An Analysis of the Phenotypic Features of Chromosome 22q11.2 Deletion Syndrome at Red Cross War Memorial Children's Hospital

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Declaration Page

I, LJ Hendricks, hereby declare that the dissertation, which I hereby submit for the degree of MMed in Paediatrics is based on independent work performed by myself. Neither the whole work nor any part of it has been, is being, or is to be submitted for another degree to any other university.

Also, this work has not been reported or published *prior to registration* for an MMed degree.

Lesley Jill Hendricks

Abstract

Chromosome 22q11.2 deletion syndrome (22qDS) is an inherited autosomal dominant disorder. It is the second most commonly occurring syndrome, Trisomy 21 being the most common. It is the most common microdeletion syndrome.

The clinical range of features with which affected individuals present is very broad and includes congenital heart disease (particularly conotruncal malformations), palatal abnormalities, characteristic facial features, and learning difficulties. In total, there are more than 180 different phenotypic features associated with 22qDS.

Due to the wide variability of phenotypic features that can arise in 22qDS it is often difficult to know when to test for the syndrome. Oskarsdottir's criteria are widely used in clinical recognition for 22qDS. However, in a study done previously at Red Cross War Memorial Children's Hospital, this same criteria was found to only have a positive predictive value of 14% for 22qDS. This is likely due to the fact that Oskarsdottir's study was based on a largely Caucasian study population. Our population in Southern Africa is largely non-Caucasian. Previous studies have confirmed that non-Caucasian population groups with 22qDS have different presenting phenotypic features.

For this reason, in this study we sought to describe the typical phenotypic features with which children with 22qDS present in our local population in South Africa.

A retrospective folder review was done of the phenotypic features of all patients who had had a FISH test done on the suspicion on 22qDS. A total of 144 patient folders were reviewed (72 patients who were FISH positive for 22qDS and 72 patients who were FISH negative and functioned as the control arm of this study).

A review on the phenotypic features of children with 22qDS revealed the most common presentation to be congenital heart disease (44%), failure to thrive (33%), dysmorphic features (32%) and cardiac failure (25%). A positive family history was only noted in 13 patients. Of those patients with a positive family history of 22qDS, only 5% were proven FISH positive for 22qDS themselves (less than the 10% described in the literature). Younger children presented more frequently with CHD, while older children presented with developmental delay and dysmorphic features. In general, developmental delay, palatal abnormalities and feeding difficulties were less common in our study population than described in the literature. Our particular patient population presented with the following CHD: isolated VSD (46%), tetralogy of Fallot (20.8%), truncus arteriosus (14.5%), PS/pulmonary artery stenosis (20.8%) and interrupted aortic arch (6%). Interrupted aortic arch was found to be the most sensitive marker for 22qDS in children with cardiac lesions. The cardiac lesions with the highest positive predictive value for 22qDS was non-isolated VSD (54%). Dysmorphic features with the highest sensitivity for 22qDS included bulbous nose (75%), abnormal digits (64%) and posteriorly rotated ears (68%).

Primary immune deficiency, thymus abnormalities, cleft palate and behavioural issues were described less in this study than previously described in the literature.

In conclusion, it is clear that non-Caucasian populations have some unique phenotypic expressions of 22qDS. It is imperative that clinicians maintain a high index of suspicion for patients with 22qDS.

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ABBREVIATIONS

22qDS	22q11.2 deletion syndrome
FISH	fluorescent in situ hybridisation
RCWMCH	Red Cross War Memorial Children's Hospital
CHD	congenital heart disease
VSD	ventricular septal defect
PA	pulmonary atresia
TOF	tetralogy of Fallot
DGCR	Di George critical region
TDR	typical deleted region
LCR	low copy repeat
UFD1L gene	ubiquitin fusion degradation 1-like gene
SNP	single nucleotide array polymorphism
MLPA	multiple ligation dependent probe amplification
CGH	comparative genomic hybridization
VPI	velopharyngeal insufficiency
CHOP	Children's Hospital of Philadelphia
AI disease	autoimmune disease
MAPCA	major aorta-pulmonary collateral arteries
PA	pulmonary atresia
IAA	interrupted aortic arch
TGA	transposition of the great arteries
DORV	double outlet right ventricle
BSS	Bernard-Soulier Syndrome

Introduction

Chromosome 22q11.2 deletion syndrome (22qDS) is an inherited autosomal dominant disorder and is the most common microdeletion syndrome. It is the second most commonly occurring genetic syndrome, Trisomy 21 being the most common. The vast majority of probands have a *de novo* microdeletion of chromosome 22q11.2. However, about 10 - 15% of those affected inherit the 22q11.2 deletion from a parent. Offspring of affected individuals have a 50% chance of inheriting the deletion. (1-4)

Due to the broad spectrum of disease presentation in individuals with the 22q11.2 deletion syndrome, and because many clinical characteristics are treatable, it is imperative that paediatricians have a high index of suspicion. A recent prospective study at Red Cross Children's Hospital (RCWMCH) has shown that it is frequently missed on clinical grounds, with potential adverse impacts on patient management and counselling. (62)

This syndrome was once known by many names e.g. DiGeorge Syndrome, Velocardiofacial Syndrome, Shprintzen Syndrome, Conotruncal Anomaly Face Syndrome, Cayler Cardiofacial Syndrome, Autosomal Dominant Opitz G/BBB Syndrome, etc. These were previously thought to be different disease entities but research confirmed a common underlying defect: microdeletion of a segment on the long arm of chromosome 22. This was then renamed CATCH 22 Syndrome. The negative connotations associated with the phrase (it refers to a problematic situation for which the only solution is denied by a circumstance inherent in that same problem) resulted in the syndrome being renamed chromosome 22q11.2 deletion syndrome. (1, 4-6)

The clinical range of features with which affected individuals present is very broad and includes congenital heart disease (particularly conotruncal malformations), palatal abnormalities, characteristic facial features, and learning difficulties. As many as 77% of individuals have an immune deficiency regardless of their clinical presentation. Additional findings include hypocalcaemia, feeding problems, renal anomalies, hearing loss (both conductive and sensorineural), laryngotracheoesophageal anomalies, growth hormone deficiency, autoimmune disorders, seizures and skeletal abnormalities. (2)

The 22qDS is also associated with an increased risk of malignancy, namely hepatoblastoma, renal cell carcinoma, Wilm's tumour, and neuroblastoma. (1, 2, 5)

In total, there are more than 180 different phenotypic features associated with 22qDS. (6, 7)

The 22qDS is diagnosed by confirmation of the microdeletion with fluorescence in situ hybridization (FISH). A small percentage (<1%) of individuals with clinical features of the 22q11.2 deletion syndrome have chromosomal rearrangements involving 22q11.2, such as a translocation between chromosome 22 and another chromosome. (8, 9)

The two probes commercially available for 22q11.2 FISH analysis are TUPLE1 and N25. The detection rate of FISH analyses using either probe is thought to be equivalent; however, FISH using either one of these probes is not sensitive enough to detect small deletions (<40 kb) within the 22q11.2 region. (8, 9)

In 2004, a study in Sweden by Oskarsdottir et al attempted to ascertain the most common features associated with the 22q11.2 deletion syndrome. The study included a cohort of 100 children and adolescents. Based on this information, a scoring schedule (see table 1) was developed to aid in the decision to perform a FISH test for the 22qDS. This study is a sensitive screening tool for the Caucasian population. However, it has been noted to be less sensitive in the screening of non-Caucasians for 22qDS. (10)

TABLE 1: Guidelines for FISH testing for 22qDS (10)

Two or more of the 8 clinical domains are required to consider the use of genetic testing for possible 22qDS; a typical cardiac defect found in infancy is sufficient for testing for 22qDS

CLINICAL DOMAIN	INFANTS	PRESCHOOL	CHILDHOOD TO ADOLESCENCE
1. Cardiac defect	Cardiac defect (TOF, IAA, truncus arteriosus, PA+VSD, VSD+arch anomaly)	Cardiac defect (TOF, IAA, truncus arteriosus, PA+VSD, VSD+arch anomaly)	Cardiac defect (TOF, IAA, truncus arteriosus, PA+VSD, VSD+arch anomaly)
2. Immunodeficiency	Thymus aplasia/hypoplasia Immunodeficiency Infection	Infection (respiratory tract infection, middle ear infection)	Infection Autoimmune phenomena
3. Abnormal calcium metabolism	Hypocalcaemia	Hypoparathyroidism	Hypoparathyroidism
4. Poor feeding	Poor feeding	Poor feeding	Poor feeding
5. Cleft palate	Cleft palate	Speech/language impairment VPI	VPI
6. Developmental issues	Developmental delay	Developmental delay Behavioural abnormalities	Learning difficulties Behavioural abnormalities
7. Other abnormalities (skeletal, clubfoot, hernia, etc)	Other abnormalities (skeletal, clubfoot, hernia, etc)	Other abnormalities (skeletal, clubfoot, hernia, etc)	Other abnormalities (skeletal, clubfoot, hernia, etc), including scoliosis
8. Dysmorphic features	Dysmorphic features	Dysmorphic features	Dysmorphic features

The aim of this study is to ascertain the most common phenotypic features noted in our population for 22qDS which might suggest the need for FISH testing (or are predictable of a positive FISH test). We also calculate the sensitivity and specificity of the most commonly occurring phenotypic features as well as the positive and negative predictive values thereof.

Using this information, we hope that it will lead to the development of a new phenotypic algorithm to better identify 22qDS patients in Sub Saharan Africa. An accurate algorithm will require further multifactorial analyses of the data in the current study.

Literature review

Introduction

After Trisomy 21, 22qDS is the second most common genetic syndrome in man. It is also the most common microdeletion syndrome. A microdeletion is defined as a very small deletion, usually involving only a fraction of a single chromosomal band. Certain syndromes, such as 22qDS, are due to deletions that encompass portions of several adjacent unrelated genes. The overall incidence of 22qDS is approximately 1 in 4000 to 1 in 8000 live births. However, this figure might be an underestimate due to the high rate of perinatal deaths secondary to severe congenital cardiac defects. (3) A study done in Sweden showed the mean annual incidence of 22qDS to be 14 per 100 000 while the prevalence of 22qDS was 23 per 100 000. This increased prevalence was likely due to an increased awareness of the syndrome. (1, 10, 11)A study done in Atlanta found the incidence to be 1 in 6000 to 1 in 6500 in white, black and Asian populations. The incidence was 1 in 3800 in the Hispanic population.(12) This was however a small study population and data regarding the Latin American population and 22qDS are scarce. Similarly, in another small study done in Singapore the incidence was found to be 1 in 10 000. (1)

Male and female genders are affected equally.

Table 2. Compared prevalence data of 22qDS in different studies around the world. (12)

Population area	France	West Sweden	Belgium	United Kingdom	United States	Singapore
Birth years	1989-1993	1991-2000	1992-1996	1994-1995	1994-1999	2000-2003
Data sources	Birth defect registry	Hospital-based	4 genetic centres	Regional genetic and paediatric cardiology	Population-based	National birth defects registry
Number of cases	12	24	51	9	43	17
Rate per 10000 births	1.0	1.4	1.6	1.3	1.7	1.02
No. with cardiac anomalies	11	14	37	6	35	16
% of cases with deletion	92%	58%	73%	67%	81%	94%
Reference	Tezenas du Montcel, et al	Oskarsdottir et al	Devriendt et al	Goodhship et al	Botto et al	Tan et al

Brief history

As cited in McDonald McGinn et al, probable cases of 22qDS have been recorded as early as 1829, where it was noted that a syndromic looking child had an absent thymus. (5) Sedlackova and Lobdell noted the combination of thymic aplasia and congenital hypoparathyroidism in 1955 and 1959, respectively. In 1965 DiGeorge described a group of infants with congenital absence of the thymus and parathyroid glands, resulting in T cell dysfunction. (5, 6) This became known as Di George Syndrome. Shortly thereafter, Kinouchi et al described conotruncal anomaly face syndrome (also known as Takao syndrome), which consisted of typical dysmorphism, velopharyngeal insufficiency (VPI), CHD and learning disabilities. Almost simultaneously, Sprintzen et al described velocardiofacial syndrome (VCFS) in children with VPI, CHD and dysmorphic features. (6) These syndromes, with their broad range of phenotypic features, became known by many names, e.g. DiGeorge Syndrome, Velocardiofacial Syndrome, Sprintzen Syndrome, Conotruncal Anomaly Face Syndrome , Cayler Cardiofacial Syndrome, Autosomal Dominant Opitz G/BBB Syndrome, etc.(5, 6) Further studies suggested a common denominator between all these syndromes - microdeletion of a segment on the long arm of chromosome 22. In 1993, the term CATCH 22 syndrome (an acronym for cardiac features, abnormal facies, thymic hypoplasia, cleft palate, hypocalcaemia and 22q deletions) was coined by Wilson et al, but due to the negative connotations associated with the phrase the syndrome was renamed chromosome 22q11.2 deletion syndrome.(1, 6)

Genetics

The reason for the phenotypic variation in 22qDS remains unclear. There is uncertain genotype-phenotype correlation even in familial cases. (4, 8, 13)

Most cases of 22qDS occur de novo. However in 7 - 10% of cases it is inherited from one or both parents as an autosomal dominant trait.(1, 4) If one or both parents are affected, there is a 50% chance that their offspring will inherit the syndrome. The risk of recurrence to two unaffected parents with a previously affected child is less than 1%. (1, 5)

Delio et al suggested a maternal origin of de novo deletions. A study done on 389 confirmed 22qDS patients showed de novo deletion was of maternal origin in 56% of probands and of paternal origin in 44% of probands. This was a statistically significant maternal bias for parent of origin of 22qDS.(14) Previous studies, however, did not show this same result (though these studies were all smaller in number).

The characteristic microdeletion found in chromosome 22q11.2 is at least 10 times more common than the next frequent human deletion syndrome, Williams syndrome. (5) A total of 5 different 22q11.2 critical regions have been identified for 22qDS.

Linkage analysis has identified a critical region containing at least 25-30 candidate genes called the Di George critical region (DGCR). The typically deleted region (TDR) is usually 3Mb in size, which encompasses about 30 genes. These typical deletions occur in the majority of cases (90%) (3, 8, 15, 16). However, smaller deletions of less than 1.5Mb have also been noted in 8% of cases. These typically occur in the centromeric region of the TDR. This encompasses approximately 24 genes. (4) A majority of patients appear to be deleted for the entire DGCR. (13, 17-19) Notably, the clinical severity of the syndrome has no correlation with the size of the deletion. (3, 20, 21) Both large and smaller deletions appear to be the result of homologous recombination between non-allelic flanking low copy repeat sequences (LCRS) located within the 22q11.2 region. In a group of 300 patients, 86% of the deletions were found to span the same 3 Mb region from LCR-A to LCR-D. In the same cohort, 7.3% had a smaller approximately 1.5 Mb deletion extending from LCR-A to LCR-B. There were also nine deletions with atypical endpoints.(9, 22-25)

A study investigating the molecular analysis in 142 patients with phenotypic features suggestive of 22qDS found that 110 (77%) were carrying the deletion. Of these, 88% carried the 3Mb deletion in the typically deleted region. The remaining 12% carried smaller or atypical deletions. No significant genotype-phenotype correlation was found between the deletion type and the clinical findings. (9, 13)

10-15% of deletions are atypical and are usually located distally to TDR. (14, 22, 26)

Individuals with 22q11 duplication*s* have also been identified but they seem to be fewer than anticipated based upon the prevalence of deletions. Nonetheless they share phenotypic similarities to patients with 22qDS. (22, 25)

Less than 1% of individuals with features of 22qDS have chromosomal rearrangements between chromosome 22 and another chromosome, as seen in the Philadelphia chromosome (translocation of 22q-9q). (1, 23)

The T box transcription factor 1 (TBX1) gene expression remains of importance in 22qDS. TBX1 is expressed in the pharyngeal mesenchyme and endodermal pouch and encodes a T box transcription factor, which is essential in the role of early vertebral development, amongst other functions. (24, 27)

Haploinsufficiency for the TBX1 gene results in decreased proliferation of endodermal cells in the branchial arches and impaired pharyngeal artery development. Branchial arches are the origin of the anterior facial structures, thyroid gland, thymus and parathyroid glands. (6, 15) TBX1 also drives expression of other downstream targets e.g. FGF8, FGF10 which are important in neural crest formation and MYF5, MYOD which regulate development of branchiomeric muscle, possibly explaining swallowing abnormalities which arise in 22qDS. TBX1 is expressed in the anterior heart field, which becomes cardiomyocytes in the outflow tract. (19, 28)

Furthermore, TBX1 is expressed in developing brain mesoderm and sclerotomes, which may account for behavioural, cognitive and psychiatric disturbances, which are common as well as skeletal abnormalities. (1, 5, 24, 27)

However, the TBX1 locus is not always found in deleted region of 22q11.2, suggesting that TBX1 haploinsufficiency is not wholly responsible for the full phenotypic picture. (5, 29)

Other deleted genes also contribute to the phenotype of affected patients: Haploinsufficiency for GPIb(beta) may contribute to thrombocytopenia , while COMT haploinsufficiency is implicated as a contributing factor to behavioural and psychiatric problems and may be related to a slight increased risk of malignancy. CRKL gene has been implicated in cardiac anomalies as well as thymic hypoplasia and craniofacial abnormalities. (4, 5, 19, 28)

Atypical deletions and duplications

In theory, duplications should occur as frequently as deletions. Duplications and deletions arise simultaneously from interchromosomal meiotic exchange in the 22q11.2 region. This results in the unequal crossover of low copy repeats. Consequently, a 3Mb deletion occurs on one homolog and reciprocal duplication on the other. (30)

It is likely that patients with duplications are identified less frequently due to the uncommon nature of their occurrence. They present with a less severe phenotype and are thus less frequently referred for testing. Even when tested, these patients often remain undetected as both FISH testing and SNP arrays are less sensitive to duplications than deletions.

The duplication phenotype, though usually less severe than 22qDS, most commonly presents with developmental delay and neuropsychological issues. They can also present with CHD, palatal abnormalities and hypocalcaemia. Additional findings include seizures, micro or macrocephaly and hearing loss. (5, 25, 30)

Atypical deletions are fairly uncommon and occur in less than 10% of cases. (4)

Diagnosis of 22qDS

Due to the wide variability of phenotypic features that can arise in 22qDS it is often difficult to know when to test for the syndrome. In our population it is difficult to diagnose 22qDS as patients often present with less recognisable facial features of the syndrome than what is described in the literature. This is most likely because the majority of our population is non-Caucasian. Few studies have been done on the phenotypic features of 22qDS in non-Caucasian population groups. Thus, it is important to have a high index of suspicion for this syndrome.

The use of the terms Caucasian and non-Caucasian in previous studies are assumed to mean White and non-white races. In the traditional biological anthropology sense of the phrase, Caucasian race refers to phenotypically similar groups from various regions that share similar cranial morphology. These regions include Europe, western and south Asia and North Africa. Non-Caucasian races include the Mongoloid, Australoid and Negroid races. Obviously, to classify patients based on their biological anthropology in medical research would be tedious and cumbersome. When referring to the phenotypic features of Caucasians in the context of 22qDS research, what is meant is that the facial phenotypic features of the so-called Caucasians are easier to recognise as 22qDS than non-Caucasians.

The most commonly used method for diagnosing 22qDS is fluorescent-in-situ hybridization (FISH). FISH testing is performed using two probes: the first chromosome 22-specific probe identifies ('tags') chromosome 22 and the second probe then hybridises to a gene known to be present in the commonly deleted region on that same chromosome. A (FISH "positive") diagnosis of 22qDS is established if the second probe is absent from a tagged chromosome, thereby indicating that the gene target is deleted. (5)

The probes commercially available for 22qDS FISH analysis are TUPLE1 and N25. The detection rate of FISH analysis using either probe is thought to be equivalent; however, neither probe is sensitive enough to detect small deletions (<40 kb) within the 22q11.2 region. (1)

The main challenges with FISH testing are:

1. The turnaround time for testing lies between 3 and 14 days, and is labour intensive.

2. The possibility of a false negative result: not all deletions have the typical endpoints. Thus, FISH testing will not detect atypical deletions if the gene target is not deleted despite the presence of a deletion (i.e. the targeted gene is not included in the deletion).

3. The FISH test is not readily available in many middle to low income countries, which often causes unnecessary delays in, or lack of, a diagnosis. Also, FISH testing is not always cost effective in resource-restricted countries. Lastly, FISH testing is often not done due to lack of awareness of attending practitioners that such a test for microdeletions is available in the

first place. In fact, in previous years in our own referral centre, only patients with truncus arteriosus were traditionally tested for 22qDS. This practice was only changed once research on 22qDS at RCWMH found it to be incorrectly limited to a single cardiac phenotype. .

Two alternatives to FISH testing are now more readily available, due to cost reductions in genetic testing techniques:

1. A PCR based testing known as the multiple ligation dependent probe amplification (MLPA) assay. This detects gene dosage abnormalities by relative quantifications of DNA. Other diagnostic tests include array CGH (comparative genomic hybridization) and multiplex qualitative real time PCR. These tests are less available and more expensive than FISH testing, but have the added advantage of having a rapid turnaround time. (1, 9, 13)

2. Single nucleotide polymorphism (SNP) arrays are now also available in some centres. These tests have the same turnaround time as FISH testing but are very expensive.

While genetic testing in general has plummeted in price, in low and middle-income countries there still exists a lack of testing infrastructure.

The advantage of these newer tests is that atypical deletions can be readily identified. (5, 22, 31, 32).

FISH analysis and SNP arrays can also be performed antenatally on both cultured amniocytes and chorionic villi. Thus, affected parents are able to test their offspring for the syndrome as early as 10-12 weeks gestation. (1, 17)

In all offspring diagnosed with 22qDS, it is recommended that both parents be screened regardless of any dysmorphism or family history of 22qDS. All siblings of affected individuals should also be screened for the syndrome.

It is also recommended that routine cytogenetic testing be done at the time that the FISH test is performed, as approximately 1% of patients with 22qDS may have chromosomal rearrangements involving 22q11.2 i.e. translocation between chromosome 22 and another chromosome. (1)

Specific phenotypic features of 22qDS

It is frequently difficult to make a firm *clinical* diagnosis of 22qDS. The 22qDS phenotype is extremely variable, even within affected families, and is the most phenotypically diverse syndrome known. No phenotypic features are pathognomonic for 22qDS and more than 180 different phenotypic features are known. Caucasian patients' facial features appear different to those of non-Caucasians; in addition, patients with atypical deletions present more subtly than those with typical deletions. (19, 33)

Aside from the typical facial dysmorphisms, the most common abnormalities are:

1. Congenital heart disease: 80% of patients with 22qDS present with CHD. 20% of patients will be asymptomatic.

2. Seizures: Seizures occur in 20% of patients with 22qDS. 40 – 70% of seizures are due to hypocalcaemia in the neonatal period. However, seizures may also arise as a result of other causes such as sepsis, hypoglycaemia and poor feeding.

3. Mild immune deficiencies arise in patients with 22qDS, associated with absent or hypoplastic thymus.

4. Failure to thrive (FTT) can arise as a result of feeding difficulties or CHD. FTT occurs in 30% of patients with the deletion.

5. Developmental delay is quite prevalent and occurs in 80% of patients with 22qDS.

6. Auto-immune disease occurs in 10-20% of patients with 22qDS.

7. Cleft palate occurs in 10% of patients. Often clefts are not identified as they are subtle submucous cleft palates with VPI.

8. Hearing loss occurs frequently. A large percentage of hearing loss occurs as a result of recurrent otitis media. This common infection occurs in 52% of patients with 22qDS. Hearing loss occurs in about 31% of cases and is usually conductive in nature.

9. Learning difficulties are a common occurrence in 22qDS. 25% of patients with the deletion have a normal intelligence quotient (IQ). 33% have a borderline IQ and 29% mild mental retardation.

10. Psychiatric manifestations typically present later in life, but may arise in early childhood. As many as 25 – 50% of patients develop schizophrenia. There is also an increased risk of autism spectrum disease and attention deficit and hyperactivity disorder (ADHD), which in particular, is linked to the development of schizophrenia in 30% of patients. There is also an increased risk of generalized anxiety disorder, obsessive-compulsive disorder and mood disorder. (34)

An early systematic review found that adults with 22qDS have higher rates of palatal anomalies, learning disabilities and mental retardation as compared to paediatric patients. This may be due to the difficulty in identifying the signs and symptoms earlier in life.(35)

Treatment of patients with 22qDS should be targeted to suit the individual patient and their particular set of phenotypic features, severity of disease and need for treatment. Early diagnosis of 22qDS provides ample opportunity to minimise illness and optimise outcomes. This includes the screening for, and management of associated conditions.

McDonald-McGinn et al described the major phenotypic features of 22qDS, as noted in Table 3.

Table 3: The major phenotypic features in 22qDS in cohort of 906 patients at Children's Hospital of Philadelphia (CHOP)(5)

		Frequency
Cardiac lesions		77%
	Teratology of Fallot	20%
	VSD	21%
	Interrupted aortic arch	12%
	Truncus arteriosus	6%
	Vascular abnormalities	6%
Immune deficiency		77%
Palatal issues	VPI	42%
	Submucous cleft palate	16%
	Overt palate	11%
	Cleft lip and palate	2%
Weschler IQ score	Average	18%
	Low average	20%
	Borderline	32%
	Mental retardation	30%

In a cohort of 906 patients, McDonald-McGinn described CHD in 74% of patients with 22qDS while 69% had varying degrees of palatal abnormalities. 77% of individuals were noted to have immune deficiency, irrespective of the clinical presentation. Additional features included hypocalcaemia, feeding problems, renal abnormalities, as well as autoimmune disorders (AI), seizures and skeletal anomalies. (1)

Oskarsdottir et al in 2005 described the major presenting phenotypic features of 22qDS. Patients were divided into two groups: those diagnosed at less than 2 years of age and those between the ages of 2 and 20. Majority of patients were diagnosed later in childhood (26% of patients were diagnosed in infancy, while 74% were diagnosed in the older age group). The 8 major clinical features investigated were congenital heart disease, thymus involvement/ recurrent infections/auto immune disease, hypocalcaemia, feeding issues, VPI/speech and language delay, developmental/behavioural abnormalities, dysmorphism, and other deformities associated with 22qDS. (10)

As a result of this study new diagnostic considerations were drafted for the testing of children suspected of 22QDS (see table 1). (10) However, this was a study done with a majority Caucasian population. We attempted using the Oskarsdottir criteria in a study at Red Cross War Memorial Children's Hospital to screen for patients with 22qDS. In our study population (which is largely non-Caucasian), the positive predictive value of this criteria was only found to be 14%. This emphasized the difficulty in clinical recognition of 22qDS in the Southern African context. (62)

Brunet et al described a study where 4% of 295 children and adolescents tested positive for 22qDS. All of these patients presented with dysmorphisms and VPI. Gross motor delay, short stature and skeletal abnormalities were also frequently noted. (36)

Digilio et al investigated 165 patients with confirmed 22qDS and found that 100% of patients had facial dysmorphisms, 82% presented with CHD, 80% of patients had speech or learning difficulties, 73% had neonatal hypocalcaemia and 69% had T cell deficiencies.(37)

Dysmorphic features include a long face, malar flattening, hypertelorism, hooded eyelids, narrow palpebral fissures, prominent nasal root, bulbous nasal tip, hypoplastic alae nasae, microglossia, micrognathia, and abnormal helices.(38-40) Interestingly, patients with atypical distal deletions seem to differ from those with the "typical" 22qDS phenotype. They tend to present with arched eyebrows, deep set eyes, a thin upper lip with a smooth philtrum and a small pointed chin. Distal deletions are also associated with postnatal growth restriction and short stature. (26)

Oskarsdottir et al published a study in 2008 describing the typical facial characteristics found in 80 patients with 22qDS. They found that at least 50% of patients had malar flatness, hooded eyelids, broad nasal bridge with broad or round nasal tip, round ears, thick or overfolded helices and low set ears. At least 30% of patients had short palpebral fissures, hypertelorism, hypoplastic alae nasae, small round nares, a small mouth and hypoplastic ear lobes. The most common feature found overall was the round shape of the ears. Based on these findings a guideline was proposed for the screening of 22qDS (Table 4). (39)

Table 4: Guideline for screening of 22qDS(39)

	Feature	**Score: 0 or 1**
Malar area	Malar flatness	
Eye region	Fullness of eyelids (= Hooded eyelids)	
Nose	Broad nasal bridge Broad/round nasal tip	
Ears	Round/broad Thick/overfolded helix Low-set	
		Total score =

- One point is allocated for each part of the face/head if one or more of the features in each area are present.
- A total score of 3 or 4 in combination with clinical features commonly associated with the 22q11 deletion syndrome indicates increased likelihood of 22qDS. A lower score does not exclude the diagnosis (39)

Veerapandiyan et al described the dysmorphic features of patients with 22qDS in the black American population (See Table 5). (38) This study comprised 50 patients and indicated that 96% of the study population had involvement of at least one system. Most common dysmorphic features noted were ear abnormalities, skeletal abnormalities, cardiovascular abnormalities and developmental delay. Less commonly, immune deficiency and endocrine dysfunction were also described.(38)

Table 5: Dysmorphic features of 50 African American patients by Veerapandiyan et al (38)

Ear abnormalities (including small ears and abnormal helices)	64%
Skeletal abnormalities (including long fingers, camptodactyly, post axial polydactyly, scoliosis, flat feet, clubbed feet and clinodactyly)	64%
Developmental delay	58%
Cardiovascular abnormalities (namely tetralogy of Fallot, VSD and truncus arteriosus)	52%
Nasal abnormalities (e.g. widened nasal bridge)	50%
Eye abnormalities (including short palpebral fissures, hypertelorism, epicanthic folds)	40%
Palatal abnormalities (e.g. high palate and submucous cleft palate)	38%
The nervous system abnormalities (seizures, hypotonia and microcephaly)	38%
Renal abnormalities	30%
Micrognathia	16%

The largest study on the Latin American population to date was a Chilean study involving 208 patients with confirmed 22qDS, investigated for phenotypic features of the deletion (see table 6). These findings were then compared to 6 other published studies from Europe, Japan and USA that used similar data collection techniques. The frequency of CHD was found to be 60%, with TOF being the most commonly occurring cardiac lesion. This was lower than described in other studies done previously (except for Oskarsdottir et al, which yielded a result of 70% frequency of CHD). The types of CHD described were similar to previously published data, namely TOF, VSD, interrupted aortic arch type B, ASD, truncus arteriosus and coarctation of the aorta. Palatal abnormalities were found in 79% of patients, which was higher than the 56% shown in previously done studies.

Feeding difficulties occurred in one third of patients with 22qDS. Hypocalcaemia occurred in 35% of cases, mild to moderate mental retardation occurred in 39% of cases, developmental delay in 85% of patients and learning difficulties in 92% of cases. Hypothyroidism was found in 18% of patients with 22qDS. No cases of juvenile idiopathic arthritis were found. Hernias (umbilical, epigastric and inguinal) occurred in 14% of cases, skeletal abnormalities in 31 % of cases and primary immunodeficiency in 4% of cases. Dysmorphic features were similar in all studies.(41)

Table 6: Clinical features of Chilean patients with 22qDS (Repetto et al) (41)

Number of patients	208
Developmental delay	85%
Palatal abnormalities	79% • VPI 40% • Submucus cleft palate 23% • Cleft lip and palate 1.4%
Congenital heart disease	60% • Tetralogy of Fallot 20% • VSD 12% • IAA type B 7% • ASD 3% • Truncus arteriosus 2.4% • Coarctation 2%
Mental retardation	39%
Hypocalcaemia	35%
Skeletal Abnormalities	31%
Hypothyroidism	18%
Hernias (umbilical, epigastric and inguinal)	14%
CNS abnormalities	12%
Renal abnormalities	11%

A study done in India showed that the combination of extracardiac features had 93% agreement with presence of 22qDS when found in conjunction with CHD. The combination of low set ears, abnormal pinna, bulbous nose and long fingers were present in 91% of patients with 22qDS with cardiac lesions. (31)

Less common clinical features of 22qDS include dysphagia, growth hormone deficiency, auto-immune disease, hearing loss and psychiatric illness. (1)

Not infrequently, structural anomalies occur, including skeletal anomalies, genito-urinary tract anomalies, airway anomalies, eye abnormalities, CNS abnormalities and GIT anomalies.(1)

Prevalence of structural heart disease

Only 20% of patients with 22qDS have a structurally normal heart. The typical cardiac defects that occur in 22qDS are conotruncal abnormalities and aortic arch defects. Typical examples include tetralogy of Fallot, interrupted aortic arch type B (IAA), VSD, and truncus arteriosus. CHD significantly affects the morbidity and mortality of patients with 22qDS. A right-sided aortic arch, aberrant subclavian artery, cervical origin of the subclavian artery, abnormal pulmonary arteries and major aorto-pulmonary collateral (MAPCA) vessels are associated cardiovascular abnormalities in 22qDS. (1, 4, 16, 28, 40, 42, 43)

Goldmuntz et al described the prevalence of 22qDS in patients with CHD. 251 patients with conotruncal CHD were tested for 22qDS. 22qDS was found in 50% of patients with interrupted aortic arch, in 34% of patients with truncus arteriosus and in 16% of patients with tetralogy of Fallot. No patients with transposition of the great arteries were found to carry the deletion. The frequency of 22qDS increased with the presence of vascular anomalies, namely anomalies of the pulmonary artery, or the aortic arch and its branches.(44)

A study by Peyvandi et al described the prevalence of 22qDS in CHD in a cohort of 1610 patients. 13% of patients tested positive for 22qDS. The highest prevalence was found in patients with interrupted aortic arch type B (56%) while the prevalence is lowest in DORV and TGA (<1% each). Pulmonary atresia, abnormal aortic arch and MAPCA's occurred more commonly in deleted patients. In fact, patients with an abnormal aortic arch anomaly were 3 times more likely to test positive for 22qDS. Alternatively, DORV and TGA are unlikely to have the deletion unless an aortic arch anomaly is present. (42)

Foksteun et al investigated 110 patients with CHD. 17% were confirmed FISH positive for 22qDS. All of the patients with 22qDS and CHD also had extracardiac features of 22qDS. TOF, PA with VSD, truncus arteriosus and interrupted aortic arch were the most commonly occurring cardiac lesions in the FISH positive study population. All patients with isolated CHD (i.e. isolated cardiac malformation with the absence of any other syndromic features) tested negative for 22qDS. This suggests that isolated CHD is not likely to be due to 22qDS.(45)

Smaller studies done by Giray et al, and Wozniak et al showed similar results.(21, 46)

About 14-18% of patients with 22qDS have VSDs. McElhinney et al investigated the prevalence of different types of VSDs in 22qDS. 125 patients with perimembranous VSD (80%), conoseptal VSD (9%) and posterior malalignment of VSD (11%) were included in the study. 22qDS was detected in 10 of patients. Common anatomic abnormalities noted in all 22qDS positive patients included right sided aortic arch, abnormal branching of the aortic arch, a cervical aortic arch and discontinuous pulmonary arteries. Of the 20 patients with anatomical abnormalities, 45% tested positive for 22qDS. No correlation was found between the type of VSD and 22qDS. However, only certain types of VSD were included in the study.(47)

Botto et al. also noted that most children with CHD and 22qDS also had multiple minor features. Conversely, the syndrome was unlikely in children with CHD without these features.(48)

A study done in India showed that of 250 patients with CHD, 21% had conotruncal defects associated with a chromosomal abnormality. Of these patients 94% had 22qDS. The types of CHD described were as follows: tetralogy of Fallot (TOF), TOF with pulmonary atresia, DORV, truncus arteriosus and IAA. The frequency of 22qDS in conotruncal defects was 19%. Also, a right-sided aortic arch seems to be associated with increased risk of 22qDS in patients with TOF and TOF/pulmonary atresia.(16, 31) Halder et al also described the increased probability of 22qDS in patients with conotruncal cardiac defects. This North Indian study found 6% of 146 patients with cardiac malformation to have the syndrome.(49)

A French study rendered similar results: 261 fetuses suspected of having conotruncal malformations on antenatal ultrasound were tested for the deletion in utero. 20% of fetuses with conotruncal malformations were found to have 22qDS. Of those with 22qDS, TOF was found in 14% of cases, pulmonary atresia/VSD was noted in 21% of cases, truncus arteriosus in 31% of cases, interrupted aortic arch in 45% of cases and transposition of the great arteries in 12% of cases.(50, 51)

A Korean multicentre study reviewed 222 patients with 22qDS. 190 patients had CHD. The most common cardiac lesions were TOF (63%), isolated VSD (20%), IAA type B (5%), DORV (3%), isolated ASD (3%) and TA (1%). Right sided aortic arch was found in 50% of patients, while aberrant subclavian artery was found in 30% of patients. Overall, CHD was found in 85% of patients with 22qDS. This study showed the prevalence of various types of CHD in Asia seemed to differ from those found in the western world. DORV, heterotaxy syndrome and subarterial VSD prevalence were shown to be higher in Asians than Caucasians. TOF with or without PA was more common in the Asian population with 22qDS than the Caucasian population with 22qDS while IAA and TA occurred less commonly.(52)

The mortality of patients with 22qDS is largely attributed to cardiac disease.(5, 43) Thus all patients with CHD suspicious of 22qDS must be screened for the syndrome.(3)

22qDS patients with CHD are also associated with significant morbidity, particularly in the area of neurodevelopment, with higher rates of motor and mental delay than other patient with CHD.(7)

Dysmorphic features

Dysmorphic features are variable and often non-specific. Patients with 22qDS may present with ear abnormalities, nasal abnormalities, hypertelorism, asymmetric crying facies, and craniosynostosis. The asymmetric crying facies is secondary to hypoplasia of the depressor anguli oris muscle and occurs in 20% of patients with 22qDS.Other dysmorphism includes polydactyly, syndactyly, clubfoot and overlapping toes. (1, 37, 40)

Digilio et al. described 100% occurrence of facial dysmorphism in their study population of 165 patients with proven 22qDS. Neonates were found to have more subtle facial dysmorphism compared to older children with 22qDS. Facial dysmorphism ranged from mild to severe and included the following: narrow, up slanting palpebral fissures, large bulbous nose with hypoplastic nares, microstomia, and small dysmorphic ears.(37)

Palatal and speech abnormalities

About 8% of individuals with a cleft palate may have 22qDS, making 22qDS the most common genetic syndrome associated with cleft palate. Palatal abnormalities include overt or submucosal cleft palate, velopharyngeal incompetence (VPI) and speech delay. As early as 1997 McDonald- McGinn suggested that detection of 22qDS in cardiac patients may be a risk factor for VPI.(2)

In a European collaborative study, 496 patients with 22qDS were investigated for palatal abnormalities. 14% of patients had an overt cleft palate or a submucous cleft palate. 32% of patients had VPI. Zori et al confirmed that 38% of the 16 patients with VPI tested positive for 22qDS.(54)

McDonald-McGinn et al showed that 69% of patients with 22qDS had palatal involvement. The most common problem was velopharyngeal insufficiency (VPI) occurring in 27% of patients.(1) Digilio et al showed a similar result, with 66% of patients with 22qDS having palatal abnormalities. The spectrum of palatal involvement was wide with the majority of patients presenting with VPI in the absence of a cleft palate.(37)

CHD combined with cleft palate has a high sensitivity for 22qDS. A study done in Norway showed 33% of patients with CHD and cleft palate had 22qDS.(30, 55)

A study by Sprintzen et al. showed in a sample of 580 known 22qDS patients that included overt, submucous and occult submucous cleft palates, that 7% patients had major CHD and 3% had minor CHD. However, in a study by Friedman et al, no relationship was found between CHD and cleft palates in patient population of 316 known 22qDS patients. (16, 55)

Other oral manifestations of 22qDS include abnormalities in dental enamel, tooth shape or number and dental caries.(56)

Feeding issues

A third of children present with feeding difficulties requiring some sort of intervention (i.e. nasogastric feeding or PEG placement). Feeding issues may arise with or without CHD or palatal abnormalities. This is likely due to dysmotility, which occurs in the pharyngo-oesophageal area. This area is derived from the third and fourth pharyngeal pouches. This results in nasopharyngeal reflux, as well as prominence of the cricopharyngeal muscle with abnormal cricopharyngeal closure.(1) Feeding difficulties are often overlooked.

ENT Abnormalities

Numerous ear abnormalities occur in 22qDS. These include abnormal helices, protruberant ears, preauricular pits or tags and narrow external auditory meati. Patients with 22qDS are often noted to have a bulbous nose with hypoplastic alar nasae and nasal dimples. Stridor can occur secondary to a vascular ring, laryngeal web or laryngomalacia. Chronic otitis media and sinusitis are common.

Hearing loss is a major contributor to speech delay. Both conductive and sensorineural hearing loss may occur. Conductive hearing loss is more common and occurs in 45% of patients. It is often associated with palatal abnormalities. Sensorineural hearing loss occurs in 2-15% of patients with 22qDS.(1, 5, 37, 40)

Calcium homeostasis

Hypocalcaemia is especially common during the neonatal period as well as pre- and post-cardiac surgery. It is seen in 17-60% of patients with 22qDS. This is most commonly secondary to hypoparathyroidism. Most patients do not require prolonged calcium supplementation. Hypocalcaemia tends to improve in the first years of life. This is due to parathyroid gland hypertrophy. (1, 29, 37, 40)

Late onset hypocalcaemia has been described quite frequently in the literature, often occurring during periods of metabolic stress e.g. puberty, infection or pregnancy.(4, 5) During baseline periods, calcium and parathyroid hormone levels remain normal.

Developmental delay

The degree of developmental delay is highly variable. It can range from mild learning disabilities to mental retardation.(4)

Mathematical skill, visuo-spatial memory and abstract reasoning are most commonly affected. Expressive language and speech skills are also delayed while receptive skills remain near normal. The mean age of onset of speech is 30 months. (5, 40, 57)

Mean IQ is 75 while severe mental retardation is uncommon. 35 – 40% of patients with 22qDS have mild to moderate mental retardation. Learning disabilities and concentration disabilities occur frequently. These are often subtle and may not be detected by the school system.(4, 57-59)

Immune deficiency

The immune system is affected in 77% of patients with 22qDS. This is the result of thymic aplasia or hypoplasia. Commonly, infants with low T cell counts improve over the first year of life. Thereafter the T cell count will decline as per usual in unaffected children. Many adult patients have T cell counts comparable to unaffected peers. Impaired T-cell production occurs in 67% of patients while 19% have impaired T-cell function, 23% have humoral defects, and 13% have IgA deficiency.(1, 33, 37, 40)

Regarding upper respiratory tract infections, 25% to 33% of patients experience recurrent ear infections or sinusitis while less than ten percent of patients develop recurrent episodes of lower respiratory tract infections.(29) However, these may also be secondary to VPI (velopharyngeal insufficiency) and aspiration events.

Associated abnormalities like CHD, gastro oesophageal reflux, palatal abnormalities and aspiration pneumonia also contribute to recurrent infection.(1)

The fetal thymus can also be a useful additional marker of 22qDS. Chaoui et al showed the sensitivity for predicting 22qDS in a fetus with signs of CHD and a hypoplastic or absent thymus on fetal ultrasound was found to be 90%, while the specificity for prenatal detection of 22qDS was 99%. (51)

Autoimmune disease

Auto immune (AI) disease is seen in about 10% of patients. The most common autoimmune disease in 22qDS are juvenile idiopathic arthritis (JIA) and haemolytic auto immune disease like idiopathic thrombocytopenic purpura (ITP).(1, 29, 33)

JIA occurs in 22qDS at a frequency that is 20 times greater than that of the general population. ITP occurs 22 times more frequently in patients with 22qDS than in the general population. Other AI disease includes Grave's disease, vitiligo, haemolytic anaemia, autoimmune neutropaenia, aplastic anaemia and celiac disease.(1)

Children with JIA also have an increased risk of selective IgA deficiency (2-4% versus 0.25% incidence in the general population).(27)

Allergic disease has also been noted to be increased in 22qDS.

Eye anomalies

Refractory errors in 22qDS are more common than in the general population. Astigmatism, hypermetropia, posterior embryotoxon and tortuous retinal vessels are frequent eye problems.(5)

Forbes et al investigated 90 patients with 22qDS for eye abnormalities. Almost 50% of patients had posterior embryotoxon, 34% tortuous retinal vessels, 20% hooded eyelids (upper, lower or both lids affected), 18% strabismus, 4% ptosis, 4% amblyopia (which is similar to that observed in the general population) and 1% tilted optic nerves.

Patients also presented with refractory errors, namely hyperopia. With increasing age the prevalence of hyperopia decreased while the prevalence of myopia increased. The majority of patients also had mild astigmatism.(1, 60)

Thus, all children with 22qDS should be evaluated for eye abnormalities and refractory errors at diagnosis and be followed up accordingly.

Behavioural and psychiatric issues

Psychiatric disorders arise commonly in 22qDS. These include attention deficit and hyperactivity disorder, poor impulse control, bipolar mood disorder, autism spectrum disorder, schizophrenia/schizoaffective disorder. The latter occurs in 10-30% of older patients.(5, 59) However, not many studies have been done on the prevalence of psychiatric disorders in young children.

Behavioural disorders and psychiatric disease occur in conjunction with developmental delay. Autism spectrum disorders occur in approximately 20% of patients with 22qDS. Also, anxiety and social withdrawal have been commonly noted.(1)

Renal and gastrointestinal anomalies

Renal and gastro intestinal abnormalities have a significant impact on morbidity.

Renal abnormalities occur in about 30-40% of patients with 22qDS. These include single kidney, multicystic dysplastic kidneys, renal calculi, horseshoe kidney, duplex collecting system, hydronephrosis, enuresis and renal tubular acidosis. GIT abnormalities include constipation, imperforate anus, malrotation, Hirschsprung disease, diaphragmatic hernia and umbilical/inguinal hernia.(1, 61)

Other associations

Most patients with 22qDS reach normal adult height. However, a study including 95 children showed that 41% were below the 5th centile in height for age. All of these children had low levels of insulin growth factor (IGF1) and IGFBP3. Three of these children had growth hormone deficiency.(1)

22qDS is associated with Bernard-Soulier syndrome (BSS). BSS is an autosomal recessive disorder resulting in thrombocytopaenia and giant platelets. It is caused by a mutation in one of 4 genes, of which one (GP1BB) maps to chromosome 22q11.2. BSS will thus arise in 22qDS patients who have the mutation on their non-deleted chromosome 22. These patients are particularly susceptible to bleeding after surgical procedures. (1)

Malignancies have been reported in 22qDS but it is uncertain if a causal relationship exists between 22qDS and certain malignancies. Malignancies specifically mentioned in the literature include hepatoblastoma, Wilms tumour, renal cell carcinoma and neuroblastoma.(1)

It is imperative that all patients known with 22qDS should be closely followed up, even as adults, to ensure minimum morbidity and mortality.

In 2011, Bassett et al published guidelines on the management of 22qDS. The guidelines were based on two international 22qDS consensus meetings, as well as a systematic review of 239 publications on 22qDS. Published guidelines included a list of the multi system features, recommendations at diagnosis and considerations for genetic counselling.(13) (see appendix for Bassett guidelines)

STUDY DESIGN

Aims:

Overall objective: To describe the typical phenotypic features with which individuals with 22qDS present in our local population in South Africa.

Specific objectives include:

1. To review all the phenotypic features with which these patients present, including immunological and endocrine features.
2. To perform a classification analysis of these phenotypic features in confirmed cases of 22qDS, including calculation of sensitivity and specificity of the most commonly occurring features as well as the positive and negative predictive values thereof.

Ultimately, the goal of this study is to use this information to develop a new phenotypic algorithm (or scoring system) to improve the screening sensitivity for 22qDS in our local population.

Methods:

A) Setting:

Red Cross War Memorial Children's Hospital

B) Methodology:

A retrospective folder review was done of all patients with a confirmed (FISH positive) 22qDS. All FISH tests were recorded on a database at the NHS cytology laboratory at Groote Schuur Hospital in Cape Town. The database was accessed and all patients who were tested for 22qDS were noted. It was from this pool of data that patients were selected to form both part of the control group (confirmed FISH negative) and the experimental group (confirmed FISH positive). Once this data was extracted from the database, these patients' folders were accessed at Red Cross Hospital and a full retrospective folder review was done.

The study population presented at various ages between the years 1990 and 2014. 769 patients were included on the FISH database, of which 120 patients tested FISH positive for 22qDS. However, not all 120 patient folders were retrievable. A total of 72 FISH positive patients' records were found. Thus, 72 patients with a positive FISH test were compared to 72 patients with a negative FISH test (the control group), with regards to their phenotypic presenting features. Demographic data and family history was noted. All possible phenotypic features noted in the folders were recorded.

These included dysmorphic features, cardiac lesions and any other phenotypic features, which may be associated with 22qDS. Using these data, the most common presenting features were noted in both FISH positive and FISH negative patients. The sensitivity and specificity as well as the positive and negative predictive values of these presenting features were then calculated. The most common cardiac lesions were also recorded, and the sensitivities, specificities, PPV and NPV were calculated for each cardiac variable. All possible dysmorphic features found in patients' folders were noted. These features were then compared in FISH positive and FISH negative patients. The sensitivity, specificity, PPV and NPV were calculated for each dysmorphic feature. The dysmorphic features included hypertelorism, truncal hypotonia, low set ears, flat nasal bridge, abnormal digits, micrognathia, epicanthic folds, posteriorly rotated ears, microcephaly, bulbous nose and high arched palate. Of the 180+ phenotypic features associated with 22qDS, only 18 were recorded in the available patient folders.

I retrospectively examined those features recorded by the attending clinicians (and subsequent investigations, e.g. echocardiography) that suggested the correct clinical diagnosis of 22qDS. These data were then compared with clinical features and investigations that also suggested testing for the microdeletion, but failed to detect 22qDS.

Other reasons for failure of detection are also discussed, specifically the possibility of false negative tests.

Inclusion criteria included:

All children at Red Cross Hospital screened for 22qDS with the FISH test were included in this study. The age of these children ranged from birth to 18 years of age.

All patients tested at other referral hospitals and subsequently referred to Red Cross Hospital for further management were also included in the study population. These also were children that ranged from infancy to 18 years of age.

Exclusion criteria included:

Any patients with incomplete or missing folders were excluded from this study. Also, patients who had another clear syndromic diagnosis were excluded from the study.

As classification analysis was being used for the purpose of pattern recognition (and therefore not for hypothesis testing), a sample size calculation (power calculation) was not required.

Each patient (in both the experimental arm as well as the control arm) was assigned a study number randomly. No names were entered on the electronic database which allowed for anonymous analysis and reporting.

Ethical approval was obtained from the Human Research Ethics committee of the Faculty of Health Sciences at the University of Cape Town (reference number HREC/REF; 685/2014).

Results

120 FISH positive patients were identified from the NHS Cytogenetics laboratory database. However, only 72 of these patients' folders were located. The missing folders were generally of patients who had not been to Red Cross Hospital in the last 10-15 years. These folders had been archived and unfortunately have become inaccessible. For this reason, the remaining 72 folders were used for this study. A further 72 patients were then randomly selected from those who were proven FISH negative from the same cytogenetics laboratory database. These 72 FISH negative patients were then used as a control arm for the study. All data related to the possible phenotypic features of 22qDS were recorded from the folders.

The ages of patients ranged from day 1 of life to 18 years of age. The mean age of presentation was 11 months.

Gender of the enrolled patients was not equally distributed. This had no effect on the study as it is well documented in the literature that gender does not affect the prevalence or expression of the disease.(1, 4)

Table 7: Demographics of study

	FISH positive (n=72)	FISH negative (n=72)	Total no. patients
Male	22	44	72
Female	50	28	72
Mean age of presentation	12.4 months	8.8 months	
Positive family history of 22qDS	7	6	13

A positive family history of 22qDS was noted in only 13 of the 144 patients. Of those 13 patients only 5% (7 out of 144 patients) were proven FISH positive for 22qDS themselves. The remainder were FISH negative patients.

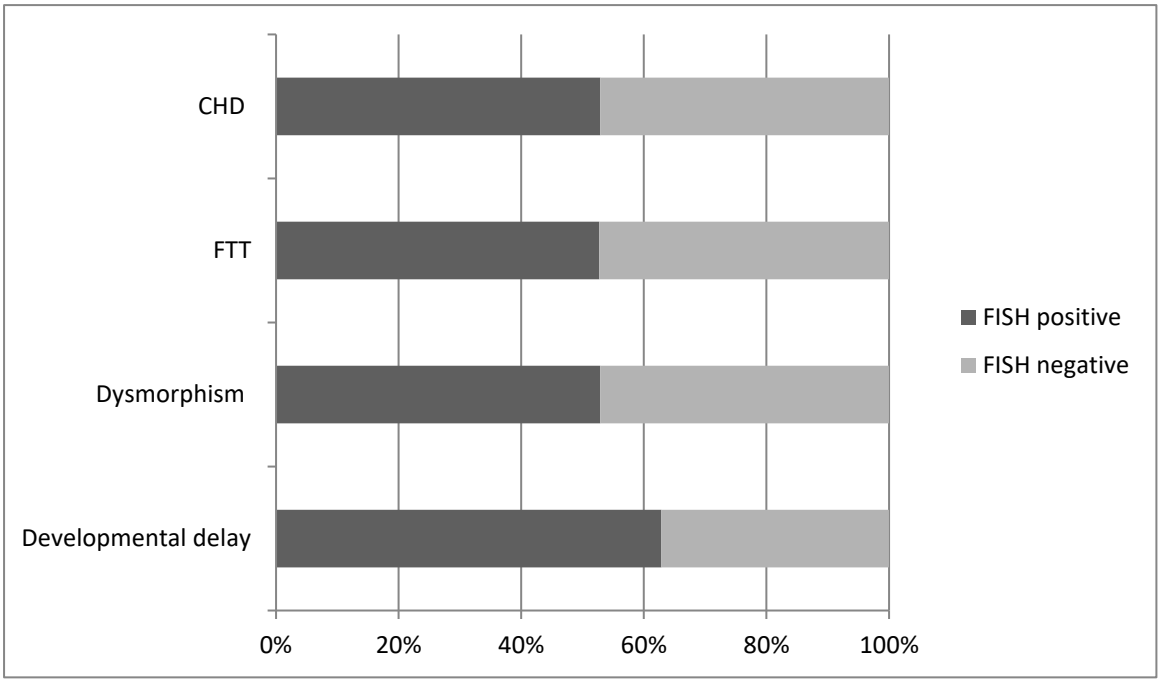
It was difficult to ascertain racial demographic data in this study. The race was not always noted in the patients' folders. In some patients, race could be reasonably assumed based on the birth name. However, this was not always clear. Particularly, Coloured and White racial groups have similar last names in South Africa. In the FISH positive group, 29 patients were noted to be Black and 19 patients Coloured. However, the remainder FISH positive patients racial group was unknown. For this reason, we could not compare phenotypic features accurately between different racial groups. The majority of our patient population was clearly non-Caucasian.

The most common presenting complaints in both groups (FISH positive and FISH negative) of patients are listed in table 8.

Table 8. The main presenting problem in our study population

PRESENTING PROBLEM	No. Patients (%)	FISH positive	FISH negative
1. CHD	121 (84%)	64 (44%)	57 (40%)
- cyanosis	69 (48%)	36 (25%)	33 (23%)
- CCF	63 (44%)	36 (25%)	27 (19%)
2. FTT	91 (63%)	48 (33%)	43 (30%)
3. Dysmorphic features	87 (60%)	46 (32%)	41 (28%)
4. Developmental delay	70 (49%)	44 (31%)	27 (19%)

Figure 4. Most common presenting features of 22qDS



The majority of patients presented to our centre with a congenital cardiac lesion. This included cyanotic and acyanotic cardiac lesions. 44% of the FISH tested patients were proven to have 22qDS with a positive FISH test. Other common presenting complaints included failure to thrive, dysmorphism, developmental delay, cyanosis and congestive cardiac failure. There was minimal missing data with the variables, cyanosis, CHD and congestive cardiac failure. This is likely because the majority of patients were transferred to our centre with CHD and were closely screened for cardiac signs and symptoms. Some of the missing data in the categories FTT and developmental delay were also attributed to the fact that some patients presented in the neonatal period, when these parameters are not yet detected. Developmental delay occurred commonly as a presenting feature in 22qDS FISH positive patients occurring in 30% of patients with 22qDS. Only 19% of FISH negative patients were noted to have developmental delay.

Sensitivity and specificity as well as positive and negative predictive values were calculated for all the common presenting complaints.

30% of patients in this study were acutely ill on admission and required admission to the intensive care unit. 19% of these were FISH positive for 22qDS while the remainder were FISH negative.

The positive predictive value (PPV) was highest for congenital heart disease, developmental delay and failure to thrive at 88%, 77.1% and 73%, respectively. The negative predictive value was highest for cardiac failure (61.9%).

The presenting complaint with the highest sensitivity for 22qDS was developmental delay, with a sensitivity of 62.8%. The specificity of developmental delay was also good at 68%. The specificity of congenital heart disease was also good at 65%. The variable of cyanosis was not found to be particularly useful, as the sensitivity, specificity and predictive values were all just above 50%.

Table 9. Sensitivity, Specificity, PPV and NPV of presenting complaints

PRESENTING COMPLAINT	SENSITIVITY (95% confidence interval)	SPECIFICTY (95% confidence interval)	POSITIVE PREDICTIVE VALUE (95% confidence interval)	NEGATIVE PREDICTIVE VALUE (95% confidence interval)
Congenital heart disease	52.8% (43.6% to 62%)	65.2% (42.7% to 83.6%)	88.8% (79.2% to 95%)	20.8% (12.1% to 32%)
Developmental delay	62.8% (50.4% to 74.1%)	68.2% (51.9% to 81.9%)	77.1% (64.1% to 87.2%)	51.8% (37.8% to 65.5%)
Failure to thrive	52.7% (42% to 63.3%)	58.5% (42.1% to 73.6%)	73.8% (61.4% to 83.9%)	35.8% (24.4% to 48.4%)
Dysmorphism	52.8% (41.8% to 63.6%)	52.9% (38.4% to 67%)	65.7% (53.4% to 76.6%)	39.7% (28% to 52.3%)
Cardiac failure	57.1% (44% to 69.5%)	55% (43.4% to 66.1%)	50% (37.9% to 62%)	61.9% 49.6% to 73.2%)
Cyanosis	52.1% (39.8% to 64.3%)	52% (40% to 63.6%)	50% (37.9% to 62%)	54.1% (42% to 65.9%)

Cardiac lesions

The majority of patients recruited in this study had some sort of cardiac lesion. This may be because most patients were referred with cardiac lesions, and cardiologists have a higher index of suspicion and are more likely to screen patients for 22qDS. All cardiac lesions were congenital and ranged from acyanotic to cyanotic lesions. 121 patients were found to have cardiac disease in this study. Of these patients, 44% were proven FISH positive patients with 22qDS. Current literature reports prevalence of conotruncal CHD between 20-30%, with non-conotruncal anomalies occurring in a further 11-20% of patients. Thus, our study seemed to reflect the trend of current literature. 5% of our study population had no cardiac abnormalities at all, which is somewhat less than the reported percentage in the literature (up to 20%). There was no missing data with regards to congenital heart disease.

Table 10. Prevalence of CHD lesions in study

Congenital Heart Disease	Number (%) of patients affected
Non-isolated VSD	66 (46%)
PDA	33 (22.9%)
Tetralogy of Fallot	30 (20.8%)
Pulmonary stenosis/ Pulmonary artery stenosis	30 (20.8%)
ASD	22 (15.2%)
Truncus arteriosus	21 (14.5%)
Coarctation of the Aorta	9 (6%)
Interrupted Aortic arch	9 (6%)
Transposition of the great arteries	2 (1.4%)
Aortic stenosis	2 (1.4%)
Other	27 (18.7%)

Few cardiac defects occurred in isolation. Patients with tetralogy of Fallot were also found to have other associated cardiac abnormalities, including a right-sided aortic arch, ASD or MAPCA's. Non-isolated VSD's were associated with ASD, right-sided aortic arch or interrupted aortic arch, PDA, left pulmonary artery stenosis, double outlet right ventricle and pulmonary stenosis or PPS. 6 patients also had a truncus arteriosus with VSD present, which is to be expected. PDA's were found in both cyanotic and acyanotic heart lesions, namely coarctation of the aorta, VSD, ASD, pulmonary stenosis, transposition of the great arteries and univentricular heart. Only 2 patients had TGA and tricuspid atresia.

Other lesions included AVSD, dilated main pulmonary arteries, MAPCA's, small pulmonary arteries, TAPVD and univentricular heart.

The sensitivity, specificity, PPV and NPV were calculated for the most common occurring cardiac lesions in 22qDS.

Table 11. Sensitivity, specificity, PPV and NPV of cardiac variables

CARDIAC LESION	SENSITIVITY (95% confidence interval)	SPECIFICITY (95% Confidence interval)	POSITIVE PREDICTIVE VALUE (95% Confidence interval)	NEGATIVE PREDICTIVE VALUE (95% Confidence interval)
VSD	59% (46.2% to 71%)	57.6% (45.9% to 68.8%)	54.1% (42% to 65.9%)	62.1% (50.3% to 73.6%)
Tetralogy of Fallot	53.3% (34.3% to 71.6%)	50.8% (41.3% to 60.3%)	22.2% (13.2% to 33.5%)	80.5% (69.5% to 88.9%)
Truncus arteriosus	71.4% (47.8% to 88.7)	53.6% (44.4% to 62.6%)	20.8% (12.15 to 32%)	91.6% (82.7% to 96.8%)
PDA	42.4% (25.4% to 40.7%)	47.7% (38.1% to 57.4%)	19.4% (11% to 30.4%)	73.6% (61.9% to 83.3%)
Pulmonary stenosis/Pulmonary artery stenosis	40% (22.6% to 59.4%)	47.3% (37.9% to 56.9%)	16.6% (8.9% to 27.3%)	75% (63.4% to 84.4%
ASD	45.4% (24.3% to 67.7%)	49.1% (40% to 58.3%)	13.8% (6.8% to 24%)	83.3% (72.7% to 91%)
Interrupted aortic arch	77.1% (39.9% to 97.1%)	51.8% (43% to 60.5%)	9.7% (4% to 19%)	97.2% (90.3% to 99.6%)
Coarctation	11.1% (0.2% to 48.2%)	47.4% (38.7% to 56.1%)	1.3% (0.04% to 7.5%)	88.8% (79.2% to 95%)
TGA	0% (0 to 84.1%)	49.3% (38.7% to 56.1%)	0% (0 to 4.9%)	97.2% (90.3% to 99.6%)
Aortic stenosis	0% (0 to 97.5%)	49.6% (41.1% to 58.1%)	0% (0 to 4.9%)	98.6% (92.5% to 99.9%)

The cardiac variables with the highest sensitivity for 22qDS were truncus arteriosus (71.4%) and interrupted aortic arch (77.1%) while the lowest sensitivities were associated with TGA and aortic stenosis (which were both 0%). The highest specificity of 22qDS in congenital heart disease was non-isolated VSD (57.6%).

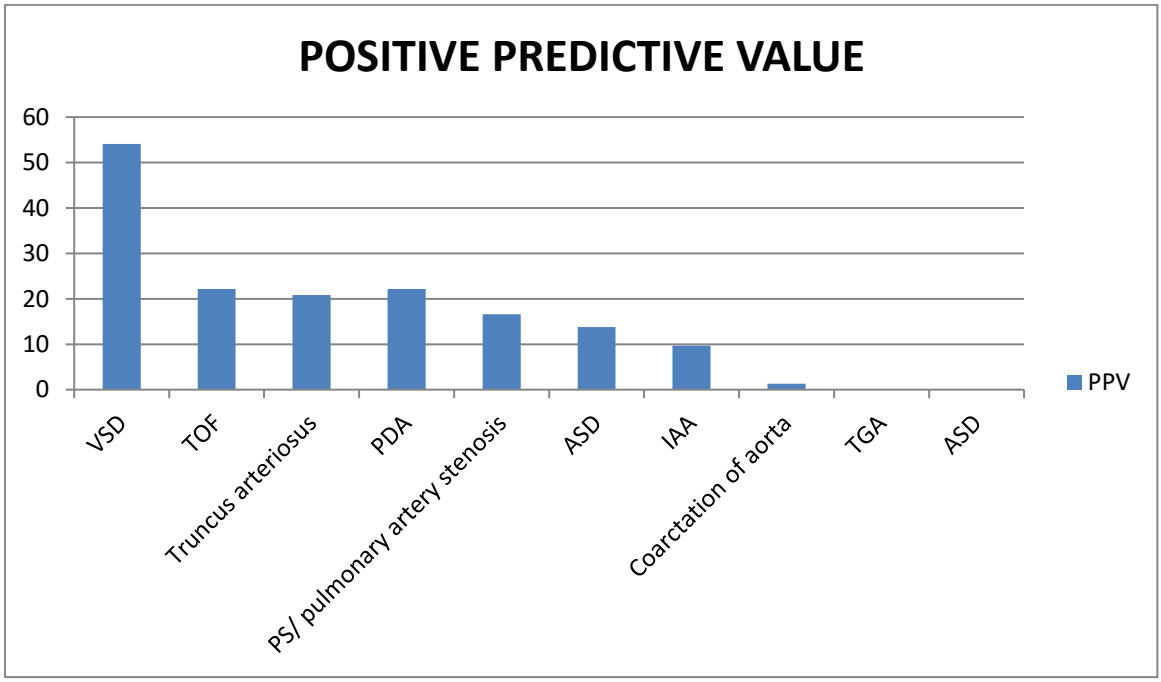
The specificity of truncus arteriosus and interrupted aortic arch were 53.6% and 51.8%, respectively. All other cardiac variables ranged in specificity between 47% and 50%.

The cardiac variable with the highest positive predictive value for 22qDS was non-isolated VSD (54.1%). The cardiac variables with the lowest PPV were coarctation of the aorta (1.3%), and TGA and aortic stenosis (both 0%).

Finally, the highest negative predictive value for 22qDS was attributed to TGA (97.2%), aortic stenosis (98.6%), interrupted aortic arch (97.2%) and truncus arteriosus (91.6%).

The cardiac variables with the lowest NPV included PDA (73.6%) and non-isolated VSD (62.5%).

Figure 5. Comparison of the cardiac variables with the highest PPV for 22qDS



Phenotypic features

The most commonly found phenotypic features of 22qDS in this study are listed in Table 12. The most common phenotypic features in this study population included cardiac lesions, dysmorphic features, failure to thrive, recurrent infections, developmental delay and speech/language abnormalities. The FISH result was more likely to be positive in children with cardiac disease, recurrent infections, developmental delay and speech/language abnormalities. In the case of failure to thrive and dysmorphic features, FISH positive patients and FISH negative patients were similar in number.

Table 12. Most common phenotypic features recorded in study

Phenotypic feature	FISH positive	FISH negative	Total Patients
1. VPI	17 (89%)	2	19
2. Immune deficiency	14 (87%)	2	16
3. Thymic aplasia/ hypoplasia	10 (83%)	2	12
4. Apnoea	6 (75%)	2	8
5. Hypocalcaemia	28 (73.6%)	10	38
6. Seizures	17 (73.9%)	6	23
7. Speech/ language problems	39 (72.2%)	15	54
8. Cleft palate	9 (69.2%)	4	13
9. Recurrent infections	55 (63.2%)	32	87
10. Developmental delay	44 (62.8%)	26	70
11. Recurrent otitis media	17 (60.7%)	11	28
12. Feeding problems	29 (60.4%)	19	48
13. Behavioural issues	9 (60%)	6	15
14. Stunting	8 (57.1%)	6	14
15. Dysmorphic features	46 (52.8%)	41	87
16. Failure to thrive	48 (52.7%)	43	91
17. Upper airway obstruction	12 (52.1%)	11	23
18. Cardiac lesion	54 (44.6%)	57	121

Dysmorphic features

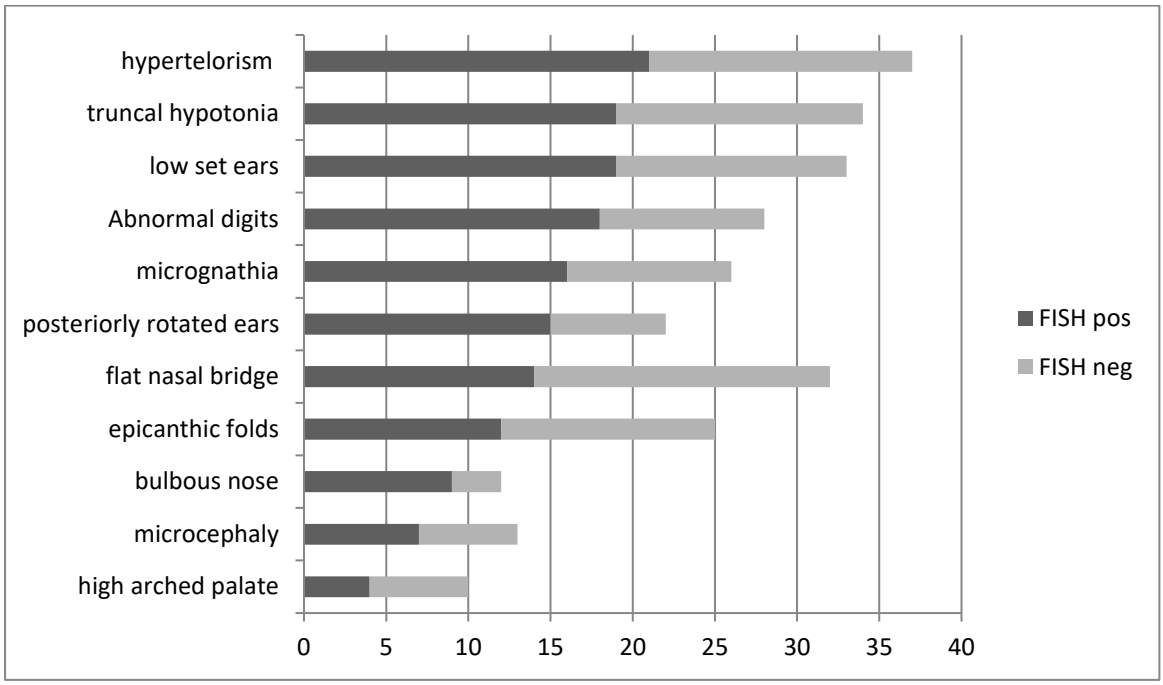
Dysmorphic features were noted in 87 patients in this study. 32% of these patients were confirmed FISH positive for 22qDS. Minimal data was missing from the folders (4%).

35 dysmorphic features noted to occur in association with 22qDS was cross-referenced with all 144 patients in our study population. The most commonly occurring dysmorphic features are recorded in table 13.

Table 13. Common dysmorphic features associated with 22qDS

Dysmorphic features	**FISH positive**	**FISH negative**	**Total**
Hypertelorism	21 (15%)	16 (10%)	37
Decreased truncal tone	19 (13%)	15 (10%)	34
Low set ears	19 (13%)	14(10%)	33
Abnormal digits	18 (13%)	10 (7%)	28
Micrognathia	16 (11%)	10 (7%)	26
Flat nasal bridge	14 (10%)	18 (13%)	32
Posteriorly rotated ears	15 (10%)	7 (4%)	22
Epicanthic folds	12 (8%)	13 (8%)	25
Bulbous nose	9 (6%)	3 (2%)	12
Microcephaly	7 (5%)	6 (5%)	13
High arched palate	4 (3%)	6 (4%)	10

Figure 3. Dysmorphic features in FISH positive patients versus FISH negative patients for 22qDS



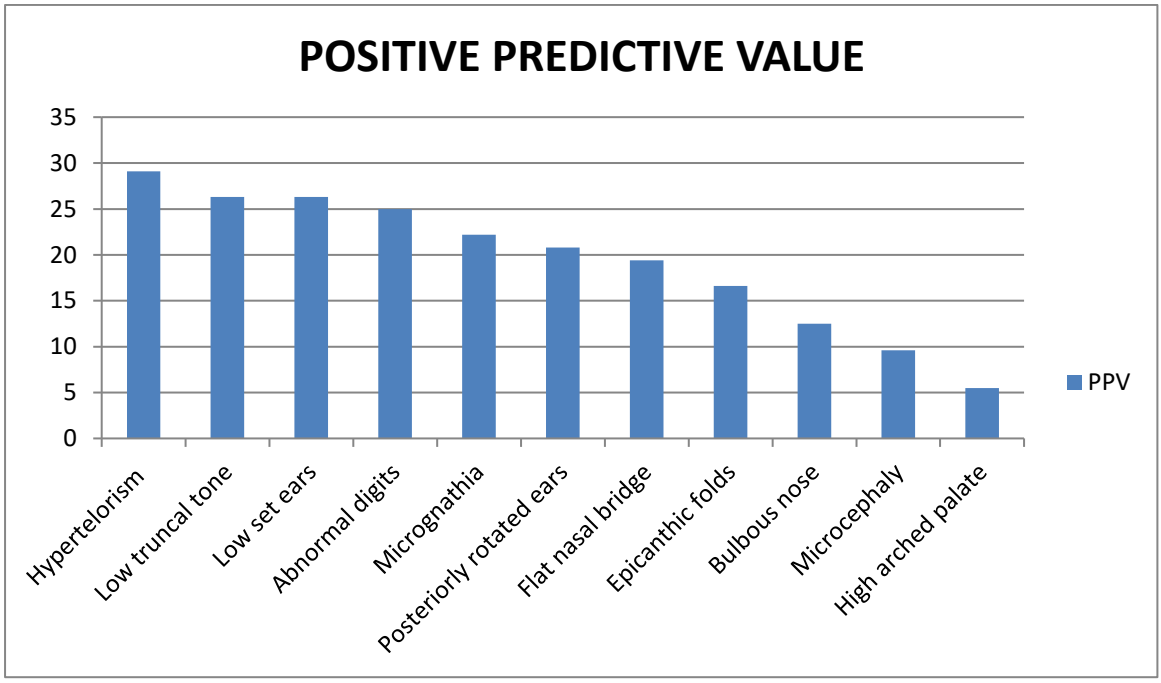
In general, these common dysmorphic features occurred more in the FISH positive arm of the study. Hypertelorism, abnormal digits and posteriorly rotated ears were far more common in FISH positive than in FISH negative patients. The only features that occurred in equal proportion in both arms (FISH positive and FISH negative patients) were microcephaly, abnormal digits, and epicanthic folds.

The least commonly occurring dysmorphic features included the presence of a third fontanel, a thin long face, macrocephaly, low hairline, short sternum and short limbs. Other uncommon features in FISH positive patients with 22qDS included large ears, pes planus, synophrys and increased nuchal skin.

Table 14. Sensitivity, specificity, positive predictive value and negative predictive value of dysmorphic features in 22qDS

DYSMORPHIC FEATURES	SENSITIVITY (95% confidence interval)	SPECIFICITY (95% confidence interval)	POSITIVE PREDICTIVE VALUE (95% confidence interval)	NEGATIVE PREDICTIVE VALUE (95% confidence interval)
Hypertelorism	56.7% (39.4% to 72.9%)	52.3% (42.6% to 62%)	29.1% (19% to 41%)	77.7% (66.4% to 86.7%)
Truncal hypotonia	55.8% (37.8% to 72.8%)	51.8% (42% to 61.4%)	26.3% (16.7% to 38.1%)	79.1% (67.9% to 87.8%)
Low set ears	57.8% (39.2% to 74.5%)	52.2% (42.5% to 61.8%)	26.3% (16.7% to 38.1%)	80.5% (69.5% to 88.9%)
Abnormal digits	64.2% (44% to 81.3%)	53.4% (43.9% to 62.7%)	25% (15.5% to 36.6%)	86.1% (75.9% to 93.1%)
Micrognathia	61.5% (40.5% to 79.7%)	52.5% (43.1% to 61.8%)	22.2% (13.2% to 33.5%)	86.1% (75.9% to 93.1%)
Posteriorly rotated ears	68.1% (45.1% to 86.1%)	53.2% (44% to 62.3%)	20.8% (12.1% to 32%)	90.2% (80.9% to 96%)
Flat nasal bridge	43.7% (26.3% to 62.3%)	48.2% (38.6% to 57.8%)	19.4% (11% to 30.4%)	75% (63.4% to 84.4%)
Epicanthic folds	48% (27.8% to 68.6%)	49.5% (40.2% to 58.8%)	16.6% (8.9% to 27.3%)	81.9% (71.1% to 90%)
Bulbous nose	75% (42.8% to 94.5%)	52.2% (43.4% to 61%)	12.5% (5.8% to 22.6%)	95.8% (88.3% to 99.1%)
Microcephaly	53.8% (25.1% to 80.7%)	49.2% (402% to 58.2%)	9.7% (4% to 19%)	91.3% (82% to 96.7%)
High arched palate	40% (12.1% to 73.7%)	49.2% (40.5% to 58%)	5.5% (1.5% to 13.6%)	91.6% (82.7% to 96.8%)

Figure 4. The PPV of various dysmorphic features in 22qDS



Dysmorphic features with the highest sensitivity for 22qDS were bulbous nose (75%), posteriorly rotated ears (68%) and abnormal digits (64%). Posteriorly rotated ears and abnormal digits were also associated with the high specificity for 22qDS (53.2% and 53.4%, respectively). Other features with similar specificity included hypertelorism (52.3%), bulbous nose (52.2%), micrognathia (52.2%) and low set ears (52.2%).

Lowest sensitivity for the microdeletion syndrome was associated with high arched palate (40%), flat nasal bridge (43.7%) and epicanthic folds (48%).

The highest positive predictive value for 22qDS was with hypertelorism (29.1%), followed by truncal hypotonia, low set ears (26.3% each), and abnormal digits (25%). Microcephaly only had a PPV of 9.7%.

The highest negative predictive value for 22qDS was associated with bulbous nose (95.8%), high palate (91.6%), microcephaly (91.3%) and posteriorly rotated ears (90.2%). The lowest negative predictive values were attributed to truncal hypotonia (79%), hypertelorism (77%) and flat nasal bridge (75%).

Discussion

This study comprised a folder review of 144 FISH tested patients - 72 patients confirmed FISH positive for 22qDS and another 72 patients with features suggestive of 22qDS but FISH negative results. The FISH negative patients functioned as the control arm of this study. The range of age of presentation of the above-mentioned patients was quite variable (D1 of life to 18 years of age). The mean age of presentation was 11 months.

Even though the FISH database had listed over 120 FISH positive patients with 22qDS over the last 20 years, only 72 folders were retrievable. The remaining folders had been archived at a separate facility to which we had no access, or the folders had been lost.

The study population was quite small. For this reason, specific phenotypic features in each age group were not done. The neonatal patients were well represented while others were poorly represented (namely, the adolescent and older childhood age groups). Thus it was decided instead to look at the overall phenotypic features with which children presented at our institution. In a follow up study, it would be helpful to attempt a full cluster analysis and compare phenotypic features between different age groups. Literature specifically pertaining to multi-dimensional analyses in 22qDS is quite scarce. Also, as both genders are equally represented in 22qDS, I did not compare findings in males and females.

In this study, a positive family history of 22qDS was noted in 13 patients (including FISH positive and FISH negative patients). Of these 13, only 5% of patients were then proven FISH positive for the same syndrome. This was slightly lower than reported in the literature, where 7-10% of patients inherit the syndrome from one or both of their parents.(1, 4) This may be a falsely low prevalence of disease as the numbers of this study population was quite low. Also, family history was not always noted in patients' folders, so this prevalence may be underestimated.

The main presenting complaints were (in order of decreasing prevalence): CHD, FTT, dysmorphic features, developmental delay, cyanosis and congestive cardiac failure. Except for cyanosis, all other complaints occurred more commonly in the FISH positive group as compared to the FISH negative group. 44% of all the patients with CHD in this study were noted to be FISH positive. However, in FISH positive patients, the prevalence of CHD was 88%. The prevalence of CHD in our study is noted to be higher than other studies, including Oskarsdottir et al, where 54% of FISH positive patients had CHD (many which occurred in the younger age group) and McDonald-McGinn et al, whose research at CHOP yielded a prevalence of 77% for CHD in 22qDS.(5, 10) Studies in non-Caucasian populations also yielded lower rates of CHD of 50-60%.(38, 41) The high prevalence of CHD in our population may be attributed to bias as most our patients were recruited from the cardiology department at RCWMCH. Our hospital is a major referral hospital for children in Sub Saharan Africa.

The majority of patients were referred to our centre for further management of their cardiac issues. The high prevalence of cardiac disease in FISH positive patients in this study may also be due to increased screening of cardiac defects in patients known with 22qDS. Other presenting complaints, like developmental delay or failure to thrive were less likely to be referred to our institution as they can be managed effectively at their presenting hospitals.

The prevalence of FTT in FISH positive patients was 66%, while the prevalence of developmental delay in FISH positive patients was 61%. 63% of FISH positive patients presented with dysmorphic features. However, this data may be somewhat skewed as some of the data pertaining to these features were missing from some folders.

66% of patients presented with failure to thrive. This phenotypic feature is not described well in the literature. It may well be that the high prevalence of failure to thrive be due to the poor social circumstances of this patient population and the suboptimal economic climate of our country, South Africa being a low to middle income country.

Developmental delay was noted in 61% of FISH positive patients in this current study. This was lower than described by Oskarsdottir et al where developmental delay occurred in 96% of their study population. 20% of these cases involved behavioural issues.(10) Veerapandiyan et al found that, in the African- American population, developmental delay occurred in 58% of patients with 22qDS.(38) Repetto et al, in a Chilean study on 22qDS, noted developmental delay in 85% of patients with the syndrome.(41) Our study yielded a lower prevalence of developmental delay than that of the current literature, with the exception of the study done by Veerapandiyan et al. This may be due to population bias, with our neonatal study population numbers being greater than the older childhood/adolescent population numbers.

Palatal involvement and feeding abnormalities were not as common a presenting feature in this study as compared to other studies. Recurrent infections were common in this study, but it was uncertain if this was due to an underlying immune deficiency or due to possible invasive procedures performed at our centre i.e. cardiac catheterization, post-operative complication of cardiac surgery, recurrent aspiration secondary to VPI or truncal hypotonia and severe malnutrition. Prevalence of immune deficiency and thymic abnormalities was not evaluated as most patients had this data missing from their folders. This is likely because many clinicians find these issues to be of less diagnostic value for 22qDS and are more likely to screen patients for these problems once the diagnosis has been confirmed, and patients present with signs or symptoms suggestive of these disorders.

Because this was a descriptive study, the aim was to focus on the phenotypic features of 22qDS which would be most helpful in the clinical screening of the syndrome. The presenting complaint with the highest sensitivity and specificity for 22qDS was developmental delay (62.8% and 68%, respectively). The specificity of CHD for 22qDS was also good as at 65%. Positive predictive value was highest for CHD and FTT at 88% and 73%, respectively. The negative predictive value was highest for cardiac failure. The variable of cyanosis was not found to be particularly useful, as the sensitivity, specificity and predictive values were all just above 50%.

121 out of 144 patients were found to have cardiac disease. Of these patients, 44% were proven FISH positive patients with 22qDS. Current literature reports prevalence of conotruncal CHD between 20-30%, with non-conotruncal anomalies occurring in a further 11-20% of patients. Thus, our study seemed to reflect the trend of current literature. 5% of the study population had no cardiac abnormalities at all, which is somewhat less than the reported percentage in the literature (up to 20%).

The most common cardiac lesions associated with FISH positive patients in this study included: non-isolated VSD (27%), tetralogy of Fallot (11%), truncus arteriosus (10%), PS/pulmonary artery stenosis (8%), ASD (7%) and interrupted aortic arch (5%). No AS, TGA or TAPVD was noted in any FISH positive patients in this study. The current study compared to previous studies as follows: the most common cardiac lesion described in patients with 22qDS was VSD (27%). This was similar to the prevalence quoted by McDonald-McGinn (21%).(1) Prevalence of TOF (11%) was described in this study as comparable to the prevalence of 13% by Peyvandi et al.(42) However, studies done by both McDonald McGinn and Repetto et al had a slightly higher prevalence of TOF in their study population (both were 20%).(1, 41) In this study, truncus arteriosus was described in 10% of patients with 22qDS. This is much lower than described in Peyvandi et al (35%).(42) However, it is only slightly higher than described in McDonald-McGinn and Repetto et al.(1, 41) IAA was reported far less than was reported in studies by McDonald- McGinn and Peyvandi et al, but had a similar prevalence to the study done by Repetto et al.(1, 41, 42)

Few cardiac lesions occurred in isolation in patients with 22qDS. Cardiac lesions associated with right sided aortic arch, DORV, MAPCA's were more likely to be FISH positive patients. This is in agreement with the current literature.

In this study, the cardiac lesion with the highest sensitivity for 22qDS was truncus arteriosus (71.4%). IAA was also associated with a high sensitivity for 22qDS (77.7%). The least sensitive cardiac lesion was noted to be PS/Pulmonary artery stenosis (40%). The cardiac lesions with the highest specificity for 22qDS were VSD and TA (57.6% and 53.6%, respectively).

The cardiac lesion with the highest positive predictive value for 22qDS was non-isolated VSD (54%), while the lowest PPV was attributed to TGA and AS (both 0%). The lesions with the highest NPV included AS (98.6%), TGA (97.2%) and IAA (97.2%).

More than 180 phenotypic features are associated with 22qDS.(6) Not all of this data was available from patients' folders as many folders were already archived at a remote facility, incomplete or missing.

Oskarsdottir et al found that 35% of children less than 2 years of age and 53% of children older than 2 years of age with 22qDS were noted to be dysmorphic.(10) McDonald-McGinn et al described dysmorphic features as important minor phenotypic features of the syndrome. These included bulbous nose, hooded eyelids and micrognathia (60%, 25% and 21%, respectively).(1, 5) Another multi-centre study done by Veerapandiyan et al on African-American patients with 22qDS showed 64% of patients presented with ear abnormalities, including low set ears, posteriorly rotated ears and abnormal helices of the ears. 40% of patients had a wide nasal bridge. 22% of patients had short palpebral fissure. Less commonly described were patients with micrognathia (16%), hypertelorism (14%), bulbous nose (12%) and hooded eyelids (12%).(38) Finally, Oskarsdottir et al in another study in 2008, described the facial features of 90 children with 22qDS. More than 50%of patients presented with malar flatness, hooded eyelids, bulbous nose, round or broad ears, thick or overfolded helices of the ear and low set ears.(39)

In this study at Red Cross War Memorial Children's Hospital, 87 of the 144 patients in our study were noted to be dysmorphic. Of these, 32% were confirmed 22qDS with positive FISH test. Considering the majority of our study population were younger children and infants, this number is in keeping with the current literature. The most common dysmorphism features in patients with 22qDS were noted to be hypertelorism (15%), truncal hypotonia (13%), low set ears (13%), abnormal digits (13%), micrognathia (11%) and posteriorly rotated ears (10%). The presence of a bulbous nose was only noted to be present in 6% of the patients with 22qDS. (62) These figures correlated more with the study by Veerapandiyan, in the African American population with 22qDS, though ear abnormalities occurred far less than described in the aforementioned study. This may be because the majority of our study population is also non-Caucasian.(38)

The dysmorphic features with the highest sensitivity for 22qDS were bulbous nose (75%) and posteriorly rotated ears (68%), while the high arched palate had the lowest sensitivity at 40%. The highest specificity was noted to be abnormal digits and posteriorly rotated ears (53% for both). Again, the lowest specificity was associated with high arched palate and microcephaly (both 49%). The highest PPV for 22qDS was associated with hypertelorism (29%) and truncal hypotonia (26%).

Lastly the highest NPV was associated with bulbous nose (95.8%), high arched palate (91.6%) and microcephaly (91.3%). The features with the lowest NPV included a flat nasal bridge (75%) and hypertelorism (77.7%).

Current guidelines for the screening of 22qDS include the Oskarsdottir score (See table below).(10) However, at present, this guideline has been largely ineffective in our particular population at RCWMCH. This is likely due to the fact that the above mentioned study was performed in Sweden and consisted of a largely Caucasian population group. It is well documented that non-Caucasian population groups often have different phenotypic features. Our current review on the phenotypic features of children with 22qDS has revealed the most common presentations to be CHD, FTT, dysmorphic features as described above, and cardiac failure. Younger children present more frequently with CHD, while older children present with developmental delay and dysmorphic features. Our particular 22qDS patient population presented with the following CHD: non-isolated VSD, tetralogy of Fallot, truncus arteriosus, PS/pulmonary artery stenosis and interrupted aortic arch. Interrupted aortic arch was found to be a sensitive marker for 22qDS in children with cardiac lesions.

Table 15. Guidelines for testing for 22qDS (10)

CLINICAL DOMAIN	INFANTS	PRESCHOOL	CHILDHOOD TO ADOLESCENCE
1. Cardiac defect	Cardiac defect (TOF, IAA, truncus arteriosus, PA+VSD, VSD+arch anomaly)	Cardiac defect (TOF, IAA, truncus arteriosus, PA+VSD, VSD+arch anomaly)	Cardiac defect (TOF, IAA, truncus arteriosus, PA+VSD, VSD+arch anomaly)
2. Immunodeficiency	Thymus aplasia/hypoplasia Immunodeficiency Infection	Infection (respiratory tract infection, middle ear infection)	Infection Autoimmune phenomena
3. Abnormal calcium metabolism	Hypocalcaemia	Hypoparathyroidism	Hypoparathyroidism
4. Poor feeding	Poor feeding	Poor feeding	Poor feeding
5. Cleft palate	Cleft palate	Speech/language impairment VPI	VPI
6. Developmental issues	Developmental delay	Developmental delay Behavioural abnormalities	Learning difficulties Behavioural abnormalities
7. Other abnormalities (skeletal, clubfoot, hernia, etc)	Other abnormalities (skeletal, clubfoot, hernia, etc)	Other abnormalities (skeletal, clubfoot, hernia, etc)	Other abnormalities (skeletal, clubfoot, hernia, etc), including scoliosis
8. Dysmorphic features	Dysmorphic features	Dysmorphic features	Dysmorphic features

2 or more of the following 8 clinical domains are required to consider the use of genetic testing for possible 22qDS; If a typical cardiac defect is found in infancy, this is sufficient for testing for 22qDS.

Other important phenotypic features shown to be less prevalent in our population included primary immune deficiency, thymus abnormalities, cleft palate, velo-pharyngeal insufficiency, feeding abnormalities and behavioural issues. It is not certain whether this is a true reflection of our population's features as there was missing data in a large portion of folders regarding these particular features.

This study has several strengths: The phenotypic features of 22qDS in our local population were described for the first time. This study also had a control arm of FISH negative patients, and thus comparative analysis could be done to ascertain the features that best predicted a clinical diagnosis of 22qDS. A further study on the phenotypic features of 22qDS using multi dimensional analysis in this same population group will aid clinical recognition of the syndrome. There are very few studies published on multi dimensional analysis to date.

However, there were also several limitations to this study. The study population was small and there were several folders that were archived and unobtainable for review. Also, many folders were incomplete with variable amounts of missing data. This is likely because many folders were from specialist clinics and thus focussed on particular complications of 22qDS as opposed to thorough assessment of all the features of the syndrome. Another limitation was the inability to describe the demographics of our study population accurately. Many patient folders did not specify the race of the patient. While most patients were clearly non-Caucasian by way of their birth names, it was particularly difficult to separate Caucasian patients from Coloured patients as their names were often very similar. Lastly, DORV was intermittently recorded in patients' folders as occurring in the presence of a VSD. This does not occur pathophysiologically however. Thus the data for VSD positive patients with 22qDS may not be completely accurate and may in fact, have a slightly lower prevalence in 22qDS.

In conclusion, it is clear that non-Caucasian populations have some unique phenotypic expressions of 22qDS. It is imperative that clinicians maintain a high index f suspicion for patients with 22qDS. All children with conotruncal abnormalities should be screened for 22qDS. Also, all children with CHD as well as IAA should also be screened for 22qDS as this has been shown to be a sensitive marker for this syndrome. Patients presenting with CHD, FTT, dysmorphic features and developmental delay should also be screened for 22qDS as these have been shown to be the most common phenotypic expression of disease in our geographical context.

There are very few studies on multidimensional analysis of 22qDS, particularly in non-Caucasian population groups. It would be helpful in a follow up study to further describe the phenotypic features of this syndrome, specifically looking at clusters of features that may occur within our population that may prove to be more sensitive for 22qDS. We also hope to do a complete cluster analysis on these same features and finally, to develop an algorithm to better identify patients with 22qDS in our setting. A future study should ascertain which cluster of phenotypic features are most common in each particular age group.

This could be done using classification trees to analyse each individual variable, and in this way establishing whether certain features cluster together in 22qDS patients in our setting. This can then be converted to a possible scoring system which could be tested in a prospective study.

APPENDIX

Bassett el al (20) suggested some practical guidelines in the management of patients with 22qDS. Different screening tests are suggested at various ages.

In 2011, Bassett et al published guidelines on the management of 22qDS. The guidelines were based on two international 22qDS consensus meetings, as well as a systematic review of 239 publications on 22qDS. Guidelines published included a list of the multi system features, recommendations at diagnosis and considerations for genetic counselling.(13)

Guidelines as proposed by Bassett et al:

Table I. Multisystem features of 22q11.2 deletion syndrome

Common features*	Relevant age groups: Prenatal	Infant to child	Teen to adult	Selected rarer features†	Management: Standard‡	Special considerations or attention	Specialties commonly involved (in addition to family medicine, pediatrics, general internal medicine, radiology)
General genetics • Dysmorphic features (>90% of cases)‖ • Multiple congenital anomalies • Learning disability/mental retardation/developmental delay (90%) • Poly-hydramnios (16%)	✓	✓	✓	• Fetal loss or infant death	✓	• Genetic counseling • Medical management • Gynecological and contraceptive services	• Medical genetics • Obstetrics and gynecology
Cardiovascular (conotruncal/other) • Any congenital defect (including minor) (50%-75%) • Requiring surgery (30%-40%)	✓	✓	✓	• Vascular ring • Dilated aortic root • Arrhythmias	✓	• Echocardiogram • Irradiated blood products for infant surgeries • Calcium level	• Cardiovascular surgery • Cardiology
Palatal and related (75%) • Hypernasal speech (crying) and/or nasal regurgitation (>90%) • Velopharyngeal insufficiency ± submucous cleft palate (overt cleft palate/cleft lip is less common) • Chronic and/or secretory otitis media • Sensorineural and/or conductive hearing loss (30%-50%)	✓	✓	✓	• Laryngeal web • Tracheo-esophageal fistula • Esophageal atresia • Preauricular tags/pits** • Microtia/anotia**	✓	• Speech therapy • Palatal surgery	• Speech pathology • Plastic surgery/Cleft palate team • Otorhinolaryngology • Audiology
Immune-related§ • Recurrent infections (35%-40%) • T-cells low and/or impaired function • Autoimmune diseases		✓	✓	• Immunoglobulin A deficiency • Severe immunodeficiency (0.5%-1%)	✓	• Influenza vaccinations • Special protocol¶ for infants	• Immunology • Rheumatology • Otolaryngology • Allergy • Respirology
Endocrine • Hypocalcemia and/or hypoparathyroidism (>60%) • Hypothyroidism (20%), hyperthyroidism (5%) • Obesity (35%, adults)		✓	✓	• Growth hormone deficiency • Type 2 diabetes	✓	• Vitamin D and calcium supplementation‖ • Growth hormone • Dietary/exercise counseling	• Endocrinology • Dietician
Gastroenterological • Gastro-esophageal reflux • Dysmotility/dysphagia (35%) • Constipation • Cholelithiasis (20%) • Umbilical/inguinal hernia	✓	✓	✓	• Aspiration • Imperforate anus • Intestinal malrotation • Hirschsprung's • Diaphragmatic hernia	✓	• Tube feeding • (Gastrostomy Nissen)	• Gastroenterology • General surgery • Feeding team • Respirology
Genitourinary • Structural urinary tract anomaly (31%) • Dysfunctional voiding (11%)	✓	✓	✓	• Echogenic/hypoplastic kidneys • Duplex kidney • Hydronephrosis	✓	• Ultrasound • Transplant	• Urology • Nephrology • Gynecology
• Structural urinary tract anomaly (31%) • Dysfunctional voiding (11%) • Unilateral renal agenesis (10%) • Multicystic dysplastic kidneys (10%)				• Duplex kidney • Hydronephrosis • Hypospadias • Cryptorchidism • Absent uterus • Nephrocalcinosis		• Transplant	• Nephrology • Gynecology • Radiology
Ophthalmology • Strabismus (15%) • Refractory errors • Posterior embryotoxon, tortuous retinal vessels**		✓		• Sclerocornea • Coloboma • Ptosis	✓	• Eye exam	• Ophthalmology

(continued)

(13)

Table I. Continued

Common features*	Relevant age groups: Prenatal	Infant to child	Teen to adult	Selected rarer features†	Management: Standard‡	Special considerations or attention	Specialties commonly involved (in addition to family medicine, pediatrics, general internal medicine, radiology)
Skeletal • Scoliosis (45%; 6% requiring surgery) • Cervical spine anomalies/thoracic butterfly vertebrae • Idiopathic leg pains in childhood • Sacral sinus	✓	✓	✓	• Cervical cord compression • Craniosynostosis • Upper/lower extremity pre and post axial polydactyly	✓	• Radiographs • Orthotics	• Orthopedics • Neurosurgery • Radiology • General surgery • Hand surgery • Physiotherapy
Hematology/Oncology • Thrombocytopenia (30%) • Splenomegaly (10%)		✓	✓	• Idiopathic thrombocytopenia • Bernard-Soulier • Autoimmune neutropenia • Leukemia, lymphoma, hepatoblastoma	✓	• Surveillance	
Neurologic • Recurrent (often hypocalcemic) seizures (40%, adults) • Unprovoked epilepsy (5%)		✓	✓	• Polymicrogyria • Cerebellar abnormalities • Neural tube defects • Abdominal migraines	✓	• Calcium, magnesium levels • Electroencephalogram • Magnetic resonance imaging	• Neurology
Growth and development • Failure to thrive • Motor and/or speech delays (>90%) • Learning disabilities (>90%); mental retardation (~35%) • Short stature (20%)	✓	✓	✓		✓	• Early intervention • Sign language • Educational supports • Vocational counseling	• Developmental pediatrics • Speech language pathology • Occupational/physical therapy • Neuropsychology • Educational psychology
Neuropsychiatric disorders • Psychiatric disorders (60%, adults) • Childhood disorders (eg, attention-deficit, autism spectrum disorders) • Anxiety and depressive disorders • Schizophrenia and other psychotic disorders (>20%)		✓	✓		✓	• Surveillance • Standard treatments	• Psychiatry • Developmental pediatrics
Other • Non-infectious respiratory disease (10-20%) • Seborrhea or dermatitis (35%); severe acne (25%) • Patellar dislocation (10%) • Dental problems—enamel hypoplasia/chronic caries • Varicose veins (10%)		✓	✓		✓		• Respirology/Pulmonary/Anesthesia • Dermatology • Rheumatology • Orthopedics • Dentistry • Vascular surgery

*Rates are estimates only of lifetime prevalence of features for 22q11DS and will vary depending on how cases are ascertained and age of the patient. Features included have prevalence >1% in 22q11DS and significantly higher than general population estimates.
†A selected (and to some extent arbitrary) set of rarer features of note in 22q11DS, emphasizing patients needing active treatment.
‡Standard surveillance, investigations, and management according to involved condition(s).
§Characteristic facial features include long narrow face, malar flatness, hooded eyelids, tubular nose with bulbous tip, hypoplastic alae nasae, nasal dimple or crease, small mouth, small protuberant ears with thick overfolded/crumpled helices, and asymmetric crying facies.
¶Infants only: minimize infectious exposures; initially withhold live vaccines; cytomegalovirus-negative irradiated blood products; influenza vaccinations; respiratory syncytial virus prophylaxis.
||All patients should have vitamin D supplementation; patients with documented hypocalcemia, relative or absolute hypoparathyroidism, or both may have to have prescribed hormonal forms (eg, calcitriol) supervised by endocrinologist.
**May be important for diagnostic purposes.

(13)

Table II. Recommended assessments for 22q11.2 deletion syndrome*

Assessment	At diagnosis	Infancy (0-12 months)	Preschool age (1-5 years)	School age (6-11 years)	Adolescence (12-18 years)	Adulthood (>18 years)
Ionized calcium, parathyroid hormone†	✓	✓	✓	✓	✓	✓
Thyrotropin (thyroid-stimulating hormone)†	✓		✓	✓	✓	✓
Complete blood cell count and differential (annual)	✓	✓	✓	✓	✓	✓
Immunologic evaluation‡	✓	✓§	✓§			
Ophthalmology	✓		✓			
Evaluate palate¶	✓	✓	✓			
Audiology	✓	✓	✓			✓
Cervical spine (>age 4 years)			✓‖			
Scoliosis examination	✓		✓		✓	
Dental evaluation			✓	✓	✓	✓
Renal ultrasound	✓					
Electrocardiogram	✓					✓
Echocardiogram	✓					
Development**	✓	✓	✓			
School performance				✓	✓	
Socialization/functioning	✓	✓	✓	✓	✓	✓
Psychiatric/emotional/behavioral††	✓		✓	✓	✓	✓
Systems review	✓	✓	✓	✓	✓	✓
Deletion studies of parents	✓					
Genetic counseling‡‡	✓				✓	✓
Gynecologic and contraceptive services					✓	✓

*These recommendations are proposed as at year end 2010. Each ✓ refers to a single assessment except as stated above and below. We have tended to err on the side of overinclusiveness. Local patterns of practice may vary.
†In infancy, test calcium levels every 3 to 6 months, then every 5 years through childhood, and every 1 to 2 years thereafter; thyroid studies annually. Check calcium preoperatively and postoperatively and regularly in pregnancy.
‡In addition to complete blood cell count with differential, in newborns: flow cytometry; and at age 9 to 12 months (before live vaccines): flow cytometry, immunoglobulins, T-cell function. Expert opinion is divided about the extent of needed immune work-up in the absence of clinical features.
§Evaluate immune function before administering live vaccines (see †).
¶In infancy, visualize palate and evaluate for feeding problems, nasal regurgitation, or both; in toddlers to adults, evaluate nasal speech quality.
||Cervical spine films to detect anomalies: anterior/posterior, lateral, extension, open mouth, skull base views. Expert opinion is divided about the advisability of routine radiography. Symptoms of cord compression are an indication for urgent neurological referral.
**Motor and speech/language delays are common; rapid referral to early intervention for any delays can help to optimize outcomes.
††Vigilance for changes in behavior, emotional state, and thinking, including hallucinations and delusions; in teens and adults, assessment would include at-risk behaviors (sexual activity, alcohol/drug use, etc).
‡‡See text for details.

(13)

Table III. Important cautions and considerations for patients with 22q11DS

Feature	Management suggestions
Aspiration pneumonia	Suctioning and chest physiotherapy may be necessary as preventions; small food portions may help; tube feeding frequently necessary
Autonomic dysfunction	Careful monitoring perioperatively and postoperatively and at times of major biological stress (eg, infections, major medical crises); provision of necessary support
Surgical complications of all types at a somewhat elevated likelihood compared to other patients (bleeding, atelectasis, seizures, difficult intubation)	Careful monitoring perioperatively and postoperatively, including ionized calcium, oxygen levels; availability of small intubation equipment
Narrow lumens (eg, airway, spinal canal, ear canals)	May need smaller sized intubation equipment Often need regular ear syringing to maximize hearing
Aberrant anatomy (anywhere)	Preparatory investigations and consideration before surgery
Aberrant vascular anatomy	Consider magnetic resonance angiography before pharyngoplasty
Adenoidectomy may worsen velopharyngeal insufficiency	Consider risk/benefit
Posterior pharyngeal flap intervention may cause sleep apnea	Consider risk/benefit
Hypocalcemia risk elevated at times of biological stress (eg, surgery, infection, burn, peripartum)	Monitoring of ionized calcium levels and consideration of increased dose of vitamin D, calcium treatment, or both
Hypocalcemia worsening factors (eg, alcohol, fizzy drinks, pancreatitis)	Minimize alcohol and pop intake; extra caution with pancreatitis; monitor calcium levels more closely
Hypocalcemia treatments may cause nephrocalcinosis	Carefully monitor therapy
Seizure diathesis	Consider myoclonic, absence or generalized seizures with apparent clumsiness/tripping, poor concentration or falls, respectively; investigate low calcium and magnesium levels and ensure adequate treatment; consider anticonvulsant medications as adjunctive medications for other medications that often lower the seizure threshold (eg. clozapine, other antipsychotic medications)
Sensitivity to caffeine	Reduce caffeine intake, especially cola, "energy" drinks, and coffee; consider as a contributory factor to anxiety and/or agitation and/or tremor
Developmental delays common in all aspects of development, structural and functional	Anticipating a slower trajectory and changing capabilities over time, with necessary supports provided, can help reduce frustrations and maximize function; a good match between the expectations and demands of the environment and the social and cognitive capabilities of the individual will minimize the risk of chronic stress and of exploitation
Increased need for sleep	Regular, early bedtime and more hours of sleep than other same-aged individuals can help reduce irritability and improve learning and functioning
Increased need for structure, routine, certainty, sameness	Environmental adjustments to improve stability and limit changes can help reduce anxiety and frustration
Constipation	Consider with verbal and especially non-verbal patients as a cause of agitation, pain, or both; routine measures, including hydration, exercise, fiber, bowel routine
Tendency to form cysts of all types	Routine
Pregnancy complications	Consider as a biological stressor for the individual in the context of their associated features and risks (eg, hypocalcemia, adult congenital heart disease, psychiatric diseases, seizure diatheses, and social situation)

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