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Congenital and Obstructive Heart Defects: A Systematic Review and Meta-Analysis

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Abstract: *Congenital heart defects (CHDs) are the most usually happening birth imperfection and most cases have no known reason. There is considerable proof to propose hereditary danger factors assume an unmistakable part in CHD improvement and numerous examinations have been fruitful distinguishing CNVs related with CHD hazard. Tragically, CNVs hazard factors are frequently uncommon and only from time to time repeated across various investigations. To deliberately audit distributed examinations directed to distinguish the relationship between duplicate number variations and non-syndromic congenital heart defects in populace considers. For our deliberate audit, we utilized three information bases to distinguish up-and-comer articles: PubMed, Web of Science and Cochrane Database of Systematic Reviews. We looked for all investigations distributed through November 21th, 2019 and aggregated elite of extraordinary examinations for assessment. Every information base was looked for contemplates containing at any rate one of the chose congenital heart deformity terms and one duplicate number variation term. The quest uncovered 1,893 one of a kind articles for audit. Our essential consideration measures were case-control or case-information base investigations of duplicate number variation hazard factors for non-syndromic congenital heart defects. Contextual investigations, case arrangement, family-based examinations were taken out from thought. Studies assessing syndromic CHD were prohibited. Approaches that assessed the predominance of a particular pathogenic CNV and were not essentially purposed for CNV disclosure were barred. A sum of 1,893 exceptional articles were at first arranged dependent on incorporation and rejection measures by auther. Articles meeting the consideration rules were fundamentally evaluated and incorporated for introduction. After investigation, 36 examinations were distinguished that met the consideration models and 27 were remembered for the quantitative survey.*

Keywords: Congenital heart defects, Obstructive heart defects, Copy number variants

Introduction

Congenital heart defects (CHDs) are the most common major birth defect in the world affecting 0.5-1.3% of live birth (Bird, Hobbs, Cleves, Tilford, & Robbins, 2006; Ferencz et al., 1985; Khoshnood et al., 2012; Oyen et al., 2009; Reller, Strickland, Riehle-Colarusso, Mahle, & Correa, 2008; Tanner, Sabrine, & Wren, 2005; Wren et al., 2012; Wu et al., 2010). Advances in the surgical and medical management of these complex congenital anomalies have resulted in a steady decrease in CHD associated childhood mortality (Gilboa, Salemi, Nembhard, Fixler, & Correa, 2010). As a result, the population of those living with CHDs has continued to rise leading to a steadily increasing prevalence of individuals living with CHD and an aging CHD population (Marelli et al., 2014; Marelli, Mackie, lonescu-lttu, Rahme, & Pilote, 2007). Despite improvements in management, CHD still remains the most common cause of infant mortality in developed countries and the most common cause of death due to a birth defect worldwide (Wren et al., 2012). The burden of these common and devastating defects poses a formidable challenge to the research community to understand the causes underpinning CHDs, the long-term outcomes in the growing adult population, and the impact they have on biological fitness.

The underlying causes and risk factors for CHD are varied and have been the focus of robust investigation for decades. There are both genetic and non-genetic etiologies underpinning non-syndromic CHD. Non-genetic risk factors for CHD are numerous and include: advanced maternal age, diabetes mellitus, febrile illness, hypertension, obesity, infertility/use of artificial reproductive technology, low socioeconomic status, and maternal exposures to teratogenic compounds (dioxins, polychlorinated biphenyls, pesticides, alcohol, isotretinoin, thalidomide, anti-folates, cigarette smoking, cocaine) (Patel & Burns, 2013). The impact of these non-genetic risk factors should not be taken lightly and it is estimated that these non-genetic risk factors could be responsible for upwards of 30% of CHDs (Wilson, Loffredo, Correa-Villasenor, & Ferencz, 1998). Nevertheless, the majority of CHDs currently have no known etiology. Genetic risk factors have been identified for many cases of CHD and are presumed to be at least partially responsible for a large portion of CHD cases with unknown etiology. Support for the genetic basis of CHD has a long lineage of support and has been reviewed elsewhere for interested readers (Andersen, Troelsen Kde, & Larsen, 2014; Fahed, Gelb, Seidman, & Seidman, 2013; Gelb, 2015; Zaidi & Brueckner, 2017). To summarize some of the important evidence supporting the contribution of genetic variation to CHD risk:

1. Monozygotic twins have higher rates of CHD concordance compared to dizygotic twins. (Nora et al., 1969)

2. Epidemiological surveys reveal that CHDs cluster within families and recur in family members, suggesting inherited genetic risk factors. (Oyen et al.,2009)
3. ln addition to recurrence of the same CHD in family units, familial aggregation of different CHDs (discordant CHDs) has been observed and suggests that different CHDs may arise through shared genetic pathways. (Oyen et al., 2010)
4. Consanguineous parentage substantially increases the risk of CHD risk, implicating the role of autosomal recessive genetic risk factors for CHD. (Shieh, Bittles, & Hudgins, 2012)
5. CHDs are common malformations listed in the phenotypic distribution of common genetic syndromes including DiGeorge syndrome, Down syndrome, Turner syndrome, Edwards syndrome, Pataeu syndrome, Williams-Beuren syndrome, Cri-Du-Chat, Jacobsen and many others. The type of CHD and risk of CHDs in these syndromes are variable, but the contribution of genetic factors is nevertheless undeniable with many having CHDs in over 50% of the affected population. (Fahed et al., 2013)
6. The advent of the molecular genetics era has identified many genes responsible for appropriate heart development that are sensitive to genetic variation. (Prendiville, Jay, & Pu, 2014)
7. The utilization of whole genome surveillance methods has revealed disproportionate numbers of large structural variations, *de novo* mutations, rare variants and damaging SNPs in CHD populations. (Cooper et al., 2011; Glessner et al., 2014; Homsy et al., 2015; Priest et al., 2016; Warburton et al., 2014).

Taken together, the role genetic variants play in CHD risk is substantial and their full impact on CHD risk is unfolding in parallel with the advancement of genomic technology. Copy number variants (CNVs), deletions and duplications in the genome, are etiological components that have been a centerpiece of the genetic investigation of CHD phenotypes. Copy number variants (CNVs) are unbalanced rearrangements of DNA that cause either an increase or decrease of DNA content in a particular genome. The rearrangement involves a continuous strand of DNA and can vary in size from 50 to millions of nucleotide bases in length (MacDonald, Ziman, Yuen, Feuk, & Scherer, 2014). Rearrangements smaller than 50 base pairs in size are classified as insertions or deletions (indels). Large structural genomic variants, namely aneuploidy, were the earliest known genetic contributors to CHD risk. The aneuploidy syndromes, trisomy 21, trisomy 18, trisomy 13 and Turner's syndrome, are all associated with substantial CHD risk and are responsible for ~9-18% of all CHDs (Hartman et al., 2011). Furthermore, the 22q11.2 deletion syndrome, DiGeorge Syndrome, is the most common human microdeletion syndrome and is responsible for a number of neurodevelopmental and structural abnormalities, including congenital heart defects, in affected children. A genetic survey of 254 lndian children that contained an abnormal aortic arch (interrupted aortic arch, coarctation of the aorta), identified pathogenic structural variants in 52 children (21%), 49 of which (94%) were 22q11.2 deletions (Anilkumar et al., 2011).

Larger studies have further delineated the prevalence of 22q11.2 deletion among CHD subtypes. A study of 1,610 patients with conotruncal defects identified 187 patients (13%) with 22q11.2 deletion syndrome. They observed higher association of 22q11.2 with specific CHDs including interrupted aortic arch – B (56.2%), truncus arteriosus (35.5%), pulmonary atresia (21.3%) and tetrology of Fallot (13.2%) which all had significant proportions of 22q11.2 deletion in the study population (Peyvandi et al., 2013). Taken together, aneuploidy syndromes and 22q11.2 deletion syndrome act as harbingers of the significant role genomic structural variation play in CHD risk. Herein we propose to review and evaluate the considerable work done interrogating the impact of CNVs on non-syndromic CHD risk.

Methods

Search Criteria: PubMed was queried for all articles containing any of the of the selected phenotype keywords and at least one of the exposure keywords on November 21st, 2019. The keywords were searched in all fields and MeSH terms (Figure 1). The phenotype terms used for in the search included: congenital heart defect, congenital heart disease, heart defect, cardiac defect, congenital heart anomaly, congenital heart, cardiovascular malformation, heart development, cardiovascular development, heart malformation. The exposure terms used in the search were: copy number variant, copy number variation, copy number, structural variant, structural variation, chromosomal imbalance. The same keywords were adapted for searching the Web of Science and Cochrane review database.

Inclusion and exclusion criteria: Articles were selected that used case- control population-based study design to study copy number variant risk factors for non- syndromic congenital heart defects. Case-database studies were also included in the study. A case-database study is a type of case-control study that, in lieu of a control population, a database of controls or reported genomic variants are used for comparison. The most common database used as a standard in CNV association studies is the Database of Genomic Variants, often referred to as the DGV (MacDonald et al., 2014). Studies were removed from consideration if they were case series, case reports, or family-case series. These observational studies were excluded

from consideration because it would allow potential CNV risk factors to be introduced into the final list of CNV risk factors that were not compared against a control or database population. Studies that evaluated syndromic congenital heart defects were also excluded. Animal based studies were also removed from consideration. Studies evaluating diagnostic efficacy of arrays were included only if they met all the inclusion criteria and the patients were not selected because they had a likely genetic diagnosis. For example, studies that evaluated the diagnostic efficacy of arrays in cases with multiple congenital anomalies that included congenital heart disease were removed from consideration. Studies that did include multiple phenotypes were included if CHDs were the focus of the study (all patients had a CHD) and measures were taken to ensure syndromic cases were excluded from the study population. For example, one of the earliest studies investigating structural variants in CHD was conducted by Thienpont et al. in 2007 and included patients with additional major malformations, mental handicap and other minor physical anomalies **(**Thienpont et al., 2007**)**. To ensure the cases in the study were not syndromic, each case was evaluated by an expert dysmorphologist, routine karyotyping was performed to exclude known genomic syndromes, and additional investigations were conducted to exclude well-defined genetic conditions. Studies that did not have a specific control population were excluded if they did not utilize an online control database like the database of genomic variants a reference dataset in their analysis. Studies that made an effort to exclude syndromic cases, but, through the analysis, identified syndromic microdeletions or microduplications, were not excluded if this occurred in a minority of the patients (<10%). After evaluation of each of the 909 articles, 45 articles were identified that met the above described inclusion and exclusion criteria.

Variants included in quantitative review: Additional selection criteria were implemented when choosing variants to include in the quantitative review including 1) the study identified a putative CNV risk factor for CHD and reported the coordinates, 2) studies utilizing lower density arrays (<100,000 probes) were excluded because of reduced CNV breakpoint resolution, 3) studies had to utilize a control population or online databases to characterize the pathogenicity of the identified CNVs. Only variants that were identified as putative CHD risk factors were included in the final collection of CNVs. The genomic coordinates for each CNV risk factor that was not mapped to the hg19 build were converted to the hg19 coordinates using the UCSC Liftover tool (Kuhn, Haussler, & Kent, 2013). Some CNVs after conversion from a previous genome build to hg19 were removed because the region is no longer included in the hg19 build of the human genome.

Population studies of CNV risk factors for CHD

Overview of included studies: The PubMed literature search identified 1,114 articles to review. An additional 1,273 articles were pulled from the Web of Science and Cochrane reviews (Figure 2). After removal of duplicates, 1,893 articles were included in the total study for review. Title and abstract screening excluded 1,759 articles, leaving 134 for critical review. Review of the 134 full text articles excluded an additional 79. The final set of 56 articles were examined against inclusion and exclusion criteria to finalize the final set of articles to include in both the qualitative and quantitative review. Thirty-six articles were included in the final qualitative synthesis and 27 studies were included in the quantitative synthesis (Table 1) (An et al., 2016; Breckpot et al., 2012; Carey et al., 2013; Costain et al., 2016; Dimopoulos et al., 2017; Giannakou et al., 2018; Giannakou et al., 2017; Glessner et al., 2014; Glidewell et al., 2015; Goldmuntz et al., 2011; Greenway et al., 2009; Hanchard et al., 2017; Hitz et al., 2012; Lalani et al., 2013; Liu et al., 2019; Luyckx et al., 2019; Moosmann et al., 2015; Payne, Chang, Koenig, Zinn, & Garg, 2012; Sanchez-Castro et al., 2016; Serra-Juhe et al., 2012; Silversides et al., 2012; Soemedi et al., 2012; Warburton et al., 2014; White et al., 2014; H. Xie et al., 2019; H. M. Xie et al., 2017; L. Xie et al., 2014). An additional 9 articles were excluded from the quantitative review, but were discussed elsewhere in the review due to their relevance to the topic (Bittel et al., 2014; Breckpot et al., 2011; Erdogan et al., 2008; Fotiou et al., 2019; Kim et al., 2016; Richards et al., 2008; Shi et al., 2018; Thienpont et al., 2007; Zhao et al., 2013) (Table 1). The PRlSMA flow diagram designed by Moher and colleagues for the for the systematic review is depicted in Figure 2.1 (Moher, Liberati, Tetzlaff, Altman, & Group, 2009). Data retrieved from the 27 articles for quantitative analysis includes reported CNV risk factors from each of the included studies, which included 773 CNVs across multiple phenotypes made up of 456 deletions and 318 duplications (one region contained both a deletion and a duplication) (Supplementary table 1). The CNVs ranged from 99 bases in length to 89 megabases in size. The coordinates for each were converted using the UCSC Liftover tool to hg19 coordinates for comparison between studies.

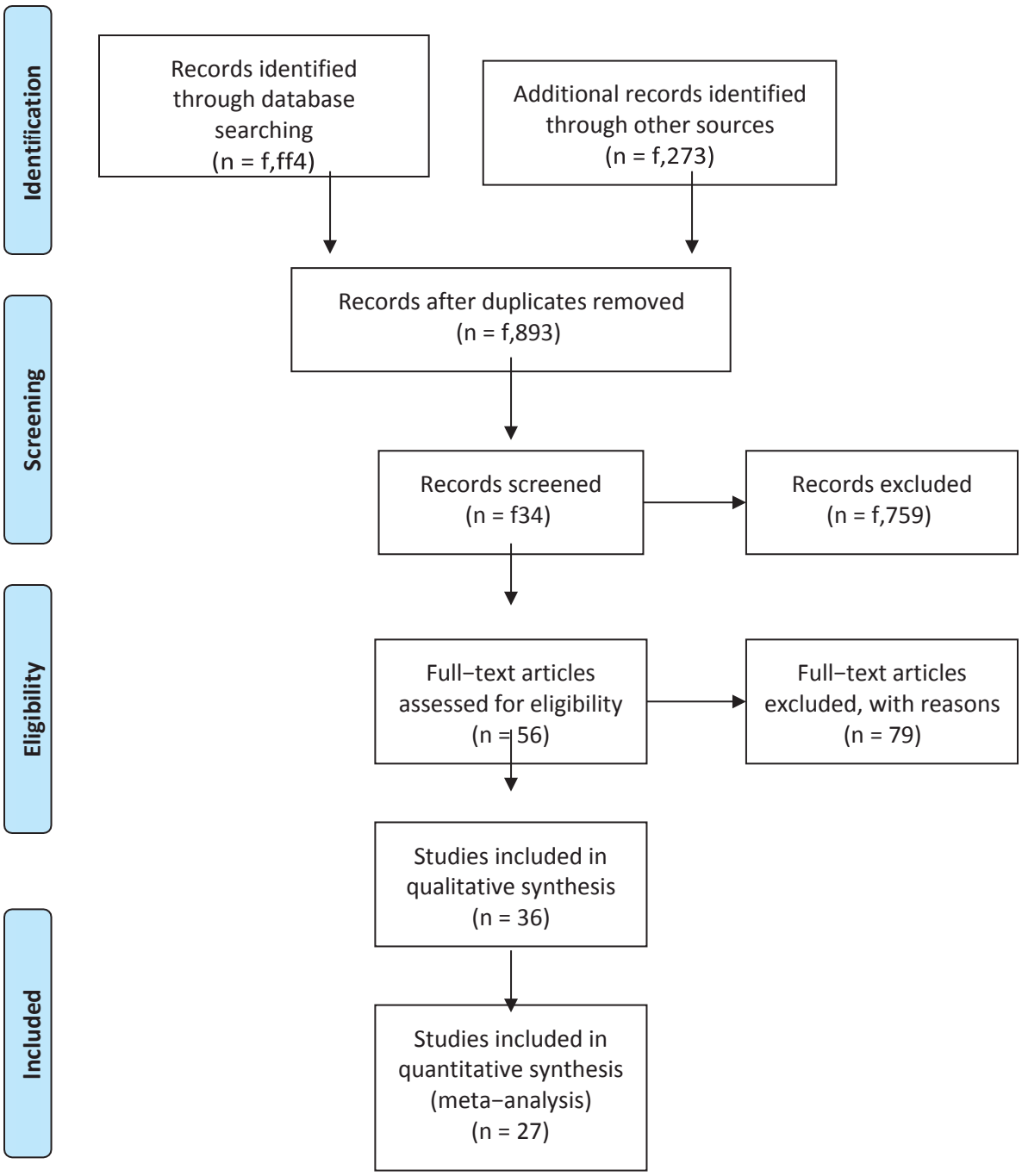

Figure 1: Prisma flow diagram of the articles reviewed in the present study.

Table 1: Thirty-six studies included in the qualitative and quantitative review.

Author I Year	Phenotype Studied	Case Population	Genotyping method	Quantitative Review	Reason for Exclusion
Xie et al. 2019	*Sporadic* pulmonary atresia with ventricular septal defect and tetralogy of Fallot	100	Whole-exome sequencing	Yes	

Luyckx et al. 2019	Bicuspid aortic valve / Thoracic Aortic Aneurysm	95	SNP Microarray	Yes	
Liu et al. 2019	Non syndromic CHD - from the Pediatric Cardiac Genomics Consortium.	760 family trios	Whole-exome sequencing	Yes	
Fotiou et al. 2019	Non-syndromic CHD	4634	Meta-analysis of Previously Reported Variants	No	Meta-analysis.
Shi et al. 2018	Total anomalous pulmonary venous connection (TAPVC)	78	Whole exome sequencing	No	No reported CNVs to include in quantitative review.
Giannokau et al. 2018	Hypoplastic right heart syndrome	42	SNP Microarray	Yes	
Xie et al. 2017	Non-syndromic CHD	973	SNP Microarray	Yes	
Hanchard et al. 2017	Left sided cardiac lesions	797 probands, 1047 parents and siblings (phase 1). 342 probands (phase 2)	SNP Microarray	Yes	
Giannakou et al. 2017	Ebstein anomaly	60	SNP Microarray	Yes	
Dimopoulos et al. 2017	Hypoplastic right heart syndrome	32	SNP Microarray	Yes	
Sanchez-Castro et al. 2016	Coarctation of the Aorta, transposition of great	316	aCGH	Yes	

	arteries, and Tetralogy of Fallot				
Kim et al. 2016	Non-syndromic CHD requiring surgical correction	422	SNP Microarray	No	Aim of study was surgical outcomes rather than CNV discovery.
Costain et al. 2016	Transposition of the great arteries and tetralogy of Fallot	101	SNP Microarray	Yes	
An et al. 2016	Ventricular septal defects	154	aCGH	Yes	
Moosmann et al. 2015	Coarctation of the aorta	70 with sporadic CoA and 13 with familial CoA	SNP Microarray	Yes	
Glidewell et al. 2015	Hypoplastic left heart syndrome	70	SNP Microarray	Yes	
Xie et al. 2014	Pulmonary atresia	82 parent trios	SNP Microarray	Yes	
White et al. 2014	Left sided cardiac defects.	257	SNP Microarray	Yes	
Warburton et al. 2014	Hypoplastic left heart syndrome and conotrucal heart defects.	213	SNP Microarray	Yes	
Glessner et al. 2014	Non-syndromic CHD	538 parent trios	SNP Microarray and Whole Exome Sequencing	Yes	
Bittel et al. 2014	Tetralogy of Fallot	34	aCGH	No	No reported CNVs to include in quantitativ

					e review.
Zhao et al. 2013	Non-syndromic CHD	100	aCGH	No	Patients with 22q11.2 deletions not removed from study.
Lalani et al. 2013	Non-syndromic CHD with extracardiac anomalies	203 (discovery), 511 (replication)	aCGH	Yes	
Carey et al. 2013	Single ventricle heart defects	223	aCGH	Yes	
Soemedi et al. 2012	Non-syndromic CHD	2256	SNP Microarray	Yes	
Silversides et al. 2012	Tetralogy of Fallot	433	SNP Microarray	Yes	
Serra-Juhé et al. 2012	Multiple congenital anomalies, including non-syndromic CHD.	33	aCGH	Yes *	
Payne et al. 2012	Hypoplastic left heart syndrome	43	aCGH	Yes	
Hitz et al. 2012	Left-sided cardiac defects	174	SNP Microarray	Yes	
Breakpot et al. 2012	Non-syndromic CHD	6 twin pairs discordant for CHD	aCGH	Yes	
Goldmuntz et al. 2011	Non-syndromic CHD	58	SNP Microarray	Yes	
Breakpot et al. 2011	Non-syndromic sporadic CHD	46	SNP Microarray	No	lncludes syndromic CHD
Greenway et al. 2009	Tetralogy of Fallot	114 (discovery), 398 (replication)	SNP Microarray	Yes	

Erdogan et al. 2008	lsolated CHD	105	aCGH	No	Poor resolution of variant reporting
Richards et al. 2008	Non-syndromic CHD with additional birth defects	20	aCGH	No	Poor resolution of variant reporting
Thienpont et al. 2007	CHD of unknown etiology	60	aCGH	No	lncludes syndromic CHD

Evolving Genomic Technology: Any discussion regarding genomic risk factors demands a brief review of the rapidly changing landscape of genomic technology. The human genome project was completed in 2003, less than two decades ago, and truly ignited a revolution of new genomic methodologies. Most relevant to this study are the production of whole genome surveillance arrays. Using whole genome variation data derived from the human genome project, the first whole genome surveillance arrays, array comparative genomic hybridization (aCGH), were developed in the early 2000s. The principle behind aCGH arrays predated the human genome project and involves fluorescent labeling of test DNA and reference DNA that is allowed to hybridize to metaphase chromosomes (Kallioniemi et al., 1992). Hybridization is measured by the strength of the different fluorescent signals of the test DNA compared to the reference DNA at given chromosomal positions. This strategy allows for the detection of deletions and duplications relative to the test chromosome. This technology was further developed from the utilization of the metaphase chromosome to microarrays containing genomic clones and cDNAs for hybridization. Moving from the metaphase chromosome to an array based platform improved genomic resolution and reduced error rates. A microarray can combine thousands and even millions of these probes into a singular platform, allowing for rapid surveillance of the entire human genome and was first described in 2003 (Albertson & Pinkel, 2003). Shortly thereafter, aCGH was used to map large scale CNVs in a human genome in two 2004 publications (lafrate et al., 2004; Sebat et al., 2004). Since these pioneering reports, the power of aCGH arrays has grown through the production of higher density arrays, leading to the detection of smaller CNVs with greater accuracy.

Two other important events in genomics led to the development of a different type of array that, instead of using cDNA, uses probes that hybridize to single nucleotide polymorphisms. The human genome project revealed substantial genetic variation in the form of single nucleotide polymorphisms (SNPs) with the identification of 1.4 million SNPs in its initial draft (Lander et al., 2001). Following completion of the human genome project, the international HapMap project was formed in 2003 to further explore and catalogue human genetic sequence variation in populations around the world (lnternational HapMap, 2003). The specific goal of the HapMap project was to identify common SNPs in 270 individuals from Asian, African and European populations. The second phase of the HapMap project concluded in 2007 and reported the identification of >3.1 million common human SNPs with a density of one SNP per kilobase (lnternational HapMap et al., 2007). The next major genetic milestone project was the 1000 Genomes Project which began in 2007 and concluded in 2015. ln their final publication, the project reported 84.7 million SNPs from 26 populations including Africa, East Asia, Europe, South Asia and the Americas (Genomes Project et al., 2015). The data gleaned from these international endeavors provided the foundation on which to build SNP microarrays. SNPs are positions in the genome in which there are two different nucleotides that occur in a human population. These two nucleotides are often referred to as alleles and regularly referenced as the A and B allele. lndividuals can be homozygous (AA or BB) or heterozygous (AB) for a specific SNP. Utilizing the growing wealth of data from the HapMap and 1000 Genomes project, companies like lllumina and Affymetrix have produced high density SNP microarrays that allow for investigation of single nucleotide variation and large structural variation across an entire genome.

Recurrent CNVs: There are a number of variables that challenge the reproducibility of many population studies identifying CNV risk factors for non-syndromic CHD. First, rare CNVs have been a focus of study with many studies first selecting CNVs for interpretation by their rarity (Soemedi et al., 2012; Warburton et al., 2014; H. Xie et al., 2019). Rare and *de novo* rare CNVs that are only observed in a case population of non-syndromic CHD patients are hypothesized to have higher penetrance and greater likelihood of pathogenicity (Fahed et al., 2013;

Glessner et al., 2014). The rarity of these CNVs makes reproduction of these same defects in analogous populations difficult and limits statistical association analysis to studies that have large populations. Second, the rapid advancement of genomic array and sequencing technology has resulted in variability in the reported breakpoints of putative CNV risk factors. Another consequence of the rapid improvement in genomic technologies is the development of higher density SNP arrays that are capable of surveying the genome at higher resolutions. For example, the latest Illumina™ arrays, the Omni5 platform, provide a resolution of ~1 probe per kilobase of the human genome with over 4.5 million probes per SNP beadchip. These newly developed high density SNP arrays offer increased genomic resolution and allow for the identification of CNVs that were missed in previous studies. Finally, the heterogeneity of CNVs challenges both association analysis and reproducibility. For example, CNVs can occur as deletion(s) or duplication(s) of a given locus. Furthermore, the length of CNVs are variable and not always consistent, even within known syndromic deletions. Finally, the penetrance of CNVs is variable with different phenotypes occurring in patients with the same pathogenic CNV. All of these CNV factors must be taken into consideration when conducting a statistical analysis of CNV risk factors and reproducing the findings. We therefore sought to evaluate the reproducibility of reported CNV risk factors for non- syndromic CHD.

A total of 773 CNV risk factors were identified from the 27 included studies. Reproducibility was evaluated by identifying CNVs that were overlapped by a CNV reported in another study by more than 80%. The choice of an 80% overlap was selected through trial and error. A higher value was not chosen because of the variability in microarray design used by the included studies which contributes to different reporting of CNV breakpoints. In total, 64 CNVs (8.3%) of the 773 CNV risk factors were replicated by at least one other study of non-syndromic CHD (Supplementary Table 2). There were 2 recurrent CNVs that were observed in 2 or more studies (Table 1). The two recurrent CNVs included duplications in 1q21.1 and deletions in 16p13.11. It is important to mention that the replication clusters were limited to CNVs that shared 80% of the same genomic coordinates for *both* CNVs tested. There are many CNVs within the cohort that were completely overlapped by larger CNVs that, as a result of the significant size difference between the two CNVs, were not considered a replication. If we expand our replication criteria to include CNVs overlapped by comparably larger CNVs, many more replications are identified with 256 of the 773 (33%) CNVs being replicated within another study. Reported genomic coordinates are aligned with the hg19 build of the human genome. ToF, Tetralogy of Fallot; MA, mitral valve atresia; VSD, ventricular septal defect; SVD, isolated single ventricle defect; HLHS, hypoplastic left heart syndrome; PA, pulmonary atresia.

There were ten 1q21.1 duplications observed in the cohort. Seven of the ten duplications were discovered in Tetralogy of Fallot patients, one was reported in a mitral valve atresia patient and two were observed in hypoplastic left heart syndrome patients. Four of the reported variants were reported by Silversides and colleagues in a study of 433 unrelated adults with Tetralogy of Fallot and pulmonary atresia (Silversides et al. 2012). Considering only the Tetralogy of Fallot patients, 1q21 duplications were observed in 4/388 or 1.03% of the test population. Another study included in the review was conducted by Greenway and colleagues also investigated CNV risk factors in 512 non-syndromic Tetrology of Fallot patients. In their study, they identified 5 duplications in 1q21.1 (0.98% of total population) (Greenway et al., 2009). Several of these CNVs were unable to be included in the dataset due to changes between the hg18 and hg19 genome build, thus limiting the number of replications between the studies. The primary gene of interest impacted by these CNVs is GJA5, or cardiac gap junction protein connexin 40. Mouse models in which GJA5 has been deleted or knocked down result in numerable complex CHDs in the offspring, primarily impacting the cardiac outflow tract (Gu, Smith, Taffet, & Delmar, 2003). Likewise, in a genetic analysis of 178 patients with non-syndromic TOF, a missense mutation (c.793C > T) was discovered in GJA5 that was not found in any of the 1568 controls. Microinjection of GJA5 containing the missense mutation into zebrafish was found to disrupt the development of the primitive heart tube in 37% of the tested embryos (Guida et al., 2013). Another study by Mefford and colleagues reviewed 22 studies of 1q21.1 duplication cases in order to further characterize the phenotypic manifestations and observed numerous clinical features including autism spectrum disorder, CHD, macrocephaly, and schizophrenia. A total of 107 patients were included in the study. They observed significant enrichment of Tetralogy of Fallot in the study population (P = 0.004) (Dolcetti et al., 2013). While not represented in the replication algorithm, Glessner et al. in 2014 identified 2 de novo duplications that overlapped GJA5. The CNVs identified by Glessner and colleagues were too small to be considered true replications of the above described CNVs, but they did impact the gene of interest, GJA5. It is clear that duplications and other genetic variations within 1q21.1 can have a negative impact on the developing fetus and present with variable clinical phenotypes. The prevalence of damaging CNVs in this locus in particular CHD populations, namely Tetralogy of Fallot, suggest it is a significant genetic contributor to

CHD risk.

Analysis of the reported CNVs also revealed four deletions in 16p13.11 in four separate studies (An et al., 2016; Carey et al., 2013; Warburton et al., 2014; L. Xie et al., 2014) and in four unique CHD phenotypes (ventricular septal defect, hypoplastic left heart syndrome, isolated single ventricle defect and pulmonary atresia). Compared to the previously discussed locus, 1q21.1, the impact of CNVs on 16p13.11 is much more unknown and subject to variability. Nagamani et al. sought to bring some clarity to the phenotypic distribution associated with 16p13.11 CNVs by studying 10 patients with duplications and 4 patients with deletions impacting 16p13.11 (Nagamani et al., 2011).

These samples were identified from >14,000 clinical aCGH arrays performed at Baylor College of Medicine. Clinical manifestations of patients with 16p13.11 deletion included developmental delay, microcephaly, and craniofacial dysmorphisms. Interestingly, they observed CHDs in their cohort of patients with 16p13.11, including Tetralogy of Fallot, coarctation of the aorta, and transposition of the great vessels. Another report detailing the clinical manifestations of five patients with 16p13.11 deletions described numerous neuropsychiatric manifestations in patients, microcephaly and epilepsy, but did not report any known CHDs (Hannes et al., 2009). The studies only contained 9 total cases with16p13.11 deletions and perhaps did not have a sample population large enough to observe the cardiac impact of these CNVs. Deletions in 16p13.11 clearly have variable phenotypic impact on patients, but the exact impact on CHD risk remains unclear.

Further investigation of this locus in human populations and animal models will be required to better understand this complex region.

Genes impacted by replicated CNVs: In total, 64 of the 773 CNVs were replicated by at least one other study. These 64 CNVs involved 491 unique genes. We entered this gene list into the DAVID functional annotation tool to see if any unique gene ontologies were enriched in this gene set. There was one enriched gene ontology (GO) term in the set: defense response to bacterium (p-value = 1.1e-7, Benjamini-Hochberg corrected p-value = 18e-4). This GO term involved 17 genes including 15 defensin proteins, *SPN* (Sialophorin) and *NLRC4* (Caspase recruitment domain family, member 12). CNVs in the þ-defensin gene have been shown to alter risk for diseases like psoriasis and Crohn's disease (Fellermann et al., 2006; Hollox et al., 2008). Additionally, it is well described that maternal febrile illness is associated with adverse neonatal outcomes, including increased risk of congenital heart defects (Botto, Lynberg, & Erickson, 2001). The enrichment of the GO term "defense response to bacterium" could imply a potential gene-environment interaction wherein CNVs involving these genes related to the immune response to bacteria are compromised and thereby conferred an increased risk of febrile illness or autoimmune disease in the mother.

Pathogenic CNVs and Outcomes: Understanding the CNV hazard factors that add to CHD danger may help in anticipating results in the influenced patients. There is restricted information in this field, however two articles distinguished in the audit give some knowledge into this creating field. One investigation directed by Kim et al. in 2016 looked to assess the effect of pathogenic CNVs on transfer free endurance in non-syndromic CHD patients (Kim et al., 2016). A control populace of 500 solid control kids selected from a similar clinical site (Children's Hospital of Philadelphia, CHOP) were utilized to assess the CNV trouble in non-syndromic CHD patients. Obviously, they noticed an essentially more prominent rate of pathogenic CNVs for their situation CHD populace (12.1%) contrasted with their control populace (5.0%) (P-esteem = 0.00016). The 12.1% of the CHD populace affected by pathogenic CNVs had an expected 3.43-crease more serious danger of death contrasted with non-syndromic CHD patients in the very populace that didn't have pathogenic CNVs (P=0.00009, 95% CI 1.66-7.09). Another investigation directed via Carey et al. in 2013 planned to assess the effect of pathogenic CNVs on newborn children with single ventricle heart absconds (Carey et al., 2013). This examination used 223 cases with single ventricular heart absconds and 270 controls and chose uncommon CNVs that were >300kb in size for their investigation. Like the discoveries saw by Kim and associates, Carey et al. noticed a more prominent extent of pathogenic CNVs for the situation populace bunch with 13.9% of the subjects with single ventricle heart abandons and 4.4% in the control populace containing pathogenic CNVs (P=0.0003). Fourteen-month results for the cases were outlined and those with pathogenic CNVs were seen to be more limited in stature (P = 0.031) and have less fortunate psychomotor improvement list scores (P=0.032). These outcomes give proof that the function of CNVs for CHD hazard reach out past the frequency of CHD and assume a part in the general result of the influenced patients.

All over again CNV hazard factors: Four examinations were recognized that required extra exertion to assess

anew CNVs for non-syndromic hazard (Glessner et al., 2014; Greenway et al., 2009; Warburton et al., 2014; L. Xie et al., 2014). These investigations revealed repetitive again CNVs in a few loci including 1q21.1, 3p25.1, 7q11.13, 7q11.23, 8p23.1, 11q24-25, 15q11.2, 16p13.11, 17q11.2, and 22q11.2. The two loci that were talked about before because of the intermittent reproduced CNVs distinguished in our audit are spoken to here: 1q21.1 and 16p13.11. Glessner and associates utilized information from SNP microarrays to notice an enhancement of again CNVs for their situation populace (22 anew CNVs in 462 CHD threesomes) contrasted with their control populace (9 once more CNVs in 841 controls) (OR = 4.6, P-esteem = 7x10-5). They additionally analyzed again CNVs recognized utilizing entire exome sequencing and noticed a comparative advancement of anew CNVs for their situation CHD populace (OR = 3.5, P-esteem = 6x10-4). Warburton et al. additionally noticed a higher pace of once more CNV transformation in their CHD populace with 9% and 2% of their cases and controls, separately, containing anew CNVs. Xie and associates didn't report a contrast between once more CNV transformation rates among case and control populaces. Scenic route examined the once more transformation rates between their case and control populace and noticed expanded paces of uncommon again CNVs for their situation populace (11/114 versus 4/98), yet the thing that matters was not critical (P-esteem = 0.18).

At the point when we consider hereditary variations related with sickness hazard, variations are frequently named latent or prevailing or, on account of danger, high penetrance or low penetrance. Acquired variations are promptly delegated latent or low penetrant on the grounds that their capacity to be acquired demonstrates their impact didn't upset the wellness of the parent or, due to the variable penetrance saw in numerous CNV hazard factors, didn't prompt an aggregate in the guardians. Alternately, all over again CNVs are viewed as bound to be penetrant or autosomal prevailing due to their quality in the proband and their nonattendance in the guardians whose conceptive wellness was not upset (Chung and Rajakumar, 2016). This worldview seems to assume some part in CHD hereditary qualities proposed that numerous non-syndromic CHD cases are brought about by all over again CNVs that have higher penetrance or work under an autosomal predominant model.

Conclusions:

Thus we checked on 27 investigations that recognized 773 CNV hazard factors for non-syndromic CHD. Under 10% of these CNVs were recreated in different examinations in non-syndromic CHD. There are a few difficulties to reproducing CNV hazard factors that should be thought of. Most examination bunches have a one of a kind pipeline their group has assembled to recognize and define CNVs by probability of pathogenicity. While numerous gatherings have assembled imaginative techniques for the disclosure and choice of possibly pathogenic CNVs, the changeability of these strategies probably add to the low reproducibility of these variations across various non-syndromic CHD techniques. The ACMG distributed rules for the clinical understanding of CNVs which have supported huge numbers of the procedures utilized by research gatherings (Kearney et al., 2011). The ACMG rules incorporate examination with set up conditions, thought of CNV size, and thought of genomic content in the CNV of premium, and correlation with inward and outside information bases. These rules remain genuinely significant and essential to the understanding of CNVs, however the changeability of utilization every proposal gets in CNV disclosure examines makes between study correlations profoundly testing. The normalization of CNV hazard factor ID through open source pipelines and ACMG embraced CNV disclosure suites would consider normalization of CNV revelation across different investigations.

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