	(5.2)	0.4	I	I	-	-	0/10 (0)	10/10 (100)
Hens and Vander Poorten, co-authors	Honig	17	4/17 (24)	7.5 (3-23)	3.0 (1.0-7.8)	3/17 (17)	4/6 (67)	10/14 (71)	1/17 (6)	-	1/17 (6)
Widdershoven 2008	Honig	25	-	7.1 (3.8-13.6)	5	15/25 (60)	4/10 (40)	-	0/25 (0)	0/25 (0)	4/25 (16)
Spruijt 2011	Honig	44	23/40 (58)	6.0 (3.4-13.9)	7.0 (1.0-19.4)	23/44 (52)	14/27 (52)	34/36 (94)	-	0/44 (0)	8/44 (18)
Baylis 2008	SP	2	2/2 (100)	<6.1 (<4.8-7.4)	-	0/2 (0)	-	0/2 (0)	0/2 (0)	-	-
Milczuk 2007	SP	3	1/3 (33)	9.3 (5.7-12.5)	0.9 (0.3-1.3)	I	I	I	-	0/3 (0)	0/3 (0)
Sie 1998	SP	3	1/3 (33)	8.9 (5.2-12.7)	1.2 (0.6-1.8)	1/3 (33)	1/3 (33)	-	0/3 (0)	0/3 (0)	1/3 (33)
Witt 1998	SP	4	4/4 (100)	6.3 (5-8)	-	-	-	-	-	-	0/4 (0)
Ysunza 2009	SP	9	9/9 (100)	(8 (5-25))	>0.5	0/9 (0)	-	-	0/9 (0)	0/9 (0)	-
Witt 1999	SP	19	17/19 (89)	8.7 (4-16)	1	18/19 (95)	-	-	0/19 (0)	1/19 (5)	1/19 (5)
Losken 2006	SP	32	6/32 (18)	6.7 (1-15)	2.1	I	-	-	1/32 (3)	1/32 (3)	7/32 (22)
Milczuk 2007	SP + Furlow	9	0/9 (0)	9.5 (6.8-11.5)	1.4 (0.2-3.7)	I	I	I	-	0/9 (0)	0/9 (0)

First author, publication year	Proce-dure	n	Palatal anomaly (%)	Age at surgery (mean years, range)	Follow-up (mean years, range)	Normal resonance (%)	Normal nasal emission (%)	Under-stand-able (%)	Hyponasal (%)	OSA (%)	Needing further surgery (%)
Hens and Vander Poorten, co-authors	Hynes	2	0/2 (0)	6.5 (5-8)	1.8 (1.2-2.3)	1/2 (50)	1/2 (50)	1/2 (50)	0/2 (0)	-	0/2 (0)
Sie 1998	Hynes	6	0/6 (0)	8.7 (4.7-13.4)	1.1 (0.2-2.9)	3/6 (50)	4/6 (67)	-	0/6 (0)	0/6 (0)	0/6 (0)
Mehendale 2004	Hynes	16	0/16 (0)	6.7	(4 (0.6-9.17))	3/16 (19)	6/16 (38)	-	2/16 (13)	0/16 (0)	3/16 (19)
Mehendale 2004	Hynes + IVP	11	11/11 (100)	6.7 (2.4-15.3)	(4 (0.6-9.17))	4/11 (36)	6/11 (55)	-	3/11 (27)	0/11 (0)	11/11 (100)

- : not reported;

I: improvement;

IVP: intravelar veloplasty;

PF: pharyngeal flap;

likely PF: pharyngoplasty not otherwise specified;

SP: sphincter pharyngoplasty;

^: include intermittent closure.

Table 5.S1: Validity assessment. Criteria based on the Cochrane Collaboration's tool for assessing risk of bias (2011).

Study	Genetic confirmation	Included all patients	Random-ized	Follow-up >1 year	Loss to follow-up (%)	Blinded	Reso-nance	Vali-dated speech test	Points (max 7)	Design	Level of Evi-dence
Argamaso 1994	-	no[a]	no	yes	-	n/a	yes	-	2	Outcomes research	2c
Arneja 2008	FISH	no[b]	no	yes	-	n/a	yes	yes	4	Outcomes research	2c
Baylis 2008	FISH	no[c]	no	-	-	yes	yes	yes	4	Cohort study	4
Brandao 2011	no (6 clinical signs)	no[e]	no	-	-	no	yes	yes	2	Outcomes research	2c
d'Antonio 2001	FISH	no[b]	no	-	-	-	-	-	1	Outcomes research	2c
Goorhuis 2003	FISH	yes	-	-	-	n/a	yes	-	3	Outcomes research	2c
Hens and Vander Poorten, co-authors	yes	yes	no	yes	10/29 (34)	no	yes	no	4	Outcomes research	2c
Leuchter 2009	-	no[b]	no	no	-	n/a	no	no	1	Outcomes research	2c
Lipson 1991	yes	yes	no	no	8/32 (25)	n/a	yes	-	3	Cohort study	4
Losken 2006	-	yes	no	-	-	n/a	no	-	1	Outcomes research	2c
MacKenzie 1987	-	no[d]	no	-	-	n/a	yes	-	1	Cohort study	4
Mehendale 2004	FISH	no[e]	no	no	5/47 (11)	yes	yes	yes	5	Outcomes research	2c
Milczuk 2007	FISH	no[e]	no	no	3/14 (21)	yes	no	no	2	Outcomes research	2c
Nicolas 2011	if available	no[b]	no	yes	0/6 (0)	n/a	no	no	3	Outcomes research	2c

Study	Genetic confirmation	Included all patients	Random-ized	Follow-up >1 year	Loss to follow-up (%)	Blinded	Reso-nance	Vali-dated speech test	Points (max 7)	Design	Level of Evi-dence
Perkins 2005	no	no[f]	no	no	-	n/a	no	-	0	Outcomes research	2c
Rottgers 2011	FISH	yes	No	no	-	no	no	yes	3	Outcomes research	2c
Rouillon 2009	FISH	no[g]	no	yes	-	n/a	no	no	2	Outcomes research	2c
Sie 1998	-	no[b]	no	no	6/30 (20)	no	yes	-	1	Outcomes research	2c
Spruijt 2011	FISH	no[b]	no	yes	10/54 (19)	n/a	yes	no	4	Outcomes research	2c
Swanson 2011	FISH	yes	no	no	7/40 (18)	n/a	yes	yes	5	Outcomes research	2c
Tatum 2002	FISH	no[d]	no	no	0/20 (0)	n/a	yes	-	3	Outcomes research	2c
Wang 2009	-	yes	no	-	-	n/a	-	yes	2	Outcomes research	2c
Widdershoven 2008	FISH	yes	no	-	-	n/a	yes	no	3	Outcomes research	2c
Widdershoven 2011	FISH/MLPA	yes	no	yes	-	no	yes	yes	5	Outcomes research	2c
Witt 1998	FISH	no[d]	no	-	-	no	-	-	1	Outcomes research	2c
Witt 1999	FISH	no[b]	no	yes	-	n/a	yes	-	3	Outcomes research	2c
Ysuzna 2009	FISH	no[h]	no	no	-	no	yes	yes	3	Outcomes research	2c

-: not reported

a: only those with asymmetric VPD

b: only those undergoing this technique

c: only those responding to a questionnaire

d: only those with abnormal carotid arteries

e: only those without previous palate surgery

f: only those with sagittally orientated m. levator palatini

g: only those without a palatal anomaly

h: only those without severe neurological disorders or abnormal hearing

n/a: not applicable.

Table 5.S2: Imaging modalities and assessments.

First author, publication year	Procedure	Imaging modality	Pharyngeal lateral wall movement	Velar movement	Gap size	Closure pattern	Tailored
Leuchter 2009	fat injection	N	-	good	minimal	-	yes
Nicolas 2011	fat injection	-	-	-	-	-	yes
Milczuk 2007	Furlow	N	%	%	<50%	yes	yes
d'Antonio 2001	Furlow	N + X	-	good	minimal	-	yes
Rottgers 2011	Furlow	N + X	-	good	-	-	yes
Perkins 2005	Furlow	N	%	%	3 ps	no	yes
Mehendale 2004	IVP	X	-	V*	yes	-	yes
Brandao 2011	IVP	N	-	-	6 ps	-	-
MacKenzie 1987	PF	N + X	-	-	-	-	yes
Argamaso 1994	PF	N + X	yes	-	-	-	yes
Baylis 2008	PF	-	-	-	-	-	-
Rottgers 2011	PF	N + X	-	poor	-	-	yes
Witt 1998	PF	N + X	satisfactory	poor	narrow to moderate	yes	yes
Brandao 2011	PF	N	-	-	6 ps	-	-
Arneja 2008	PF	N + X	yes	yes	yes	-	-
Rouillon 2009	PF	N	-	-	-	-	-
Goorhuis 2003	PF	-	-	-	-	-	-
Tatum 2002	PF	N + X	%	%	-	-	yes
Ysunza 2009	PF	N + X	%	%	%	yes	yes
Swanson 2011	PF	X	%	-	3 ps	yes	yes
Wang 2009	PF	X	yes	-	yes	no	yes
Lipson 1991	(likely PF)	X	-	yes	-	-	-
Widdershoven 2011	PF (33), SP (7)	N + X	3 ps	3 ps	yes	yes	yes
Rottgers 2011	PF + Furlow	N + X	-	good	-	-	yes
Hens and Vander Poorten, co-authors	Honig	N + X	yes	yes	-	-	no
Widdershoven 2008	Honig	N	4 ps	4 ps	4 ps	-	no
Spruijt 2011	Honig	N	3 ps	3 ps	-	-	no
Baylis 2008	SP	-	-	-	-	-	-
Milczuk 2007	SP	N	%	%	large	yes	yes
Sie 1998	SP	N + X	6 ps	6 ps	3 ps	yes	yes
Witt 1998	SP	N + X	poor	poor	large	yes	yes
Ysunza 2009	SP	N + X	%	%	%	yes	yes
Witt 1999	SP	N + X	poor	active	large	yes	yes
Losken 2006	SP	N + X	yes	yes	yes	yes	yes
Milczuk 2007	SP + Furlow	N	%	%	>50%	yes	yes

First author, publication year	Procedure	Imaging modality	Pharyngeal lateral wall movement	Velar movement	Gap size	Closure pattern	Tailored
Hens and Vander Poorten, co-authors	Hynes	N + X	yes	yes	-	-	-
Sie 1998	Hynes	N + X	6 ps	6 ps	3 ps	yes	yes
Mehendale 2004	Hynes	N + X	-	V*	large	-	yes
Mehendale 2004	Hynes + IVP	N + X	-	V*	large	-	yes

- : not reported; %: measured as a percentage value; I: improvement; IVP: intravelar veloplasty; N: nasendoscopy; PF: pharyngeal flap; likely PF: pharyngoplasty not otherwise specified; ps: point scale; SP: sphincter pharyngoplasty; V*: closure ratio, extended length, velocity of closure, lift; X: X-ray cephalograms or (video)fluoroscopy.

At most centers the data accrued from imaging studies were used to tailor the surgery. Only patients who underwent a Honig velopharyngoplasty did not have a tailored surgery, Therefore, no subanalyses were performed comparing the outcomes of patients whose surgeries were tailored to those whose surgeries were not tailored.

In total, postoperative outcome was reported for 525 patients. Nearly half of the patients underwent a PF procedure. Lipson et al (Lipson et al 1991) did not specify what kind of pharyngoplasty was performed, but this was likely a PF since this was the most popular procedure in the early 1990s. Postoperative follow-up ranged from 0.2-19.4 years. Resonance was rated based on perceptual assessments by speech therapists using 2 to 20 point scales. Nasal emissions were assessed by auscultation or with mirrors and rated on 2 to 20 point scales. In some studies nasometry was used to assess the percentage of nasal resonance. Understandability was rated based on perceptual speech using 2 to 5 point scales or percentage scores. OSA was inventoried based on patient history, with subsequent polysomnography if necessary (Rottgers et al 2011; Swanson et al 2011; Widdershoven et al 2011b; Witt et al 1999a). In some studies speech outcome was reported following primary surgery for VPD and further surgery was recommended (D'Antonio et al 2001a; MacKenzie-Stepner et al 1987; Witt et al 1999a; Witt et al 1998a), while in other

studies speech outcome was reported following further surgery (Hens and Vander Poorten, co-authors) (Leuchter et al 2009; Losken et al 2006; Mehendale et al 2004; Nicolas et al 2011; Rottgers et al 2011; Sie et al 1998; Spruijt et al 2011; Swanson et al 2011; Widdershoven et al 2008b; Widdershoven et al 2011b; Witt et al 1998a).

Quantitative data synthesis

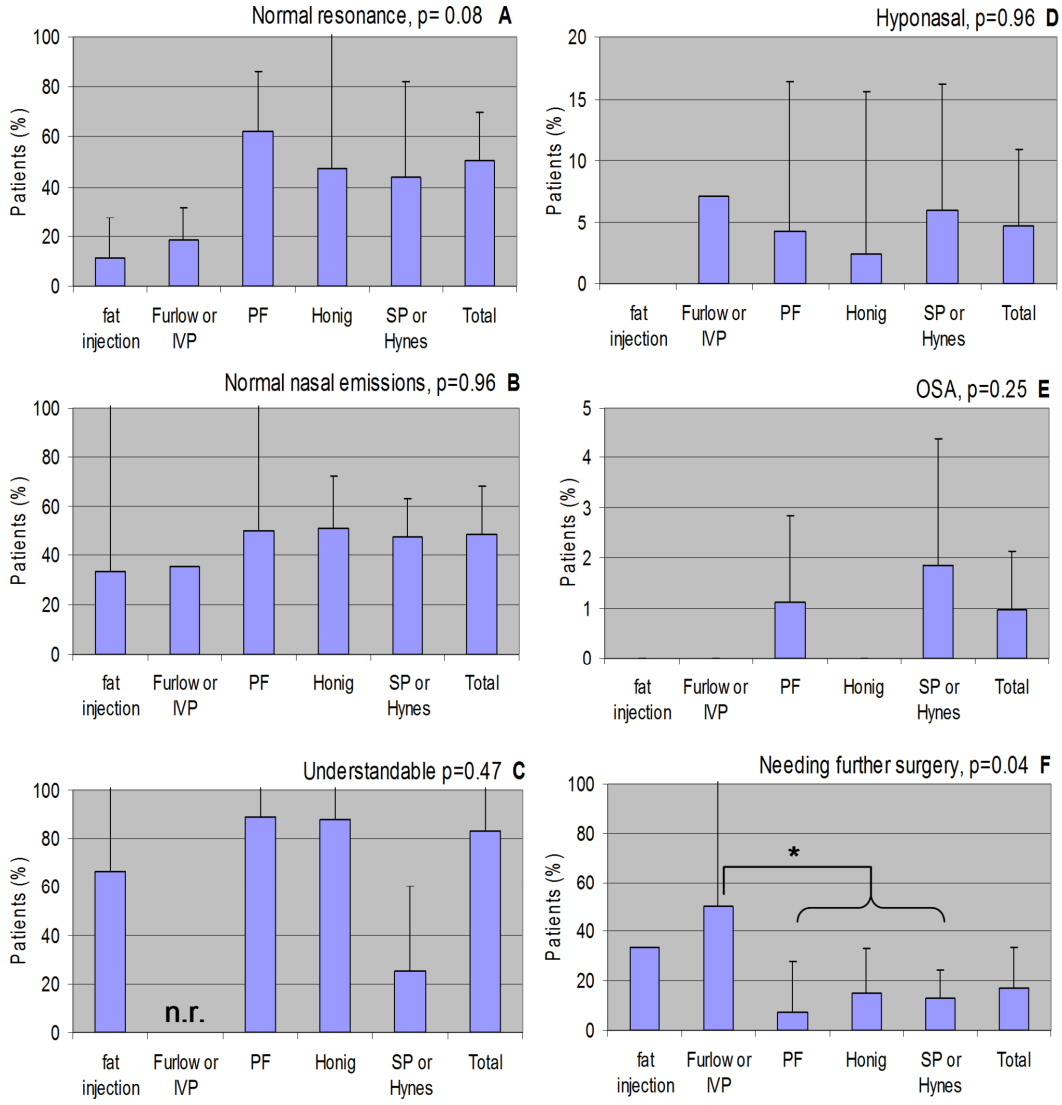
Overall, 50% of patients attained normal resonance, 48% attained normal nasal emissions scores, and 83% had understandable speech postoperatively. However, 5% became hyponasal, 1% had obstructive sleep apnea, and 17% required further surgery (Table 5.2, Figure 5.2). The standard deviations were large for many outcomes, and the variability between the standard deviations was large for the percentages with OSA and those needing further surgery (Levene's test $p<0.05$).

Table 5.2: Outcomes by procedure. Mean percentage of patients ± standard deviation (number of studies, number of patients).

	Fat injection	**Furlow or IVP**	**PF**	**Honig**	**SP or Hynes**	**All**
Normal resonance	11 ± 24 (2, 9)	18 ± 19 (2, 33)	62 ± 79 (11, 175)	48 ± 100 (3, 86)	44 ± 108 (8, 68)	50 ± 98 (26, 371)
Normal nasal emissions	33 ± 141 (2, 9)	36 ± 0 (1, 14)	50 ± 124 (5, 118)	51 ± 37 (3, 43)	47 ± 35 (5, 38)	48 ± 79 (16, 222)
Understand-able	67 ± 71 (2, 9)	- (0, 0)	89 ± 102 (2, 27)	88 ± 73 (2, 50)	25 ± 50 (2, 4)	83 ± 77 (8, 90)
Hyponasal	0 ± 0 (1, 3)	7 ± 0 (1, 14)	4 ± 35 (8, 143)	2 ± 19 (2, 42)	6 ± 30 (9, 100)	5 ± 29 (21, 302)
OSA	0 ± 0 (2, 9)	0 ± 0 (4, 45)	1 ± 6 (11, 181)	0 ± 0 (2, 69)	2 ± 8 (9, 108)	1 ± 6 (28, 412)
Needing further surgery	33 ± 0 (2, 9)	50 ± 154 (6, 54)	7 ± 66 (10, 142)	15 ± 31 (3, 86)	13 ± 34 (9, 94)	17 ± 92 (30, 385)

IVP: intravelar veloplasty; PF: pharyngeal flap; SP: sphincter pharyngoplasty.

Figure 5.2: Outcomes by procedure. A) Normal resonance. B) Normal nasal emissions. C) Understandable. D) Hyponasal. E) OSA. F) Needing further surgery.



The diversity of quality, design, and patient populations of the included studies precluded a fixed or randomized meta-analysis. The heterogeneity could not be corrected for using a meta-regression since much data was missing, such as the amount of velopharyngeal movement. However, in an attempt to gain insight into overall trends in outcome, data was pooled according to the surgical procedures, grouping Furlow with IVP since both are palatoplasties, and SP with Hynes since the techniques differ only slightly. Widdershoven et al (Widdershoven et al 2011b) report on 33 patients who underwent a PF and 7 patients who underwent an SP, but do not report the outcomes separately. The outcomes of all 40 patients were included in the PF group. For patients who underwent both a palatoplasty and a pharyngoplasty, most outcome measures were counted toward the pharyngoplasty groups. However, the need for further surgery was counted toward the palatoplasty group when this was part of the two-staged approach (Mehendale et al 2004; Rottgers et al 2011).

Weighted ANOVA testing showed no significant differences with regard to speech outcome between the five procedure groups. There was a trend for the patients who attained normal resonance to differ between the groups ($p=0.08$), with a lower percentage of patients attaining normal resonance after a fat injection or palatoplasty (11-18%) than after the more obstructive pharyngoplasties (44-62%).

Only patients who underwent PFs or SPs incurred OSA, yet this was not statistically significantly more often than after other procedures ($p=0.25$).

The need for further surgery differed significantly between the five procedure groups ($p=0.04$). Further testing with the contrasts and Bonferroni correction revealed that the difference was only significant between the patients who underwent a palatoplasty and those who underwent a pharyngoplasty (50% versus 7-13%, $p=0.03$).

Discussion

By systematically reviewing the available literature, data were presented and analyzed from 27 studies including 525 patients with 22q11DS and VPD who

underwent surgical correction. All surgeries except the Honig were tailored based on preoperative imaging. Overall, 50% of patients attained normal resonance. Fewer patients who underwent only a palatoplasty tended to attain normal resonance and more needed had a greater need for further surgery compared to than patients who underwent a pharyngoplasty. Therefore, the evidence suggests that for patients with 22q11DS and VPD the morbidity of further surgery can be minimized when the cleft team decides a pharyngoplasty should be performed directly instead of only a palatoplasty. This is also the feeling the senior surgeon authors (ABM, VVP, GH) of this manuscript hold. VVP almost always chooses an extensive modified Honig procedure with supraperiosteal retropositioning and a cranially-based large PF in patients with 22q11DS.

Limitations

As aforementioned, questions about treatment efficacy should ideally be answered in a clinical trial. In a multi-center randomized controlled trial with nonsyndromic patients, 146 patients per procedure group were calculated to be required to find a 20% difference in outcomes between patients who underwent a PF and those who underwent an SP. However, the trial was terminated prematurely due to lower referral rates than predicted, changes in preoperative assessment leading to referrals for more nonsurgical interventions, and surgeons' growing preference for palate re-repair (Abyholm et al 2005). Among patients with 22q11DS, larger variance is expected, necessitating even more patients per procedure group.

Given logistic hurdles, a practical solution to gain insight into trends requires turning to lower level evidence which is confounded by bias. For example, the 22q11.2 deletion was not genetically confirmed in all studies, most studies only included a specific subgroup of patients with 22q11DS who required surgery to treat their VPD, and speech was only tested blindly and using a validated test in two studies (Baylis et al 2008; Mehendale et al 2004) (Table 5.S1). The outcome of some pharyngoplasties may have been wrongly attributed to those pharyngoplasties since some patients underwent palatoplasties or multiple pharyngoplasties, either prior to being referred for the reported procedure (Tatum et al 2002) or as further

surgery (Spruijt et al 2011; Witt et al 1998a). Unfortunately, there was no data on the duration and intensity of postoperative speech therapy. Finally, when data are pooled there is a chance that the conclusions are misleading (Reade et al 2008). Therefore, raw data from each study are presented to allow readers to draw their own conclusions.

Patients

When considering the management of VPD in patients with 22q11DS, as for all patients with VPD, there are both conservative and surgical options. No randomized studies have been conducted to compare the effect of the natural history of speech development to the effect of intervention since leaving VPD untreated is considered ethically unacceptable (Riski 1979). Anecdotal experiences with older patients with VPD who have not have surgery due to limited resources in developing countries show that VPD does not resolve spontaneously. Clinical observations indicate that even minor amounts of VPD do not generally correct themselves and tend to increase with age (Graham et al 1973).

Patients underwent surgery between the ages of 2.4 and 31 years. One may postulate that those undergoing surgery at an older age may be disadvantaged since compensations are more ingrained and their brains have less plasticity to relearn speaking techniques. Yet, when tested, age was not found to predict speech outcome (Spruijt et al 2011) nor the need for further surgery (Losken et al 2006).

All children receive speech and language therapy. When this insufficiently corrects VPD due to anatomic deficits, the velopharyngeal gap can be decreased in size by obturation with a prosthesis, inserting autologous or synthetic materials, or surgically. The clinical and radiological characteristics of the patient and the velopharyngeal function guide the clinician's treatment choice. Prosthetics are bothersome and less effective than surgery, as proven in a randomized controlled trial among syndromic and nonsyndromic children with moderate to severe VPD (Marsh & Wray 1980) or with a hypodynamic pharynx (Witt et al 1995b).

In many studies, the indication for surgery was not specified beyond "VPD." When the degree of preoperative VPD was reported (n=13 studies), the lack

of a universal scale hampered comparison between studies. However, in three studies the outcomes after different procedures were reported (n=3 studies) (Hens and Vander Poorten, co-authors) (Mehendale et al 2004; Rottgers et al 2011), allowing comparison of baseline VPD between patients that underwent different procedures. In the study by Rottgers et al. (Rottgers et al 2011), patients who primarily underwent a Furlow procedure had an average Pittsburgh Weighted Speech Score of 18.4, while patients who primarily received a PF the average score was 26.8 (Rottgers et al 2011). In the studies by Hens and Vander Poorten (co-authors) and Mehendale et al. (Mehendale et al 2004) no group averages were reported, but each patient was rated on a 5 point scale, making it more difficult to summarize the data. In the study by Hens and Vander Poorten (co-authors), 50% of the patients who underwent a Hynes procedure (n=2) had severely hypernasal speech, while 65% of the patients who underwent a Honig procedure (n=17) had severely hypernasal speech. In the study by Mehendale et al. (Mehendale et al 2004), there was one patient with severely hypernasal speech in each group. One patient who underwent both an IVP and a Hynes procedure was not hypernasal and did not have any nasal emission or turbulence preoperatively but only had mild VPD on nasendoscopy. These baseline differences likely affect outcome: a greater degree of preoperative nasalance is prognostic for an increased need for further surgery (Losken et al 2006).

Imaging

At some centers, preoperative imaging is assessed with the assumption that the velopharyngeal closure pattern should dictate the procedure choice (Armour et al 2005) or the amount of velopharyngeal movement should affect the operative technique. However, both the assessment of the imaging and the extrapolation to a specific surgical procedure are imperfect. Using standardized assessment of nasendoscopic views of velopharyngeal movement (Golding-Kushner et al 1990), interrater reliability was only 0.4 for semi-quantitative judgment of velar and lateral wall motion, and even lower for characteristics that were measured qualitatively (Sie et al 2008). Similarly, interrater agreement was <0.5 among routine assessors of

videofluoroscopy (Witt et al 1998b), but >0.8 in another center (Karling et al 1999a; b).

Furthermore, both the amount and pattern of velopharyngeal motion (Argamaso et al 1980; Karling et al 1999a; Lewis & Pashayan 1980; Perkins et al 2005; Shprintzen et al 1980; Witt et al 1998b; Zwitman 1982) and the dimensions of a PF (Vandevoort et al 2001) change after surgery, compromising the logic of tailoring procedures and/or techniques based on preoperative findings. Among syndromic and nonsyndromic children the amount of lateral wall adduction is not correlated with outcome (Vandevoort et al 2001). In this systematic review, patients who had more favorable velopharyngeal movement underwent less obstructive surgeries (Table 5.S2), Compared to their counterparts who had less favorable velopharyngeal movement and underwent more obstructive pharyngoplasties, fewer patients who underwent less obstructive fat injections or palatoplasties attained normal resonance and more patients required further surgery.

Surgical procedures

Ideally, an operation is based on anatomic and physiologic knowledge and clinical trials to test the hypothesis (Marsh et al 1989). In a cadaver study, Huang et al (Huang et al 1998) reason that the palatoplasty is the most physiological solution to restore velopharyngeal function when there is a cleft palate with maloriented muscles as it reinstates the sling mechanism of the levator veli palatini muscles. When there is VPD despite the correct positioning of the palatal muscles, a pharyngoplasty is often required. An SP is said to preserve the sphincter function of the superior constrictor while augmenting the thickness of the pharyngeal walls, decreasing the velopharyngeal port size (Milczuk et al 2007). Creating a PF, conversely, disrupts the pharyngeal sphincter mechanism by dividing the superior constrictor muscle (Huang et al 1998). However, the flap donor site on the posterior pharyngeal wall heals by circular contraction (Shprintzen 1988), possibly causing the muscle fibers to migrate medially (Barone et al 1994).

The results from trials among patients without 22q11DS should not be simply be extrapolated to this unique group (Rottgers et al 2011; Ysunza et al 2009).

Lipson et al (Lipson et al 1991) lament that a standard repair of an overt or submucous cleft was never adequate to prevent VPD in patients with 22q11DS. Having VPD and any syndrome is associated with having a hypodynamic velopharynx (Witt et al 1995b) and is prognostic for poorer postoperative outcome (Perkins et al 2005). Lower primary success rates for all patients with hypodynamic velopharynges, including those with 22q11DS, supports the logic of segregating this group (which constitutes up to 25% of the population with VPD) from the larger cleft palate population (Witt et al 1995b). In general, the speech outcome after surgery has been reported to be worse in patients with 22q11DS than in patients without the syndrome (D'Antonio et al 2001a; D'Antonio et al 2001b; Losken et al 2003; Losken et al 2006; Nicolas et al 2011; Sie et al 1998; Sie et al 2001; Widdershoven et al 2008b), but some patients with 22q11DS fare as well as their non-syndromic counterparts (Argamaso et al 1994; Brandao et al 2011; Meek et al 2003; Milczuk et al 2007; Perkins et al 2005; Pryor et al 2006; Rouillon et al 2009).

Treating VPD in any patients with hypo- or adynamic velopharynges, including nonsyndromic patients and patients with other syndromes, is a challenge. A study comparing outcomes reported 42% (n=15/36) failure after primary treatment among patients with a hypodynamic velopharynx and only 13% (n=16/119) failure among patients with a dynamic velopharynx (Witt et al 1995b). Treatment algorithms suggest creating an SP in patients with a hypodynamic pharynx (Marsh 2003; Sie & Chen 2007). However, in patients with neurologic VPD, PFs and SPs have similar outcomes (Davison et al 1990; Peat et al 1994).

The choice which surgical technique to employ is largely based on the surgeon's preference (Witt et al 1995b). Forty-eight percent of surgeons who answered a questionnaire (n=13/27) create PFs in over half of their patients with 22q11DS (Witt et al 1998a). This systematic review confirms this predilection for PFs. Some prefer to create a PF (Ysunza et al 2009), stating the outcome is superior because the procedure is simpler and the results are less variable than after an SP (Chegar et al 2007). Others prefer an SP above a wide PF because the latter has an increased risk of OSA (Armour et al 2005; Losken et al 2006; Marsh 2003; Mehendale et al 2004; Witt et al 1995b). Finally, one center recommends a two-

staged approach and waiting six months between a palatoplasty and pharyngoplasty to determine whether the need for a pharyngoplasty has been resolved or allow a less obstructive pharyngoplasty to be created (Mehendale et al 2004; Sommerlad et al 2002).

Surgical techniques

Not all palatoplasties, PFs, or SPs are the same. A palatoplasty can include a Z-plasty (D'Antonio et al 2001a; Milczuk et al 2007; Perkins et al 2005; Rottgers et al 2011; Sie et al 2001) or varying degrees of dissection and repositioning of the levator veli palatini muscles (Mehendale et al 2004). A PF can be cranially (Rouillon et al 2009; Wang et al 2009a) or caudally based (Goorhuis-Brouwer et al 2003). A palatoplasty with supraperiosteal retropositioning of the velar sling can be combined with a PF in a (modified) Honig procedure (Mink van der Molen et al 2008l; Seagle et al 2002). The PF donor site can be closed (Arneja et al 2008; Chegar et al 2007; Karling et al 1999b; Shprintzen et al 1980; Swanson et al 2011) or left to heal by secondary intention (Arneja et al 2008) thereby allowing scar constriction to decrease the pharyngeal width (Karling et al 1999a). The width of PFs can be varied by lining (Argamaso et al 1980; Swanson et al 2011) or shortening (Argamaso et al 1994) the flap to prevent tubing (Lin et al 1999). Even then, the eventual flap width is unpredictable (Argamaso et al 1994; Karling et al 1999b; Keuning et al 2009; Vandevoort et al 2001), compromising the logic of tailoring the technique based on velopharyngeal movement. During an SP, the width of the flaps (Sie et al 1998; Ysunza et al 2009), the height of inset (Mehendale et al 2004; Sie et al 1998; Witt et al 1999a; Ysunza et al 2009) and the amount of overlap of the two lateral flaps (Losken et al 2003; Losken et al 2006) can be varied.

In this systematic review, despite the differences in technique, Furlows and IVPs were not separated since both are palatoplasties in which no material is added and the levator veli palatini muscles are positioned as physiologically as possible. SPs and Hynes were not separated since in both procedures lateral flaps are created, rotated, and inset on the posterior pharyngeal wall. For both SP and Hynes, the height of inset was tailored to the level of attempted velopharyngeal contact.

Outcome

Definitions of success differ (Sie et al 1998). Since the indication for a corrective surgery is VPD, the goal should be resolution of VPD while avoiding overcorrection and the need for further surgery (Losken et al 2006). As Furlow Jr. so strongly stated, "there are no points for 'significant improvement'… near-miss successes in one institution may not be classified the same in another; they make inter-institutional comparisons of questionable validity" (Perkins et al 2005). Certainly this systematic review has questionable validity due to the differences in reporting between centers. We attempted to bypass the different definitions by including only numbers of patients with normalized resonance. Undoubtedly, the definition of normalcy also differs between centers.

None of the interventions in current use is completely successful in correcting VPD. The low rate of normal resonance may be attributed to the short postoperative follow-up after which the full effect of speech therapy has not yet been achieved (Spruijt et al 2011).

The low rate of normal resonance may reflect the purposeful creation of less obstruction to prevent OSA. OSA is a possible serious complication following pharyngoplasty (Graham et al 1973) and is associated with pharyngeal hypotonia (Goldberg et al 2005). Patients with 22q11DS with hypotonia who undergo surgical correction of VPD are therefore particularly at risk for developing OSA (Agarwal et al 2003; Kravath et al 1980; Sher et al 1986; Shprintzen 1988).

Despite surgeons' fears of inducing OSA, we found only 4 cases in these studies. Interestingly, OSA did not occur more frequently among patients receiving PFs (n=2) than those receiving SPs (n=2). In one case, the OSA resolved within 3 weeks on nasally applied continuous positive airway pressure (Witt et al 1999a). The others had further surgery to increase the velopharyngeal port size. No OSA occurred when a palatoplasty and pharyngoplasty were performed in one stage (Milczuk et al 2007) nor at centers where the two-stage approach is employed (Mehendale et al 2004; Rottgers et al 2011).

Further surgery may be needed when there is residual VPD (D'Antonio et al 2001a; Leuchter et al 2009; MacKenzie-Stepner et al 1987; Mehendale et al 2004;

Nicolas et al 2011; Rottgers et al 2011; Spruijt et al 2011; Widdershoven et al 2008b; Witt et al 1999a) or OSA (Losken et al 2006; Swanson et al 2011; Widdershoven et al 2011b). Whether it is carried out depends on the recommendation of the cleft team and the patients' or their family's desires (Sie et al 1998; Swanson et al 2011; Witt et al 1999a). The increased need for further surgery among patients who underwent a palatoplasty is affected by the deliberate two-staged approach.

There were no significant differences in speech outcomes or morbidity between the groups that underwent different types of pharyngoplasties. It is unclear whether this reflects the appropriateness of tailoring based on velopharyngeal movement, or whether the procedures have similar efficacy despite differences in velopharyngeal movement.

Conclusion

Based on outcomes research (level 2c evidence) and poor quality cohort studies (level 4 evidence), a Grade C recommendation (Phillips 1998) can be made to minimize the morbidity of further surgery for patients with 22q11DS and VPD by choosing to perform a pharyngoplasty directly. Only performing a palatoplasty resulted in a greater need for further surgery. Higher level evidence is needed to confirm or refute these findings. While a randomized controlled trial seems unfeasible, by conducting prospective cohort studies at multiple centers and uniformly documenting patient characteristics, velopharyngeal movement, and outcome measures, a meta-analysis could be performed with correction for the various factors.

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CHAPTER 6:
Self-Reported Speech Problems in Adolescents and Young Adults with 22q11.2 Deletion Syndrome: A Cross-sectional Cohort Study

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Abstract

Background: Speech problems are a common clinical feature in 22q11.2 deletion syndrome. It is unclear how many patients undergo speech and language therapy and pharyngoplasty and whether these interventions normalize the speech. The objectives of this study were to 1) inventory the speech history and current self-reported speech rating of adolescents and young adults, and 2) examine possible variables influencing the current speech ratings including cleft palate, surgery, speech and language therapy, intelligence quotient, and age at assessment.

Methods: In this cross-sectional cohort study, 50 adolescents and young adults with 22q11.2 deletion syndrome (ages 12-26 years, 67% female) filled in questionnaires. A neuropsychologist administered an age-appropriate intelligence quotient test. The demographics, histories and intelligence of patients with normal speech (speech rating =1) were compared to those with different speech (speech rating >1).

Results: Of the 50 patients, a minority (26%) had a cleft palate, nearly half (46%) underwent a pharyngoplasty, and all (100%) had speech and language therapy. Poorer speech ratings were correlated with more years of speech and language therapy (Spearman correlation=0.418, p=0.004, 95%CI 0.145-0.632). Only 34% had normal speech ratings. The groups with normal and different speech were not significantly different regarding demographic variables, a history of cleft palate, surgery or speech and language therapy, and intelligence quotient.

Conclusions: All adolescents and young adults with 22q11.2 deletion syndrome had undergone speech and language therapy and nearly half underwent pharyngoplasties. Only 34% attained normal speech ratings. Those with poorer speech ratings had speech and language therapy for more years.

Introduction

Speech problems are one of the most common clinical features in 22q11.2 deletion syndrome (22q11DS, OMIM #192430/188400). They are distressing for patients and their caregivers, but a generalization has been made that ultimately most patients learn to speak and communicate effectively (Kobrynski & Sullivan 2007; McDonald-McGinn & Sullivan 2011). Naturally, parents of young, newly diagnosed patients inquire what to expect regarding the clinical course and therapy.

The incomplete closure of the velopharyngeal valve, also known as velopharyngeal dysfunction (VPD), manifests as feeding difficulties in infants and speech problems once older. In patients with 22q11DS VPD may be caused by a cleft palate, different velopharyngeal proportions (platybasia and a wide, deep pharynx), and often includes a neuromuscular component (Baylis et al 2008; D'Antonio et al 2001b; Ford et al 2000; Goorhuis-Brouwer et al 2003; Persson et al 2003; Spruijt et al 2012; Widdershoven et al 2008a). Speech problems include hypernasal speech, audible nasal emission/turbulence and weak pressure consonants, glottal articulation, and laryngeal phonation (Rommel et al 1999). The prevalence among patients with 22q11DS is cited as 27-92% (Cohen et al 1999; Dyce et al 2002; McDonald-McGinn et al 1999; McDonald-McGinn & Sullivan 2011; Oskarsdottir et al 2005b; Ryan et al 1997). This wide range may reflect variable age ranges and patient selection strategies. To date it is unclear how many 22q11DS patients undergo speech and language therapy (SLT) and surgery (palatoplasties and pharyngoplasties) and whether these interventions ameliorate the dysfunction. Surgery is recommended for patients with VPD that is not amendable to SLT.

In the Netherlands, speech-language pathologists who assess patients with 22q11DS in our tertiary referral center advise community-based speech-language therapists how to tailor the therapy for each patient using principles described previously (Golding-Kushner & Shprintzen 2009). In patients with low intelligence and/or delayed language, spoken language is combined with sign language. For patients with isolated problems with articulation or resonance, only articulation

therapy is advised. However, if patients become frustrated, sign language can be added.

Many studies in which the speech of patients with 22q11DS was assessed only include children up to 11 years old (Baylis et al 2008; Botto et al 2003; D'Antonio et al 2001b; Ford et al 2000; Gerdes et al 1999; Goorhuis-Brouwer et al 2003; McDonald-McGinn et al 1999; Oskarsdottir et al 2005a; Oskarsdottir et al 2005b; Persson et al 2003; Ryan et al 1997; Scherer et al 2001; Solot et al 2001). One study showed improvement of speech with increasing age, which is perhaps a corollary of development (D'Antonio et al 2001b) albeit delayed (Bassett et al 2011; Kobrynski & Sullivan 2007; Lima et al 2010; McDonald-McGinn & Sullivan 2011; Persson et al 2006; Rommel et al 1999). In studies including older patients, speech outcome was not reported separately (Ford et al 2000; McDonald-McGinn et al 1999; Oskarsdottir et al 2005b; Persson et al 2003; Ryan et al 1997; Solot et al 2001; Wang et al 2009a), preventing conclusions regarding the further course of speech problems. In a study with adults age 18 years or older, 41% had VPD or hypernasal speech (Cohen et al 1999). No mention was made of previous SLT and/or surgery and the relation with the outcome. While acquiring data for this study, findings were published on speech and hearing in adults with 22q11DS (aged 19-38 years) in which 66% were reported to have mild to severe VPD (Persson et al 2012). Again, SLT was not inventoried. Surgery was mentioned, but not correlated to the speech.

To date it is unknown to what extent speech improvement can be obtained through SLT and pharyngoplasty in 22q11DS. This study aims to fill the gap between young children and adults by focusing on speech in adolescents and young adults with 22q11DS, and exploring the relationship between speech rating and SLT and surgery. In this cross-sectional cohort study, 1) the speech history and current self-reported speech of 50 adolescents and young adults with 22q11DS was inventoried through questionnaires, and 2) possible variables influencing the current speech ratings (including cleft palate, surgery, SLT, full-scale intelligence quotient (FSIQ), and age at assessment) were examined.

Materials and Methods

Research Plan

To study this, a cohort of adolescents and young adults with 22q11DS and their caregivers completed questionnaires to inventory the speech history and current speech rating, and a neuropsychologist administered a FSIQ test. The demographics, histories and intelligence of patients with normal speech (speech rating =1) were compared to those with different speech (speech rating >1).

Patients

To limit selection bias, all adolescents and young adults with genetically confirmed 22q11DS in the tertiary hospital's database were invited for analysis. Data analysis was performed after the first 50 patients (aged 12-26 years, mean 18 years) and their caregivers attended the outpatient clinic at the department of psychiatry for a concomitant study on genetics and psychopathology. The University Medical Center Utrecht institutional review board approved this study, and written informed consent was obtained from all patients. When patients were minors, written informed consent was also obtained from their guardians.

Speech Evaluation

Patients were given questionnaires (Supplementary Materials and Methods) to complete with their caregivers to assess the speech using the rating used by the Dutch Association for Cleft and Craniofacial Anomalies (Meijer 2003) (Table 6.1). This scale is used to assess two aspects the Speech Parameters Group for reporting speech outcomes in individuals with cleft palate determined to be socially important: speech understandability and acceptability (Henningsson et al 2008). The score ranges from 1 to 5. Patients with a speech rating of 1 are deemed to have normal speech, a rating greater than 1 indicates the speech differs from others, and a rating greater than 3 indicates the speech is difficult to understand.

Table 6.1: Perceptual speech rating (Dutch Association for Cleft and Craniofacial Anomalies) (Meijer 2003).

Rating	*Description*
1	The speech is understandable and normal.
2	The speech differs from others. This does not lead to comments and the speech is understandable.
3	The speech differs from others. This does lead to comments and the speech is understandable.
4	The speech is understandable with some difficulty.
5	The speech is not understandable.

When a speech-language pathologist clinically rates the speech using the Dutch Association for Cleft and Craniofacial Anomalies (Meijer 2003), the five-point overall speech rating is preceded by sub-questions specifying how frequently others (including the caregiver, the patient's teacher/employer, adults who do not know the patient, and the patient's peers) understand the patient's speech, how frequently the patient receives comments on his/her speech, and how frequently the caregiver needs to explain to others what the patient says. Likewise, in our questionnaire, the question on the five-point overall speech rating was preceded by these sub-questions. We also posed an open question inviting the caregiver to add any explanation.

Determinants

Possible determinants of speech were inventoried through the questionnaire, including demographics, cleft palate (overt and submucous), previous palatoplasty or pharyngoplasty, amount of SLT, ear infections, and the use of hearing aids. A neuropsychologist administered the age-appropriate WISC-III (third edition of the Wechsler Intelligence Scale for Children) or WAIS (Wechsler Adult Intelligence Scale) tests to assess the current FSIQ.

Statistics

Descriptive statistics were calculated for the factors inventoried through the questionnaire. To determine whether factors were related to speech, the cohort was divided into patients with normal speech ratings (speech rating =1) and those with

different speech (speech rating >1). The significance of each of the potential determinants was calculated using the variable-appropriate Mann Whitney U-test (for continuous variables) or chi-square test (for binomial variables).

Since cleft palates and pharyngoplasties are important factors in speech outcome, we performed sub-analyses by re-dividing the cohort into groups of patients with and without cleft palates and into groups of patients who had pharyngoplasties and those who did not. Their questionnaire answers and FSIQs, were compared again using the Mann Whitney U- and chi-square tests where appropriate.

We recognized that the categorical classification of speech as either normal or different does not justify the dimensional scale of speech quality, and therefore also tested the continuous spectrum of speech ratings against possible determinants (the continuous variables age at assessment, years of SLT, age at pharyngoplasty, and FSIQ) by calculating Spearman correlations. All statistical analyses were two-tailed and performed with IBM SPSS Statistics software for Windows (Version 20.0. Armonk, NY, USA), with statistical significance defined as $p<0.05$. All p-values are nominal, without corrections for multiple testing.

Results

Overall results

Some caregivers did not answer all the questions, and FSIQs were not available for two patients. A minority (26%, n=13/50) reported having any form of cleft palate, which was repaired either in isolation or in combination with a pharyngoplasty (Figure 6.1). Nearly half (46%, n = 23/50) had a pharyngoplasty. Of those who had a pharyngoplasty, 26% (n = 6/23) had VPD following a previous cleft palate repair. All (100%, n = 50/50) had SLT; the median duration was 6 years, once weekly, for 30 minutes per session. Most (66%) stopped SLT because the speech was sufficiently understandable, but some (19%) quit due to lack of progress. Many (69%) had a history of frequent ear infections and received grommets, with some reporting fewer ear infections following pharyngoplasty. A considerable number

(18%) wore at least one hearing aid. Only 34% of patients (n=17/50) had normal speech ratings. Even those who were reported by the caregivers as having normal speech, were not always understood by the caregiver, the patient's teacher/employer, adults who do not know the patient, and the patient's peers. They still received comments about their speech and the caregiver sometimes needed to explain what they were trying to say (Table 6.2).

Table 6.2: Questionnaire answers for all participants, and subgroup analyses for those with normal speech compared to those with different speech.

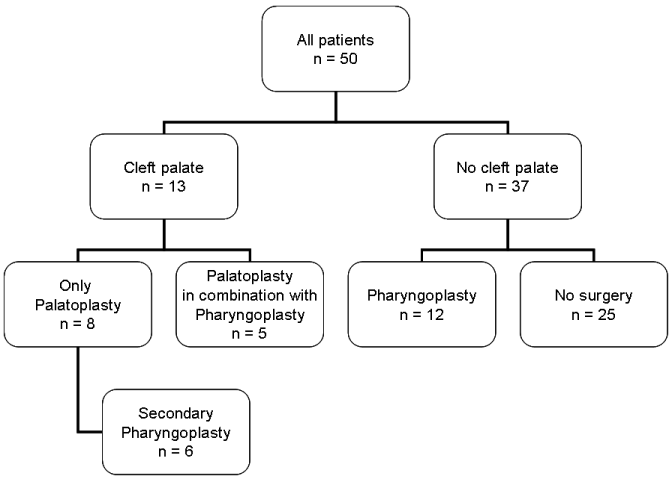
Factor (n, %)	***All (n = 50)***	***Normal speech (n = 17)***	***Different speech (n = 33)***	***Signifi-cance (p-value)***
Female	30 (67)	14 (82)	17 (52)	0.06 [a)]
Age (median, range)	18 (12 - 26)	20 (12 - 26)	18 (14 - 25)	0.66 [b)]
• *Age under 16 years*	15 (33)	4 (23)	11 (33)	0.53 [a)]
Caucasian	39 (87)*	13 (87)*	29 (91)*	0.50 [a)]
Cleft palate	13 (26)	2 (13)	11 (37)	0.17 [a)]
• *Age repair (median years, range)*	5.7 (0.2 - 11.7)	6.2 (2.6 - 11.7)	5.0 (0.2 - 10.5)	0.60 [b)]
Pharyngoplasty	23 (46)	8 (47)	15 (46)	1.00 [a)]
• *Age (median years, range)*	6.0 (2.2 - 12.4)	6.4 (3.7 - 11.7)	6.0 (2.2 - 12.4)	0.75 [b)]
• *Pre-operative speech rating (median, range)*	4 (2 - 5)	4 (3 - 5)	4 (2 - 5)	0.53 [b)]
• *Expected normalization*	10 (50)*	4 (50)	7 (54)*	1.00 [a)]
• *Changed expectations*	5 (24)*	1 (13)	4 (29)*	0.61 [a)]
• *Satisfied*	17 (71)*	8 (100)	10 (71)*	0.16 [a)]
Speech and language therapy	50 (100)	17 (100)	33 (100)	
• *Years (median, range)*	6.0 (1 - 17)	3.0 (1 - 17)	6.5 (1 - 14)	0.11 [b)]
• *Sessions/week (median, range)*	1 (1 - 5)	1 (1 - 4)	1 (1 - 5)	0.89 [b)]
• *Minutes/session (median, range)*	30 (15 - 60)	30 (20 - 60)	30 (15 - 60)	0.12 [b)]
• *Currently continuing*	8 (16)	1 (6)	7 (21)	0.24 [a)]
• *Stopped after sufficiently understandable*	21 (66)*	11 (85)*	11 (46)*	
• *Stopped due to no progress*	6 (19)*	1 (8)*	7 (29)*	
• *Stopped for another reason*	5 (16)*	1 (8)*	5 (21)*	

Factor (n, %)	*All (n = 50)*	*Normal speech (n = 17)*	*Different speech (n = 33)*	*Signifi-cance (p-value)*
Frequent ear infections	31 (69)	12 (71)	23 (70)	1.00 [a)]
• *Grommets*	27 (79)*	9 (69)	21 (81)*	0.45 [a)]
• *Fewer after pharyngoplasty*	4 (27)*	2 (40)*	2 (13)	0.25 [a)]
• *Hearing aid*	8 (18)	4 (24)	5 (15)	0.47 [a)]
Speech rating (median, range)	2.0 (1 - 4)	1 (1)	2 (1.5 - 4)	
• *Always understood by care-giver*	23 (46)	15 (88)	8 (24)	0.00 [a)]
• *Always understood by teacher/employer*	21 (42)	14 (82)	7 (21)	0.00 [a)]
• *Always understood by adults*	14 (28)	11 (65)	3 (9)	0.00 [a)]
• *Always understood by peers*	19 (38)	13 (77)	6 (18)	0.00 [a)]
• *Never receive comments*	19 (38)*	13 (77)	6 (19)*	0.00 [a)]
• *Never need to explain*	18 (36)	14 (82)	4 (12)	0.00 [a)]
Normal understandability and acceptability	17 (39)*	17 (100)	0 (0)	0.00 [a)]
Full-scale intelligence quotient (median, range)	65 (45 - 89)*	70 (45 - 89)*	64 (46 - 88)*	0.43 [b)]

*Percentages are based on questionnaires answered; missing data was excluded.

[a)] Chi-square test (for binomial variables), [b)] Mann Whitney U-test (for continuous variables).

Figure 6.1: Flowchart of palate status and subsequent surgeries. All patients with a cleft palate (n = 13/50) had a palatoplasty; some also had a pharyngoplasty. Nearly half of all patients had a pharyngoplasty (n = 23/50). Twenty-five patients did not have a cleft palate and did not have surgery.



Speech characteristics

Answers to the open question on speech included many specifications that the speech was hypernasal, too quiet, poorly articulated, more like mumbling, monotonous, and poorer when the patient was tired. Some offered reasons for the different speech including "losing air through a small opening somewhere," the muscles not working, or the palate being too short. Many pointed out that language choice (only using key words, short phrases, incomplete sentences, and a poor storyline) and shyness further limited understandability. Mentally handicapped peers tended to understand them better; adults who were unfamiliar with their speech had more difficulty understanding them. Even those familiar with the different speech sometimes had difficulty understanding what was being said without knowing the context, and often needed to ask the patients to speak more clearly or repeat themselves. One caregiver wrote, "We get used to her speech. Over the phone, the understandability is poor. When speaking to strangers, she does her best to be understood. Apparently it requires extra effort."

Normal vs different speech

Comparing the questionnaire answers from the group with normal speech (speech rating =1) to the group with different speech (speech rating >1) showed no significant differences apart from speech understandability (Table 6.2). There was a trend for more participants with normal speech to be females (p=0.06). Neither a history of a cleft palate nor pharyngoplasty were associated with the speech rating (p=0.34 and p=1.00, respectively). The FSIQs ranged from 45 to 89 in both groups, with a median around 65 (p=0.43).

Cleft palate surgery and pharyngoplasty

Regrouping the patients for the sub-analysis comparing patients who had cleft palates to those who did not, showed no significant difference in the numbers of patients in each group with normal speech (15% vs 41%, p=0.17), and the mean speech ratings were the same (2.2 vs 2.0, p=0.39).

Regrouping the patients for the sub-analysis comparing patients who had pharyngoplasties to those who did not, showed that equal numbers of patients in

each group had normal speech (35% vs 33%, p=1.00), and the mean speech ratings were the same (1.9 vs 2.1, p=0.60). The only differences between these groups were that the patients who had pharyngoplasties tended to have had more years of SLT (7.5 vs 5.4, p=0.10) and have higher FSIQs (68 vs 62, p=0.06).

Comparing patients who had a pharyngoplasty following a cleft palate repair to those who had a primary pharyngoplasty, showed no difference in the prevalence of normal speech (33% vs 35%, p=1.00).

Correlations

After testing the continuous spectrum of speech ratings against possible determinants, no correlation was found between speech rating and age at assessment (Spearman correlation = - 0.061, p=0.68, 95% confidence interval -0.336 – 0.224), speech rating and age at pharyngoplasty (Spearman correlation 0.013, p=0.95, 95% confidence interval -0.401 – 0.422), nor speech rating and FSIQ (Spearman correlation = - 0.151, p=0.31, 95% confidence interval -0.419 – 0.142). However, speech rating was correlated with the number of years of SLT (Spearman correlation = 0.418, p=0.004, 95% confidence interval 0.145 - 0.632), showing that those with poorer speech ratings continued SLT for more years.

Discussion

In the current study we found that only about one-third of adolescents and young adults with 22q11DS had normal speech. About half had pharyngoplasties and all had speech therapy. No determinants were found for a normal speech rating, indicating that a history of cleft palate or surgery, age at surgery, FSIQ, and age at assessment are unlikely to be strong predictors of speech outcome in 22q11DS. The only difference detected was that those with poorer speech ratings continued SLT for more years. Factors that can account for the fact that we did not find any predictors in this study are 1) self-reported speech rating on a five-point scale is not sensitive enough to pick up important but subtle differences in speech outcome, and 2) given the multi-factorial etiology of speech problems in 22q11DS (Baylis et al

2008; D'Antonio et al 2001b; Goorhuis-Brouwer et al 2003; Widdershoven et al 2008a), many more patients will likely need to be recruited to find predictors of speech outcome. A strong point of this study is the fact that we recruited patients from the department of psychiatry, independent of anyone involved in speech therapy or surgery. While the overall speech rating remains "different" in patients with 22q11DS compared to the general population, surgeons and speech-language pathologists should not be disheartened: "Success" is ultimately determined by improvement (Dudas et al 2006). In 1960 a surgeon attested that "The effect of even a little improvement on the personalities of these discouraged and misunderstood patients [with cleft-type speech] has been very dramatic" (Randall et al 1960).

Questionnaire

Data acquisition was challenging in this group of patients. Some patients spend hours traveling to the tertiary hospital, where they then spend many hours doing different tests and seeing different specialists. To minimize their burden, we did not extend their hospital visit with a formal speech test administered by a speech-language pathologist, but used questionnaires. Performing a survey in this manner allowed us to study a much larger cohort of patients with 22q11DS than would otherwise have been possible. Additionally, ratings as seen through our patients' eyes are truly what matter most.

Although we attempted to limit selection bias by collaborating with researchers who invited all adolescents and young adults for a concomitant study on genetics and psychopathology, it is still possible that patients who volunteered to participate in these studies may not be representative for the entire 22q11DS population. Perhaps those with a more severe phenotype were more motivated to participate.

Furthermore, questionnaires have limitations. Our questionnaire was not validated; rather, it resembled a structured interview (Supplementary Materials and Methods). Inherently, there was an element of recall bias where caregivers were asked to remember, for example, whether patients had frequent ear infections and

how much SLT they had. We could not confirm assertions via medical files because patients were treated at other centers.

The speech rating measure is subjective: Different speech assessors have different standards. Some parents may be very satisfied with their child's imperfect speech because they are proud of improvements following years of SLT. Others may continue to be disappointed with minor imperfections. A speech-language pathologist's assessment remains the gold standard for evaluating speech (Smith & Kuehn 2007). In research settings scientific credibility is increased when multiple blinded assessors score recorded speech samples (Lohmander et al 2009; Persson et al 2012). However, given constrictions in research funding and study burden for participants, in this study speech was not assessed by (multiple) speech-language pathologists. Instead, we resorted to assessing speech via the questionnaires. We feel that, while not a gold standard, this laypeople's speech assessment does provide an important measure of how patients and their caregivers perceive the quality of speech.

Speech

Our findings confirm previously reported observations in younger patients with 22q11DS: the speech is hypernasal, quiet, poorly articulated, and sometimes difficult to understand even for those who know the patient well (Golding-Kushner et al 1985; Kuehn & Moller 2000; Rommel et al 1999; Solot et al 2001). While hypernasality is partially amenable via surgery, other aspects remain abnormal in many patients (Boseley & Hartnick 2004; Kobrynski & Sullivan 2007). In a study on patients aged 1 to 54 years speech problems started early and were a major problem for many patients until age 10 years, but gradually diminished with increasing age or after pharyngoplasty (Lima et al 2010).

We found that all patients had SLT. Lack of specification about the type of therapy they had hampers drawing conclusions on the effect The median duration was 6 years, once weekly, for 30 minutes per session. This is much longer than reports from another center where, following a pharyngoplasty patients receive 20-30 minutes of SLT weekly for an average of 8 months (maximum 25 months)

(Wang et al 2009a). Others have found that greater speech improvement was attained and maintained over a longer period when SLT was more frequent than once weekly (Albery et al 1982). In the Netherlands SLT is part of the basic universal health care coverage package for all patients with a diagnosis. Children who are enrolled in special education receive intensive SLT early on which tapers off to once a month once older. The speech-language pathologists at our tertiary referral hospital advise community-based speech-language pathologists to administer blocks of therapy because the learned skills often deteriorate in patients with 22q11DS. Future research could focus on details of SLT including motor speech disorders, compensatory articulation, language disorders, and the effect of therapy.

The indication for surgery is hypernasal speech which is resistant to SLT. Nearly half of our patients had pharyngoplasties. Worldwide, among patients with 22q11DS who undergo surgery to correct VPD, only 51% achieve normalized resonance (Spruijt et al 2012). In this study we found that as adolescents and young adults, their speech ratings (median rating 2/5) were not significantly different from patients who did not have surgery. This may attest to the efficacy of surgery to improve speech in children with poor pre-operative speech ratings (median rating 4/5). However, the natural course of speech ratings in children with poor speech ratings has not been studied.

It is unclear whether a speech plateau is reached in adolescents and young adults after SLT and surgeries. Our data are surprisingly consistent with a previous, smaller study (n=24) among adults with 22q11DS (Persson et al 2012), which reported palatal anomalies in 39% (in our study 26%), pharyngoplasties in 50% (in our study 46%), and absent VPD in 33% (in our study 34% had “normal” speech). The speech-pathologists who evaluated the speech in that study also noted that the patients’ voices were quiet and monotonous. However, in a larger study with adults age 18 or older, only 41% had VPD or hypernasal speech (Cohen et al 1999).

Cleft Palate

In our study 26% of patients had some form of palatal cleft. Prevalence rates reported in other studies vary as a function of patient selection, with the greatest prevalence reported by cleft centers (D'Antonio et al 2001b) and lower rates in samples not (exclusively) derived in cleft clinics. Overt cleft palate is seen in 11% (Kobrynski & Sullivan 2007; Lima et al 2010; McDonald-McGinn & Sullivan 2011) and submucous cleft palate is seen in 2-16% (McDonald-McGinn & Sullivan 2011; Ryan et al 1997), but the latter is very difficult to identify without nasendoscopy and therefore significantly underestimated (Shprintzen 2008).

Ear Infections and Hearing

In our study 69% of patients reported having frequent ear infections, 79% had grommets, and 18% wore a hearing aid. This prevalence of ear infections is higher than the reported 2-50% (McDonald-McGinn & Sullivan 2011; Reyes et al 1999; Ryan et al 1997) in larger studies, but may be related to the age range of the patients sampled. In those large studies the prevalence of conductive hearing loss was 31-84% and sensorineural hearing loss was only 2-11% (McDonald-McGinn et al 1999; McDonald-McGinn & Sullivan 2011; Reyes et al 1999). In the small study among adults with 22q11DS 41% had a hearing impairment, half of which were conductive, one-third sensorineural, and the others mixed (Persson et al 2012). Hearing loss is thought to be secondary to palatal abnormalities (McDonald-McGinn et al 1999; Ryan et al 1997). While hearing impairment negatively affects speech intelligibility, it was not found to be related to velopharyngeal function (Persson et al 2012). In our study, 27% reported less frequent ear infections following pharyngoplasty.

Intelligence Quotient

We measured a median FSIQ of 65 (range 45-89), which is near the adult mean of 70 (Chow et al 2006; McDonald-McGinn & Sullivan 2011; van Amelsvoort et al 2004). Patients with normal speech ratings did not have higher FSIQs than those with different speech. If FSIQ affects the ability to apply techniques learned in SLT (Spruijt et al 2011), patients with lower FSIQs would be expected to have

poorer speech ratings. The lack of association between the eventual speech rating and FSIQ (Spearman correlation =-0.151, p=0.31, 95% confidence interval -0.419 – 0.142) may provide evidence that the advice to tailor SLT to the FSIQ is effective. However, since the application of this advice is dependent on implementation by the many community-based speech-language pathologists, it is more likely that the speech rating is not associated with FSIQ.

Conclusion

We specifically studied adolescents and young adults with 22q11DS to give clinicians concrete numbers to quote to caregivers of young patients who seek information about the expected course of speech problems. We confirmed earlier observations that speech problems are highly prevalent in 22q11DS: All adolescents and young adults with 22q11DS had SLT and nearly half had pharyngoplasties. Only 34% attained normal self-reported speech ratings. No determinants were found to predict speech rating, including a history of cleft palate or surgery, age at surgery, FSIQ, and age at assessment. Those with poorer speech ratings continued SLT for more years. While speech does not normalize in a majority of patients, it is generally understandable and acceptable allowing most patients to speak effectively (Kobrynski & Sullivan 2007; McDonald-McGinn & Sullivan 2011).

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Speech Questionnaire

Please answer the following questions with your parent/caregiver. Where there are multiple choices, circle your answer.

Study title: *Self-Reported Speech Problems in Adolescents and Young Adults with 22q11.2 Deletion Syndrome*

Name _____ Gender male / female

Date of birth _____ Current date _____

Race African / Asian / Caucasian / Latin American / Middle Eastern / Other _____

History

A. Was the young adult born with a cleft palate? Yes / No / Not sure

If yes, how severe was the cleft? (circle the number)

1. Both the hard and soft palate.
2. Only the hard palate.
3. Not sure.

Surgery

B. Did the young adult have surgery on his/her palate? Yes / No

If yes, When? _____ (month, year)

C. Did the young adult have a pharyngoplasty (speech-improving surgery)? Yes / No

If yes, When? _____ (month, year)

How would you rate the speech before surgery? (circle the number)

1. The speech was understandable and normal.
2. The speech differs from others. This did not lead to comments (from acquaintances or strangers) and the speech was understandable.
3. The speech differed from others. This did lead to comments and the speech was understandable.
4. The speech was understandable with some difficulty.
5. The speech was not understandable.

Did you expect the speech to normalize? Yes / No

Did your expectations change? Yes / No

Are you satisfied with the result? Yes / No

Speech and language therapy

D. Did the young adult have speech and language therapy? Yes / No

If yes, How long? ____ years

How often? ____ sessions per week

How much? ____ minutes per session

E. Is the young adult currently having speech and language therapy? Yes / No
If not, why was therapy ended? (circle the number)
1. The speech was adequately understandable.
2. The speech was not adequately understandable, but progress was no longer being made.
3. Another reason, namely _____

Hearing

F. Did the young adult have frequent ear infections? Yes / No
If yes, Did he/she have grommets? Yes / No
Did he/she have fewer ear infections after the pharyngoplasty?
Yes / No / Not applicable

G. Do you use a hearing aid? Yes / No
If yes, in left / right / both ears?

Speech understandability

H. Do you understand the young adult's speech?
Always / Often / Sometimes / Never

I. Does the young adult's teacher/employer understand his/her speech?
Always / Often / Sometimes / Never

J. Do adults who do not know the young adult understand his/her speech?
Always / Often / Sometimes / Never

K. Do the young adult's peers understand his/her speech?
Always / Often / Sometimes / Never

L. Do you or does the young adult receive comments about his/her speech?
Always / Often / Sometimes / Never

M. Do you need to explain to others what the young adult says?
Always / Often / Sometimes / Never

N. Do you have any other remarks on this topic? (for example, that the young adult often uses sign language when others do not understand his/her speech, or that you often do not understand his/her speech)
_____

O. How would you rate the current speech? (circle the number)
1. The speech is understandable and normal.
2. The speech differs from others. This does not lead to comments (from acquaintances or strangers) and the speech is understandable.
3. The speech differs from others. This does lead to comments and the speech is understandable.
4. The speech is understandable with some difficulty.
5. The speech is not understandable.

Summary and Conclusions

Understanding and managing speech problems in patients with 22q11DS is especially challenging because many clinical findings are associated with the syndrome and the presentation is heterogeneous among patients (Cirillo et al 2014; Widdershoven et al 2008a). Our objectives were to increase the understanding of the etiology of VPD in 22q11DS (Chapters 1, 2, and 3) and find prognostic factors for outcome (Chapters 4, 5, and 6).

Chapter 1. Velopharyngeal hypotonia contributes to VPD in 22q11DS. To explore a myogenic component, specimens of the pharyngeal constrictor muscle were taken from children with and without the syndrome. Histologic properties were compared between the groups, and the specimens did not differ. A myogenic component of the etiology could not be confirmed.

Chapter 2. To explore the possibility that a neurogenic defect causes velopharyngeal hypotonia in 22q11DS, we compared the histology of the nucleus ambiguus in the *Tbx1*$^{+/-}$ mouse model for 22q11DS to that of wild type mice. We did not find a difference or variability between the volumes of the nucleus ambiguus of *Tbx1*$^{+/-}$ and wild type mice. The etiology of velopharyngeal hypotonia and variable speech in children with 22q11DS warrants further investigation.

Chapter 3. Platybasia has often been described in 22q11DS, however the clinical impact is unclear. Theoretically, patients with more obtuse cranial base angles have deeper pharynges, and may therefore have more hypernasal speech. In our chart review we found that patients with hypernasal speech had a significantly more obtuse mean cranial base angle. However, the prevalence of platybasia among those patients was not significantly greater than in patients with normal resonance, and there was no correlation between resonance and the cranial base angle. The clinical significance of platybasia remains unknown.

Chapter 4. Naturally, parents are interested to know whether their child's speech will benefit from surgery. However, prognostic factors remain elusive (Losken et al 2003). In our chart review of patients with 22q11DS and VPD,

residual hypernasality persisted in many patients after velopharyngoplasty. None of the preoperative factors that were studied had prognostic value for the outcome.

Chapter 5. To determine whether a particular surgical procedure results in a greater percentage of postoperative normal resonance in patients with 22q11DS and VPD, we systematically reviewed the available literature. Sub-questions included which procedure results in less morbidity and whether tailoring the procedure to preoperative patient characteristics results in superior outcome. In the heterogeneous group of patients with 22q11DS and VPD, a grade C recommendation can be made to minimize the morbidity of further surgery by choosing to perform a pharyngoplasty directly instead of only a palatoplasty.

Chapter 6. Although speech problems are a common clinical feature in 22q11DS, it is unclear how many patients undergo speech and language therapy and pharyngoplasty and whether patients and their caregivers find that these interventions normalize the speech. Therefore we inventoried the speech history and current self-reported speech of adolescents and young adults, and examined variables that possibly influence the speech ratings. All adolescents and young adults had undergone speech and language therapy and nearly half underwent pharyngoplasties. Only 34% attained normal speech ratings. Those with poorer speech ratings had speech and language therapy for more years.

These studies have contributed to our understanding of speech problems in 22q11DS, and given clinicians some numbers to quote when informing parents and caregivers about the expected course of treatment and outcome. More research is needed before we will be able to achieve more predictable and reliable results for each patient with 22q11DS and VPD.

The etiology of VPD in 22q11DS is multifaceted (Widdershoven et al 2008a), and is likely a sum of many factors. Future studies to elucidate the etiology of the pharyngeal hypotonia in 22q11DS, could include invasive neuromuscular conduction studies of the velopharyngeal muscles. The role of the central nervous system in velopharyngeal closure could be investigated by comparing fMRI images

taken during speech or performing electrophysiological analysis in distinct regions of the brain in wild type and *Tbx1*$^{+/-}$ mice.

We did not find prognostic factors for speech outcome. This is likely due to insufficient patient numbers and incomplete data. A randomized controlled trial seems unfeasible, but by conducting prospective cohort studies at multiple centers and uniformly documenting patient characteristics, velopharyngeal movement, and outcome measures, a meta-analysis could be performed with correction for the various factors.

Nederlandse samenvatting en conclusies

Het is een uitdaging om spraakproblemen bij patiënten met het 22q11.2 deletie syndroom (22q11DS) te begrijpen en te behandelen. Het syndroom beïnvloedt meerdere orgaansystemen en kan zich zeer heterogeen presenteren bij verschillende patiënten (Cirillo et al 2014; Widdershoven et al 2008a) Als lid van een multidisciplinair team dat zorgt voor patiënten met het 22q11DS, is een plastisch chirurg betrokken bij de behandeling van een lip-, kaak- en/of gehemeltespleet en velopharyngeale dysfunctie (VPD). De pharynx is een musculaire buis die de neus- en mondholte verbindt met het strottenhoofd en de slokdarm. Het inwendige van de buis is de keelholte. Bij VPD sluit het zachte gehemelte de opening tussen de mond- en neusholte onvolledig, waardoor lucht via de neus kan wegstromen tijdens de spraak. De resulterende 'open neusspraak' valt op en kan de verstaanbaarheid negatief beïnvloeden. Onze doelen waren om meer inzicht te krijgen in de etiologie van VPD bij het 22q11DS (hoofdstukken 1, 2, en 3) en prognostische factoren te vinden voor de uiteindelijke spraak kwaliteit (hoofdstukken 4, 5, en 6).

Hoofdstuk 1. Keelspierzwakte (velopharyngeale hypotonie) draagt bij aan de VPD bij het 22q11DS. Om te onderzoeken of de keelspiercellen anders zijn in 22q11DS, werden stukjes van de bovenste circulaire pharynxspier (musculus constrictor pharyngis superior) van kinderen met en zonder het syndroom onder de microscoop bekeken. De spiercellen van kinderen met 22q11DS waren niet opvallend of meetbaar verschillend van de spiercellen van kinderen zonder het syndroom. Er lijkt geen spierziekte ten grondslag te liggen aan de keelspierzwakte die gezien wordt bij het 22q11DS.

Hoofdstuk 2. Het is mogelijk dat de keelspierzwakte in 22q11DS een gevolg is van verminderde aansturing van de spieren door de zenuwen. Om te onderzoeken of de hersenstamkern die de keelspieren aanstuurt (nucleus ambiguus) anders is in 22q11DS, werden de hersenstammen van *Tbx1*$^{+/-}$ muizen (het muizenmodel voor 22q11DS) en normale (wildtype) muizen onder de microscoop

bekeken. Er waren geen opvallende of meetbare verschillen tussen de hersenstamkernen van muizen met en zonder het syndroom. Er is meer onderzoek nodig om de oorzaak van keelspierzwakte in 22q11DS te begrijpen.

Hoofdstuk 3. Een plattere schedelbasishoek (platybasia) is vaak beschreven bij het 22q11DS, maar de klinische consequenties zijn onduidelijk. Theoretisch hebben patiënten met een plattere schedelbasis een diepere keel, waardoor de mond-neusholte minder goed kan worden afgesloten en er meer open neusspraak optreedt. Via status onderzoek hebben we de mate van open neusspraak van kinderen met het 22q11DS onderzocht. Op Röntgenfoto's hebben we de schedelbasishoeken gemeten. Er was geen correlatie tussen de mate van open neuspraak en de schedelbasishoek. De klinische relevantie van een plattere schedelbasishoek blijft onduidelijk.

Hoofdstuk 4. Natuurlijk zijn ouders nieuwsgierig of de spraak van hun kind zal verbeteren na een operatie. De uitkomsten zijn wisselend, en prognostische factoren zijn onbekend (Losken et al 2003). Via status onderzoek van patiënten met 22q11DS en VPD, vonden we dat veel patiënten een zekere mate van open neusspraak hielden na een spraakverbeterende operatie (velopharyngoplastiek). Geen van de preoperatieve factoren die werden bestudeerd had voorspellende waarde voor het resultaat.

Hoofdstuk 5. Wereldwijd worden verschillende soorten spraak-verbeterende operaties uitgevoerd. Wij hebben systematisch de literatuur beoordeeld om te onderzoeken of er een operatietechniek is waarbij een hoger percentage van patiënten met 22q11DS een normale spraak behaald. We hebben ook gekeken of er nadelen zijn aan bepaalde operaties (bijvoorbeeld, of de kans groot is dat een tweede operatie nodig zal zijn), of dat een "custom made" operatie betere resultaten geeft. Er waren veel en grote verschillen tussen de studies en de patiënten die werden behandeld. Op basis van de bevindingen wordt aanbevolen de kans op een tweede operatie te minimaliseren door in eerste instantie een operatie aan de keel te doen (pharyngoplastiek) in plaats van alleen aan het gehemelte (palatoplastiek).

Hoofdstuk 6. Hoewel spraakproblemen veel voorkomen bij het 22q11DS, is het onduidelijk hoeveel patiënten logopedie en/of een spraakverbeterende operatie

ondergaan. Het is ook onbekend of de patiënten en hun verzorgers vinden dat de spraak uiteindelijk normaal wordt. Wij hebben vragenlijsten laten invullen door jong volwassenen met het 22q11DS en hun verzorgers. Alle patiënten hadden logopedie gekregen, en bijna de helft had een spraakverbeterende operatie gehad. Slechts 34% vond de spraak uiteindelijk normaal. Degenen met slechtere spraak hadden meer jaren logopedie gehad.

Deze studies hebben bijgedragen aan ons begrip van spraakproblemen bij het 22q11DS Daarnaast geven ze de medisch specialist getallen die gebruikt kunnen worden om ouders voor te lichten over het verwachte verloop van de behandeling en het resultaat. Meer onderzoek is nodig voordat we in staat zijn om beter voorspelbare en betrouwbare resultaten te bereiken bij iedere patiënt met 22q11DS en VPD.

De etiologie van VPD in 22q11DS is veelzijdig (Widdershoven et al 2008a), en is waarschijnlijk een som van meerdere factoren. Enkele ideeën voor toekomstig onderzoek zijn om met behulp van electromyografie (EMG) de geleiding van de keelspierzenuwen te testen, en op spraak fMRI-hersenscans de rol van het centrale zenuwstelsel te analyseren.

We hebben geen prognostische factoren gevonden voor de uiteindelijke spraak. Dit is waarschijnlijk te wijten aan de kleine patiënten aantallen en onvolledige gegevens. Een gerandomiseerd gecontroleerd onderzoek lijkt echter niet haalbaar. Indien in meerdere centra op een uniforme manier patiëntkenmerken, keelspierbeweging, en spraakuitkomsten gedocumenteerd worden, zou een meta-analyse kunnen worden uitgevoerd met correctie voor verschillende van invloed zijnde factoren.

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Curriculum Vitae

Nicole Spruijt was born in the Netherlands. Through her mother she also has United States citizenship. Since her father worked for UNICEF, she grew up in Cambodia, Rwanda, Guatemala, and Nepal.

In 2002 she moved back to the Netherlands to study Biomedical Sciences. After completing her BSc, she went on to do Selective Utrecht Medical Masters (SUMMA) to become a medical doctor with an affinity for research. This PhD thesis was started during a research internship in her final year of medical school.

While gathering and analyzing data and writing these studies, she gained medical registration to work in the USA and the UK and has been working at the departments of general surgery (St. Antonius Hospital in Nieuwegein/Utrecht), cardiothoracic surgery (University Medical Center in Utrecht), and plastic surgery (St. Andrew's Centre at Broomfield Hospital in Chelmsford UK, VieCuri Medical Center in Venlo/Venray, Maastricht University Medical Center, and Maxima Medical Center in Veldhoven/Eindhoven). She hopes to become a plastic surgeon.

Oh, the depth of the riches and wisdom and knowledge of God!
How unsearchable are his judgments and how inscrutable his ways!
- Romans 11:33