GENETICS OF CONGENITAL HEART DISEASE

The genetics of congenital heart disease: The role of advanced genomic approaches

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ABSTRACT

As the leading non-infectious cause of paediatric morbidity and mortality worldwide, congenital heart disease (CHD) is a significant social and healthcare burden, especially in low- and lower-middle-income countries, including South Africa. The aetiology of CHD is poorly understood, though heritable genetic factors have been shown to contribute to the risk of CHD in individuals of European ancestry. In this review, we highlight the impact that advanced genomic approaches have had on the understanding of the role of genetics in CHD. We also summarise current knowledge of the genetics of CHD in Africa, and the challenges and opportunities for conducting genomic research in these populations. Chromosomal microarrays and next-generation sequencing platforms allow for high-throughput screening of patients for genetic mutations and show great potential for the identification of genetic causes of CHD. Advancing our understanding of the genetic architecture and risk factors associated with CHD in patients of African descent is the first step towards improving CHD diagnosis and management. Therefore, exploring the genetics of CHD has the potential to improve the quality of life for children born with CHD in Africa, by enabling clinicians to identify familial inheritance patterns and predict recurrent risks and prognostic outcomes pre- and post-surgical intervention. SAHeart 2021;18:136-147

INTRODUCTION

Congenital heart disease (CHD; Table I) is the most prevalent birth defect and the leading non-infectious cause of paediatric morbidity and mortality worldwide.[1,2] Approximately 9 per 1 000 children are born with CHD annually,[3] with an estimated 11 000 South African children born with this condition each year.[4] CHD is defined as a structural malformation of the heart and/or great blood vessels that occurs before birth.[3] The disease develops as a result of perturbations in normal cardiac development,[5] and can range from asymptomatic to life-threatening depending on the severity and complexity of the cardiac lesion(s). Tremendous strides in treatment, management, and cardiothoracic surgery have led to an increased survival rate of children born with CHD, and consequently a growing adult CHD population.[6] This poses the question of the genetic risks to the offspring of CHD patients. As a result, there is an increasing need for the incorporation of medical genetics in CHD management, with possible implications on diagnosis, recurrence risk, and family screening.[7]

Despite many advances in diagnosis and treatment of CHD, our understanding of its causes remains relatively poor, though the role of genetic mutations and chromosomal rearrangement has been demonstrated.[8] Sporadic, non-syndromic CHD is a complex genetic disorder with evidence for polygenic susceptibility constituted by an observed elevated recurrence risk amongst first-degree relatives, albeit without Mendelian segregation.[5,9] In families with an index case with CHD, the risk of the same CHD phenotype amongst siblings increases between 3-fold and 80-fold depending on the type of CHD, while the risk of another CHD phenotype increases 2- to 3-fold.[5] Advanced genetic approaches including whole-exome sequencing (WES) and chromosomal microarrays (CMAs) have

TABLE I: Abbreviations and acronyms.

Abbreviation	Meaning
ASD	Atrial septal defect
CGH	Comparative genomic hybridisation
CHD	Congenital heart disease
CMA	Chromosomal microarray analysis
CNV	Copy number variant
CVD	Cardiovascular disease
ECA	Extracardiac anomaly
FISH	Fluorescence in situ hybridisation
LMIC	Low and lower-middle-income country
MLPA	Multiplex ligation-dependent probe amplification
NAHR	Nonallelic homologous recombination
PDA	Patent ductus arteriosus
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variant
SSA	Sub-Saharan Africa
T21	Trisomy 21
TOF	Tetralogy of Fallot
TS	Turner syndrome
WES	Whole-exome sequencing
VSD	Ventricular septal defect

led to the identification of numerous rare and newly occurring single nucleotide variants (SNVs) and copy number variants (CNVs) associated with CHD.[9,10,11] Because patients with confirmed genetic syndromes are at higher risk of operative mortality and morbidity, advancing our understanding of the causes of CHD will help define disease risk, improve the way we assess and treat individuals with CHD, and facilitate prevention.[12] This review will summarise the current knowledge of the genetics of CHD, with specific consideration to ongoing research and challenges in Africa.

CONGENITAL HEART DEFECTS

The heart is the first organ to develop in the human embryo through a complex series of events reviewed in more detail elsewhere.[13] Any disruption during cardiogenesis can result in a cardiac defect.

CHD is an umbrella term for a spectrum of cardiac anomalies of differing incidence and severity. The diversity of cardiac phenotypes implicated in CHD has resulted in multiple classification systems based on anatomical, functional, and clinical features.[14-17] The wide range of cardiac lesions and underlying developmental mechanisms has made aetiological and epidemiological studies of CHD a great challenge. However, CHDs may be divided into two simple categories: isolated CHD, in which the congenital defect is limited to the heart, and CHD with extracardiac anomalies (ECAs), in which additional features of other body systems, such as neurodevelopmental delay, craniofacial and limb malformations, are observed, sometimes forming part of a syndrome.

BURDEN OF DISEASE

Cardiovascular disease (CVD) is the leading cause of death worldwide,[18] an increasingly important healthcare concern due to the widespread effect of urbanisation seen in low- and lower-middle-income countries (LMICs) over the past century.[19,20] CHD is a major cause of cardiovascular morbidity and mortality in children and a significant health burden worldwide.[21] A systematic literature review by Liu, et al.[3] reported a substantial increase in CHD birth prevalence from ~4 per 1 000 live births in 1970 to the estimated 9 per 1 000 live births seen today, largely due to improved screening and diagnosis of minor defects.[3]

Despite the significant burden of CHD, the prognosis for children born with CHD in higher-income countries has drastically improved over the last 50 years, with over 90% of affected individuals surviving to adulthood.[22] This improved outlook can be attributed to the increased availability of prenatal echocardiography and improved medical and surgical care.[3] With the increased prevalence of CHD, the issues of long-term prognosis, and the underlying causes of CHD are gaining more global attention.[6]

The socio-economic burden of CVD including CHD falls heavily on LMICs, many of which are situated in sub-Saharan Africa (SSA).[18,23,24] The majority of children with CHD are born in these countries and face a starkly different prognosis to children with CHD in higher-income countries.[22] These regional differences can be attributed to limited availability of antenatal screening leading to fewer diagnoses and increased mortality rates, inadequate access to life-saving surgeries, intervention, and cardiac care, and an insufficient health infrastructure.[22,25] Paediatric cardiac services are an extremely expensive area of medicine and are not readily available to the majority of patients living in SSA. The estimated CHD prevalence of 9 per 1 000 live births is generally accepted worldwide, with genetic, environmental, and epigenetic factors accounting for the variation seen between regions.[26] However, a recent systematic review reported a significantly lower prevalence rate

in Africa (2.315 per 1 000 live births), reflecting severe resource constraints and limited access to healthcare, leading to a low detection rate and a paucity of available estimates from Africa.[3]

Although epidemiological data in SSA are limited, single-centre reports and prevalence studies amongst neonates and school children describe similar patterns in CHD in the region, with septal defects comprising the most common CHDs, followed by patent ductus arteriosus (PDA), and cyanotic defects such as Tetralogy of Fallot (TOF) and truncus arteriosus occurring less frequently.[27-31] In all cases, TOF appears the most common cyanotic form of CHD (6% - 13%). These patterns have held in diverse countries across SSA, such as Botswana, Uganda, Nigeria and Tanzania, although a 10-year cross-sectional study in Cameroon reported pulmonary stenosis as the second most common CHD in their population.[32] A recent meta-analysis of studies from East Africa concluded that the prevalence of septal defects in the region remains lower than in global meta-analyses, but still worryingly high.[33] The estimated birth prevalence of CHD in Botswana was 2.8 - 4.95 per 1 000,[27] while prevalence amongst Nigerian school children was 6.6 per 1 000.[34] However, a recent study of 3 857 Nigerian neonates reported a high CHD prevalence of 28.8 per 1 000,[35] with severe CHD occurring in 3.4 per 1 000 births. These vastly differing results support the suggestion that the low prevalence of CHD in Africa (2.315 per 1 000) is likely due to a paucity of data from the region.

THE AETIOLOGY OF CONGENITAL HEART DISEASE

The aetiology of CHD has been the focus of numerous studies over the past decades.[1,36-38] Although our understanding of the molecular pathways involved in heart development has improved greatly, the underlying causes of most CHD cases remain unclear.[39-41] Approximately 40% of CHDs are due to known genetic causes (aneuploidy: ~23%; de novo CNVs: ~15%; de novo mutations: ~10%; inherited mutations: ~1%), but the relative contributions of epigenetic and environmental factors have not been quantified. It has long been appreciated that environmental, heritable genetic, and epigenetic factors can cause CHD, often in the context of a multifactorial disease.[1] The interaction between these risk factors is thought to increase susceptibility to the development of a heart defect.[12] With more children born with CHD surviving to adulthood and starting families of their own, improving our understanding of disease aetiology and recurrence risks has become critical. Importantly, understanding the causes of CHD will help clinicians determine the prognostic outcome for surgery or treatments, and identify patients at higher risk of operative morbidity and mortality.[40]

ENVIRONMENTAL RISK FACTORS FOR CONGENITAL HEART DISEASE

Environmental risk factors for CHD include any non-genetic factors that have been associated with the risk of developing a cardiac defect, many of which occur in utero.[40] Environmental factors that have been associated with CHD include maternal exposure to cigarette smoke, alcohol, thalidomide, isotretinoin and antiseizure medication,[38] infectious agents such as rubella,[42] and teratogens such as dioxins and pesticides.[43] However, the contribution of specific environmental factors to CHD is unknown as most associations have been derived from small observational studies which have not been replicated and may have been influenced by recall bias. Novel non-genetic causes and risk factors for CHD are continuously arising despite efforts to minimise these modifiable influences.[44]

Certain potentially modifiable risk factors for CHD such as folate deficiencies and air pollution are likely to significantly affect patients in LMICs, including South Africa, where exposure to these risk factors is at higher levels when compared to high-income countries. Although data regarding these factors could improve public health priorities worldwide, the impact of these risk factors on the incidence of CHD in SSA has been minimally explored.[45,46]

HERITABLE RISK FACTORS FOR CONGENITAL HEART DISEASE

There is a multitude of evidence that supports the role of genetics in CHD, including population-based studies, twin studies, and the recurrence risk between 2- and 80-fold for first-degree relatives of CHD patients.[5] Population-based studies have revealed an elevated incidence of certain CHD subtypes such as septal defects in consanguineous populations, suggesting a recessive genetic contribution to the development of CHD.[39,47,28] Previous studies by Wang, et al.[12] and Øyen, et al.[49,50] found an increased risk of recurrence of both similar and discordant forms of CHD amongst relatives when compared to the general population. The elevated recurrent risk in consanguineous populations and relatives, who share a genetic background, emphasises the genetic contribution to CHD pathogenesis.

CHD is a complex heterogeneous genetic disorder associated with both familial and sporadic inheritance patterns (Figure 1). Familial CHD mutations can be inherited in an autosomal dominant, autosomal recessive, or X-linked manner, and can manifest in a variety of clinical phenotypes.[51] Advances in genetic technology such as WES and CMAs have led to the identification of numerous defective genes implicated in CHD. It has been postulated that several hundred genes may be involved in

CHD pathogenesis, with many still to be discovered.[9,52] Variants across the frequency spectrum can affect CHD, from common variants with low but cumulative impacts, to rare mutations or CNVs with high impact.

For the majority of cases CHD occurs sporadically, with only 2% of CHD cases exhibiting familial disease.[50] Sporadic CHD may arise as a result of de novo genetic events, which include single-gene mutations, point mutations such as SNVs, chromosomal aberrations, and smaller CNVs of particular chromosomal regions. It is important to note that CHD is usually oligogenic or complex in its genetics and can result from a combination of these genetic factors.[5,53,54]

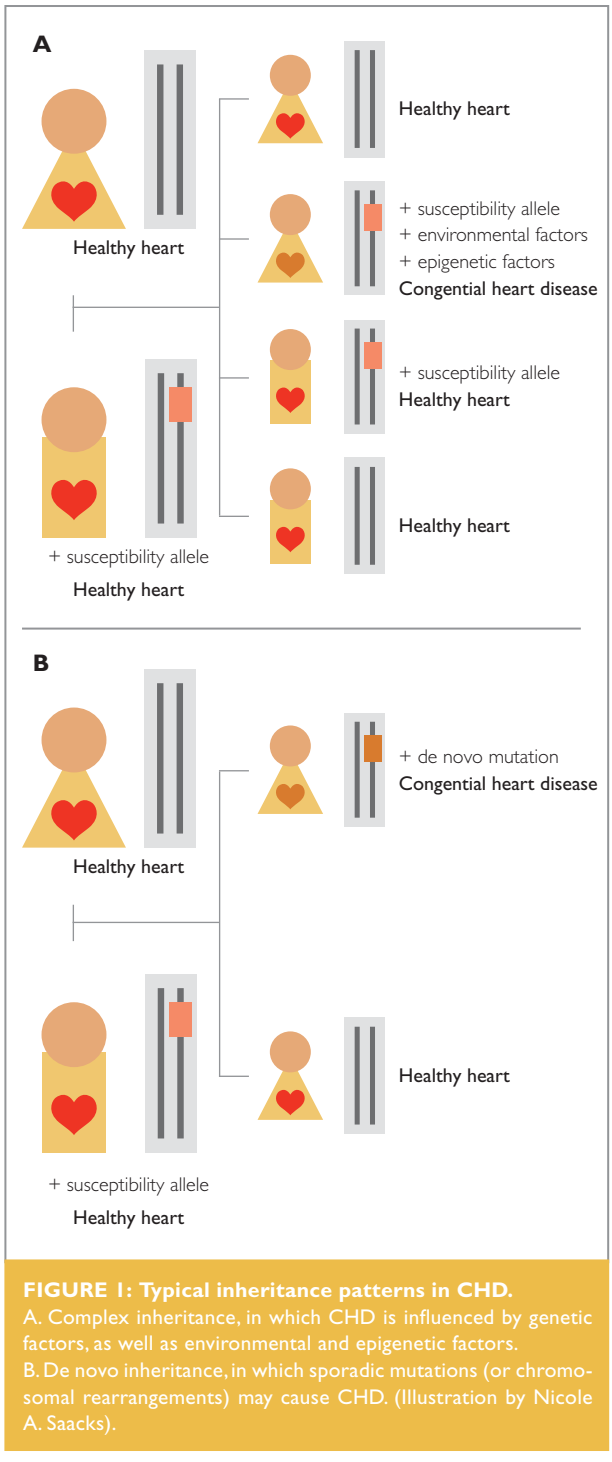

FIGURE 1: Typical inheritance patterns in CHD.
A. Complex inheritance, in which CHD is influenced by genetic factors, as well as environmental and epigenetic factors.
B. De novo inheritance, in which sporadic mutations (or chromosomal rearrangements) may cause CHD. (Illustration by Nicole A. Saacks).

SINGLE GENE MUTATIONS ASSOCIATED WITH CONGENITAL HEART DISEASE

Traditional genetic techniques such as linkage analysis and candidate gene approaches have enabled the discovery of numerous causative genes implicated in CHD pathogenesis. However, these techniques rely on multiple affected family members and an understanding of the underlying molecular pathways of cardiac development, which may be challenging in CHD. Previous studies have identified rare causal mutations in genes encoding cardiac transcription factors such as NKX2-5, GATA4, and TBX5 in patients with non-syndromic CHD.[55-57] These transcription factors control critical events during cardiac development and regulate genes important for cardiomyocyte differentiation, proliferation, and apoptosis.[55,58] Additionally, genes that encode structural proteins including cardiac actins and myosins have been linked to CHD.

CHD has been attributed to Mendelian syndromes in 3% - 5% of cases.[40] For example, truncating mutations in the T-box transcription factor, TBX5, have been linked to Holt-Oram syndrome, a Mendelian disease often associated with cardiac abnormalities.[36] Haploinsufficiency of the transcription factor TBX1 is a common finding in individuals with DiGeorge syndrome and is responsible for many of the associated cardiovascular phenotypes.[59] The NOTCH signalling pathway gene NOTCH1 has been identified as a major susceptibility gene for defects such as bicuspid aortic valve, aortic stenosis, and TOF.[10,60] Mutations in the NOTCH1 ligand JAG1, and NOTCH2 have been associated with Alagille syndrome, an autosomal dominant disorder linked to CHD.[61] The complex nature of CHD genetics, in which one gene can give rise to more than one type of CHD, and one CHD can be caused by mutations in more than one gene, has made establishing phenotype-genotype correlations a major challenge.[49]

CHROMOSOMAL ABNORMALITIES ASSOCIATED WITH CONGENITAL HEART DISEASE

A chromosomal abnormality occurs when chromosomal material is lost or gained and can cause a range of genetic disorders if dosage-sensitive genes are affected.[40] Chromosomal abnormalities contribute to approximately 8% - 20% of CHD cases.[9,53] The chromosomal causes of CHD can be divided into 2 categories: gross chromosomal anomalies (or aneuploidies), and smaller CNVs (Figure 2).[40]

ANEUPLOIDIES

Aneuploidy is typically defined as an abnormal number of chromosomes.[8] Chromosomal aneuploidies were the first genetic causes of CHD to be discovered and continue to play an important role in CHD pathology today.[5,44] The most common aneuploidy is Trisomy 21 (T21 or Down syndrome). This genetic syndrome affects approximately 1 in 800 individuals and is the most common chromosomal disorder seen in individuals with CHD.[8] Cardiac complications are the most common cause of death amongst T21 syndrome patients,[62] and approximately 40% - 50% of individuals with T21 syndrome have an associated cardiac defect. T21 syndrome patients commonly present with an atrial septal defect (ASD), ventricular septal defect (VSD), PDA, atrioventricular septal defect, or TOF.[8] Turner syndrome (TS) results as a partial or complete loss of the X chromosome in females,[54] and 33% of cases occur in conjunction with CHD, usually on the left side of the heart.[5] Bicuspid aortic valve is the most common heart defect associated with TS, with a prevalence of 15% - 30%, followed by coarctation of the aorta which has a prevalence of 7% - 18%. These cardiac anomalies can lead to serious complications for individuals with TS, including aortic dilation and dissection.[63] CHD is observed in 60% - 80% of individuals with trisomy 13 (Patau syndrome) and trisomy 18 (Edwards syndrome).[5] Another common aneuploidy is Klinefelter syndrome. Approximately 50% of males born with Klinefelter syndrome have an associated CHD, usually presenting with PDA or an ASD.[54] A selection of well-established chromosomal abnormalities associated with CHD is shown in Table II.

COPY NUMBER VARIATION

Copy number variation is a type of structural genetic variation whereby segments of the genome are duplicated or deleted. CNVs are a subgroup of structural variants comprising insertions, deletions and complex rearrangements of any size. In this review, CNVs are defined as microdeletions/microduplications of the genome that are larger than 1 kilobase (kb) in size and affect the dosage of one or more genes. CNVs typically arise from nonallelic homologous recombination (NAHR) during meiosis (Figure 3),[64] although other mechanisms responsible for CNV generation include non-homologous end-joining, fork stalling, template switching, and L1-mediated retro-transposition; these are discussed in detail elsewhere.[64,65]

Genomic microduplications and microdeletions can range in size from 1 kb to several mega-bases and are a common source

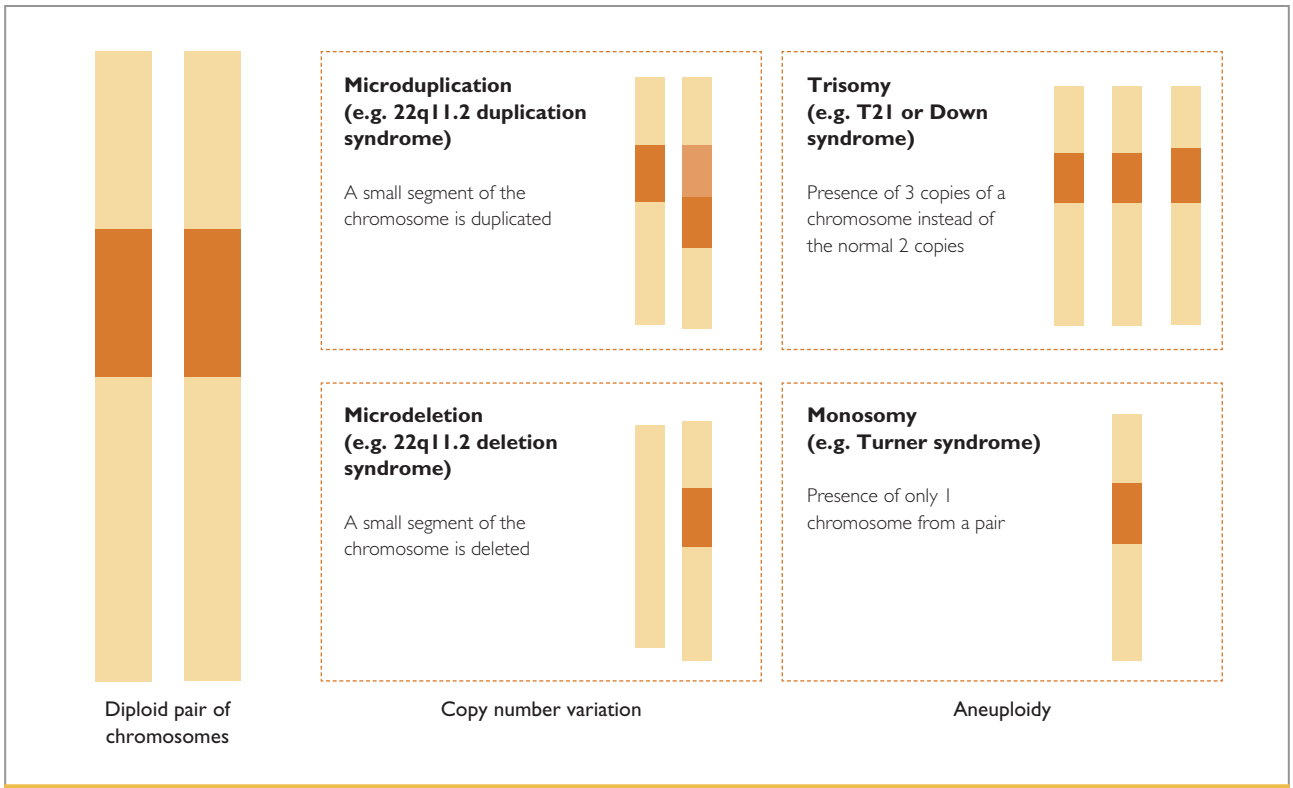

FIGURE 2: Chromosomal causes of CHD.
Representation of typical chromosomal alterations. Chromosomes are depicted in light orange, and regions of deletion or duplication are in dark orange (Illustration by Nicole A. Saacks).

TABLE II: Chromosomal abnormalities associated with congenital heart disease.*

Chromosomal anomaly	Locus	Most common CHD
Chromosomal aneuploidies		
Trisomy 8 mosaicism	Chromosome 8	VSD, PDA, CoA, TAPVR, TrA
Trisomy 9 mosaicism	Chromosome 9	PDA, LSVC, VSD, TOF, pulmonary atresia, DORV
Patau syndrome	Chromosome 13 (Trisomy 13)	ASD, VSD, PDA, HLHS
Edwards syndrome	Chromosome 18 (Trisomy 18)	ASD, VSD, PDA, TOF, DORV, TGA, CoA, BAV
Down syndrome	Chromosome 21 (Trisomy 21)	AVSD, ASD, VSD, TOF, TGA
Turner syndrome	Chromosome X (Monosomy X)	CoA, BAV, AS, HLHS
Klinefelter syndrome	Chromosome X (47- XXY)	MVP, PDA, ASD
Copy number variants		
1p36 deletion	1p36	PDA, VSD, ASD, BAV, Ebstein anomaly
1q21.1 microduplication	1q21.1	TOF, TGA, ASD, pulmonary atresia
1q41q42 microdeletion	1q41q42	BAV, ASD, VSD, TGA
1q43q44 microdeletion	1q43q44	VSD, CoA, HLHS
2q31.1 microdeletion	2q31.1	VSD, ASD, PDA
2q37 microdeletion	2q37	VSD, ASD, CoA
Wolf-Hirschhorn syndrome	4p	ASD, VSD, PDA, aortic atresia, dextrocardia, TA, TOF
Cri-du-chat syndrome	5p	VSD, ASD, PDA
Williams-Beuren syndrome	7q11.23 deletion	AS and PS, PPS
8p23.1 deletion	8p23.1	AVSD, PS, VSD, TOF
Kleefstra syndrome	9q34.3 deletion	ASD, VSD, TOF, PA stenosis
Jacobsen syndrome	11q deletion	HLHS, AS, VSD, CoA
15q11.2 microdeletion	15q11.2	TOF, BAV
15q24 microdeletion	15q24	PDA, PA stenosis, PS
16p11.2p12.2 microdeletion	16p11.2p12.2	TOF, BAV, pulmonary atresia
17q21 microdeletion	17q21	PS, ASD, VSD, BAV
Alagille syndrome	20p12 deletion	Peripheral PA hypoplasia, TOF, PS
22q11.2 deletion	22q11.2	IAA type B, TrA, TOF
22q11.2 duplication	22q11.2	TOF, HLHS, VSD, PS, TrA
Phelan-McDermid syndrome	22q13 microdeletion	PDA, VSD, ASD, TAPVR

**Adapted from Blue, et al.,[40] Pierpont, et al.,[8] and Soemedi, et al.[11]*
AS = aortic stenosis, ASD = atrial septal defect, AVSD = atrioventricular septal defect, BAV = bicuspid aortic valve, CoA = coarctation of the aorta, DORV = double-outlet right ventricle, HLHS = hypoplastic left heart syndrome, IAA type B = interrupted aortic arch type B, LSCV = persistent left superior vena cava, MVP = mitral valve prolapse, PA = pulmonary artery, PDA = patent ductus arteriosus, PPS = peripheral pulmonary stenosis, PS = pulmonary valve stenosis, TA = tricuspid atresia, TAPVR = total anomalous pulmonary venous return, TGA = transposition of the great arteries, TOF = tetralogy of Fallot, TrA - truncus arteriosus, VSD = ventricular septal defect.

of genetic variation associated with many Mendelian diseases and genetic disorders.[66] Emerging evidence has indicated that CNVs are important contributors to numerous disorders including cancer,[67] neuropsychiatric disorders,[68] neuro-developmental disorders,[69] and congenital defects including CHD.[41] CNV mutations can be inherited or de novo and can lead to altered copies of dosage-sensitive genes, the effects of which range from benign to fatal depending on the function of the genes implicated.[70] Large CNVs comprising several million base pairs can be detected by cytogenetic analyses and/or fluorescence in situ hybridisation (FISH), whereas smaller CNVs are identified using high-resolution microarrays that can detect single nucleotide polymorphisms and CNVs.[44]

CNVs occur frequently amongst healthy individuals, making up about 12% of the genome of the average person.[71] Although the majority of CNVs have no phenotypic consequence in healthy individuals, microduplications and/or microdeletions that implicate dosage-sensitive genes can be detrimental. If critical genetic regulatory elements are disrupted, dependent genes may be over- or under-expressed which can significantly contribute to disease pathogenesis.[21] It is therefore important

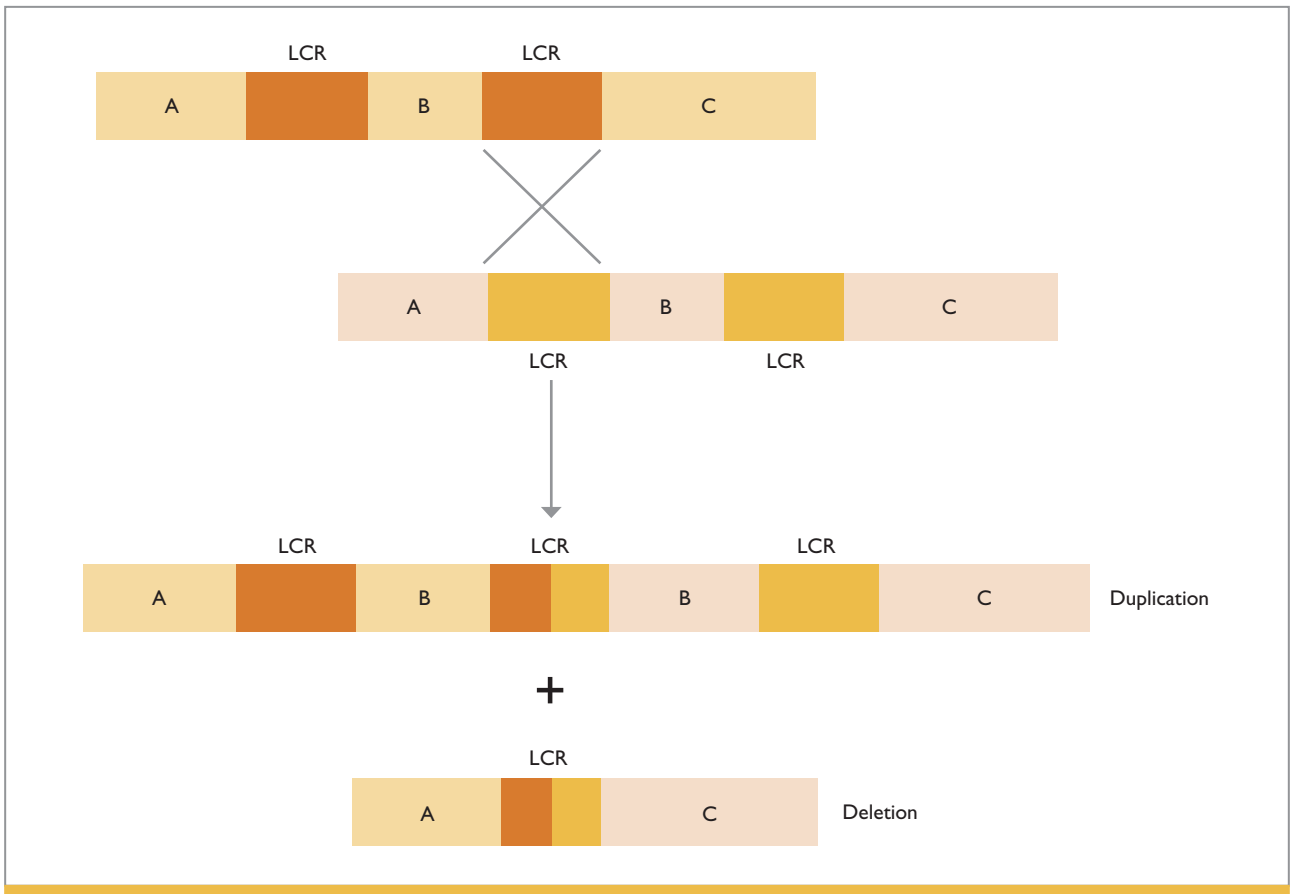

FIGURE 3: Nonallelic homologous recombination.
NAHR is a form of recombination between 2 DNA regions with high sequence similarity, and 1 of the major mechanisms underlying CNV formation. This unequal crossing over results in reciprocal deletion and duplication of the intervening sequence. If this occurs during meiosis, resultant offspring can inherit a CNV. A, B and C represent different genes. LCR = low copy repeats. (Illustration by Nicole A. Saacks).

to differentiate pathogenic CNVs from likely benign CNVs that are commonly found in the general population.

In the context of CHD, researchers and clinicians have defined CNV pathogenicity according to the following criteria: a CNV overlapping a known disease-associated region or known dosage-sensitive CHD gene; a CNV located in a gene-rich region; a CNV that comprises a large deletion or duplication; a de novo mutation or a CNV associated with a specific phenotype within a family and/or a rare CNV found in less than 1% of healthy individuals.[72] CNVs are categorised as variants of uncertain clinical significance when insufficient evidence of pathogenicity is available at the time of reporting.

The discovery of pathogenic and potentially pathogenic CNVs associated with CHD has significantly improved our understanding of the aetiology of the disease.[8] Investigation of the role of CNVs in CHD pathology has led to the identification of numerous dosage-sensitive genes that are critical for cardiac development. Previous studies of large CHD cohorts have detected a1.8-fold to 3.9-fold greater burden of CNVs in CHD cases compared to controls, with large, rare, gene-containing CNVs having a greater impact on CHD.[11,21,41,68]

COPY NUMBER VARIATION IN SYNDROMIC CONGENITAL HEART DISEASE

Cardiac defects commonly occur in conjunction with a multitude of genetic disorders (syndromic CHD) characterised by large CNVs (Table II). The most common microdeletion in humans is 22q11.2 deletion syndrome, caused by a deletion that is not visible by standard karyotyping on the long (q) arm of chromosome 22 as a result of NAHR.[5] The cardiac phenotype for 22q11.2 deletion syndrome varies but usually presents with TOF, truncus arteriosus, and/or interrupted aortic arch- type B.[15] It appears that deletion of TBX1 is the primary causal mechanism to the clinical phenotype associated with the syndrome.[73] Additional well-characterised CHD-associated CNVs include a deletion at 7q11.23, which causes Williams-Beuren syndrome,[51] a deletion at 11q24-25, which results in

Jacobsen Syndrome,[74] a deletion at 8p23, which leads to haploinsufficiency of GATA4, resulting in a variety of CHD phenotypes with neurodevelopmental delay,[75] and a 1p36 deletion, which commonly occurs in conjunction with a septal defect, and is associated with orofacial malformations, microcephaly, and mental retardation.[51] The majority of these genetic disorders cannot be identified by conventional karyotyping and require CMA or FISH for an accurate diagnosis.[21]

COPY NUMBER VARIATION IN NON-SYNDROMIC CONGENITAL HEART DISEASE

Non-syndromic CHD can occur in isolation or in conjunction with ECAs such as neurodevelopmental delay and/or dysmorphism. Several studies have demonstrated that CNV is a significant contributory factor to the development of CHD with ECAs. For example, Thienpont, et al.[76] found a rare causative CNV in 17% of 60 CHD patients, including CNVs in regions of known cardiac transcription factors (NKX2-5 and NOTCH1), suggesting an association between CNV and CHD. A similar study by Richards, et al.[77] identified rare CNVs in 25% of 40 patients with CHD who showed normal karyotypes. Half of the cohort presented with isolated CHD and the other half had CHD with ECAs. However, causative CNVs were only detected in study subjects who presented with CHD and ECAs. Syrmou, et al.[78] detected CNVs in 37 of 55 individuals with CHD (67%); 81% of the CNV-positive CHD patients presented with ECAs. Collectively, these studies demonstrate the important role of CNVs in the development of CHD with ECAs, and that the genes implicated in CHD tend to have multiple phenotypic effects. These studies also indicate that CMAs can be a useful tool to identify causative CNVs in individuals presenting with CHD and ECAs when the standard karyotype appears normal.[77]

Most CHD cases (up to 85%) occur in isolation without ECAs. However, the role of CNVs in isolated CHD has been minimally explored. Identifying isolated CHD is often challenging, as ECAs can be easily missed, or not yet present at the time of diagnosis, especially in very young study populations. Longitudinal studies with carefully phenotyped study subjects are required to define the role of CNV in isolated CHD.[21] Previous large-scale studies have investigated CNVs in cohorts which include patients with isolated CHD and patients with CHD and ECAs. Soemedi, et al.[79] found that a duplication of the gap-junction gene GJA5 increased the risk of TOF by ten-fold. This study also showed that microdeletions of the 1q21.1 region corresponded to a population-attributable risk of approximately 1% for TOF. Furthermore, a large-scale genome-wide investigation of CNV data from 2 256 individuals with CHD, 283 trio CHD-affected families, and 1 538 controls was performed by Soemedi, et al. [11] This study showed that rare deletions account for 3% - 4% of the population attributable risk for TOF and other CHDs.[11] Tomita-Mitchell, et al.[41] explored the effect of CNVs in 945 individuals diagnosed with CHD and detected pathogenic CNVs in 4.3% of their study subjects (excluding 135 patients with syndromic CHD-associated chromosomal abnormalities). Additionally, Erdogan, et al.[80] identified de novo causative CNVs in 3% of 105 patients with isolated CHD presenting with varied phenotypes. Many of the identified CNVs contained genes important for cardiac development, and/or genes critical for correct left-right patterning of the heart according to animal models. A recent study by Kim, et al.[81] identified large pathogenic CNVs (>300kb) that were significantly associated with increased postoperative mortality in non-syndromic CHD patients.

Overall, these studies highlight the significant impact of CNV in CHD pathology and indicate that as genetic testing progresses, investigating and identifying pathogenic CNVs associated with specific forms of CHD will become an increasingly useful tool in gene discovery and accurate CHD diagnosis. This is particularly important for patients with complex CHD and CHD with ECAs, whereby identifying additional anomalies not easily detected by standard karyotyping could significantly improve disease prognosis and patient outcome. Furthermore, exploring the role of CNVs in CHD will contribute to our understanding of healthy cardiac development and related perturbations, and provide knowledge relevant to clinical practice and potential therapeutic strategies.[82]

DISCOVERING GENES FOR CONGENITAL HEART DISEASE

Over the years, many genes involved in cardiac development have been discovered. However, the complete process of cardiogenesis is not fully understood.[52] Emerging sophisticated, high-throughput genetic technology such as next-generation sequencing has rapidly advanced the pace at which genes are being discovered. Next-generation sequencing technologies include gene panel tests, whole-genome sequencing, and WES. WES is a genomic technique that sequences all the protein-coding regions of the genome and has been used to identify rare, causative SNVs in CHD-associated genes as well as previously unreported genes, in both small and large patient cohorts. For example, Page, et al.[10] used WES to detect unique pathogenic variants in a cohort of 829 non-syndromic TOF patients. Similarly, a study by Zaidi, et al.[83] performed WES on 362 sporadic severe CHD patients, their parents, and 264 control trios and found a significant excess of de novo damaging mutations in genes involved in cardiac development amongst the patients.

In the past, detection of aneuploidies and gross chromosomal aberrations has relied heavily on standard karyotyping. However, smaller CNVs are not easily detected by this method and require a higher resolution CMA analysis (Figure 4). CMA is a clinical genetic technique that includes single nucleotide polymorphism (SNP) arrays or comparative genomic hybridisation (CGH) and allows for the interrogation of rare CNVs known to be associated with a disease, as well as other emerging chromosomal deletions and duplications in the genome not visible by standard karyotyping.[84] Although the resolution of CMAs depends on many factors, array CGH and SNP arrays are usually able to detect CNVs upwards of 50 - 100kb in size (Figure 4). It is likely that 5% - 15% of nonsyndromic CHD is attributable to CNVs above 100kb, although the contribution of CNVs smaller than 50kb is unclear due to the limited resolution of these techniques (Figure 4). Sequencing technologies such as WES are only able to detect insertions or deletions less than 100bp in size, but the role of CNVs between 100bp and 50kb may be delineated using long-read sequencing, which has not been utilised in CHD studies as yet. Nevertheless, CMA is becoming an increasingly important tool used in both prenatal and postnatal clinical genetic settings,[84] and has led to the identification of numerous pathogenic CNVs implicated in CHD discussed elsewhere.[41,76,77,79,80] The International Standard Cytogenomic Array Consortium recommends CMA as the first-tier cytogenetic diagnostic test for children born with congenital abnormalities including CHD, as it provides the most comprehensive coverage of the genome.[85]

GENETICS OF CONGENITAL HEART DISEASE IN SUB-SAHARAN AFRICA

CHD has been described as a "neglected" condition in South Africa, with an underestimated reported prevalence of approximately 2.35 per 1 000 live births.[3] In 2013, Zühlke, Mirabel, & Marijon provided evidence that suggested the burden of CHD is vastly underestimated as a result of poor prognoses for African children born with CHD.[26] There is a lack of African-based evidence on the genetic basis of cardiovascular disease in SSA due to poor funding and limited local expertise.[86] Consequently, the major genetic breakthroughs for CHD that have been seen in high-income countries of the world over the past few decades, have not been replicated in most LMICs including South Africa.

The epidemiology of CHD in Africa shows a spectrum of CHD phenotypes with varied prevalence.[86] However, there are relatively few genomic studies in SSA CHD populations; these studies could contribute to our understanding of the epidemiological data, and how we manage and treat African children with CHD.[86] A comprehensive review of the available literature on the genetics of CHD in SSA revealed 4 independent studies that used molecular genetic approaches to investigate the causes of CHD in African populations.[87-90] A genomic study in Rwanda was done in 2014 where echocardiography, standard karyotyping, and Multiplex Ligation-dependent Probe Amplification (MLPA) was performed on 125 patients with clinical features of genetic disorders. The study showed that 64 of the 125 study subjects had CHD, and a genetic cause was

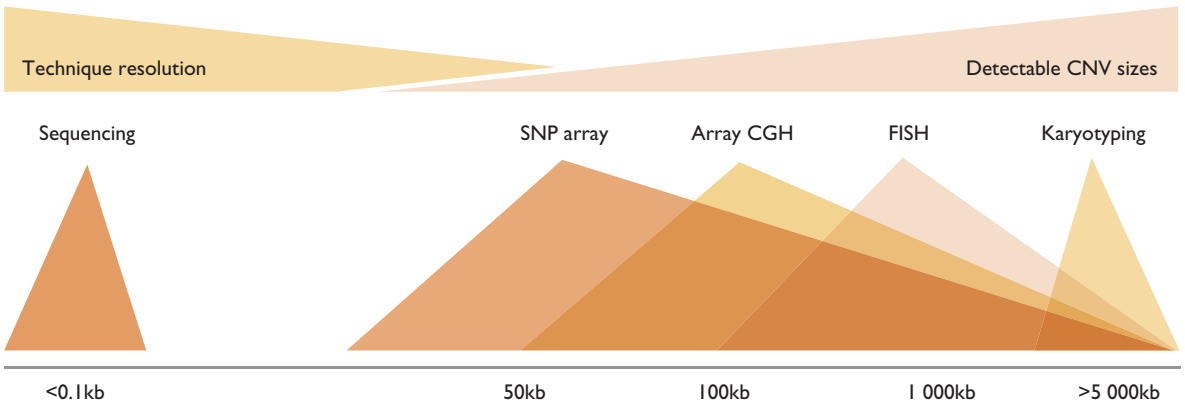

FIGURE 4: Comparison of different CNV detection methods.
Indicated are the sizes of CNVs that are detectable by each genetic technique. Karyotyping is limited to the detection of whole and partial chromosomal aneuploidy, while FISH, array CGH and SNP array can be used to detect smaller CNVs. Sequencing technologies such as whole-exome sequencing and whole-genome sequencing can only detect small insertions or deletions (approximately 100 bp).
CGH = comparative genomic hybridisation, CNV = copy number variation, FISH = fluorescence in situ hybridisation, kb = kilobase, SNP = single nucleotide polymorphism (Illustration by Timothy F. Spracklen).

found for 61 of the 64 subjects, although this high detection rate may have been influenced by the selection of participants with signs of genetic syndromes. Of the 22 patients who presented with normal karyotypes, MLPA, and FISH analyses enabled Teteli and colleagues to detect a 7q11.23 duplication, a 13qter deletion, and a 22q11.2 deletion within the study cohort.[88] Two years later, De Decker, et al.[87] used FISH to determine the prevalence of 22q11.2 deletion syndrome in children with CHD at Red Cross War Memorial Children's Hospital in Cape Town. This genetic study identified deletions at the 22q11.2 locus in six out of 125 patients (4.8%). Similarly, Wonkam, et al.[89] investigated the prevalence of 22q11.2 deletion syndrome in CHD patients in Cameroon using MLPA and FISH. In this study, 22q11.2 deletion was detected in 2 of 70 patients (2.8%). Both patients had conotruncal heart defects in conjunction with ECAs.[89] Most recently, a WES study of 98 Nigerian CHD patients found disease-causing mutations in known CHD genes in 9% of the patient cohort, with 77.8% of the mutation-positive patients presenting with syndromic disease.[90] Collectively these studies illustrate the ability to diagnose syndromic CHD using standard karyotyping and FISH, and their relevance in genomic studies on the African content. However, except for the Nigerian WES study, these CHD investigations focussed mainly on individuals with known genetic disorders which contribute to a small fraction of the total burden of CHD.

To our knowledge, there has been minimal research into the genetic basis of non-syndromic CHD in Africa and implementing techniques that can identify potential causative single-gene mutations and CNVs in genes involved in cardiac development is becoming increasingly important. Advanced genetic platforms including next-generation technologies (whole-genome sequencing and WES) and high-resolution CMA allow for the interrogation of entire genomes and CNVs of all sizes in a single run.[86] Access to clinical genetic testing in Africa remains extremely limited. Although large-scale initiatives such as the Human, Hereditary and Health in Africa (H3Africa) consortium seek to address the lack of genomic information from African populations and build capacity for research in the region,[91] translating genomic information to the clinical setting is a challenge. Issues include access to and cost of sequencing or genotyping arrays, limited knowledge of the role of genetics in healthcare, and the dearth of experts and genetic counsellors across Africa.[92,93] We are not aware of any genomic centres of excellence in Africa. However, using these genomic platforms to investigate the genetic underpinnings of CHD in Africa will provide valuable insight into the complexities of CHD, by validating the disease-causing genes found in other populations, refining disease-associated loci, and possibly identifying new genes that may contribute to CHD pathogenesis.

CONCLUSION

Many large-scale genetics studies have demonstrated the contribution of CNVs and single nucleotide variants to the development of cardiac defects in individuals of European ancestry. While this approach has identified numerous pathogenic and likely pathogenic variants linked to CHD, to our knowledge, no study to date has investigated the contribution of rare CNVs to the development of CHD in African populations including South Africa. Improving our understanding of the genetic architecture and risk factors associated with CHD in patients of African descent is the first step toward improving the accuracy of CHD diagnosis, and a crucial step toward identifying potential measures to combat CVD. Moreover, exploring the genetics of CHD has the potential to improve the quality of life for children born with CHD in Africa, and will enable clinicians to identify familial inheritance patterns and predict recurrence risks and prognostic outcomes pre- and post-surgical intervention.

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