RETROSPECTIVE REVIEW OF GENETIC CONDITIONS IN PATIENTS WITH CONGENITAL HEART DEFECTS ATTENDING A QUATERNARY PAEDIATRIC CARDIOLOGY SERVICE IN JOHANNESBURG, SOUTH AFRICA

Daisy Salome Mokwele

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of

Master of Medicine in Medical Genetics

Johannesburg, 2025

DECLARATION

I, Daisy Salome Mokwele, declare that this Research Report is my own unaided work. It is being submitted for the degree of Master of Medicine in Medical Genetics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signature: 

Daisy Salome Mokwele

27th January 2025 in Johannesburg

DEDICATION

To my husband and third supervisor, Nkokone, for the support and constantly checking on the progress of this project, thank you so much.

Our sons, Madimetja and Phuti, you inspire me to go so much further.

To my mother, for the reminder that I had not used all my God-given talents.

My father, for instilling a good work ethic in his children.

PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT

1. 14th International Congress of Human Genetics, 22-26th February 2023, Cape Town South Africa: Poster presentation. Retrospective review of genetic conditions in patients with congenital heart defects attending a quaternary paediatric cardiology service in Johannesburg, South Africa. Mokwele Daisy, Ntsinjana Hopewell, Krause Amanda.

2. Southern African Society for Human Genetics Biennial Congress, 27-29 October 2024, Sun City Resort, South Africa: Poster presentation. Retrospective review of genetic conditions in patients with congenital heart defects attending a quaternary paediatric cardiology service in Johannesburg, South Africa. Mokwele Daisy, Ntsinjana Hopewell, Krause Amanda.

ABSTRACT

Congenital heart defects (CHDs) are the most common major birth defects, with an estimated incidence of 6 per 1000 livebirths. The causes of CHDs remain poorly understood. Genetic and environmental factors are known to contribute. The study aimed to retrospectively review patients with CHDs attending a cardiology service for confirmed genetic conditions. A review of 181 clinical records of children (0 months to 18 years) seen between 1 January 2019 – 31 December 2019 was conducted. Genetic testing was performed on 58/181 (32%) patients and 29/181 (16%) were confirmed to have a genetic syndrome. Trisomy 21 was the predominant chromosomal aneuploidy and 22q11.2 deletion the predominant copy number variant detected. Single gene causes and other chromosomal copy number variants were underrepresented due to unavailability or non-requisition of tests. We recommend routine, multidisciplinary genetic assessment of patients with CHDs with expanded genetic testing to improve diagnostic rates, direct management and optimize outcomes.

Keywords: congenital, heart defects, genetic.

ACKNOWLEDGEMENTS

Research Supervisors:

- Prof. A. Krause, Head, Division of Human Genetics, National Health Laboratory Service and School of Pathology, Faculty of Health Sciences, The University of the Witwatersrand, Johannesburg, South Africa.
- Prof. H.N. Ntsinjana, Head of Unit, Department of Paediatric Cardiology, Nelson Mandela Children's Hospital and Department of Paediatrics and Child Health, Faculty of Health Sciences, The University of the Witwatersrand, Johannesburg, South Africa.

Thank you for your contribution and efforts towards this project.

Nelson Mandela Children's Hospital, patients, and families

Thank you for making this work possible.

Research Statistician: Dr. Clarence Yah

Thank you for your expertise and sharing your knowledge towards this work.

Division of Human Genetics, National Health Laboratory Service and School of Pathology, Faculty of Health Sciences, The University of the Witwatersrand, Johannesburg, South Africa.

Thank you for supporting this project.

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NOMENCLATURE/LIST OF ABBREVIATIONS AND SYMBOLS

AAP	American Academy of Paediatrics
ACMG	American College of Medical Genetics and Genomics
ACTC	Actin alpha cardiac gene
AHA	American Heart Association
APVR	anomalous pulmonary venous return
ASD	atrial septal defect
AVSD	atrioventricular septal defect
CFTR	cystic fibrosis transmembrane conductance regulator
CGH	comparative genomic hybridization
CHDs	congenital heart defects
CNS	central nervous system
CNVs	copy number variants
CTD	conotruncal defects
FISH	fluorescence in situ hybridization
GIT	gastrointestinal tract
ISCA	International Standards for Cytogenomic Arrays
IQR	interquartile range
JAG	jagged gene
LEFTY	left-right determination factor gene
LMIC	low and middle-income country
LVOTO	left ventricular outflow tract obstruction
MAPK	mitogen-activated protein kinase gene
MLPA	Multiple ligation-dependent probe amplification

MYH	myosin heavy chain gene
NHLS	National Health Laboratory Service
NMCH	Nelson Mandela Children's Hospital
NOTCH	neurogenic locus notch homolog protein gene
PDA	patent ductus arteriosus
PPS	peripheral pulmonary stenosis
QF-PCR	quantitative fluorescence polymerase chain reaction
REDCap	Research Electronic Data Capture
RVOTO	right ventricular outflow tract obstruction
SNP	single nucleotide polymorphism
SNV	single nucleotide variant
WES	whole exome sequencing
WGS	whole genome sequencing
Wnt	wingless-related integration site gene
ZIC	zinc-finger in cerebellum gene

CHAPTER 1

INTRODUCTION

1.1. Epidemiology of congenital heart defects

Congenital heart defects (CHDs) are a common group of major birth defects. These are structural cardiac defects that occur due to disruption of embryonic differentiation and cardiogenesis (Zaidi and Brueckner, 2017). Following an examination of incidences of CHDs reported in 62 studies published after 1955, an incidence of moderate to severe CHDs was estimated at 6 per 1000 livebirths (Hoffman and Kaplan, 2002). These studies were performed mainly in North America, Europe and Asia, with the African continent largely not studied.

In South Africa, the burden of birth defects including that of CHDs is unknown as these are poorly captured, with deaths from congenital defects hidden among deaths caused by communicable diseases (Lebese et al., 2016). Birth defects including CHDs contribute to an increase in stillbirths, neonatal deaths and under-5 mortality, threatening the attainment of Sustainable Developmental Goals (SDG). SDG 3, target 3.2 states: **"**By 2030, end preventable deaths of newborns and children under 5 years of age, with all countries aiming to reduce neonatal mortality to at least as low as 12 per 1,000 live births and under-5 mortality to at least as low as 25 per 1,000 live births" (United Nations, https://sdgs.un.org/goals, 2015).

CHDs are a significant cause of increased mortality and morbidity, and a source of global economic burden. Data availability from low middle income countries (LMIC) on the prevalence, morbidity and mortality of congenital heart diseases is however limited (Zimmerman and Sable, 2020). Data on the genetic causes of CHDs specific to LMIC is nearly non-existent as studies focus more on phenotypes of CHDs without including genetic investigations (Thomford et al., 2018).

1.2. Aetiology of congenital heart defects

The exact mechanisms by which congenital heart defects occur remain poorly understood and are thought to be as a result of many factors. Genetic, epigenetic, and environmental factors are known to contribute (Muntean et al., 2017).

Congenital heart defects occur either as isolated defects, with no other system involvement or as part of a syndrome. A second congenital anomaly in an individual with a congenital heart defect increases the chance of making a specific genetic diagnosis (Homsy et al., 2015). In addition, severe congenital heart defects, such as, truncus arteriosus, interrupted aortic arch, tetralogy of Fallot, ventricular septal defect with aortic arch anomaly, pulmonary atresia with ventricular septal defect and atrioventricular septal defect, even when seemingly non-syndromic, were most associated with a genetic diagnosis in one study (Jerves et al., 2020).

1.2.1. Examples of genes involved in the aetiology of CHDs

The common groups of genes implicated in congenital heart disease are those involved in transcription regulation, signal transduction and cardiac structural protein functions (Cowan and Ware, 2015). Transcription factors, amongst other functions, form a complex that is necessary for cardiac septation with mutations leading to atrial or ventricular septal defects, as seen with some *GATA4* gene variants (Xiang et al., 2014). Variants in *ACTC1,* which encodes a cardiac protein, actin, have also been reported to cause atrial septal defects (Matsson et al., 2008).

Other genes implicated in CHDs are nodal signalling and notch signalling genes e.g., *NOTCH1, NOTCH2* and *JAG1* (Shabana et al., 2020). Mutations in the *NOTCH* signalling genes have been found to affect aortic valve development leading to a variety of aortic valve abnormalities e.g., bicuspid aortic valve, aortic valve stenosis and subsequent left ventricular growth failure (Shabana et al., 2020).

Laterality signalling pathways are involved in the establishment of the left-right axis and asymmetric placement of organs along the midline. Some of the genes involved in the laterality signalling pathways are *ZIC3* and *LEFTY2*. Pathogenic variants in these genes, may lead to heterotaxy syndromes (Shabana et al., 2020).

Mutations in structural cardiac protein gene *MYH6,* which codes for myosin heavy chain 6 may also lead to structural cardiac abnormalities, with hypoplastic left heart syndrome reported in one study (Theis et al., 2015).

1.2.2. The role of epigenetics in the aetiology of CHDs

Epigenetics involves changes in expression of genes without changing the DNA sequence. Epigenetic changes, some of which may be heritable, involve DNA methylation, ATP-dependent chromatin remodelling, histone modification (methylation and acetylation), and regulation by miRNA (Turnpenny and Ellard, 2017).

DNA methylation has been shown to play a role in cardiomyocyte proliferation (Muntean et al., 2017). Chromatin remodelling plays an essential role in heart development by reprogramming gene expression under different pathophysiological conditions. Chromatin regulators exert their functions in a temporal- and tissue-specific manner to direct the formation of various cardiovascular tissues (Han et al., 2011). Histone modification plays a role in cardiac septation, right outflow tract septation and terminal differentiation (Muntean et al., 2017).

It is however difficult to assess the effect of epigenetics in practice as it is not clear what the optimal time during development is, for testing gene expression. Epigenetic mapping is being explored to assist with this (Muntean et al., 2017).

1.2.3. Family history as a contributor to CHDs risk

The risk of CHDs is increased in an individual with a significant family history of congenital heart defects. In CHDs with monogenic inheritance, for example, Holt-Oram or Noonan syndrome which are both inherited in an autosomal dominant manner, the recurrence risk to offspring is up to 50%. Recurrence risk of non-syndromic, familial CHDs in the first-degree relatives is estimated between 5-10% if one parent or 2 siblings are affected, decreasing to 3% if only one sibling is affected (Cowan and Ware, 2015).

Clustering of CHDs within families may be attributed to multifactorial causes including oligogenic or polygenic factors. Multiple variants in multiple genes are thought to interact synergistically with the environment to cause these (Morrish et al., 2022). Familial clustering of cases and high heritability of CHDs are suggestive of a strong genetic component even when the mode of inheritance is not obvious (Cowan and Ware, 2015).

Non-syndromic, familial (inherited) CHDs often affect multiple family members and are mostly thought to be due to inherited single nucleotide variants (SNVs). Heritable genetic burden involving multiple genes however may also be important (Morrish et al., 2022).

1.3. Syndromic congenital heart defects

Syndromic CHDs are associated with extracardiac abnormalities, neurodevelopmental problems or growth abnormalities (overgrowth/restricted growth). Chromosomal and Mendelian single-gene syndromes occur in about 20 - 30% of congenital heart defects cases (Muntean et al., 2017). Chromosomal syndromes may arise due to chromosomal aneuploidy (loss or gain of complete chromosomes), structural changes of sub-chromosomal regions due to copy number variants and complex chromosomal abnormalities (rearrangements).

1.3.1. Chromosomal aneuploidy as a cause of syndromic CHDs

Chromosomal aneuploidy is a recognized cause of congenital heart defects. Dysregulation of many genes that occurs due to loss or gain of a whole chromosome leads to abnormal cardiac and extracardiac development. CHDs are present in 40-50% of individuals with trisomy 21, 20-50% of those with Turner syndrome (monosomy X) and more than 80% of trisomy 13 and 18 patients (Shabana et al., 2020).

1.3.2. Chromosomal copy number variants as causes of syndromic CHDs

Chromosomal copy number variants (CNVs) can be deletions or duplications ranging from 1 kilobase to several megabases of DNA, many of which are associated with CHDs. Examples of these include 22q11.2 deletion syndrome, William syndrome (chromosome 7q11.23 deletion), Wolf-Hirschhorn syndrome (chromosome 4p16.3 deletion) and 1p36 deletion syndrome (Pierpont et al., 2018).

1.3.3. Single gene conditions as causes of syndromic CHDs

Single gene disorders are caused by pathogenic variants leading to abnormal quantity or function of the protein produced by the affected gene. Examples of single gene syndromes associated with congenital heart defects include RASopathies (Noonan, LEOPARD, cardio-facio-cutaneous and Costello syndromes), Alagille, Holt-Oram, Kabuki, Cornelia de Lange and CHARGE syndromes (Pierpont et al., 2018).

1.3.4. Environmental factors as causes of syndromic CHDs

Environmental causes of CHDs are well described although not fully understood. Intrauterine exposure to maternal medication (e.g. anticonvulsants), illicit drugs, alcohol, congenital infections such as rubella and maternal diabetes are associated with an increased risk of CHDs (Nees and Chung., 2020).

1.4. Non-syndromic congenital heart defects

Non-syndromic CHDs lesions are CHDs without associated extracardiac manifestations, neurodevelopmental delay or dysmorphic features. The genetic basis of non-syndromic CHDs has been difficult to elucidate due to locus heterogeneity, non-penetrance, polygenic effects with modifier genes and other non-genetic contributors (Pierpont et al., 2018).

Locus heterogeneity refers to variants in different genes causing the same phenotype, e.g. variants in *GATA4* and *TBX5* are known to cause atrial septal defects (Pierpont et al., 2018). An example of non-penetrance is the presence of the same *NOTCH1* gene variant in a parent and child, with the child presenting with aortic stenosis and the parent without a congenital heart defect (Pierpont et al., 2018).

Most cases of non-syndromic CHDs are isolated, with no associated family history, with new dominant mutations in some individuals (Pierpont et al., 2018). This has led to a lack of appreciation of the role of genetics in non-syndromic CHDs. Advances in molecular technologies have associated an increasing number of definitive and candidate genes with CHDs, (Morton et

al., 2022). Genetic testing for genes associated with non-syndromic CHDs is being offered to affected individuals by some groups and this should assist in defining causative genes and genotype-phenotype correlation better.

1.5. Polygenic risk contribution to CHDs

Development of new strategies of gene hunting and testing are contributing to the improvement in understanding the complex multifactorial causes of CHDs. Several single nucleotide polymorphisms that influence the development of congenital heart disease have been identified (Bagger et. al., 2024).

Next generation sequencing (NGS) at high depths read of the exome and genome results in identification of thousands of single nucleotide polymorphisms. These advances in genetic testing technologies have led to improved diagnostics and identification of novel genetic causes of CHDs. As an example, the endothelial nitric oxide synthase gene polymorphism was found to be associated with pulmonary stenosis in the African-American population (Cowan and Ware, 2015).

More complex multigene and epigenetic regulatory mechanisms are thought to play a greater role than monogenic causes of CHDs. In the future, polygenic risk scores (PRS) quantifying genetic burden across multiple CHDs 'risk' alleles may provide insight into CHDs causation or modification (Morrish et al., 2022). However, a lot of work still needs to be done to define and validate PRS across various populations and assess their utility.

1.6. Description of genetic tests used for the investigation of CHDs

Genetic causes of CHDs include chromosomal aneuploidies, chromosomal copy number variants/rearrangements and single gene disorders. Genetic testing therefore needs to be directed based on the suspected underlying genetic cause, type of CHDs and associated clinical features.

1.6.1. Genetic testing for chromosomal aneuploidies

Genetic testing for chromosomal aneuploidies includes **QF-PCR aneuploidy** (quantitative fluorescence polymerase chain reaction), **FISH** (fluorescence in situ hybridization), although less often used in practice and **karyotype** analysis.

QF-PCR aneuploidy specifically looks at copy number variants of whole chromosomes or significant chromosome segments of X, Y, 13, 18 and 21 (Turnpenny and Ellard, 2017).

Karyotype analysis involves visualization of the whole chromosome set to assess for extra, missing or translocated chromosomal material. Depending on the resolution, changes of 5 megabases and above in size may be visualized.

1.6.2. Genetic testing for chromosomal copy number variants

Karyotype, chromosomal microarray, **MLPA** (multiplex ligation-dependent probe amplification), **FISH** and **WES** (whole exome sequencing) may be used to assess for copy number variants (Turnpenny and Ellard, 2017; Jerves et. al, 2020).

Karyotype may be limited in this regard depending on the size of the variant as submicroscopic (<5Mb) variants may be too small to visualize with the technique. Karyotype however has the advantage of assisting with visualization of rearrangements to give further information regarding the position of extra or missing chromosomal material, rearrangements and inversions within the genome (Ito et. al, 2017; Morrish et al., 2022).

Subtelomeric/deletion MLPA is used to detect missing or extra chromosomal segments. MLPA is however probe specific and can only assess a small segment of a chromosome at a time making it less efficient than chromosomal microarray. Methylation specific MLPA (MS-MLPA) has the benefit of assessing epigenetic changes (methylation) for certain imprinted regions of the genome (Nussbaum et. al. 2016). An example of a condition that may present with CHDs is Kagami Ogata syndrome, which is an imprinting disorder caused by absence or overexpression of genes within an imprinted cluster on chromosome 14q32. Kagami Ogata syndrome may arise from paternal UPD14 or epimutations/deletions on the maternal chromosome.

FISH, like MLPA, assesses for copy number variants. FISH is targeted, limiting its use for genome wide chromosomal copy number variant assessment although this may be assessed using chromosome painting FISH (Turnpenny and Ellard, 2017). It does however allow for the localization of the CNVs.

Chromosomal microarray is utilized for quantitative assessment of genomic gains or losses. Chromosomal microarray, however does not provide information about the position of the extra material in the genome. Copy number variants of around 100 kilobases in size may be detected by low resolution microarray. High resolution microarray may detect smaller CNVs, (Turnpenny and Ellard, 2017). Comparative genomic hybridization array (CGH) which compares the patient specimen with a control (reference) specimen may be used. Alternatively, single nucleotide polymorphism (SNP) arrays may be used. SNP arrays have an added advantage of detecting areas of homozygosity and not requiring a control, (Turnpenny and Ellard, 2017).

Whole exome sequencing (WES) and whole genome sequencing (WGS), which were initially thought to be only for single gene sequencing, are able to assess for CNVs as well (Manickam et al., 2021).

1.6.3. Genetic testing for single gene variants

Single gene mutations can be assessed via **Sanger sequencing** or **next generation sequencing (NGS)**. Sanger sequencing determines the nucleotide sequence of DNA using capillary sequencers and remains the gold standard for sequencing due to longer read length and high accuracy (Turnpenny and Ellard, 2017). It is however time consuming, limiting genetic testing to a single or few genes at a time and costly if multiple genes need to be assessed. It is phenotype driven, requiring differential diagnoses to enable a choice of genes to be assessed. Sanger sequencing also has a potential for allele dropout which may lead to inaccurate results. It has been largely replaced by high throughput next generation sequencing for the day-to-day genetic testing of single nucleotide variants (Turnpenny and Ellard, 2017).

NGS includes single gene, gene panel, whole exome or whole genome testing. WES assesses single gene mutations in the protein coding regions (exons), splice acceptor and donor sites of the DNA which account for approximately 1- 2% of the genome (Ng et al., 2009). Mutations in

exons, splice site acceptor and donor sites are estimated to account for 85% of monogenic diseases, (Hamilton et. al., 2016). WES has the ability to analyze genes simultaneously, making it an effective method for both novel disease gene discovery and the efficient diagnosis of known genetic diseases where the phenotype is not specific or in conditions known to be caused by more than one gene (genetic heterogeneity) (Hamilton et. al., 2016). Trio-WES, which includes familial comparators, is considered the most optimal if available to help contextualize rare variants. If not possible, WES can be effectively performed as proband only or duo, with diagnostic yield being slightly reduced compared with trio testing (Manickam et al., 2021).

Whole genome sequencing (WGS) provides coverage of both exon and intron targets and assesses for copy numbers and single nucleotide variants, (Bagger et al., 2024). WGS requires a comprehensive computational and storage infrastructure to allow data processing within a clinically relevant timeframe (Bagger et al., 2024). Data produced from WGS is quite substantial with a potential for increased variants of uncertain significance (VUS). These are variants with an unknown effect within actionable genes. Genetic variation in between individuals and current limited technologies lead to difficulties understanding the clinical impact of these variants as either benign or pathogenic resulting in care challenges. With increased testing of varied population groups and enrichment of variants to clarify the mutation landscape, we will likely be able to understand the role of these variants of uncertain significance (Bagger et al., 2024).

Compared with standard genetic testing, exome sequencing and genome sequencing (ES/GS) have a higher diagnostic yield and may be more cost-effective when ordered early in the diagnostic evaluation process to avoid multiple sequential genetic tests in individuals. The ACMG guidelines strongly recommend that ES/GS be considered as a first- or second-tier test for patients with congenital anomalies with onset under 1 year of age and developmental delay/ intellectual disability with onset under 18 years of age (Manickam et al., 2021).

1.7. Genetic testing in patients with congenital heart defects

The rate of genetic diagnosis for patients with congenital heart defects is variable. The diagnostic yield differs across CHDs categories. Diagnostic rates of between 25-39% have been reported in infants under 1-year of age, depending on the group of patients assessed and testing methods

used (Geddes and Earing, 2018). A population assessment of children with CHDs found a yield from karyotype of 10.8% (480/4430). In the absence of the common aneuploidies however, the yield from karyotype was 1.6% (Geddes and Earing, 2018).

Chromosomal microarray has a high diagnostic yield and is the recommended first line investigation for the evaluation of patients with major cardiovascular anomalies, critical CHDs, or congenital heart disease patients with multiple congenital anomalies, developmental delay and autism spectrum disorders (Wu et al., 2017). Patients with syndromic congenital heart defects were found to have a higher incidence of pathogenic CNVs (15-20%) when compared to those with isolated, non-syndromic congenital heart defects (4-14%) (Geddes and Earing, 2018). In a study performed in Cape Town, South Africa, which included individuals with syndromic and non-syndromic CHDs, microarray detected likely causative CNVs covering known CHD-associated genes in 5/89 (5.6%) patients, (Saacks et al., 2022). This was considered to be in keeping with other studies done in European populations which yielded 3-25% of CNVs in patients with CHDs (Blue et al., 2017).

The highest diagnostic yield of genetic testing in CHDs is in individuals with syndromic and/or a familial (inherited form) CHDs (Blue et al., 2017). In one study, actionable variants were identified using genomic sequencing in 49% of those with non-syndromic, familial forms and in 43% of those with CHDs and extracardiac manifestations (Alankarage et al., 2019). Non-syndromic, isolated CHDs reported yield is 3-10% for chromosomal microarray, 2-10% for WES and 10% for WGS (Morrish et al., 2022).

Genetic testing practices for CHDs are yet to be standardized in many centres. Clinicians are becoming increasingly aware of the benefits of genetic testing, however screening within the context of CHDs is relatively low in clinical practice especially in patients with isolated CHDs. Standardized guidelines regarding indications for genetic testing in patients with CHD are limited.

1.7.1. Utility of genetic assessment of patients with CHDs

Knowledge of the genetics of CHDs assists in risk assessment of associated complications, evaluation of associated extracardiac manifestations and provision of information about long-term prognosis (Cowan and Ware, 2015). This knowledge, allows for holistic discussions about management of the cardiac and extracardiac abnormalities in patients with CHDs. As an example, patients with 22q11.2 deletion syndrome may be prone to sepsis, present with hypocalcaemia or graft vs host disease following transfusion with non-irradiated blood products. The use of genetic information in clinical decision making has the potential to decrease morbidity and mortality as it allows clinicians to anticipate potential complications and more accurately counsel families about the expected post-operative course, prognosis, and long-term outcomes.

Cascade genetic assessment including genetic testing can be provided for family members of the affected individual. A genetic diagnosis allows for the assessment of reproductive risks and provision of family planning options. Reproductive options such as preimplantation genetic diagnosis or prenatal diagnosis may be offered to couples with a personal or family history of CHDs and a known genetic diagnosis. In the event that CHDs is detected prenatally, arrangements can be made for delivery at a centre that offers an appropriate level of care for the condition. A tertiary/quaternary hospital with access to a neonatal intensive care unit and cardiothoracic facilities, or termination of pregnancy may be considered depending on the severity of the lesion and parents/ patients' wishes.

Clinical geneticist involvement in a dedicated CHDs genetics program resulted in an additional ~8–13% of genetic diagnoses when compared to ad-hoc referrals in one study, (Geddes et. al., 2019). In this study, phenotyping by a clinical geneticist assisted with the detection of complex phenotypes with dual diagnoses and identification of additional genetic diagnoses associated with changes in care. Genetic testing utilization improved significantly decreasing the likelihood of multiple tests ordered on individual patients. Similar findings were also reported by Goldenberg et. al, 2017.

A clinical geneticist role is pedigree collation and assessment, phenotyping of patient including dysmorphology assessment and genetic counselling (with genetic counsellors). The role extends to educating the family regarding implications for testing, possible testing outcomes (including

variants of unknown significance, incidental findings, a negative or positive result), recurrence risk for offspring and siblings, cascade family testing in the event of a positive test, monitoring and anticipatory management in the event of a positive test, and cessation of monitoring in family members who test negative for the family variant and containment of patients (for both positive and negative results).

Specialised cardiac genetics clinics were recently endorsed by the American Heart Association (AHA) as crucial to best-practice cardiovascular care, (Morrish et al., 2022). Within a resource-constrained country such as South Africa, and probably other LMIC, it may not be currently possible to set up specialised cardiac genetics clinics in all centres. It should however be possible in some tertiary/ quaternary centres and should be noted as the optimal level of care to aim for and aspire towards.

1.8. Genetic risk mediators of medical and surgical outcomes of CHDs

Survival rates of patients with CHDs have increased significantly over the past number of years. Improved surgical techniques and medical care for those that are able to access care have contributed to this increase. Disease-related morbidity however remains considerable with reduced life-expectancy in these patients, (Pierpont et al., 2018).

Surgical outcomes in patients with CHDs have genetic risk mediators. The underlying genetic condition is one of the determinants of length of intubation, transfusion requirements, growth, and neurodevelopmental outcomes. Studies of patients with CHDs have shown that abnormal genetic tests correlate to poorer surgical outcomes (Pierpont et al., 2018).

As a group, for example, patients with pathogenic CNVs have poorer outcomes than those without CNVs. Patients with CHDs with 22q11.2 deletion have longer cardiopulmonary bypass times, more re-operations and longer intensive care stays than patients with CHDs without this deletion, (Putotto et al., 2022). Patients with CHDs with other large CNVs (>300 kb) have a higher risk of death or need for transplantation than patients with CHDs without large CNVs, (Kim et al., 2016).

Single gene variants are also known to contribute. Variants in *MYH6* are associated with ventricular performance abnormalities and an increased risk of perioperative mortality (Geddes and Earing, 2018).

The investigation of underlying genetics in patients presenting with CHDs needs to become routine standard of care to improve medical and surgical outcomes (Cowan and Ware, 2015). With the shortage of medical geneticists to actively evaluate each individual patient, it is imperative for other healthcare providers to gain some familiarity with a standardized approach to the genetic assessment of a patient with congenital heart disease.

1.9. Aim of the study

The study aimed to retrospectively review patients with CHDs attending a newly established paediatric cardiology service for confirmed genetic conditions, and assess whether clinical and molecular genetic assessment can be expanded in this group of patients.

1.10. Study objectives

1. To describe congenital heart defects seen in patients presenting at the paediatric cardiology clinic.
2. To categorise these conditions as syndromic or non-syndromic based on retrospective data available.
3. To document if genetic testing was done on these patients, the type of genetic testing done and results thereof.
4. To recommend an approach to genetic assessment of patients with congenital heart defects in a resource-limited setting.

CHAPTER 2

RESEARCH METHODOLOGY

2.1. Subjects and study setting

Nelson Mandela Children's Hospital (NMCH), Johannesburg South Africa, is a tertiary/quaternary referral-only hospital. It offers cardiac care in the form of cardiology and cardiothoracic surgery to paediatric patients within Gauteng province, and those from the neighbouring provinces of the North-West, Limpopo and on occasion, Mpumalanga.

A retrospective file review and data analysis of the clinical records of children seen at the cardiology service at NMCH between 1 January 2019 – 31 December 2019 (1-year period) was performed. This period was chosen when the study was initiated in 2022 as it would have been the most active period since the service is newly established and the subsequent 2 years had been disrupted by the COVID19 pandemic. Children who were 0 months to 18 years at the time of consultation within the stipulated time-period were included in the study. This is the age group seen at the Nelson Mandela Children's Hospital cardiology service.

A total of 206 patients were seen over the study period. Only patients with structural CHDs were included in the study. Individuals with isolated cardiomyopathy and acquired heart diseases were excluded. A total of 181 patient records were included, with 25 excluded due to failure to meet study criteria. The study was approved by the Wits Human Research Ethics Committee (Medical), clearance certificate no. M220840 (**Appendix A**).

2.2. Data sources

Data was obtained from a variety of sources including patient files, electronic database, medical reports and medical image storage systems based at NMCH cardiology clinic.

2.3. Data collection

Data was collected and collated by the researcher. Nominal data, viz. sex, type of cardiac lesion, reported dysmorphic features or minor anomalies, other extracardiac structural abnormalities, associated neurodevelopmental abnormalities, genetic testing done and results were categorised

and coded for analysis. Continuous data, viz. age at presentation to NMCH for quaternary care and weight were also recorded and analysed. Data collected was captured onto a Research Electronic Data Capture (REDCap) database hosted at the University of the Witwatersrand, Division of Human Genetics. A data collection sheet had been designed by the researcher for this purpose and is attached (**Appendix B**).

Cardiac lesions were classified using Botto level 3 classification (National Birth Defects Study) with additional categories for those not represented by the Botto level 3 classification (Botto et al., 2007). The Botto level 3 categories included septal defects, atrioventricular septal defects (AVSD), conotruncal defects, heterotaxy syndromes, left ventricular outflow tract obstruction (LVOTO), right ventricular outflow tract obstruction (RVOTO), complex cardiac conditions and anomalous pulmonary venous return (APVR). Other categories described in this cohort but not included in the Botto level 3 classification are patent ductus arteriosus (PDA) and valvular lesions.

Records of patients were anonymized by the assignment of a study number as an identifier. The patients' names and hospital numbers were delinked and kept separately from the study data by the researcher via a password. Only the researcher and supervisors have access to this reference chart.

2.4. Data analysis

Descriptive statistics were utilized to present the patients' characteristics and clinical variables using proportions (%) for categorical data, with median and interquartile ranges (IQR) for continuous data. Age at presentation to NMCH was analysed using inter-quantile ranges.

Types of congenital heart defects presenting were categorized. Presence of reported dysmorphic features/minor anomalies, additional structural extracardiac anomalies (categorized per organ system) were also described. The number of patients who demised during this period was reported. The number of patients with neurodevelopmental delay and growth restriction were reported on. The number of genetic tests done, number of positive genetic test results and genetic diagnoses in those with positive genetic tests were also reported on.

If the patients presented with CHDs only, with no other associated anomalies, they were considered as non-syndromic. Syndromic CHDs were those associated with extracardiac structural abnormalities, reported dysmorphic features or minor anomalies. It should however be noted that

neurodevelopmental delay and growth restriction may occur as a result of the underlying genetic syndrome or as a consequence/ complication of the CHDs. Due to lack of longitudinal data, we were not able to define the cause of growth restriction/ failure to thrive precisely. Neurodevelopmental delay was used to classify CHDs as syndromic, however growth restriction was not unless it was intrauterine growth restriction.

Stata Statistics/Data analysis 18.0 Stata Corp LLC software for Microsoft Windows, was used for the analysis of data.

CHAPTER 3

RESULTS

3.1. Patient demographics

A total of 181 patient records were examined. Patient characteristics are shown in **Table 3.1**. The median age at presentation to NMCH was 7 months (IQR 0.7-24 months), age range 1 day – 17 years.

Table 3.1: Characteristics of patients with CHDs (n=181)

Characteristics	N	%
Sex		
Male	103	56.9
Female	78	43.1
Reported dysmorphic features/ minor anomalies	38	21.0
Congenital heart defects		
Isolated	108	59.7
Syndromic	73	40.3
Mortality	21	11.6

3.2. Types of cardiac lesions diagnosed in patients with CHDs

The types of presenting cardiac lesions [as per Botto level 3 classification described by the National Birth Defects Prevention study (Botto et al., 2007)], are shown in **Table 3.2.**

Table 3.2. Types of presenting CHDs as per Botto level 3 classification (n=139)

Type of defect	N	%
Conotruncal defects	40	22.1
Septal defects	32	17.7
LVOTO	27	14.9
RVOTO	17	9.4
AVSD	14	7.7
APVR	5	2.8
Heterotaxy	4	2.2

Other categories not included in the Botto level 3 classification were PDA 28 (15.5%), complex cardiac conditions 10 (5.5%), valvular lesions 3 (1.7%), and peripheral pulmonary stenosis (PPS) 1 (0.6%).

3.3. Dysmorphic features/ minor anomalies

Dysmorphic features/ minor anomalies were reported in 38 (21%) individuals. In 50% (19/38) of the patients' files, the dysmorphic features were unspecified including those occuring in children with a molecular diagnosis of Down syndrome. In cases where the dysmorphic features were specified, the majority had 2 or more features described.

3.4. Major structural abnormalities

Thirty-one major structural abnormalities were reported in 25 (13.8%) individuals. These involved the gastrointestinal 17/31 (54.8%), respiratory 4/31 (12.9%), genitourinary 4/31 (12.9%), skeletal 3/31 (9.7%), CNS 2/31 (6.5%) and ectodermal 1/31 (3.2%) systems.

Additional structural extracardiac abnormalities are represented in **Table 3.3.**

Table 3.3: Types of additional structural abnormalities in patients with CHDs (n=31)

System	N	%	Type of structural abnormality
Gastrointestinal	17	54.8	Cleft palate Oesophageal atresia Tracheo-oesophageal atresia Congenital diaphragmatic hernia Hypertrophic pyloric stenosis Duodenal atresia Anorectal malformation
Respiratory	4	12.9	Choanal atresia Subglottic web Right main bronchus hypoplasia Trilobed left lung
Genitourinary	4	12.9	Unilateral renal agenesis Chordee, hypospadias Unspecified urethral abnormality Cryptorchidism
Skeletal	3	9.7	Unilateral limb reduction defect Limb length discrepancy Missing 12^{th} rib
CNS	2	6.5	Neural tube defects -lumbosacral myelomeningocoele -spina bifida occulta
Ectodermal	1	3.2	Extracranial, occipital haemangioma

Four of the patients were reported to have more than one additional major structural abnormality (up to 3 systems in one case), see **Table 3.4**.

Table 3.4. Patients presenting with >1 additional extracardiac structural abnormality

Patient	No. of additional structural abnormalities	Type of structural abnormality
1	2	Tracheo-oesophageal fistula Cleft palate
2	2	Choanal atresia Genitourinary abnormality (cryptorchidism, micropenis)
3	2	Congenital diaphragmatic hernia Unilateral limb reduction defect
4	3	Spina bifida occulta Genitourinary abnormality (cryptorchidism, hypospadias, chordee) Skeletal (limb length discrepancy, missing 12^{th} rib)

3.5. Mortality rate of patients with CHDs in the cohort

Of the cohort, 160 (88.4%) were still alive and 21 (11.6%) had demised at the time of the study. Some patients demised perioperatively and these would have been investigated as per protocol of surgical/ anaesthetic deaths. The balance of patients demised at the base hospital or at home with post-mortem reports of the cause of death not provided.

3.6. Neurodevelopmental abnormalities reported in patients with CHDs

Neurodevelopmental abnormalities were reported in 30 (16.6%) cases. The level of developmental delay was however not reported.

3.7. Growth restriction reported in patients with CHDs

Growth restriction (failure to thrive and/ or stunting) can occur as a result of the CHDs, malnutrition or underlying genetic condition. Growth restriction/ failure to thrive was reported in 52 (28.7%) cases. Due to the absence of longitudinal data, it was not possible to assess the underlying cause further.

3.8. Genetic testing in patients with CHDs

A total of 67 genetic tests were performed on 58 (32%) individuals.

3.8.1. Types of genetic tests performed on patients with CHDs

Access to genetic testing in the study period was limited to QF-PCR aneuploidy, karyotype, FISH (22q11.2 FISH mostly requested), subtelomeric/deletion MLPA and MS-MLPA. Chromosomal microarray, single gene sequencing, WES and WGS were mostly inaccessible therefore limiting a wide range of genetic diagnoses.

The most frequent genetic test performed was QF-PCR aneuploidy 26/67 (38.8%). This was followed by subtelomeric/ microdeletion MLPA 17/67 (25.4%), FISH for 22q11.2 deletion testing 14/67 (20.9%), karyotype 7/67 (10.5%), chromosomal microarray 1 (1.5%), single gene sequencing 2/67 (3.0%) for RASopathy panel and *CFTR* gene for suspected cystic fibrosis. Genetic tests performed are represented in **Figure 3.1.**

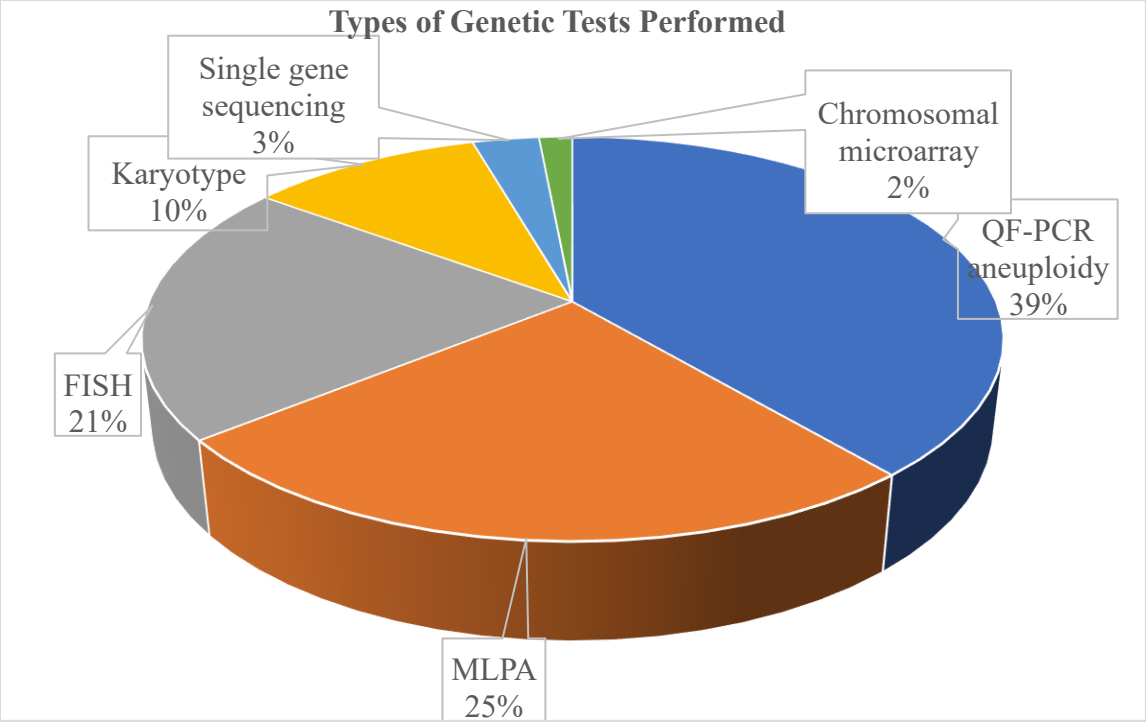

Figure 3.1: Types of genetic tests performed in a subset of patients with CHDs (n=67)

Of the 38 patients assessed with dysmorphic features/ minor anomalies, 29/38 (76.3%) received genetic testing. Two tests were performed per individual in three patients. As an example, a female neonate who presented with pulmonary atresia, VSD, PDA, left anotia, brachycephaly, widely spaced nipples and anorectal malformation had QF-PCR aneuploidy and 22q11.2 deletion FISH performed. Of note are the multiple tests performed in some individuals, up to 4 genetic tests/ individual in some cases.

3.8.2. Genetic test results recorded in patients with CHDs

Of the 67 tests performed on 58 patients, 29 (43.3%) were positive, 36 (54%) were negative and 2 (3.0%) equivocal as shown in **Figure 3.2**.

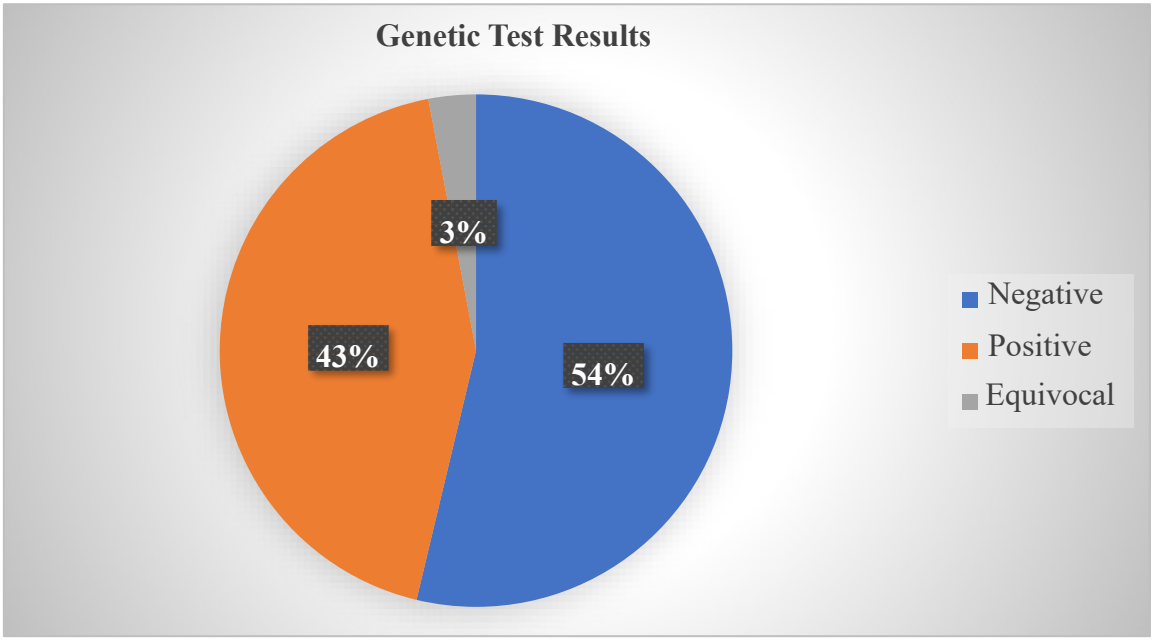

Figure 3.2: Genetic test results in patients with CHDs

The equivocal results included a heterozygous deletion of a single probe on chromosome 15q (*SNRPN*-u1b gene at 15q11.2) and a heterozygous deletion of a single probe at chromosome 16p (*CREBBP* gene at 16p13.3) using the P245 probe mix on subtelomeric/ microdeletion MLPA in both cases. No further testing was done for the above-mentioned patients.

Of the 29 positive genetic tests results, 22 (75.9%) were trisomy 21, 6 (20.7%) copy number variants [4 (13.8%) 22q11.2 deletion, 2 (6.9%) William syndrome], and 1 (3.5%) single gene *PTPN11* (Noonan syndrome), see **Figure 3.3.**

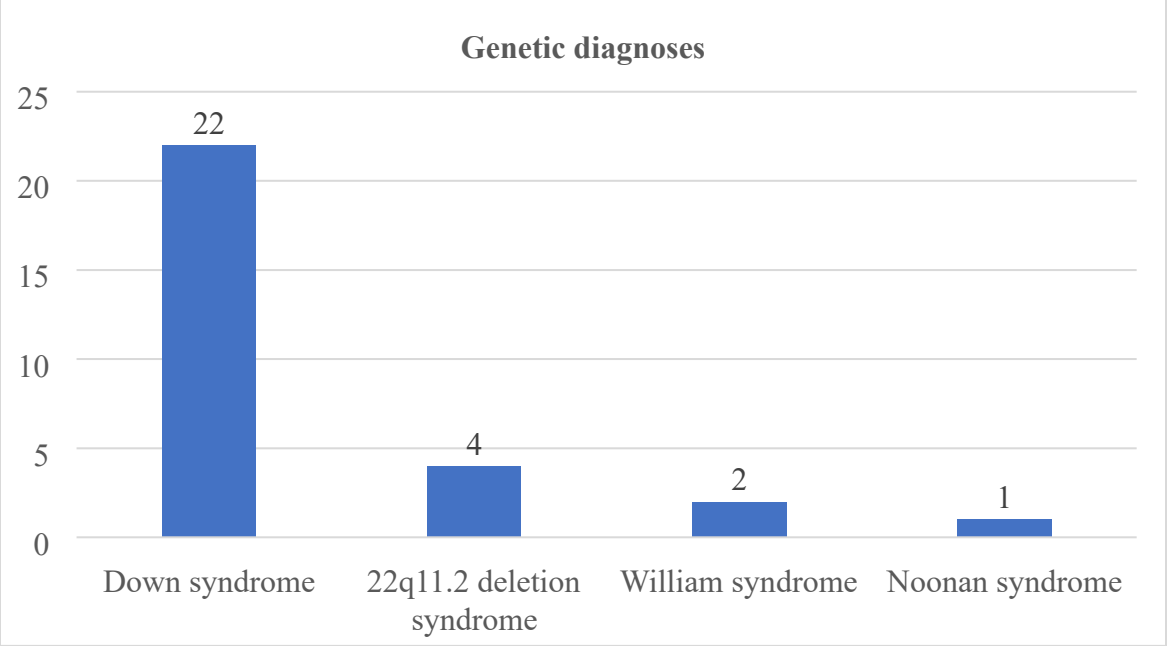

Figure 3.3: Genetic diagnoses obtained after genetic testing

Twelve out of the fourteen (85.7%) patients presenting with AVSD had genetic testing performed. Of those tested, 11/12 (91.7%) had Down syndrome and 1/12 (8.3%) Noonan syndrome. Chromosomal copy number variants (deletions/duplications) were also found as a cause of syndromic CHDs, with 4/29 (13.8%) of positive tests detecting 22q11.2 deletion and 2/29 (6.9%) 7q11.2 deletion (William syndrome).

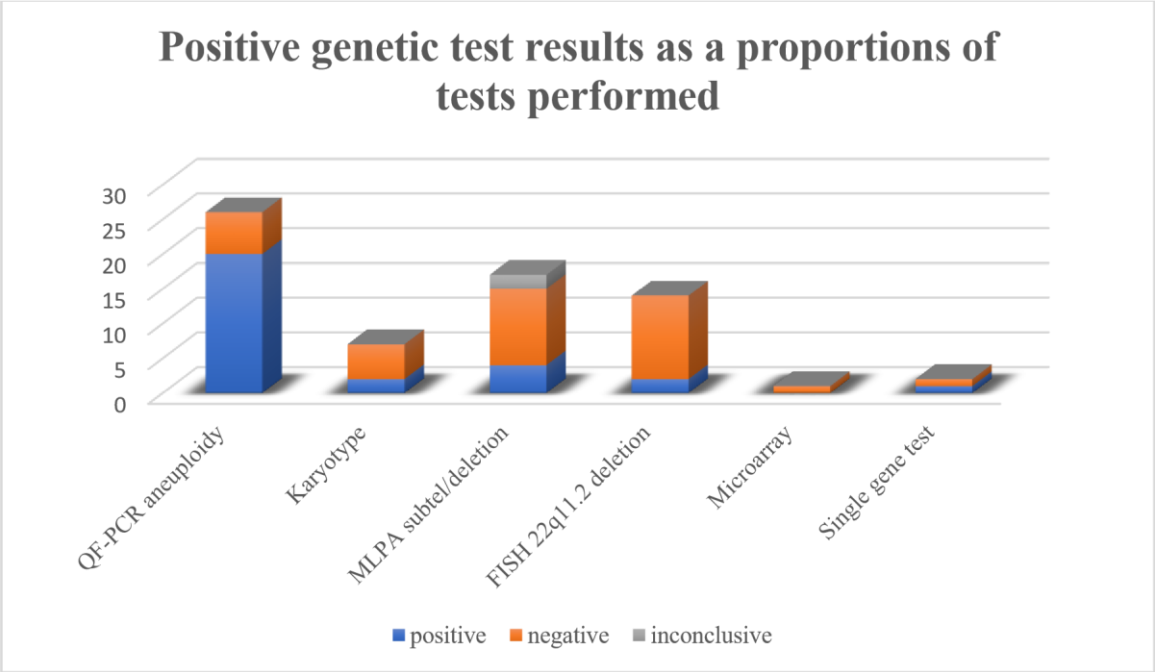

Figure 3.4. Positive genetic test results as a proportion of tests performed on a subset of patients with CHDs

3.9. Syndromic vs non-syndromic CHDs

CHDs were categorized in this study as non-syndromic if there were no associated dysmorphic features/ minor anomalies, developmental delay or major structural extracardiac abnormalities documented. Apparently non-syndromic CHDs were found in 108 (59.7%) patients. Seventy-three (40.3%) patients presented with syndromic CHDs.

Down syndrome, 22q11.2 deletion, William and Noonan syndrome were syndromic causes confirmed by genetic testing. A large amount of syndromic and non-syndromic CHDs remained without a genetic diagnosis.

CHAPTER 4
DISCUSSION

4.1. Age at presentation for care in patients with CHDs

The median age at presentation for tertiary/quaternary care including for cardiothoracic surgery in this cohort was 7 months. Patients present at later stages of disease and at older ages in low-resource settings in comparison to those in high-income countries, (Thomford et al., 2018). Widespread use of prenatal ultrasound screening including fetal anomaly scan, allows for the prenatal diagnosis of CHDs in high-income countries. This allows for planning of delivery in a hospital equipped to handle complex cardiac cases. In our setting, very few children with CHDs are diagnosed prenatally with some of those with critical CHDs succumbing in the neonatal period or infancy, (Saacks et al., 2022).

If not diagnosed prenatally, infants born at a local clinic, primary or secondary hospital, without prior knowledge of the underlying CHDs, need to be stabilized and transferred in specialized ambulances to tertiary/ quaternary care centres for further care including cardiothoracic surgery as needed. This is a challenge within a limited-resource setting due to the limited availability of specialized ambulances and personnel.

4.2. Types of presenting cardiac lesions in patients with CHDs

A wide range of structural cardiac abnormalities was seen in this cohort as was expected for a centre that sees a significant number of patients with complex medical needs. Conotruncal defects (22.1%) were the most common in this cohort, followed by septal defects (17.7%). Septal defects are reported to represent the commonest CHDs in patients with moderate to severe CHDs, (Hoffman and Kaplan, 2002). Our result is most likely due to selection bias as the majority of patients seen at NMCH are those needing cardiothoracic surgical intervention and thus may have more complex cardiac presentations.

4.3. Reported dysmorphic features/minor anomalies in patients with CHDs

Dysmorphic features/minor anomalies were reported in 21% of cases. In their paper, Ahrens-Nicklas et al. report a high genetic test yield in the presence of eye abnormalities e.g. epicanthic folds, hypertelorism; cleft palate and cranial abnormalities (for example microcephaly) and presence of 3 fontanelles, (Ahrens-Nicklas et al., 2016). This finding highlights the importance of thorough dysmorphology examination and documentation in patients with CHDs as this may assist in the diagnosis of underlying genetic conditions.

Data from our cohort was however sparse in the description of dysmorphic/ minor anomalies findings. In 19/38 (50%) of patients with reported dysmorphic features/ minor anomalies, dysmorphic features were unspecified, including in those with Down syndrome. In the other 50% (19/38) patients, dysmorphic features were specified and these assisted with the choice of genetic test requested. A minimum of two features per patient were noted and these were typical of the clinical and molecular diagnoses. As an example, in a patient who was found to have William syndrome on subtelomeric/ microdeletion MLPA, the features described were, elfin facies, periorbital fullness, broad mouth, and prominent ears which are consistent. Similarly, brachycephaly, epicanthic folds, low-set ears and brachydactyly were described in a patient with Down syndrome.

The frequency of major structural defects was noted to be increased 5-fold that of the general population if 2 dysmorphic features/ minor anomalies were present (i.e. 11% in those with 2 dysmorphic features/minor anomalies vs 2.2% in the general population). This increase is even higher in those with 3 dysmorphic features/ minor anomalies (90% of those with 3 dysmorphic features/ minor anomalies were found to have major structural defects) (Marden et al., 1964).

4.4. Additional major structural abnormalities reported in patients with CHDs

Additional major structural abnormalities were noted in 13.8% of individuals in this cohort of patients. The commonest were gastrointestinal abnormalities with over half of the patients with additional structural abnormalities presenting. Anorectal malformations and congenital diaphragmatic hernia (CDH) were the most common GIT abnormalities.

The majority of the patients with additional GIT abnormalities did not receive a genetic diagnosis. Helm and Ware identified a genetic diagnosis in 35.1% of those with gastrointestinal/abdominal wall abnormalities (Helm and Ware, 2024).

One of the three patients with duodenal atresia had Down syndrome, and one of the 5 patients with anorectal malformation received a diagnosis of Down syndrome as well. Of the 5 patients with congenital diaphragmatic hernia, only 1 had genetic testing, a QF-PCR aneuploidy, which was negative with no reported dysmorphic features suggestive of the common aneuploidies.

There are genetic conditions known to cause both CDH and CHDs, for example, Cornelia de Lange syndrome, Fryns syndrome, Pallister Killian syndrome and chromosomal aneuploidies, trisomy 13 and 18. The choice of genetic test in the above depends on the clinical picture. If phenotype is specific for a monogenic condition for example Cornelia de Lange syndrome, one can proceed with sequencing (e.g. gene panel, WES). If, however not specific or suggestive of a chromosomal condition, one can proceed with chromosomal microarray/ aneuploidy testing.

In another study, Egbe et al. reported extra-cardiac/ major structural abnormalities to occur in approximately 13% of newborns with CHDs (Egbe et al., 2014). Ahrens-Nicklas et al. reported from their study findings that the presence of neurological (corpus callosum abnormalities, hypotonia) or musculoskeletal (syndactyly, clinodactyly and vertebral abnormalities) increased the odds of finding a genetic diagnosis (Ahrens-Nicklas et al., 2016). The majority of the patients in our cohort did not have brain imaging and we are therefore unable to comment with regards to structural brain involvement. There were however two patients reported with neural tube defects. One with lumbosacral myelomeningocoele, associated Chiari II malformation and no genetic testing performed. Another with spina bifida occulta with associated genitourinary and skeletal abnormalities who also had no genetic testing.

4.5. Mortality rates in patients with CHDs

A hundred and sixty (88.4%) patients were still alive and 11.6% had demised at the time of the study. Not all details regarding the final cause of death were available however as some deaths occurred at the primary hospital or at home. Patients who demised intra/ post-operatively were investigated further and a post-mortem report shared with the medical team looking after the

patient and next of kin. Associations between type of CHD, genetic condition, extracardiac structural abnormalities and mortality rates were unable to be drawn due to scanty retrospective data, lack of access to post-mortem reports and small sample size.

4.6. Neurodevelopmental abnormalities rates reported in CHDs patients

Neurodevelopmental abnormalities were reported in 16.6% cases. Neurodevelopmental abnormalities are reported to occur in patients with CHDs, either as a consequence of the underlying genetic condition, complication of the congenital heart defect or related procedures/ interventions. In this study, the presence of neurodevelopmental delay assisted in classifying the CHDs as likely syndromic. The degree of severity of neurodevelopmental delay was however not documented.

4.7. Associated growth restriction rates reported in CHDs patients

Growth restriction was reported in 28.7% cases and may be as a result of the CHDs, malnutrition or the underlying genetic condition. The exact contributors to faltering weight and stunting could not be accurately determined due to the cross-sectional nature of the data.

4.8. Genetic testing of patients with CHDs

Access to genetic testing in the study period was limited to QF-PCR aneuploidy, karyotype, subtelomeric/ deletion MLPA and 22q11.2 FISH. Chromosomal microarray, single gene sequencing, WES and WGS were mostly inaccessible therefore limiting a wide range of genetic diagnoses.

In the NHLS/NMCH setting, chromosomal microarray is now the first line test for patients with multiple congenital abnormalities and intellectual disability, where the phenotype is not specific for the common aneuploidies (T13, 18 or 21). WES is only accessible via research studies and WGS is mostly inaccessible in this setting.

Different provinces in South Africa have different arrangements with the Department of Health and local health authorities regarding broader access to genetic testing. In the Western Cape as an example, a budget is allocated per annum for the medical geneticists to prioritize genetic testing. They are then able to access local, national and overseas testing should this be required. This resource is however dwindling and no longer available for some centres. Gauteng and other provinces which are able to access the Johannesburg centres for clinical assessment and phenotyping, were until March 2024 able to access limited single gene testing via the inherited disease gene panel. This option is unfortunately no longer available due to laboratory logistical problems.

All provinces within South Africa have access to QF-PCR aneuploidy, karyotype, FISH, chromosomal microarray, MLPA and MS-MLPA via specializes NHLS laboratories in the country (Braamfontein, Groote Schuur, Sefako Makgatho and Bloemfontein). Turn around times are however long.

Sixty-seven genetic tests were performed on 32% of the patients. Genetic testing relied on the treating physician as there was no protocol to guide genetic testing in patients with CHDs at the time. A significant number of patients who presented with AVSD, 12/14 (85.7%) or had phenotypic features of Down syndrome received genetic testing.

Conotruncal defects have long been associated with genetic conditions and guidelines have existed to guide the genetic assessment of individuals presenting with conotruncal defects. Conotruncal defects are known to have a high genetic test yield and would routinely be referred for genetic testing for CNVs, specifically 22q11.2 deletion. This historically included mainly 22q11.2 deletion FISH and subtelomeric/ deletion MLPA as chromosomal microarray was not routinely available at the time of the study. Fifteen (15/40, 37.5%) of those who presented with conotruncal defects were tested in this cohort.

Of the patients with dysmorphic features/ minor anomalies, 23.7% (9/38) did not receive genetic testing. As an example, a 1-year-old female patient with VSD, large forehead, webbed neck, and developmental delay did not receive any genetic testing. Similarly, a 3-month-old male with ASD, PDA, TAPVD, microtia, micrognathia, growth restriction, rectourethral fistula received no further genetic testing after QF-PCR aneuploidy test detected no common aneuploidies.

Array CGH is recommended in syndromic CHDs as per ACMG guidelines as 1st line to rule out copy number variants. Single gene panel testing is an option where the phenotype is suggestive of a recognizable phenotype, e.g. RASopathy panel in a patient with features of Noonan syndrome. WES/WGS is recommended if the phenotype is not recognizable or where single gene panel/ array CGH testing yielded no results. The clinical details documented were not sufficient to enable suggestions for further testing in those patients with negative genetic testing. We do recommend following guidance as recommended (flow sheet attached and refer to genetics clinic as necessary, Appendix D).

In this cohort, all patients with syndromic CHDs, critical CHDs, and familial CHDs should have received genetic testing. At least 73 (40.3%) patients (15 more than the number tested) were categorized as syndromic and therefore genetic testing would be recommended. Family history of CHDs was not however not documented, therefore making it difficult to assess the total number of tests recommended for this cohort. This underscores the importance of multidisciplinary clinical and molecular genetic assessment including the involvement of medical geneticist as part of the team to collate and interpret family pedigree, phenotype and direct genetic testing.

4.9. Genetic test results in patients with CHDs

Twenty-nine (43.3%) of the sixty-seven tests requested were positive, yielding a genetic diagnosis in 16.0% of the patients. This is lower than expected for those centres which involve medical geneticists closely in the care of patients with CHDs (Geddes et al., 2019). In these studies, a diagnostic rate of 25% was reported (Ahrens-Nicklas et al., 2016) with a higher genetic testing rate.

Down syndrome was overrepresented with 75.9% of the positive tests detecting trisomy 21. It is not surprising that trisomy 21 was the most common diagnosis as it is a common genetic condition. Down syndrome phenotype is easily recognizable to most health care practitioners and therefore would mostly be referred for genetic testing and similarly for CHDs assessment and surgical correction if required. Genetic testing for Down syndrome is also readily accessible. Atrioventricular septal defect (AVSD) had a high genetic diagnostic yield. This may be as a

result of its association with Down syndrome. The majority of patients presenting with AVSD received a molecular diagnosis of Down syndrome.

Trisomy 13/18 patients with multiple congenital anomalies assessed as likely to have poor prognosis due to limited lifespan would generally not access the cardiothoracic services in this setting. This group of children are therefore not represented in this cohort.

It is surprising that Turner syndrome is not represented in this cohort. This may be due to the fact that the phenotype is not easily recognizable in the younger age group. Recognition and therefore diagnosis of Turner syndrome tends to occur at a later age with other presentations viz. primary amenorrhoea, short stature and bicuspid aortic valvular disease.

Of the 17 tests done in 15 patients with conotruncal cardiac defects, 6 were positive, with a 40% (6/15) diagnostic rate. Trisomy 21 was detected in 3 patients, and the other 3 had 22q11.2 deletion. The tests performed were however limited viz. subtelomeric/ deletion MLPA, QF-PCR aneuploidy and FISH for 22q11.2 deletion.

Chromosomal microarray is the preferred test for chromosomal copy number variants (microdeletions/ duplications) as it is broader and therefore is more like to pick up a wider group of CNVs. Chromosomal microarray is considered a first-tier clinical diagnostic test for individuals with congenital abnormalities or developmental disabilities, (Miller et al., 2010) and should be offered to patients with CHDs as these are a common feature of chromosomal copy variants disorders.

Genetic testing in individuals with isolated CHDs, where a likely genetic change would be a single gene variant, and therefore requiring single gene panel, WES or WGS was low. This was both as a result of non-requisition by clinicians and unavailability of genetic tests at the NHLS at the time of the study. Medical geneticist involvement in the assessment of patients with CHDs has been shown to contribute to improved care as it assists in the expedient diagnosis of genetic conditions if associated and the choice of appropriate genetic testing (Morrish et al., 2022). The traditional view of CHDs, especially if there are no associated abnormalities, as a ‘non-genetic’ leads to the under-referral of patients and their families for clinical genetics assessments.

Noonan syndrome, was the only single gene condition detected in this cohort. This patient was diagnosed as part of a research study and the result has since been validated. Other single gene

conditions that may be associated with CHDs (e.g. Alagille, other RASopathies and CHARGE syndrome) were not detected. This again emphasizes the importance of medical geneticist involvement to assist with phenotyping and directing appropriate genetic test request and the need for broad availability of genetic tests.

Screening for genetic conditions in patients with CHDs is not standardized. Genetic testing in patients with CHDs is underutilized (Helm and Ware, 2024). Recent studies looking at hospitalized infants with CHDs show the benefits of standardization of genetic evaluations, leading to early diagnosis of genetic conditions, specific management and family risk assessment (Helm and Ware, 2024).

4.10. Syndromic and non-syndromic CHDs

Apparently non-syndromic CHDs were found in the majority of the patients in this cohort. This is expected as the majority of patients with CHDs are reported to have an isolated cardiac defect without the involvement of other organ systems (Garg, 2006). Adequate information regarding family history of CHDs was not provided therefore making it difficult to classify cases into non-syndromic, familial or non-syndromic, sporadic (non-familial). It is clear from various observations that extracardiac abnormalities cannot solely be used as a driver to initiate genetic testing as individuals with non-syndromic CHDs with an underlying genetic cause, will be missed (Helm and Ware, 2024). An increased number of de novo protein-truncating or missense variants in autosomal dominant CHD-associated genes were found in patients with non-syndromic CHDs highlighting the need for genetic testing in non-syndromic CHDs (Nees and Chung, 2020).

Chromosomal copy number variants (deletions/duplications) were also found as a cause of syndromic CHDs. The prevalence of CNVs is reported to be varied depending on the cohort and method of detection used with reports of 3-25% (Nees and Chung, 2020). In our cohort, with the use of limited genetic testing (subtelomeric/ microdeletion MLPA and 22q11.2 deletion FISH), and minimal chromosomal microarray, the reported prevalence is 6/181 (3.3%). Chromosomal CNVs are reported more in patients with extracardiac manifestations as compared to those with no extracardiac manifestations, (Wu et al., 2017). Wolf-Hirschhorn and 1p36 deletion syndrome

are some of the CNVs commonly seen with CHDs that are not represented in this cohort. This is surprising as some of these conditions would have been picked up by subtelomeric/ microdeletion MLPA.

No intervention was undertaken during this study. As part of the reporting of the study, an algorithm was formulated to assist with future genetic assessment of patients with congenital heart defects. This will be handed to the cardiology unit at the Nelson Mandela Children's Hospital.

CHAPTER 5

5.1. Limitations

This was a retrospective review of patients' records, and thus complete data were not available on all patients. Some records were kept at the primary/ referring hospitals.

The study centre is a referral centre and sees a select cohort of complex patients needing tertiary or quaternary medical and surgical care. Only patients who have been assessed to likely benefit from the cardiology and cardiothoracic services offered are referred to NMCH for further management. Patients with less complex presentations managed at primary and secondary level of care centres were not fully represented in this cohort, therefore introducing selection bias.

Patients, who have been assessed to have a poor prognosis, those that would benefit from palliative care only and are therefore not candidates for the level of care offered, were not referred by their primary institutions to NMCH and therefore are also not represented in this cohort.

Factors that influenced genetic testing in our cohort were availability of genetic tests, type of CHDs, associated congenital anomalies, neurodevelopmental problems and recognizable phenotype. There seemed to be limited recognition of other rare genetic causes of CHDs as requested genetic testing was limited to commonly recognizable syndromes. Selective genetic testing was mainly requested in those patients whose phenotype resembled a common recognizable genetic syndrome (e.g. Down syndrome, William syndrome) and in those with AVSD.

As part of the study, we wanted to look at the likely syndromic cases with negative genetic tests to suggest a possible diagnosis/ test based on the phenotype. An attempt was made but was unsuccessful due to scanty clinical details provided in the patient records.

5.2. Recommendations

Genetic testing protocols have been shown to reduce cost and increase the yield of genetic diagnoses in CHDs (Durbin et al., 2023). The average time to resolve a diagnostic odyssey in

rare diseases as documented by the EURORDIS-led Community Engagement Task Force in Europe has been estimated to be around 5.6 years. Timeous genetic diagnosis likely to be provided by clear genetic assessment guidelines and multidisciplinary care, has been shown to shorten the time to diagnosis and access to appropriate care including targeted therapeutic interventions or aiding transition to palliative care. The net healthcare expenditure is reduced by limiting the number of genetic tests performed per patient and the number of visits with various specialists to investigate the underlying diagnosis (Faye et al., 2024). We recommend the use of genetic testing protocols to realize these benefits. We have included recommendations to guide the approach to genetic assessment of patients with CHDs in a resource-limited setting (**Figure 5.1**).

ISCA (International Standard Cytogenomic Array Consortium) consensus statement supports the use of CMA as the first-tier cytogenetic diagnostic test for patients with developmental delay/ intellectual disability, autism spectrum disorder or multiple congenital anomalies (Miller et al., 2010). The American College of Medical Genetics and Genomics updated guidelines recommend WES/WGS as first-tier/second-tier test in paediatric patients with congenital anomalies or intellectual disability. WES/WGS has a higher diagnostic yield and is cost-effective if ordered early in the diagnostic evaluation of this group of patients with findings that are not specific for a known syndrome (Manickam et al., 2021). We recommend the availing of extended genetic testing to include uninterrupted availability of chromosomal microarray, single gene panels and WES to allow broader access.

There is a limited number of genes associated with CHDs on the inherited disease panel, these can be virtually assessed. This is subject to a patient being assessed and phenotyping done by a medical geneticist. At present, IDP is not available due to laboratory logistical problems.

Other factors that influence genetic testing, e.g. family history of CHDs, dysmorphic features/ minor anomalies and major anomalies were not adequately documented in this cohort. This underscores the importance of involving medical genetics specialists as part of the team to care for patients with CHDs to assist with patient phenotyping, comprehensive family pedigree assessment and appropriate choice of genetic tests to improve diagnosis of less recognizable syndromes.

A more in-depth analysis of the data with a probably larger cohort of patients would improve its value, further research in genetic conditions in CHDs is needed.

Medical genetics involvement will also allow for genetic counselling and follow up to allow pre- and post-test counselling, family cascade testing, recurrence risk assessment, reproduction planning with discussions around prenatal or preimplantation testing options.

5.3. Conclusions

There is a need for broader recognition of genetic conditions and genetic assessment of patients with CHDs. Medical genetics involvement is important to assist in the detailed phenotyping, recognition of rare genetic syndromes which allows for directed testing to allow timely diagnosis and intervention. Care for patients with CHDs should include detailed phenotyping, documentation of dysmorphic features/ minor anomalies, additional structural and non-structural abnormalities, comprehensive family pedigree and broader genetic testing with increased medical genetics involvement.

The types of genetic tests requested in this cohort reflect the available tests within the constrained LMIC setting. Access to genetic testing remains restricted in our setting, although improved. Universal genetic testing of patients with CHDs may allow us, in the long-term, to define those patients that would benefit from genetic testing, especially within the understudied African population.

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APPENDIX A: Human Research Ethics Committee (Medical) University of the Witwatersrand Clearance Certificate no. M220840

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Dr DS Mokwele

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M220840

NAME: (Principal Investigator)	Dr DS Mokwele
DEPARTMENT:	School of Pathology Division of Human Genetics Medical School University
PROJECT TITLE:	*Retrospective review of genetic conditions in patients with congenital heart defects attending a quaternary paediatric cardiology service in Johannesburg, South Africa*
DATE CONSIDERED:	2022/08/26
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Professors A Krause and H Nsinjana
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2022/10/07

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. I agree to submit a yearly progress report. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in August and therefore reports and re-certification will be due in the month of August each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

17/10/2022
Date

APPENDIX B: Redcap-based data collection sheet

Retrospective review of genetic conditions in patients with congenital heart defects attending a quaternary paediatric cardiology service in Johannesburg, South Africa.

Participant ID	_____
Age_at_ascertainment	_____
Age_at_diagnosis	_____
Sex	○ male ○ female ○ other
Sex other	_____

CHD CLASSIFICATION		
	VSD	ASD
Septal defects	☐	☐

CHD CLASSIFICATION	
	Patent ductus arteriosus
PDA	☐

CHD CLASSIFICATION	
	atrioventricular septal defect
AVSD	☐

CHD CLASSIFICATION					
	Truncus arteriosus	Interrupted aortic arch	d-TGA	DORV	TOF
Conotruncal defects	☐	☐	☐	☐	☐

CHD CLASSIFICATION			
	HLHS	Coarctation of the aorta	Aortic stenosis
Left ventricular outflow tract obstruction	☐	☐	☐

CHD CLASSIFICATION

	Pulmonary stenosis	Pulmonary atresia	Tricuspid atresia
Right ventricular outflow tract obstruction	☐	☐	☐

CHD CLASSIFICATION

	Single ventricle	L-TGA	Multiple complex heart anomaly
Complex CHD	☐	☐	☐

CHD CLASSIFICATION

	Heterotaxy
Heterotaxy	☐

CHD CLASSIFICATION

	Other
Other	☐

Dysmorphic features
- ◯ Yes
- ◯ No

Dysmorphism
- ☐ Unspecified
- ☐ Head
- ☐ Face
- ☐ Neck
- ☐ Trunk
- ☐ Abdominal
- ☐ Genitalia
- ☐ Skeletal upper limbs
- ☐ Skeletal lower limbs
- ☐ Skeletal spine
- ☐ Skin/Ectoderm

Additional structural abnormalities
- ◯ Yes
- ◯ No

Additional structural abnormalities
- ☐ CNS
- ☐ Urinary tract system
- ☐ GIT
- ☐ Respiratory
- ☐ Face
- ☐ Ear
- ☐ Skeletal system
- ☐ Abdominal wall defects
- ☐ Other

Comorbid conditions
- ◯ Yes
- ◯ No

Comorbid conditions
- ☐ Unspecified
- ☐ Intellectual disability
- ☐ Growth restriction
- ☐ Neurodevelopmental
- ☐ Neuropsychiatric
- ☐ Other

Genetic test	◯ Yes ◯ No
Genetic test	☐ QF-PCR aneuploidy ☐ Karyotype ☐ FISH ☐ MLPA ☐ Microarray ☐ Single gene sequencing ☐ WES ☐ WGS ☐ Other
Genetic test results	◯ negative ◯ positive ◯ undetermined

GENETIC DIAGNOSIS

	Trisomy 21	Trisomy 18	Trisomy 13	Turner syndrome	Other
Aneuploidy	☐	☐	☐	☐	☐

GENETIC DIAGNOSIS

	22q11 deletion	William syndrome	Wolff-Hirschhorn	1p36 deletion	Cri-du-chat	other
Copy number variants	☐	☐	☐	☐	☐	☐

GENETIC DIAGNOSIS

	Noonan syndrome	Alagille	CHARGE	Holt-Oram	Other
Single gene syndromes	☐	☐	☐	☐	☐

OTHER IDENTIFIED CAUSES

	None	Teratogens drugs	Teratogens congenital infections	Other
Other aetiology	☐	☐	☐	☐

INVESTIGATIONS

	Echocardiogram	CT scan	MRI	X-rays	Ultrasound
Investigations	◯	◯	◯	◯	◯

CT Scan	☐ Brain ☐ Head bony windows ☐ Neck ☐ Chest ☐ Abdomen ☐ Spine ☐ Limbs ☐ CT angiogram

MRI	☐ Brain ☐ Neck ☐ Chest ☐ Abdomen ☐ Spine ☐ Limbs ☐ MRA
X-ray	☐ Skull ☐ Chest ☐ Abdomen ☐ Spine ☐ Limbs
Ultrasound	☐ Cranial ☐ Neck ☐ Abdomen ☐ Spine ☐ Limbs

	Syndromic	Isolated
Classification	☐	☐

Proposed new diagnosis	◯ Yes ◯ No
Proposed diagnosis	◯ copy number variation ◯ single gene condition ◯ teratogen ◯ multifactorial ◯ other
Intervention	◯ None ◯ Curative ◯ Palliative
Type of intervention	☐ Conservative ☐ Pharmacotherapy ☐ Surgical interventional catheterization ☐ Surgical cardiothoracic

OUTCOME

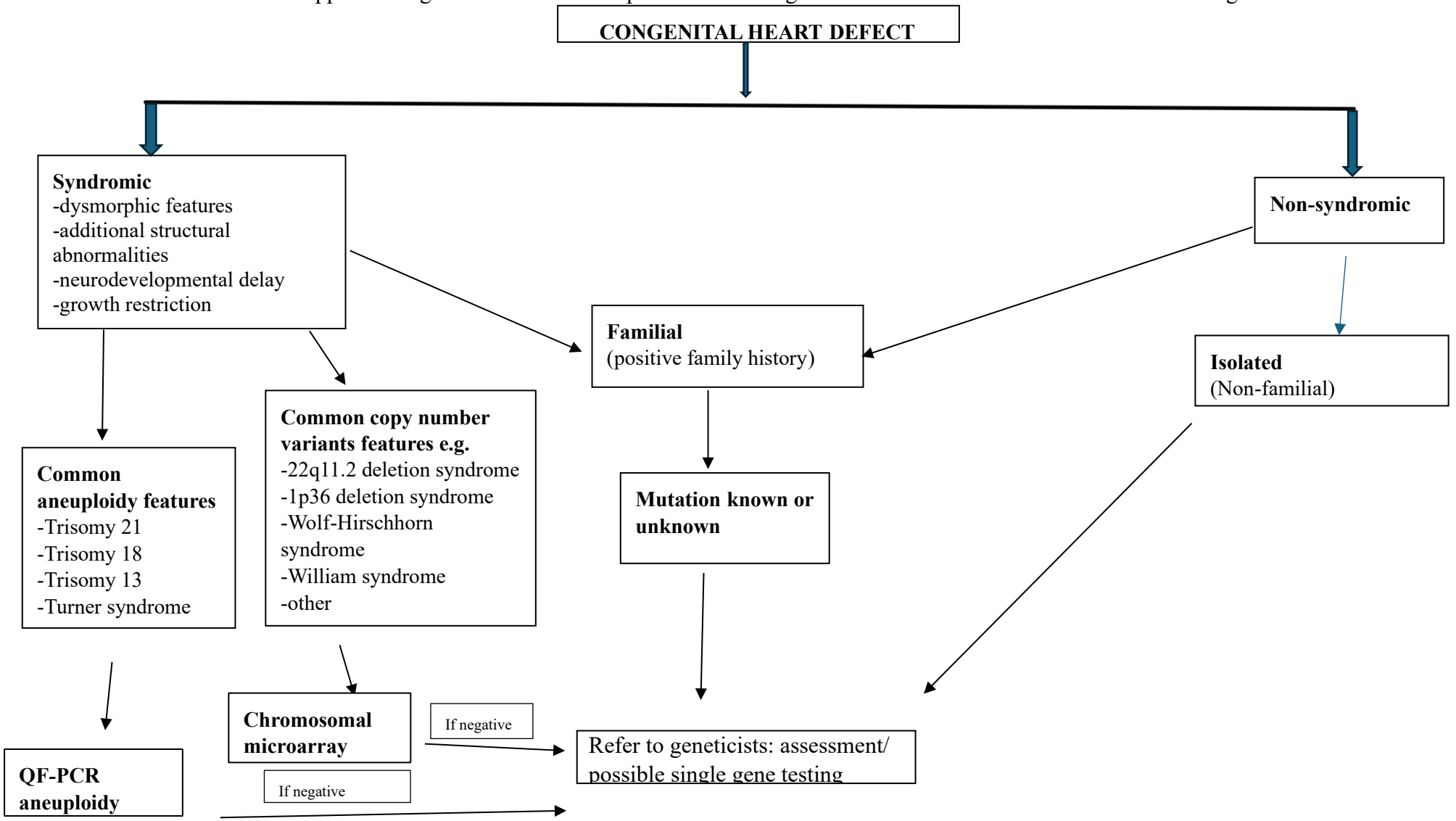
	Demised	Alive
Outcome	☐	☐

APPENDIX C: Proposed consultation form for clerking of patients with CHDs

Family history of: congenital anomalies Y/N multiple miscarriages Y/N	Elaborate:	
Type of cardiac lesion: Septal/ Conotruncal/ LVOTO/ RVOTO/ AVSD/ Complex/ Heterotaxy/ APVR		
Dysmorphic features Y/N	Elaborate	
Extracardiac abnormalities documentation: CNS: Y/N Craniofacial: Y/N Respiratory: Y/N Renal: Y/N GIT/GUT: Y/N Skeletal: Y/N Other: Y/N Neurodevelopmental delay/ ID/ ASD: Y/N Growth restriction Y/N	Elaborate	
CHD classification: Syndromic Non-syndromic: Isolated Familial (inherited)		

Suspected chromosomal aneuploidy: Trisomy 21/18/13: Y/N Turner syndrome: Y/N	Elaborate	QF-PCR aneuploidy Y/N
Suspected chromosomal copy number variant/ rearrangement: Y/N	Elaborate	Microarray Y/N
Suspected single gene disorder: Y/N	Refer for medical geneticist assessment	
Pretest genetic counselling: Y/N		

APPENDIX D: Recommended approach to genetic assessment of patients with congenital heart defects in a resource-limited setting



APPENDIX E: Letter of approval to conduct research at NMCH

Nelson Mandela Children's Hospital

27th September 2022

Dr. Daisy S Mokwele

Re: Permission Letter to Use Paediatric Cardiology Database

This letter serves to grant you permission to use the Paediatric Cardiology Database in all its forms (electronic, hardcopy, files etc) for the purposes of your research in our Department at the Nelson Mandela Children's Hospital. The undersigned is the senior custodian of all clinical data within our hospital.

Sincerely,

Dr Pinky L Chirwa MBchB, FCPaed
Director of Clinical services
Nelson Mandela Children's Hospital
Email: pinky.chirwa@nmch.co.za

NPC Registration No: 2011/008134/08
Web address: www.nelsonmandelachildrenshospital.org
Postnet Suite 258, Private Bag X2600, Houghton, 2041 | 6 Jubilee Road, Parktown, Johannesburg, 2196
Tel: (+27 11) 274-5600 Fax: (+27 11) 486-3914

APPENDIX F: Plagiarism Form

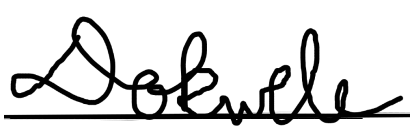
PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY

I, Daisy Salome Mokwele, Student number 9402484K am a student registered for the degree of Master of Medicine (Medical Genetics) in the academic year 2025.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 27 January 2025

APPENDIX G: Turnitin Report

MMED Ulwazi Final Writeup 27012025 Research Report Clean copy.docx

by Daisy Mokwele

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Submission ID: 2572924204

File name: MMED_Ulwazi_Final_Writeup_27012025_Research_Report_Clean_copy.docx (704.02K)

Word count: 9469

Character count: 54852

CHAPTER 1

INTRODUCTION

1.1. Epidemiology

a . These structural cardiac defects that occur due to disruption of embryonic differentiation and cardiogenesis (Zaidi and Brueckner, 2017). Following an examination of incidences of CHDs reported in 62 studies published after 1955, an incidence of moderate to severe CHDs was estimated at 6 per 1000 livebirths (Hoffman and Kaplan, 2002). These studies were performed mainly in North America, Europe and Asia, with the African continent largely not studied.

In South Africa, the burden of birth defects including that of CHDs is unknown as these are poorly captured, with deaths from congenital defects hidden among deaths caused by communicable diseases (Lebese et al., 2016). Birth defects including CHDs contribute to an increase in stillbirths, neonatal deaths and under-5 mortality, threatening the attainment of Sustainable Developmental Goals (SDG). SDG 3, target 3.2 states: "By 2030, end preventable deaths of newborns and children under 5 years of age, with all countries aiming to reduce neonatal mortality to at least as low as 12 per 1,000 live births and under-5 mortality to at least as low as 25 per 1,000 live births" (United Nations, https://sdgs.un.org/goals, 2015).

CHDs increased mortality a source of global economic burden. Data availability from low middle income countries (LMIC) on the prevalence, morbidity and mortality of congenital heart diseases is however limited (Zimmerman and Sable, 2020). Data on the genetic causes of CHDs specific to LMIC is nearly non-existent as studies focus more on phenotypes of CHDs without including genetic investigations (Thomford

. Aetiology defects

exact mechanisms by which congenital heart defects occur remain poorly understood and are thought to be many factors. , epigenetic, are known to contribute (Muntean et al., 2017).

Congenital heart defects occur either as isolated defects, with no other system involvement or as part of a syndrome. A second congenital anomaly in an individual with a congenital heart defect increases the chance of making a specific genetic diagnosis (Homsy et al., 2015). In addition, [redacted], tetralogy of Fallot, [redacted] anomaly, [redacted], even when seemingly non-syndromic, [redacted] one study (Jerves et al., 2020).

1.2.1. Examples of genes involved in the aetiology of CHDs

The common groups of genes implicated in congenital heart disease are [redacted] transcription [redacted] protein functions (Cowan and Ware, 2015). Transcription factors, amongst other functions, [redacted] with [redacted] leading to atrial or ventricular septal defects, as seen with some *GATA4* gene variants (Xiang et al., 2014). Variants in *ACTC1,* which encodes a cardiac protein, actin, have also been reported to cause atrial septal defects (Matsson et al., 2008).

Other genes implicated in CHDs are nodal signalling and notch signalling genes e.g., *NOTCH1, NOTCH2* and *JAG1* (Shabana et al., 2020). Mutations in the *NOTCH* signalling genes have been found to affect aortic valve development leading to a variety of [redacted] and [redacted] (Shabana [redacted]., 2020).

Laterality signalling pathways are [redacted] establishment [redacted]. Some of [redacted] genes involved in the laterality signalling pathways are *ZIC3* and *LEFTY2*. Pathogenic variants in these genes, may lead to heterotaxy syndromes (Shabana et al., 2020).

Mutations in structural cardiac protein gene *MYH6,* which codes for myosin heavy chain 6 may also lead to structural cardiac abnormalities, with hypoplastic left heart syndrome reported in one study (Theis et al., 2015).

1.2.2. The role of epigenetics in the aetiology of CHDs

Epigenetics involves changes in expression of genes without changing the DNA sequence. Epigenetic changes, some of which may be heritable, involve DNA methylation, ATP-dependent chromatin remodelling, histone modification (methylation and acetylation), and regulation by miRNA (Turnpenny and Ellard, 2017).

DNA methylation has been shown to [9] cardiomyocyte proliferation (Muntean ., 2017). Chromatin remodelling plays an essential role [10] (Han et al., 2011). Histone modification plays a role in cardiac septation, right outflow tract septation and terminal differentiation (Muntean et al., 2017).

It is however difficult to assess the effect of epigenetics in practice as it is not clear what the optimal time during development is, for testing gene expression. Epigenetic mapping is being explored to assist with this (Muntean et al., 2017).

1.2.3. Family history as a contributor to CHDs risk

The risk of CHDs is increased in an individual with a significant family history of congenital heart defects. In CHDs with monogenic inheritance, for example, Holt-Oram or Noonan syndrome [48] both , the recurrence risk to offspring is up to 50%. Recurrence risk of non-syndromic, familial CHDs in the first-degree relatives is estimated between 5-10% if one parent or 2 siblings are affected, decreasing to 3% if only one sibling is affected (Cowan and Ware, 2015).

Clustering of CHDs within families may be attributed to multifactorial causes including oligogenic or polygenic factors. Multiple variants in multiple genes are thought to interact synergistically with the environment to cause these (Morrish et al., 2022). Familial clustering

of cases and high heritability of CHDs are suggestive of a strong genetic component even when the mode of inheritance is not obvious (Cowan and Ware, 2015).

Non-syndromic, familial (inherited) CHDs often affect multiple family members and are mostly thought to be due to inherited single nucleotide variants (SNVs). Heritable genetic burden involving multiple genes however may also be important (Morrish et al., 2022).

1.3. Syndromic congenital heart defects

Syndromic CHDs are associated with extracardiac abnormalities, neurodevelopmental problems or growth abnormalities (overgrowth/restricted growth). [59] occur in about - 30% congenital heart defects cases (Muntean et al., 2017). Chromosomal syndromes may arise due to chromosomal aneuploidy ([32] of due to copy number variants and complex chromosomal abnormalities (rearrangements).

1.3.1. Chromosomal aneuploidy as a cause of syndromic CHDs

Chromosomal aneuploidy is a recognized cause of congenital heart defects. Dysregulation of many genes that occurs due to loss or gain of a whole chromosome leads to abnormal cardiac and extracardiac development. CHDs are present in 40-50% of individuals with trisomy 21, 20-50% of those with Turner syndrome (monosomy X) and more than 80% of trisomy 13 and 18 patients (Shabana [9]

. Chromosomal as causes of syndromic CHDs

Chromosomal copy number variants (CNVs) [12] kilobase several megabases of DNA, many of which are associated with CHDs. Examples of these include 22q11.2 deletion syndrome, William syndrome (chromosome 7q11.23 deletion), [28] (chromosome) and 1p36 deletion syndrome ([28]).

1.3.3. [redacted] conditions as causes of syndromic CHDs

Single gene disorders are caused by pathogenic variants leading to abnormal quantity or [redacted] produced [redacted] affected [redacted]. Examples [redacted] single gene syndromes associated with congenital heart defects include RASopathies (Noonan, LEOPARD, cardio-facio-cutaneous and Costello syndromes), Alagille, Holt-Oram, Kabuki, Cornelia de Lange and CHARGE syndromes (Pierpont et al., 2018).

1.3.4. Environmental factors as causes of syndromic CHDs

Environmental causes of CHDs are well described although not fully understood. Intrauterine exposure to maternal medication (e.g. anticonvulsants), illicit drugs, alcohol, congenital infections such as rubella and maternal diabetes [redacted] CHDs (Nees [redacted] Chung., 2020).

1.4. Non-syndromic congenital heart defects

Non-syndromic CHDs lesions are CHDs without associated extracardiac manifestations, neurodevelopmental delay or dysmorphic features. [redacted] non-syndromic CHDs [redacted] due [redacted] locus heterogeneity, non-penetrance, polygenic effects with modifier genes and other non-genetic contributors (Pierpont et al., 2018).

Locus heterogeneity refers to [redacted] causing [redacted], e.g. [redacted] and [redacted] known to cause atrial septal defects (Pierpont et al., 2018). An example of non-penetrance is the presence of the same *NOTCH1* gene variant in a parent and child, with the child presenting with aortic stenosis and the parent without a congenital heart defect (Pierpont et al., 2018).

Most cases of non-syndromic CHDs are isolated, with no associated family history, with new dominant mutations in some individuals (Pierpont et al., 2018). This has led to a lack of appreciation of the role of genetics in non-syndromic CHDs. Advances in molecular

technologies have associated an increasing number of definitive and candidate genes with CHDs, (Morton et al., 2022). Genetic testing for genes associated with non-syndromic CHDs is being offered to affected individuals by some groups and this should assist in defining causative genes and genotype-phenotype correlation better.

1.5. Polygenic risk contribution to CHDs

Development of new strategies of gene hunting and testing are contributing to the improvement in understanding the complex multifactorial causes of CHDs. Several single nucleotide polymorphisms that influence the development of congenital heart disease have been identified (Bagger et. al., 2024).

Next generation sequencing (NGS) at high depths read of the exome and genome results in identification of thousands of single nucleotide polymorphisms. These [36] improved diagnostics identification novel genetic causes of CHDs. As an example, the endothelial nitric oxide synthase gene [6] pulmonary stenosis the African-American (Cowan and Ware, 2015).

More complex multigene and epigenetic regulatory mechanisms are thought to play a greater role than monogenic causes of CHDs. [1] CHDs 'risk' CHDs (Morrish et al., 2022). However, a lot of work still needs to be done to define and validate PRS across various populations and assess their utility.

1.6. Description of genetic tests used for the investigation of CHDs

Genetic causes of CHDs include chromosomal aneuploidies, [52] /rearrangements . Genetic testing therefore needs to be directed based on the suspected underlying genetic cause, type of CHDs and associated clinical features.

1.6.1. Genetic testing for chromosomal aneuploidies

Genetic testing for chromosomal aneuploidies includes [30] **aneuploidy** ( ), although less often used in practice and **karyotype** analysis.

QF-PCR aneuploidy specifically looks at copy number variants of whole chromosomes or significant chromosome segments of X, Y, 13, 18 and 21 (Turnpenny and Ellard, 2017).

Karyotype analysis involves visualization of the whole chromosome set to assess for extra, missing or translocated chromosomal material. Depending on the resolution, changes of 5 megabases and above in size may be visualized.

1.6.2. Genetic testing for chromosomal copy number variants

Karyotype, [47] and **WES** (whole exome sequencing) may be used to assess for copy number variants (Turnpenny and Ellard, 2017; Jerves et. al, 2020).

Karyotype may be limited in this regard depending on the size of the variant as submicroscopic (<5Mb) variants may be too small to visualize with the technique. Karyotype however has the advantage of assisting with visualization of rearrangements to give further information regarding the position [49] inversions within the genome (Ito et. al, 2017; Morrish et al., 2022).

Subtelomeric/deletion MLPA is used to detect missing or extra chromosomal segments. MLPA is however probe specific and can only assess a small segment of a chromosome at a time making it less efficient than chromosomal microarray. Methylation specific MLPA (MS-MLPA) has the benefit of assessing epigenetic changes (methylation) for certain imprinted regions of the genome (Nussbaum et. al. 2016). An example of a condition that may present with CHDs is Kagami Ogata syndrome, which is an imprinting disorder [16] an . Kagami Ogata syndrome may arise [16] .

FISH, like MLPA, assesses for copy number variants. FISH is targeted, limiting its use for genome wide chromosomal copy number variant assessment although this may be assessed using chromosome painting FISH (Turnpenny and Ellard, 2017). It does however allow for the localization of the CNVs.

Chromosomal microarray is utilized for quantitative assessment of genomic gains or losses. Chromosomal microarray, however does not provide information about the position of the extra material in the genome. Copy number variants of around 100 kilobases in size may be detected by low resolution microarray. High resolution microarray may detect smaller CNVs, (Turnpenny and Ellard, 2017). Comparative genomic hybridization array (CGH) which compares the patient specimen with a control (reference) specimen may be used. Alternatively, [redacted] may be used. [redacted] an [redacted] detecting areas of homozygosity and not requiring a control, (Turnpenny and Ellard, 2017).

[redacted]), which were initially thought to be only for single gene sequencing, are able to assess for CNVs as well (Manickam et al., 2021).

1.6.3. Genetic testing for single gene variants

Single gene mutations can be assessed via **Sanger sequencing** or **next generation sequencing (NGS)**. Sanger sequencing determines the nucleotide sequence of DNA using capillary sequencers and remains the gold standard for sequencing due to longer read length and high accuracy (Turnpenny and Ellard, 2017). It is however time consuming, limiting [redacted] and [redacted] if multiple genes need to be assessed. It is phenotype driven, requiring differential diagnoses to enable a choice of genes to be assessed. Sanger sequencing also has a potential for allele dropout which may lead to inaccurate results. It has been largely replaced by high throughput next generation sequencing for the day-to-day genetic testing of single nucleotide variants (Turnpenny and Ellard, 2017).

NGS includes [redacted], gene [redacted] testing. WES assesses single gene mutations in the protein coding regions (exons), splice acceptor and donor sites of

the DNA which account for approximately 1- 2% of the genome (Ng et al., 2009). Mutations in exons, splice site acceptor and donor sites , (Hamilton ., 2016). WES has , making it where the phenotype is not specific or in conditions known to be caused by more than one gene (genetic heterogeneity) (Hamilton et. al., 2016). Trio-WES, which includes familial comparators, is considered the most optimal if available to help contextualize rare variants. If not possible, WES (Manickam et al., 2021).

Whole (WGS) exon intron and assesses for copy numbers and single nucleotide variants, (Bagger et al., 2024). allow (Bagger et al., 2024). Data produced from WGS is quite substantial with a potential for increased variants of uncertain significance (VUS). These are variants with an unknown effect within actionable genes. Genetic variation in between individuals and current limited technologies lead to difficulties understanding the clinical impact of these variants as either benign or pathogenic resulting in care challenges. With increased testing of varied population groups and enrichment of variants to clarify the mutation landscape, we will likely be able to understand the role of these variants of uncertain significance (Bagger et al., 2024).

Compared with standard genetic testing, (ES/GS) process to avoid multiple sequential genetic tests in individuals. The ACMG with onset under 1 year of age and under).

1.7. Genetic testing in patients with

rate diagnosis patients defects variable. diagnostic yield differs across CHDs categories. Diagnostic rates of between 25-39% have been reported in infants under 1-year of age, depending on the group of patients assessed and testing methods used (Geddes and Earing, 2018). A population assessment of children with CHDs found a yield from karyotype of 10.8% (480/4430). In the absence of the common aneuploidies however, the yield from karyotype was 1.6% (Geddes and Earing, 2018).

Chromosomal microarray has a and recommended investigation the evaluation of major cardiovascular , critical CHDs, or congenital heart disease patients with multiple congenital anomalies, (Wu ., 2017). Patients syndromic congenital heart defects %) when compared those congenital heart defects (%) (Geddes and Earing, 2018). In a study performed in Cape Town, South Africa, which included individuals with syndromic and non-syndromic CHDs, microarray detected (5.6%) , (Saacks et al., 2022). This was considered to be in keeping with other studies done in European populations which yielded 3-25% of CNVs in patients with CHDs (Blue ., 2017).

highest testing CHDs is in individuals with syndromic and/or a familial (inherited form) CHDs (Blue et al., 2017). In one study, actionable variants were identified using genomic sequencing in 49% of those with non-syndromic, familial forms and in 43% of those with CHDs and extracardiac manifestations (Alankarage et al., 2019). Non-syndromic, isolated CHDs reported yield is 3-10% for chromosomal microarray, 2-10% for WES and 10% for WGS (Morrish et al., 2022).

Genetic testing practices for CHDs are yet to be standardized in many centres. becoming , however CHDs low patients with isolated CHDs. Standardized guidelines regarding indications for genetic testing .

1.7.1. Utility of genetic assessment of patients with CHDs

Knowledge of the genetics of CHDs assists in risk assessment of associated complications, evaluation of associated extracardiac manifestations and provision of information about long-term prognosis (Cowan and Ware, 2015). This knowledge, allows for holistic discussions about management of the cardiac and extracardiac abnormalities in patients with CHDs. As an example, patients with 22q11.2 deletion syndrome may be prone to sepsis, present with hypocalcaemia or graft vs with . The use of in as it allows anticipate potential complications and the .

Cascade genetic assessment including genetic testing can be provided for family members of the affected individual. A genetic diagnosis allows for the assessment of reproductive risks and provision of family planning options. may couples with personal or family history of CHDs and a known genetic diagnosis. In the event that CHDs prenatally, centre that offers for condition. /quaternary and cardiothoracic facilities, depending on the severity of the lesion and parents/ patients' wishes.

involvement CHDs resulted in one study, (Geddes et. al., 2019). In this study, phenotyping by a clinical geneticist assisted with and identification additional . Genetic testing utilization improved significantly decreasing ordered patients. Similar findings were also reported by Goldenberg et. al, 2017.

A clinical geneticist role is pedigree collation and assessment, phenotyping of patient including dysmorphology assessment and genetic counselling (with genetic counsellors). The role extends to educating the family regarding implications for testing, possible testing outcomes

(including variants of unknown significance, incidental findings, a negative or positive result), recurrence risk for offspring and siblings, cascade family testing in the event of a positive test, monitoring and anticipatory management in the event of a positive test, and cessation of monitoring in family members who test negative for the family variant and containment of patients (for both positive and negative results).

Specialised cardiac genetics clinics were [redacted], (Morrish et al., 2022). Within a resource-constrained country such as South Africa, and probably other LMIC, it may not be currently possible to set up specialised cardiac genetics clinics in all centres. It should however be possible in some tertiary/ quaternary centres and should be noted as the optimal level of care to aim for and aspire towards.

1.8. Genetic risk mediators of medical and surgical outcomes of CHDs

Survival rates of patients with CHDs have increased significantly over the past number of years. Improved surgical techniques and medical care for those that are able to access care have contributed to this increase. Disease-related morbidity however remains considerable with reduced life-expectancy in these patients, (Pierpont et al., 2018).

Surgical outcomes in patients with CHDs have genetic risk mediators. The underlying genetic condition is one of the determinants of length of intubation, transfusion requirements, growth, and neurodevelopmental outcomes. Studies of patients with CHDs have shown that abnormal genetic tests correlate to poorer surgical outcomes (Pierpont et al., 2018).

As a group, for example, patients with pathogenic CNVs have poorer outcomes than those without CNVs. [redacted], (Putotto et al., 2022). [redacted], (Kim et al., 2016).

Single gene variants are also known to contribute. [redacted] abnormalities [redacted] (Geddes [redacted] Earing, 2018).

The investigation of underlying genetics in patients presenting with CHDs needs to become routine standard of care to improve medical and surgical outcomes (Cowan and Ware, 2015). With the shortage of medical geneticists to actively evaluate each individual patient, it is imperative for other healthcare [redacted] the [redacted] a patient [redacted] congenital heart disease.

1.9. Aim of the study

The study aimed to retrospectively review patients with CHDs attending a newly established paediatric cardiology service for confirmed genetic conditions, and assess whether clinical and molecular genetic assessment can be expanded in this group of patients.

1.10. Study objectives

1. To describe congenital heart defects seen in patients presenting at the paediatric cardiology clinic.
2. To categorise these conditions as syndromic or non-syndromic based on retrospective data available.
3. To document if genetic testing was done on these patients, the type of genetic testing done and results thereof.
4. To recommend an [redacted] congenital heart defects in a resource-limited setting.

CHAPTER 2

RESEARCH METHODOLOGY

2.1. Subjects and study setting

[redacted] a tertiary/quaternary referral-only hospital. It offers cardiac care in the form of cardiology and cardiothoracic surgery to paediatric patients within Gauteng province, and those from the neighbouring provinces of the North-West, Limpopo and on occasion, Mpumalanga.

A retrospective file review and data analysis of the clinical records of children seen at the cardiology service at NMCH between 1 January 2019 – 31 December 2019 (1-year period) was performed. This period was chosen when the study was initiated in 2022 as it would have been the most active period since the service is newly established and the subsequent 2 years had been disrupted by the COVID19 pandemic. Children who were 0 months to 18 years at the time of consultation within the stipulated time-period were included in the study. This is the age group seen at the Nelson Mandela Children's Hospital cardiology service.

[redacted] 206 [redacted] seen over [redacted]. Only patients with structural CHDs were included in the study. Individuals with isolated cardiomyopathy and acquired heart diseases were excluded. A total of 181 patient records were included, with 25 excluded due to failure to meet study criteria. [redacted] certificate no. M220840 (**Appendix A**).

2.2. Data sources

Data was obtained [redacted] patient files, [redacted] database, medical reports [redacted] medical image storage systems based at NMCH cardiology clinic.

2.3. Data collection

Data was collected and collated by the researcher. Nominal data, viz. sex, type of cardiac lesion, reported dysmorphic features or minor anomalies, other extracardiac structural abnormalities,

associated neurodevelopmental abnormalities, genetic testing done and results were categorised and coded for analysis. Continuous data, viz. age at presentation to NMCH for quaternary care and weight were also recorded and analysed. Data collected was captured onto the Witwatersrand, Division of Human Genetics. A data collection sheet had been designed by the researcher for this purpose and is attached (**Appendix B**).

Cardiac lesions were classified using Botto level 3 classification (National Birth Defects Study) with additional categories for those not represented by the Botto level 3 classification (Botto et al., 2007). The Botto level 3 categories included septal defects, defects, syndromes, obstruction (obstruction ( ), complex cardiac conditions anomalous pulmonary venous return (APVR). Other categories described in this cohort but not included in the Botto level 3 classification are patent ductus arteriosus (PDA) and valvular lesions.

Records of patients were anonymized by the assignment of a study number as an identifier. The patients' names and hospital numbers were delinked and kept separately from the study data by the researcher via a password. Only the researcher and supervisors have access to this reference chart.

to present patients' characteristics and clinical variables using proportions (%) , with . Age at presentation to NMCH was analysed using inter-quantile ranges.

Types of congenital heart defects presenting were categorized. Presence of reported dysmorphic features/minor anomalies, additional structural extracardiac anomalies (categorized per organ system) were also described. The number of patients who demised during this period was reported. The number of patients with neurodevelopmental delay and growth restriction were reported on. The number of genetic tests done, number of positive genetic test results and genetic diagnoses in those with positive genetic tests were also reported on.

If the patients presented with CHDs only, with no other associated anomalies, they were considered as non-syndromic. Syndromic CHDs were those associated with extracardiac structural abnormalities, reported dysmorphic features or minor anomalies. It should however be noted that neurodevelopmental delay and growth restriction may occur as a result of the underlying genetic syndrome or as a consequence/ complication of the CHDs. [41] longitudinal [redacted] were [redacted] define the cause of growth restriction/ failure to thrive precisely. Neurodevelopmental delay was used to classify CHDs as syndromic, however growth restriction was not unless it was intrauterine growth restriction.

Stata Statistics/Data analysis 18.0 Stata Corp LLC software for Microsoft Windows, was used for the analysis of data.

181 patient records were examined. **3.1.** at presentation to NMCH was 7 months (IQR 0.7-24 months), age range 1 day – 17 years.

Table 3.1: Characteristics of patients with CHDs (n=181)

Characteristics	N	%
Reported dysmorphic features/ minor anomalies	38	21.0
Congenital heart defects		
Isolated	108	59.7
Syndromic	73	40.3
Mortality	21	11.6

3.2. Types of cardiac lesions diagnosed in patients with CHDs

The types of presenting cardiac lesions [as per classification described (Botto)], are .

. Types presenting CHDs as per Botto level 3 classification (n=139)

Type of defect	N	%
Conotruncal defects	40	22.1
Septal defects	32	17.7
LVOTO	27	14.9
RVOTO	17	9.4
AVSD	14	7.7
APVR	5	2.8
Heterotaxy	4	2.2

Other categories not included in the Botto level 3 classification were PDA 28 (15.5%), complex cardiac conditions 10 (5.5%), valvular lesions 3 (1.7%), and peripheral pulmonary stenosis (PPS) 1 (0.6%).

3.3. Dysmorphic features/ minor anomalies

Dysmorphic features/ minor anomalies were reported in 38 (21%) individuals. In 50% (19/38) of the patients' files, the dysmorphic features were unspecified including those occuring in children with a molecular diagnosis of Down syndrome. In cases where the dysmorphic features were specified, the majority had 2 or more features described.

3.4. Major structural abnormalities

Thirty-one major structural abnormalities were reported in 25 (13.8%) individuals. These involved the gastrointestinal 17/31 (54.8%), respiratory 4/31 (12.9%), genitourinary 4/31 (12.9%), skeletal 3/31 (9.7%), CNS 2/31 (6.5%) and ectodermal 1/31 (3.2%) systems.

Additional structural extracardiac abnormalities are represented in 21 .

[redacted]: Types [redacted] additional structural abnormalities [redacted] with CHDs (n=31)

System	N	%	Type of structural abnormality
Gastrointestinal	17	54.8	Cleft palate Oesophageal atresia Tracheo-oesophageal [redacted] [redacted] [redacted] [redacted] Anorectal malformation
Respiratory	4	12.9	Choanal atresia Subglottic web Right main bronchus hypoplasia Trilobed left lung
Genitourinary	4	12.9	Unilateral renal agenesis Chordee, hypospadias Unspecified urethral abnormality Cryptorchidism
Skeletal	3	9.7	Unilateral limb reduction defect Limb length discrepancy Missing 12th rib
CNS	2	6.5	Neural tube defects -lumbosacral myelomeningocoele -spina bifida occulta
Ectodermal	1	3.2	Extracranial, occipital haemangioma

Four of the patients were reported to have more than one additional major structural abnormality (up to 3 systems in one case), see **Table 3.4**.

Table 3.4. Patients presenting with >1 additional extracardiac structural abnormality

Patient	No. of additional structural abnormalities	Type of structural abnormality
1	2	Tracheo-oesophageal fistula Cleft palate
2	2	Choanal atresia Genitourinary abnormality (cryptorchidism, micropenis)
3	2	Congenital diaphragmatic hernia Unilateral limb reduction defect
4	3	Spina bifida occulta Genitourinary abnormality (cryptorchidism, hypospadias, chordee) Skeletal (limb length discrepancy, missing 12^{th} rib)

3.5. Mortality rate of patients with CHDs in the cohort

Of the cohort, 160 (88.4%) were still alive and 21 (11.6%) had demised at the time of the study. Some patients demised perioperatively and these would have been investigated as per protocol of surgical/ anaesthetic deaths. The balance of patients demised at the base hospital or at home with post-mortem reports of the cause of death not provided.

3.6. Neurodevelopmental abnormalities reported in patients with CHDs

Neurodevelopmental abnormalities were reported in 30 (16.6%) cases. The level of developmental delay was however not reported.

3.7. Growth restriction reported in patients with CHDs

Growth restriction (failure to thrive and/ or stunting) can occur as a result of the CHDs, malnutrition or underlying genetic condition. Growth restriction/ failure to thrive was reported in 52 (28.7%) cases. Due to the absence of longitudinal data, it was not possible to assess the underlying cause further.

3.8. Genetic testing in patients with CHDs

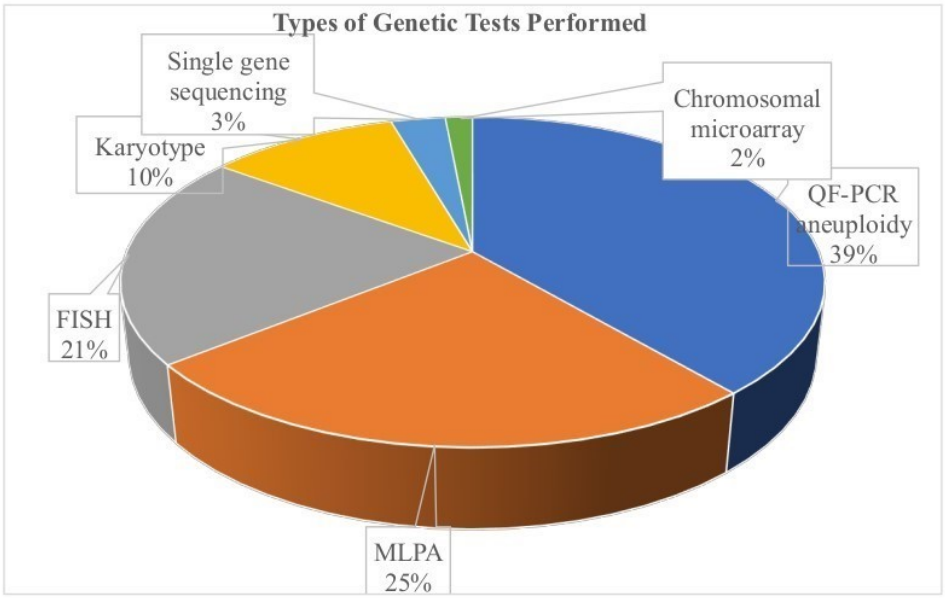
A total of 67 genetic tests were performed on 58 (32%) individuals.

3.8.1. Types of genetic tests performed on patients with CHDs

Access to genetic testing in the study period was limited to QF-PCR aneuploidy, karyotype, FISH (22q11.2 FISH mostly requested), subtelomeric/deletion MLPA and MS-MLPA. Chromosomal microarray, single gene sequencing, WES and WGS were mostly inaccessible therefore limiting a wide range of genetic diagnoses.

The most frequent genetic test performed was QF-PCR aneuploidy 26/67 (38.8%). This was followed by subtelomeric/ microdeletion MLPA 17/67 (25.4%), FISH for 22q11.2 deletion testing 14/67 (20.9%), karyotype 7/67 (10.5%), chromosomal microarray 1 (1.5%), single gene sequencing 2/67 (3.0%) for RASopathy panel and *CFTR* gene for suspected cystic fibrosis. Genetic tests performed are represented in **Figure 3.1.**

Figure 3.1: Types of genetic tests performed in a subset of patients with CHDs (n=67)

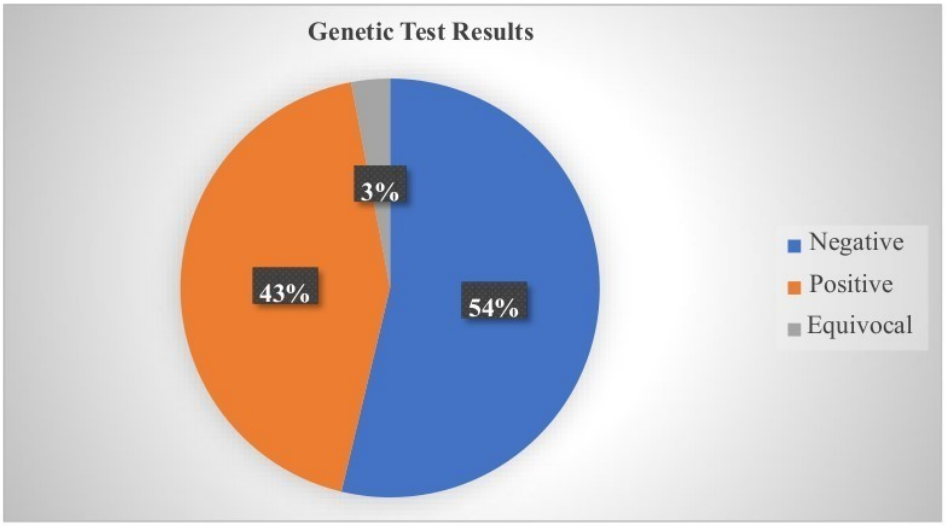


Of the 38 patients assessed with dysmorphic features/ minor anomalies, 29/38 (76.3%) received genetic testing. Two tests were performed per individual in three patients. As an example, a female neonate who presented with pulmonary atresia, VSD, PDA, left anotia, brachycephaly, widely spaced nipples and anorectal malformation had QF-PCR aneuploidy and 22q11.2 deletion FISH performed. Of note are the multiple tests performed in some individuals, up to 4 genetic tests/ individual in some cases.

3.8.2. Genetic test results recorded in patients with CHDs

Of the 67 tests performed on 58 patients, 29 (43.3%) were positive, 36 (54%) were negative and 2 (3.0%) equivocal as shown in **Figure 3.2**.

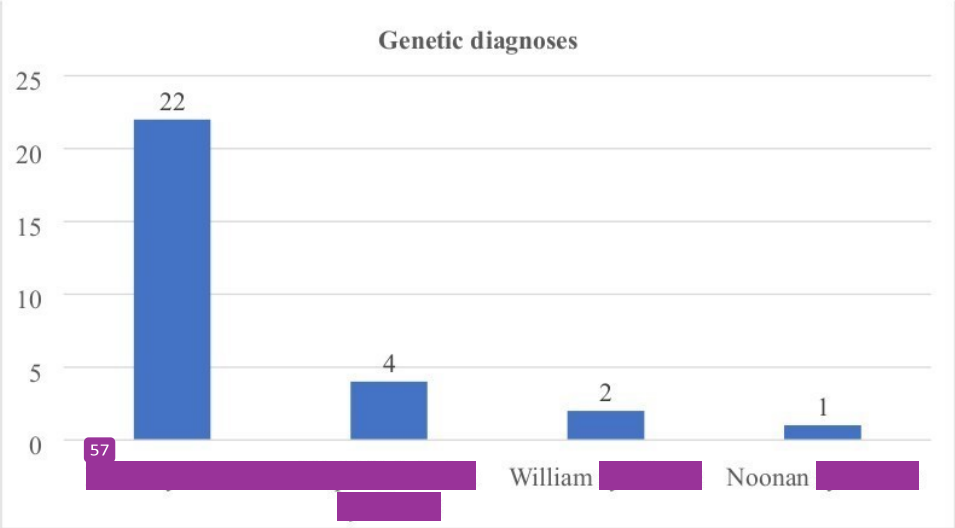
Figure 3.2: Genetic test results in patients with CHDs



The equivocal results included 23 on chromosome 15q (*SNRPN*-u1b gene at 15q11.2) 23 at chromosome 16p (*CREBBP* gene at 16p13.3) using the P245 probe mix on subtelomeric/ microdeletion MLPA in both cases. No further testing was done for the above-mentioned patients.

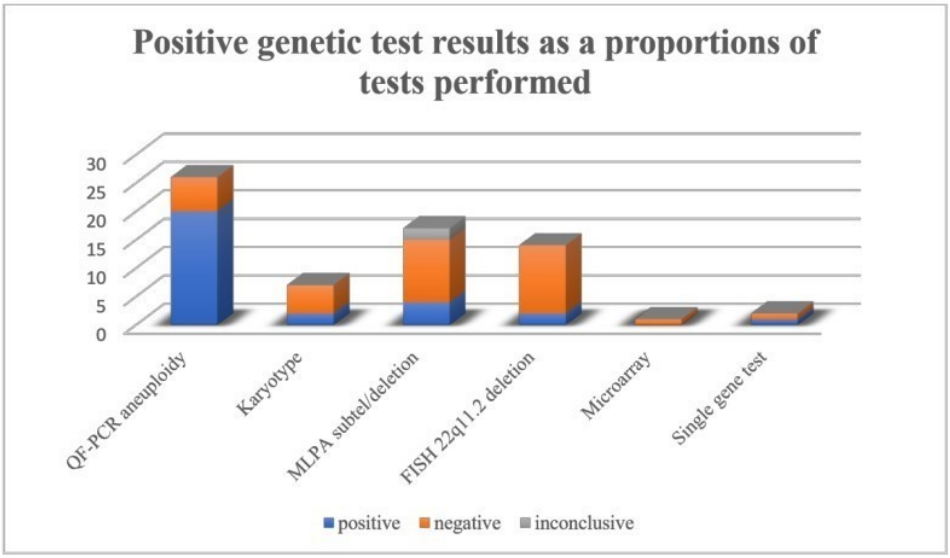
Of the 29 positive genetic tests results, 22 (75.9%) were trisomy 21, 6 (20.7%) copy number variants [4 (13.8%) 22q11.2 deletion, 2 (6.9%) William syndrome], and 1 (3.5%) single gene *PTPN11* (Noonan syndrome), see **Figure 3.3.**

Figure 3.3: Genetic diagnoses obtained after genetic testing



Twelve out of the fourteen (85.7%) patients presenting with AVSD had genetic testing performed. Of those tested, 11/12 (91.7%) had Down syndrome and 1/12 (8.3%) Noonan syndrome. Chromosomal copy number variants (deletions/duplications) were also found as a cause of syndromic CHDs, with 4/29 (13.8%) of positive tests detecting 22q11.2 deletion and 2/29 (6.9%) 7q11.2 deletion (William syndrome).

Figure 3.4. Positive genetic test results as a proportion of tests performed on a subset of patients with CHDs



3.9. Syndromic vs non-syndromic CHDs

CHDs were categorized in this study as non-syndromic if there were no associated dysmorphic features/ minor anomalies, developmental delay or major structural extracardiac abnormalities documented. Apparently non-syndromic CHDs were found in 108 (59.7%) patients. Seventy-three (40.3%) patients presented with syndromic CHDs.

Down syndrome, 22q11.2 deletion, William and Noonan syndrome were syndromic causes confirmed by genetic testing. A large amount of syndromic and non-syndromic CHDs remained without a genetic diagnosis.

CHAPTER 4
DISCUSSION

4.1. Age at presentation for care in patients with CHDs

The median age at presentation for tertiary/quaternary care including for cardiothoracic surgery in this cohort was 7 months. Patients present at later stages of disease and at older ages in low-resource settings in comparison to those in high-income countries, (Thomford et al., 2018). Widespread use of prenatal ultrasound screening including fetal anomaly scan, allows for the prenatal diagnosis of CHDs in high-income countries. This allows for planning of delivery in a hospital equipped to handle complex cardiac cases. In our setting, very few children with CHDs are diagnosed prenatally with some of those with critical CHDs succumbing in the neonatal period or infancy, (Saacks et al., 2022).

If not diagnosed prenatally, infants born at a local clinic, primary or secondary hospital, without prior knowledge of the underlying CHDs, need to be stabilized and transferred in specialized ambulances to tertiary/ quaternary care centres for further care including cardiothoracic surgery as needed. This is a challenge within a limited-resource setting due to the limited availability of specialized ambulances and personnel.

4.2. Types of presenting cardiac lesions in patients with CHDs

A wide range of structural cardiac abnormalities was seen in this cohort as was expected for a centre that sees a significant number of patients with complex medical needs. Conotruncal defects (22.1%) were the most common in this cohort, followed by septal defects (17.7%). Septal defects are reported to represent the commonest CHDs in patients with moderate to severe CHDs, (Hoffman and Kaplan, 2002). Our result is most likely due to selection bias as the majority of patients seen at NMCH are those needing cardiothoracic surgical intervention and thus may have more complex cardiac presentations.

4.3. Reported dysmorphic features/minor anomalies in patients with CHDs

Dysmorphic features/minor anomalies were reported in 21% of cases. In their paper, Ahrens-Nicklas et al. report a high genetic test yield in the presence of eye abnormalities e.g. epicanthic folds, hypertelorism; cleft palate and cranial abnormalities (for example microcephaly) and presence of 3 fontanelles, (Ahrens-Nicklas et al., 2016). This finding highlights the importance of thorough dysmorphology examination and documentation in patients with CHDs as this may assist in the diagnosis of underlying genetic conditions.

Data from our cohort was however sparse in the description of dysmorphic/ minor anomalies findings. In 19/38 (50%) of patients with reported dysmorphic features/ minor anomalies, dysmorphic features were unspecified, including in those with Down syndrome. In the other 50% (19/38) patients, dysmorphic features were specified and these assisted with the choice of genetic test requested. A minimum of two features per patient were noted and these were typical of the clinical and molecular diagnoses. As an example, in a patient who was found to have William syndrome on subtelomeric/ microdeletion MLPA, the features described were, elfin facies, periorbital fullness, broad mouth, and prominent ears which are consistent. Similarly, brachycephaly, epicanthic folds, low-set ears and brachydactyly were described in a patient with Down syndrome.

The frequency of major structural defects was noted to be increased 5-fold that of the general population if 2 dysmorphic features/ minor anomalies were present (i.e. 11% in those with 2 dysmorphic features/minor anomalies vs 2.2% in the general population). This increase is even higher in those with 3 dysmorphic features/ minor anomalies (90% of those with 3 dysmorphic features/ minor anomalies were found to have major structural defects) (Marden et al., 1964).

4.4. Additional major structural abnormalities reported in patients with CHDs

Additional major structural abnormalities were noted in 13.8% of individuals in this cohort of patients. The commonest were gastrointestinal abnormalities with over half of the patients with additional structural abnormalities presenting. Anorectal malformations and congenital diaphragmatic hernia (CDH) were the most common GIT abnormalities.

The majority of the patients with additional GIT abnormalities did not receive a genetic diagnosis. Helm and Ware identified a genetic diagnosis in 35.1% of those with gastrointestinal/abdominal wall abnormalities (Helm and Ware, 2024).

One of the three patients with duodenal atresia had Down syndrome, and one of the 5 patients with anorectal malformation received a diagnosis of Down syndrome as well. Of the 5 patients with congenital diaphragmatic hernia, only 1 had genetic testing, a QF-PCR aneuploidy, which was negative with no reported dysmorphic features suggestive of the common aneuploidies.

There are genetic conditions known to cause both CDH and CHDs, for example, [46] and chromosomal aneuploidies, trisomy 13 and 18. The choice of genetic test in the above depends on the clinical picture. If phenotype is specific for a monogenic condition for example Cornelia de Lange syndrome, one can proceed with sequencing (e.g. gene panel, WES). If, however not specific or suggestive of a chromosomal condition, one can proceed with chromosomal microarray/ aneuploidy testing.

In another study, Egbe et al. reported extra-cardiac/ major structural abnormalities to occur in approximately 13% of newborns with CHDs (Egbe et al., 2014). Ahrens-Nicklas et al. reported from their study findings that the presence of neurological (corpus callosum abnormalities, hypotonia) or musculoskeletal (syndactyly, clinodactyly and vertebral abnormalities) increased the odds of finding a genetic diagnosis (Ahrens-Nicklas [9]., 2016). the patients our did not have brain imaging and we are therefore unable to comment with regards to structural brain involvement. There were however two patients reported with neural tube defects. One with lumbosacral myelomeningocoele, associated Chiari II malformation and no genetic testing performed. Another with spina bifida occulta with associated genitourinary and skeletal abnormalities who also had no genetic testing.

4.5. Mortality rates in patients with CHDs

A hundred and sixty (88.4%) patients were still alive and 11.6% had demised at the time of the study. Not all details regarding the final cause of death were available however as some deaths occurred at the primary hospital or at home. Patients who demised intra/ post-operatively were investigated further and a post-mortem report shared with the medical team looking after the

patient and next of kin. Associations between type of CHD, genetic condition, extracardiac structural abnormalities and mortality rates were unable to be drawn due to scanty retrospective data, lack of access to post-mortem reports and small sample size.

4.6. Neurodevelopmental abnormalities rates reported in CHDs patients

Neurodevelopmental abnormalities were reported in 16.6% cases. Neurodevelopmental abnormalities are reported to occur in patients with CHDs, either as a consequence of the underlying genetic condition, complication of the congenital heart defect or related procedures/ interventions. In this study, the presence of neurodevelopmental delay assisted in classifying the CHDs as likely syndromic. The degree of severity of neurodevelopmental delay was however not documented.

4.7. Associated growth restriction rates reported in CHDs patients

Growth restriction was reported in 28.7% cases and may be as a result of the CHDs, malnutrition or the underlying genetic condition. The exact contributors to faltering weight and stunting could not be accurately determined [39].

4.8. Genetic testing of patients with CHDs

Access to genetic testing in the study period was limited to QF-PCR aneuploidy, karyotype, subtelomeric/ deletion MLPA and 22q11.2 FISH. Chromosomal microarray, single gene sequencing, WES and WGS were mostly inaccessible therefore limiting a wide range of genetic diagnoses.

In the NHLS/NMCH setting, [55] now line [44], where the not specific for the common aneuploidies (T13, 18 or 21). WES is only accessible via research studies and WGS is mostly inaccessible in this setting.

Different provinces in South Africa have different arrangements with the Department of Health and local health authorities regarding broader access to genetic testing. In the Western Cape as an example, a budget is allocated per annum for the medical geneticists to prioritize genetic testing. They are then able to access local, national and overseas testing should this be required. This resource is however dwindling and no longer available for some centres. Gauteng and other provinces which are able to access the Johannesburg centres for clinical assessment and phenotyping, were until March 2024 able to access limited single gene testing via the inherited disease gene panel. This option is unfortunately no longer available due to laboratory logistical problems.

All provinces within South Africa have access to QF-PCR aneuploidy, karyotype, FISH, chromosomal microarray, MLPA and MS-MLPA via specializes NHLS laboratories in the country (Braamfontein, Groote Schuur, Sefako Makgatho and Bloemfontein). Turnaround times are however long.

Sixty-seven genetic tests were performed on 32% of the patients. Genetic testing relied on the treating physician as there was no protocol to guide genetic testing in patients with CHDs at the time. A significant number of patients who presented with AVSD, 12/14 (85.7%) or had phenotypic features of Down syndrome received genetic testing.

Conotruncal defects have long been associated with genetic conditions and guidelines have existed to guide the genetic assessment of individuals presenting with conotruncal defects. Conotruncal defects are known to have a high genetic test yield and would routinely be referred for genetic testing for CNVs, specifically 22q11.2 deletion. This historically included mainly 22q11.2 deletion FISH and subtelomeric/ deletion MLPA as chromosomal microarray was not routinely available at the time of the study. Fifteen (15/40, 37.5%) of those who presented with conotruncal defects were tested in this cohort.

Of the patients with dysmorphic features/ minor anomalies, 23.7% (9/38) did not receive genetic testing. As an example, a 1-year-old female patient with VSD, large forehead, webbed neck, and developmental delay did not receive any genetic testing. Similarly, a 3-month-old male with ASD, PDA, TAPVD, microtia, micrognathia, growth restriction, rectourethral fistula received no further genetic testing after QF-PCR aneuploidy test detected no common aneuploidies.

Array CGH is recommended in syndromic CHDs as per ACMG guidelines as 1st line to rule out copy number variants. Single gene panel testing is an option where the phenotype is suggestive of a recognizable phenotype, e.g. RASopathy panel in a patient with features of Noonan syndrome. WES/WGS is recommended if the phenotype is not recognizable or where single gene panel/ array CGH testing yielded no results. The clinical details documented were not sufficient to enable suggestions for further testing in those patients with negative genetic testing. We do recommend following guidance as recommended (flow sheet attached and refer to genetics clinic as necessary, Appendix D).

In this cohort, all patients with syndromic CHDs, critical CHDs, and familial CHDs should have received genetic testing. At least 73 (40.3%) patients (15 more than the number tested) were categorized as syndromic and therefore genetic testing would be recommended. Family history of CHDs was not however not documented, therefore making it difficult to assess the total number of tests recommended for this cohort. This underscores the importance of multidisciplinary clinical and molecular genetic assessment including the involvement of medical geneticist as part of the team to collate and interpret family pedigree, phenotype and direct genetic testing.

4.9. Genetic test results in patients with CHDs

Twenty-nine (43.3%) of the sixty-seven tests requested were positive, yielding a genetic diagnosis in 16.0% of the patients. This is lower than expected for those centres which involve medical geneticists closely in the care of patients with CHDs (Geddes et al., 2019). In these studies, a diagnostic rate of 25% was reported (Ahrens-Nicklas et al., 2016) with a higher genetic testing rate.

Down syndrome was overrepresented with 75.9% of the positive tests detecting trisomy 21. [redacted] 35 [redacted] trisomy 21 was [redacted] as it [redacted] a common genetic condition. Down syndrome phenotype is easily recognizable to most health care practitioners and therefore would mostly be referred for genetic testing and similarly for CHDs assessment and surgical correction if required. Genetic testing for Down syndrome is also readily accessible. Atrioventricular septal defect (AVSD) had a high genetic diagnostic yield. This may

be as a result of its association with Down syndrome. The majority of patients presenting with AVSD received a molecular diagnosis of Down syndrome.

Trisomy 13/18 patients with multiple congenital anomalies assessed as likely to have poor prognosis due to limited lifespan would generally not access the cardiothoracic services in this setting. This group of children are therefore not represented in this cohort.

It is surprising that Turner syndrome is not represented in this cohort. This may be due to the fact that the phenotype is not easily recognizable in the younger age group. Recognition and therefore diagnosis of Turner syndrome tends to occur at a later age with other presentations viz. primary amenorrhoea, short stature and bicuspid aortic valvular disease.

Of the 17 tests done in 15 patients with conotruncal cardiac defects, 6 were positive, with a 40% (6/15) diagnostic rate. Trisomy 21 was detected in 3 patients, and the other 3 had 22q11.2 deletion. The tests performed were however limited viz. subtelomeric/ deletion MLPA, QF-PCR aneuploidy and FISH for 22q11.2 deletion.

Chromosomal microarray is the preferred test for chromosomal copy number variants (microdeletions/ duplications) as it is broader and therefore is more like to pick up a wider group of CNVs. [redacted] considered [redacted] congenital abnormalities or [redacted], (Miller et al., 2010) and should be offered to patients with CHDs as these are a common feature of chromosomal copy variants disorders.

Genetic testing in individuals with isolated CHDs, where a likely genetic change would be a single gene variant, and therefore requiring single gene panel, WES or WGS was low. This was both as a result of non-requisition by clinicians and unavailability of genetic tests at the NHLS at the time of the study. Medical geneticist involvement in the assessment of patients with CHDs has been shown to contribute to improved care as it assists in the expedient diagnosis of genetic conditions if associated and the choice of appropriate genetic testing (Morrish et al., 2022). The traditional view of CHDs, especially if there are no associated abnormalities, as a 'non-genetic' leads to the under-referral of patients and their families for clinical genetics assessments.

Noonan syndrome, was the only single gene condition detected in this cohort. This patient was diagnosed as part of a research study and the result has since been validated. Other single gene conditions that may be associated with CHDs (e.g. Alagille, other RASopathies and CHARGE syndrome) were not detected. This again emphasizes the importance of medical geneticist involvement to assist with phenotyping and directing appropriate genetic test request and the need for broad availability of genetic tests.

Screening for genetic conditions in patients with CHDs is not standardized. Genetic testing in patients with CHDs is underutilized (Helm and Ware, 2024). Recent studies looking at hospitalized infants with CHDs show the benefits of standardization of genetic evaluations, leading to early diagnosis of genetic conditions, specific management and family risk assessment (Helm and Ware, 2024).

4.10. Syndromic and non-syndromic CHDs

Apparently non-syndromic CHDs were found in the majority of the patients in this cohort. This is expected as the majority of patients with CHDs are reported to [redacted] cardiac [redacted] the involvement [redacted]). Adequate information regarding family history of CHDs was not provided therefore making it difficult to classify cases into non-syndromic, familial or non-syndromic, sporadic (non-familial). It is clear from various observations that extracardiac abnormalities cannot solely be used as a driver to initiate genetic testing as individuals with non-syndromic CHDs with an underlying genetic cause, will be missed (Helm and Ware, 2024). [redacted] or [redacted] were found [redacted] patients with [redacted]-syndromic CHDs highlighting the need for genetic testing in non-syndromic CHDs (Nees and Chung, 2020).

Chromosomal copy number variants (deletions/duplications) were also found as a cause of syndromic CHDs. The prevalence of CNVs is reported to be varied depending on the cohort and method of detection used with reports of 3-25% (Nees and Chung, 2020). In our cohort, with the use of limited genetic testing (subtelomeric/ microdeletion MLPA and 22q11.2 deletion FISH), and minimal chromosomal microarray, the reported prevalence is 6/181

(3.3%). Chromosomal [redacted] reported [redacted] manifestations as [redacted] no extracardiac manifestations, (Wu et al., 2017). Wolf-Hirschhorn and 1p36 deletion syndrome are some of the CNVs commonly seen with CHDs that are not represented in this cohort. This is surprising as some of these conditions would have been picked up by subtelomeric/ microdeletion MLPA.

No intervention was undertaken during this study. As part of the reporting of the study, an algorithm was formulated to assist with future genetic assessment of patients with congenital heart defects. This will be handed to the cardiology unit at the Nelson Mandela Children's Hospital.

CHAPTER 5

5.1. Limitations

This was a retrospective review of patients' records, and thus complete data were not available on all patients. Some records were kept at the primary/ referring hospitals.

The study centre is a referral centre and sees a select cohort of complex patients needing tertiary or quaternary medical and surgical care. Only patients who have been assessed to likely benefit from the cardiology and cardiothoracic services offered are referred to NMCH for further management. Patients with less complex presentations managed at primary and secondary level of care centres were not fully represented in this cohort, therefore introducing selection bias.

Patients, who have been assessed to have a poor prognosis, those that would benefit from palliative care only and are therefore not candidates for the level of care offered, were not referred by their primary institutions to NMCH and therefore are also not represented in this cohort.

Factors that influenced genetic testing in our cohort were availability of genetic tests, type of CHDs, associated congenital anomalies, neurodevelopmental problems and recognizable phenotype. There seemed to be limited recognition of other rare genetic causes of CHDs as requested genetic testing was limited to commonly recognizable syndromes. Selective genetic testing was mainly requested in those patients whose phenotype resembled a common recognizable genetic syndrome (e.g. Down syndrome, William syndrome) and in those with AVSD.

As part of the study, we wanted to look at the likely syndromic cases with negative genetic tests to suggest a possible diagnosis/ test based on the phenotype. An attempt was made but was unsuccessful due to scanty clinical details provided in the patient records.

5.2. Recommendations

the yield of CHDs (Durbin et al., 2023). The average time to resolve a diagnostic odyssey in rare diseases as documented by the EURORDIS-led Community Engagement Task Force in Europe has been estimated to be around 5.6 years. Timeous genetic diagnosis likely to be provided by clear genetic assessment guidelines and multidisciplinary care, has been shown to shorten the time to diagnosis and access to appropriate care including targeted therapeutic interventions or aiding transition to palliative care. The net healthcare expenditure is reduced by limiting genetic performed number visits with various specialists to investigate the underlying diagnosis (Faye et al., 2024). We recommend realize these benefits. We have included recommendations to guide the CHDs in a resource-limited setting (**Figure 5.1**).

ISCA (supports the use of CMA patients disorder). updated WES/WGS / paediatric . WES/WGS has is - effective if of group of patients with findings that are not specific for a known syndrome (Manickam et al., 2021). We recommend the availing of extended genetic testing to include uninterrupted availability of chromosomal microarray, single gene panels and WES to allow broader access.

There is a limited number of genes associated with CHDs on the inherited disease panel, these can be virtually assessed. This is subject to a patient being assessed and phenotyping done by a medical geneticist. At present, IDP is not available due to laboratory logistical problems.

Other factors that influence genetic testing, e.g. family history of CHDs, dysmorphic features/ minor anomalies and major anomalies were not adequately documented in this cohort. This underscores the importance of involving medical genetics specialists as part of the team to care for patients with CHDs to assist with patient phenotyping, comprehensive family pedigree

assessment and appropriate choice of genetic tests to improve diagnosis of less recognizable syndromes.

A more in-depth analysis of the data with a probably larger cohort of patients would improve its value, further research in genetic conditions in CHDs is needed.

Medical genetics involvement will also allow for genetic counselling and follow up to allow pre- and post-test counselling, family cascade testing, recurrence risk assessment, reproduction planning with discussions around prenatal or preimplantation testing options.

5.3. Conclusions

There is a need for broader recognition of genetic conditions and genetic assessment of patients with CHDs. Medical genetics involvement is important to assist in the detailed phenotyping, recognition of rare genetic syndromes which allows for directed testing to allow timely diagnosis and intervention. Care for patients with CHDs should include detailed phenotyping, documentation of dysmorphic features/ minor anomalies, additional structural and non-structural abnormalities, comprehensive family pedigree and broader genetic testing with increased medical genetics involvement.

The types of genetic tests requested in this cohort reflect the available tests within the constrained LMIC setting. Access to genetic testing remains restricted in our setting, although improved. Universal genetic testing of patients with CHDs may allow us, in the long-term, to define those patients that would benefit from genetic testing, especially within the understudied African population.

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CERTIFICATE OF SUBMISSION FOR EXAMINATION SIGNED BY SUPERVISORS OF HIGHER DEGREES CANDIDATES

Full name	Daisy Salome Mokwele
Student number	9402484K

Candidate for the degree of: Master of Medicine in Medical Genetics
has submitted her research report

Entitled: Retrospective review of genetic conditions in patients with congenital heart defects attending a quaternary paediatric cardiology service in Johannesburg, South Africa.

Contact no	0825705742	E-mail	daisy.mokwele@wits.ac.za mokweled@gmail.com

Mark with an X on appropriate box	Yes	No
Has this thesis/dissertation/research report been submitted with the acquiescence of the supervisor?	X	
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List all publications, which your student has published in peer-reviewed journals from his/her postgraduate research report/dissertation/thesis during the course of his/her studies in the Faculty of Health Sciences (Include authors, year, title of paper, name of journal, volume number and page numbers). This information is mandatory.

No publications as yet.

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Name of Supervisor 1: Amanda Krause

Telephone: 082-809-5797 Email: amanda.krause@wits.ac.za

Signature: AKrause Date: 07/10/2024

Name of Supervisor 2: Hopewell Ntsinjana

Telephone: 0730736832 Email: hopewell.ntsinjana@wits.ac.za

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Signature:  Date: 07/10/2024

Name of Supervisor 3: _____

Telephone: _____ Email: _____

Signature: _____ Date: _____

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IMPORTANT NOTICE WITH REGARD TO THE SENATE STANDING ORDERS:

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If the Supervisor is not prepared to agree to the submission of a thesis, the candidate shall still be entitled, if he or she wishes, to submit it for examination. When a thesis is submitted against the advice of the Supervisor, this should be recorded in the minutes of the Faculty Graduate Studies Committee. In such a case, no internal examiners are appointed but a Supervisor's report will still be required. After the examination process, the external examiner(s) will be advised by the Chairperson of the Faculty Graduate Studies Committee that the thesis was submitted against the advice of the Supervisor.

A.24 *Nomination of Examiners*:

Nomination of examiners should take place at least six weeks before submission of the thesis or dissertation. *(The Postgraduate Office will not accept any submission for examination without the confirmed appointment of the nominated examiners.*)

A.25 Confidentiality of names of examiners (both external and internal)

The names of the examiners should be confidential during the examination process and may only be revealed to the candidate with the acquiescence of the examiner once the final version of the thesis has been submitted to the Faculty and the process has been completed.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

CERTIFICATE OF SUBMISSION FOR EXAMINATION OF MASTERS RESEARCH REPORT SIGNED BY HIGHER DEGREES CANDIDATES

Full name	Daisy Salome Mokwele		
Student number	9402484K		
Title of submitted Research Project: Retrospective review of genetic conditions in patients with congenital heart defects attending a quaternary paediatric cardiology service in Johannesburg, South Africa. *NB: If this title is different to your previously approved title, no further action can be taken by the Faculty Office until a change of title has been approved.*			
Contact no	0825705742	E-mail	daisy.mokwele@wits.ac.za mokweled@gmail.com

1. If you are likely to move in the next 6-12 months, please give the anticipated date of move: N/A
2. I hereby submit my **Masters (research report)** for **examination** (Select whichever is applicable)
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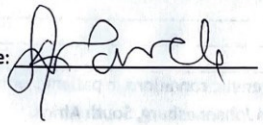
      If yes: I have included a copy of the animal ethics committee clearance certificate as an appendix in this document **N/A**
   d) Done research using human subjects, human tissue or patient records **Yes**
      If yes: I have included a copy of the human ethics clearance certificate as an appendix to the research document **Yes**

7. I understand that I may not graduate unless my University fees have been paid in full.
8. My Supervisors names, departments, telephone numbers and email addresses are as follows:

Name	Prof. Amanda Krause		
Department	Division of Human Genetics, School of Pathology, National Health Laboratory Service and Faculty of Health Sciences, The University of the Witwatersrand, Johannesburg, South Africa		
Telephone	011 489 9211 082 809 5797	E-mail	amanda.krause@wits.ac.za amanda.krause@nhls.ac.za
Name	Prof. Hopewell Ntsinjana		
Department	Paediatric Cardiology Unit, Nelson Mandela Children's Hospital, Department of Paediatrics and Child Health, Faculty of Health Sciences, The University of the Witwatersrand, Johannesburg, South Africa		
Telephone	010 133 0670 0737356832	E-mail	hopewell.ntsinjana@nmch.org.za Hopewell.ntsinjana@wits.ac.za

List all publications, which you have published in peer-reviewed journals from your postgraduate research report during the course of your studies in the Faculty of Health Sciences (Include authors, year, title of paper, name of journal, volume number and page numbers). This information is mandatory.

No publications

Signature of candidate:  Date: 02 October 2024

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