

## REVIEW

# An Update on the Genetic Aspects in Congenital Ventricular Septal Defect

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Ventricular septal defects (VSDs) are the most common type of heart malformation and may occur like a part of a syndrome or as an isolated form. Clinical manifestations are related to the interventricular flow, which is determined by the size of the defect. Aiming at the identification of genetic causes is important in both syndromic and non-syndromic forms of VSD, to estimate the prognosis and choose the optimal management. Other reasons of the identification of genetic factors in the etiopathogenesis include the assessment of the neurodevelopmental delay risk, recurrence in the offspring, and association with extracardiac malformations. The diagnostic process has been improved, and currently the use of the most suitable and accessible technique in the clinical practice represents a challenge. Additional advantages in genetic testing were brought by next-generation sequencing technique, various testing panels being available in many laboratories.

**Keywords:** ventricular septal defects, genetic analysis, updates

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## Introduction

According to the current studies, the most common congenital anomalies at birth, are represented by congenital heart diseases (CHDs). The prevalence of CHDs is between 2% and 3% [1,2]. It is reported that 20-30% of CHDs are associated with non-cardiac anomalies, defined as syndromic CHD, and approximately 70-80% of CHDs are an isolated form of congenital anomalies, also called non-syndromic CHDs [3].

The ventricular septal defects (VSDs) can be present as part of other anomalies, like tetralogy of Fallot or diagnosed as isolated form, in approximately 0.4% of patients [4]. Cornoet et al. reported in their study, performed on patients aged between the first week of life and three decades of life, the frequency of 32.1% of VSDs in CHDs patients [5].

Genetic and environmental factors play an important role in the pathogenesis of CHD. The genetic variants have a major role in the development of these anomalies being identified in up to 30% of CHDs [6,7]. Based on sequencing technology, in a large study on CHDs, it was identified that 8% of mutations are de novo, and approximately 2% are inherited [8]. On the other side, in 2% of cases, the environmental factors are presumed to be the etiology for CHDs [7]. Similar, the genetic etiology of VSDs is complex and heterogeneous. Furthermore, the genetic anomalies in VSDs can be classified in different categories depending on the amount of the genetic material involved: (1) abnormal number of chromosomes (aneuploidy), (2) large deletions/duplications, (3) copy number variations (CNVs) and (4) single gene mutations. According to literature, large chromosomal abnormalities and aneuploidies are detected in

9% of patients with CHDs, CNVs in 3-10% of patients and small genetic variations in 4% of cases [7,8]. On the other hand, in patients with VSDs, chromosomal anomalies are reported in 36.5% of cases, CNVs in 16.9% and small genetic variations in 2.8% [9-11].

Depending on the type of the anatomical severity in patients with isolated form of CHD, overall survival rate after cardiac surgery was improved in the last decades, but long-term complications remain a major problem [12]. Besides that, the short-term complications after surgery have a high incidence and are represented by cardiac failure, renal complications, lung, brain and vascular complications [13]. It was reported that long-term survival after surgery is strongly associated with the presence of CNVs or single nucleotide polymorphisms (SNPs) [14].

New strategies, consisting of personalized medicine according to the needs of each patient with CHD, are based on the use of the most efficient target therapies and improve the long-term outcome [15,16]. An experimental study performed on mice models with RASopathies, demonstrated that after administration of Rapamycin and threonine protein kinase inhibitors, an improvement on cardiac hypertrophy and left ventricular function was obtained [17]. Moreover, new genome editing techniques could be theoretically a therapeutic method based on their capacity to recognize and restructure the target sequence [18]. A recent publication sustains this perspective in inheritable mutations and describes the correction of a gene mutation in human embryos with hypertrophic cardiomyopathy [19]. Induced Pluripotent Stem Cells (iPSC) represent another important resource for study in CHD and allows to create human models of disease. Several experimental studies using human iPSC derived cardiomyocytes allow generating cardiac tissue like endothelial cells

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and vascular mural cells with safety and with feasible results in children with CHD [20]. Additionally, to generate disease model in iPSC the most highly efficient technique is CRISPR/Cas9 which can induce specific mutation and provides the possibility to study the underlying cause of human disease [21].

As a consequence, the advantages in genetic research need to be introduced into the clinical practice of CHDs and should evaluate the possible major risks to estimate the overall survival rate and be a part of future decision-making in assessment for these patients.

In conclusion, the results of genetic research need to be introduced in the clinical practice of CHDs, however, the potential risks must be evaluated, which should be a part of future decision-making in these patients. An additional benefit of clarifying the genetic origin is the possibility of recurrence risk assessment in the family in inherited forms.

For practical considerations, it is essential to understand the limitations and benefits of the existing genetic techniques and apply the most efficient genetic analysis in the investigation of CHDs patients [22].

We performed this study to summarize the latest genetics research and highlight the potential applications of genetic aspects in patients with VSDs.

### Chromosomal aneuploidy and large deletions/duplications

The aneuploidies that survive to term include trisomy of 13, 18 and 21 and the heterosomal monosomy Turner syndrome. Clinical management of aneuploidies requires an accurate assessment including the cardiac anatomy, trisomy 21 being frequently associated with VSD.

**Down syndrome (trisomy 21)** is frequently associated with CHDs. In this syndrome, the prevalence of CHDs is between 40% and 50% [23]. It is reported that in 50% of cases, the mortality rate of Down syndrome patients is directly associated with CHDs and the associated complications [23]. Atrioventricular septal defects (ASVDs) are reported in approximately 30% of cases, followed by VSDs with a variable percentage between 21.5 - 35% [24]. Maslen et al. [24] reported the highest incidence of VSDs (35%) on Down syndrome patients and suggest that Down syndrome patients with hemodynamically significant lesion must be surgically treated to prevent the development of pulmonary vascular lesion, respectively Eisenmenger's syndrome [24]. The overall survival rate is directly associated with Eisenmenger's syndrome development, and according to literature, the prognosis of pediatric Eisenmenger's syndrome patients is influenced by mutations in the *ACVRL1* and *TBX4* genes [25].

**Jacobsen syndrome** is caused by the deletion in the long arm of chromosome 11, the length of the deletion being estimated at 7-16 Mb, which can include the telomeric region as well [26]. Clinical features include cognitive impairment, platelet dysfunction, CHDs, ophthalmological, gastrointestinal, and genitourinary anomalies [27]. Cardi-

ovascular features are present in more than 50% of patients and one-third of them have a membranous VSD [23].

Also, VSDs are frequently identified in syndromes with aneuploidies, including trisomy 18, trisomy 13, and Klinefelter syndrome [28]. Other chromosomal abnormalities such as deletion in 4p24, duplications in 16p13, 22q11, 8q21 and partial monosomy of chromosome 18 were identified in a recent study that evaluated 151 cases diagnosed prenatally with VSDs [29].

### Copy number variations

The presence of CNVs, such as chromosomal microdeletions or microduplications, play an important role in genetic variability. There are several CNVs in the human genome, but only a small part of them is being considered pathogenic for a disease. The rest are considered benign or with an unknown or uncertain significance. The pathogenic CNVs for non-syndromic CHDs were reported by Carey et al. being present in 10 to 20% of patients [30]. In the last decades, the number of pathogenic CNVs for CHDs increased as a result of the research studies published. Future studies with a large number of patients are needed because a high number of potential pathogenic or uncertain significant CNVs are currently being reported. Russel et al. reported that potential pathogenic CNVs can be considered in patients with CHD in the absence of other extracardiac malformations and are frequently encountered compared with the healthy population [31]. Besides the fact that not all deletions are considered pathogenic, currently it is accepted that a deletion is more frequently pathogenic compared to a duplication, having more often a phenotypic impact and being associated with a genetic syndrome. Herein, we describe the pathogenic CNVs associated with different syndromes which include VSDs.

**Deletion 1p36** is the most common deletion in humans. Clinically the syndrome caused by a 1p36 deletion associates: developmental delay, vision problems, hearing loss, distinctive facial features, brain anomalies, CHD, cardiomyopathy, and renal anomalies. This deletion was reported frequently in patients with VSDs. The clinical features caused by 1p36 deletion syndrome are caused by the pathogenic deletion of the *RERE*, *CASZ1*, *ECE1*, and *LUZP1* genes [32].

**22q11.2 microdeletion syndrome** (DiGeorge syndrome) is characterized by hypoplasia of the thymus and parathyroid glands, cardiac malformation, and facial dysmorphisms.

Moreover, in chromosome 22, the deletion of a region in the long arm is described in literature to have the main role in the clinical appearance in 10 to 20% of cases with DiGeorge syndrome [33]. The frequency of the deletion confirmation increased to 45% in patients who also present VSDs and aortic arch anomaly [33]. Clinical features of this syndrome may also be a consequence of a small deletion in the short arm of chromosome 10 [23]. Also, it is known that maternal comorbidities, like diabetes mellitus,

or behavior factors like alcohol use, are directly involved in the appearance of various clinical features in the offspring [23, 34]. In 22q11 deletion syndrome, cardiovascular disease is one of the most common features, and it is estimated that 10% of patients with VSDs present this deletion [34].

Currently, for this genetic locus, reciprocal CNVs are described. Duplication of 22q11.2 region is associated with a variable disorder with a phenotype which includes learning disability and heart defects in 15% of cases, but opposite to deletion, the duplication syndrome had a protective effect for schizophrenia [35, 36].

An atypical phenotype of 22q11.2 microdeletion syndrome is described in cases with a mutation outside this region, in *GP1BB* gene. A mutation with loss of function of *GP1BB* is reported in Bernard-Soulier syndrome (BSS) being associated with a higher risk of bleeding during surgery or other invasive procedures [33].

**1q21.1 deletion** includes clinical features such as microcephaly, intellectual disability, short stature, eye abnormality, and less commonly associated skeletal malformation, genitourinary anomalies, and CHDs [37]. There are many types of CHD described in 1q21.1 deletion, including VSDs. One possible etiology for VSD is a mutation the *GJA5* gene (1q21.1) which encodes for connexin 40 - a cardiac gap junction protein [38]. Opposing, the reciprocal CNVs, such as **1q21.1 duplication**, are associated with the same features, but the opposing phenotype includes macrocephaly [35,37,38].

**8p23.1 deletion** frequently associate mild to moderate intellectual disability, behavioral problems, microcephaly, diaphragmatic hernia, and CHDs. *GATA4* encodes a transcription factor involved in heart development, and its absence/imbalance expression due to 8p23.1 deletion often leads to CHDs (>90%) [39].

### Small genetic variation - Syndromic single gene disorders

**Alagille Syndrome**, caused by a mutation in *JAG1* and *NOTCH2* gene, is an autosomal dominant syndrome characterized by mild motor and intellectual development delay, prominent forehead, hypertelorism, chronic cholestasis or even liver failure, orthopedic complications, and cardiovascular features. Most frequently, in approximately 60% of cases with Alagille syndrome branch pulmonary stenosis is described followed by arterial narrowing and structural cardiac defects (tetralogy of Fallot, VSDs and ASDs) [23, 40]. Some features, mainly the liver failure is influenced by specific types of mutations in *JAG1* and *NOTCH2* genes, but this mechanism is not similar for CHDs. Considering Alagille syndrome is recommended in the presence of characteristic cardiovascular features [41,42].

**Holt-Oram syndrome** is frequently caused by the presence of the null allele of the *TBX5* gene and in 17% of cases by a heterozygous variant of the same gene. In less than 1%, the Holt Oram syndrome is caused by a partial

or complete deletion of the *TBX5* gene [43]. The phenotype consists mostly of two common features: (1) radial ray abnormalities and (2) CHDs. In 75% of cases, a cardiac anomaly is confirmed and involves atrial or ventricular septum defects and conduction system abnormalities [44].

**Char syndrome** is caused by mutations in the *TFAP2B* gene, and in 50% of cases a heterozygous variant of this gene is detected with familial inheritance [45]. Clinical aspects consist of flat midface, broad nasal tip, flat nasal bridge, hypertelorism, hand abnormalities with a shorter middle section of the fifth finger and patent ductus arteriosus as the most common heart anomaly [46]. Also, heart defects such as VSDs and complex CHDs have been reported as associated anomalies in Char syndrome [47].

**CHARGE syndrome.** In most cases causal pathogenic variants of the *CHD7* gene, and large deletions in this gene were identified [48]. The phenotype of this condition includes coloboma, heart defect, choanal atresia, delay in growth/development, genital, and ear anomalies. The CHDs reported in CHARGE syndrome are heterogeneous; conotruncal defects and septal defects are the most frequently described CHDs [49]. The study performed by Corsten et al. described truncating variants of *CHD7* in 80% of CHD patients and missense and splice site variants in 58% of patients, identified in subjects with CHD with the presence of both genetic anomalies [50].

**Kabuki syndrome** in 75% of cases is caused by pathogenic variants of the *KMT2D* gene and in approximately 5% in the *KDM6A* gene with an X-linked and autosomal dominant inheritance [51]. Common recognizable features are intellectual disability, long palpebral fissures, depressed nasal tip, arched eyebrows, large dysplastic ear, and cleft palate. Coarctation of the aorta, atrial septal defect, and VSDs are the most common CHDs described in Kabuki syndrome [52].

**Noonan syndrome** is a part of RASopathies group disorders with autosomal dominant inheritance. In 50% of cases multiple missense variants in the *PTPN11* gene were reported, and in other 30% of cases different genes variants involved in RAS pathways were described [53]. Facial features are age-dependent, and it is difficult to correlate the genotype with the phenotype, even that in 80% of cases, structural eye abnormalities, hypothyroidism, and short stature were described. A large number of patients with Noonan syndrome (80% to 90%) have CHDs, of which 40% cases have pulmonary stenosis [54]. Other CHDs are tetralogy of Fallot, atrial septal defect, and arterial defects [54]. In Noonan syndrome cases with septal defects commonly pathogenic variants in the *SOS1* gene were described [55].

### Other syndromes commonly associated with VSD

In Table 1 are enlisted syndromes frequently associated with VSD, including the genes usually involved and other common clinical features.

Table 1. Other syndromes frequently associated with VSD

Syndrome	Gene(s)	Loci/Region	Other cardiac disease	Other Clinical Features	References
Cornelia de Lange	<i>NIPBL</i> <i>SMC1A</i> <i>HDAC8</i> <i>SMC3</i> <i>RAD21</i>	5p13 Xp11.22 Xp13.1 10q25.2 8q24.11	PS, ASD, AoCo, HCM	Growth retardation, intellectual disability, gastroesophageal reflux	56
Williams-Beuren	<i>ELN</i>	7q11.23	AS, PS, Systemic hypertension, MVP, AoCo	Facial features, intellectual disability, difficulty in visual-spatial tasks	57,23
Costello	<i>HRAS</i>	11p13.3	Arrhythmia, HCM, PS, aortic dilatation	Growth retardation, intellectual disability, rhabdomyosarcomas	58
Rubinstein-Taybi	<i>CREBBP</i> <i>EP300</i>	16p13.3 22q13.2	MVD, ASD, TOF, PS, MS	Growth retardation, microcephaly, micrognathia, broad halluces, intellectual disability	59
Beckwith-Wiedemann	<i>CDKN1C</i>	11p15.5	ASD, PS	CNS malformations, abdominal wall defects, macroglossia	60
Smith-Lemli-Opitz	<i>DCHR7</i>	11q12-13	Complex CHD, ASD, PDA,	Hypotonia, CNS malformations, hypospadias, adrenal insufficiency	61
Nance-Horan	<i>NHS</i>	Xp22.13	TOF, PDA	Epileptic encephalopathy, congenital cataract, dental anomalies, hypotonia	62
Bardet-Biedl	<i>BBS1</i> <i>BBS10</i>	4q27	AS, PS, ASD PDA	Obesity, anosmia, polydactyly, retinitis pigmentosa, anosmia	23

PS = pulmonary stenosis; ASD = atrial septal defect; AoCo = aortic coarctation; HCM = hypertrophic cardiomyopathy; AS = aortic stenosis; MVP = mitral valve prolapse; MVD = mitral valve dysplastic; TOF = tetralogy of Fallot; ASD = atrial septal defect; MS = mitral stenosis; PDA = patent ductus arteriosus; CNS = central nervous system.

### Non-syndromic single gene disorders

Genetic aspects of non-syndromic single gene disorders of VSDs are complex. A new correlation between the phenotype, genotype, and the prognosis of the patients is needed, especially in those cases where the etiology remains unknown. We admit that these correlations seem to be very difficult to be realized because of a multifactorial mechanism, with more than 50 genes involved in heart development [63]. In a study performed by Pang S et al. mutations in the *GJA1*, *SMAD2* (encode proteins involved in cell signaling), *TBX20*, *TBX5*, *GATA4*, *GATA6*, *CITED2*, (encode transcription factors involved in cardiac development) were described as pathogenic for VSDs [64].

The most important connexin proteins is connexin-43 (Cx43) encoded by the *GJA1* gene (MIM 121014) expressed mainly in the heart and liver [65]. Kosuke et al. identified a missense de novo mutation in the *GJA1* gene, c.145C>G in one patient with VSD and syndactyly type III [66]. Wang et al. screened 418 CHD patients from which 44.5% were diagnosed with VSD and they identified three heterozygous missense mutations (c. 458G>A, c. 781G>T, c.968 C>T) in the *GJA1* gene [67]. In the *SMAD2* gene (MIM 601366), two de novo point mutation involved in methylation pathway were identified by Zaidi et al. [68].

*TBX20* (MIM 606061) is implicated in cardiovascular morphogenesis. Anomalies of this process were associated with mutations in this gene. In a study performed by Rai-Tai et al. [69], a loss-of-function mutation (c.820A>T) in a familiar form of CHD with autosomal dominant inheritance was described [60]. Akiko et al. identified a novel mutation (c.991A>G) in exon 7 of the same gene in one patient with VSD and in other two patients with the same pathology they identified a variant in exon 8 (c.791G>A) of *TBX5* gene [70].

Another gene involved in CHDs, specifically in VSD1, is *GATA4* (MIM 600576), anomalies of this gene being reported in the familial form of CHD, especially the pathogenic variant c.899 A>C [71]. Additionally, an experimental study on mice models confirmed that mutations in *GATA4* can be involved in CHD pathogenesis [72]. Variants in the *GATA6* (MIM 601656) gene, a member of GATA gene family, have also been reported in familial CHDs [73]. Additionally, Allen et al. identified a de novo heterozygous inactivating mutation in 50% cases with pancreatic agenesis, and 90% of them were also diagnosed with CHDs [74]. Other findings revealed the potential role of mutations of the *CITED2* (MIM 602937) gene in CHDs, especially in VSD2; Xu et al. described 3 mutations in this gene (c.550G>A, c.573-578del6, c.574A>G) with possible pathogenic implications [75].

Mutations in *NKX2-5* (MIM 600584) are associated with VSD3 and in a study performed in 150 Egyptian children with CHDs it is reported that two polymorphisms (rs2277923, rs28936670) may be involved in the pathogenesis [76].

### Technical approach

Briefly, we can summarize the genetic techniques in three categories: (1) conventional karyotype used to identify aneuploidy, large deletions or duplications, translocations, etc.; (2) chromosomal microarray/array comparative genomic hybridization (aCGH), Fluorescent in situ hybridization (FISH) and Multiplex Ligation-dependent Probe Amplification (MLPA) useful for identification of CNVs of the DNA such as microdeletions, microduplications but also for aneuploidies, small supernumerary chromosomes and point mutations [77]; (3) gene testing, sequencing and next generation sequencing with whole exome sequencing (WES) or whole genome sequencing (WGS).



New platforms for genetic testing have expanded options with an increased resolution. Thus, choosing the best type of analysis to identify the cause of disorders can be a challenge and can lead to waste of the resources. It is confirmed that some techniques, including WGS and WES are essential tools in diagnosis and establishing the pathogenesis in CHDs and VSDs, but with a high cost for a routine practice [78].

According to the resolution of genetic tests, the pathogenic mechanisms in VSDs can be detected using all these methods. However, based mainly on new higher resolution techniques (WES, WGS), the number of pathogenic and probably pathogenic variants increased. It is important to determine the genetic etiology for VSD patients to offer additional information about diagnosis and evolution of the disease. Moreover, only after identification of the pathogenic variant, the genetic counseling for the patient and his family can be adequate. Furthermore, based on this idea, Kelle et al. analyzed 152 family members of patients with Hypoplastic left heart syndrome caused by known mutations, and they identified 11% of family members with mutations and cardiovascular malformations that have been previously undiagnosed [79]. Also, the benefit for genetic testing is higher for patients entering reproductive age because the recurrence risk is described among 3% to 8% [80].

The cytogenetic investigation in VSDs, similar with CHDs, must be considered in patients with specific phenotype for a chromosomal syndrome; patients with developmental delay, dysmorphic features or multiple malformation syndrome; patients with abnormal prenatal screening (echocardiography) which revealed a major visceral malformation or a major cardiac anomaly.

In the detection of chromosomal abnormalities including CNVs, Monteiro et al. proposed the MLPA technique as a first genetic test based on low costs, efficiency in detection, and because it is easier to perform and analyze [81]. Additionally, it has been proved that in syndromic CHD cases with abnormal cytogenetic analysis, the MLPA was a fast and efficient method for establishing the origin of a small supernumerary marker chromosome [82].

Based on a large number of genes involved in non-syndromic VSDs patients, the sequencing techniques are increasingly used and according to Pierpont et al. there are several hundreds of genes which contribute to approximately 10% of cases with severe CHD [23]. Recommendations for WGS or WES techniques in VSDs are based on (1) multiple genes that can cause or contribute to VSD; (2) the possible existence of de novo variants associated with syndromic VSD and inherited variants for non-syndromic type [83]; (3) the fact that both sporadic and inherited sequence variants can cause VSD; (4) the heterogeneity of the phenotype (type and dimensions of VSD) caused by different variants in the same gene; (5) incomplete penetrance and inheritance especially in familial form of VSD [23].

## Conclusions

Based on the new molecular techniques, the knowledge in the pathogenesis of VSD increased and had a major effect on medical care and genetic counseling. Currently, next-generation sequencing panels are available, and CNVs detection is accessible for many laboratories. All the techniques mentioned are useful for syndromic and non-syndromic VSD patients' diagnosis, increasing the accuracy of the diagnosis, but each of these techniques has different benefits and limitations. The identification of the genetic variants in some cases also had direct implications in the clinical care of the VSDs patients, in the recognition of other extracardiac anomalies, estimation of the recurrence family risk and improving the decision of the medical therapy.

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## Authors' contribution

GAC designed the study, wrote the manuscript and approved the final manuscript. FT review the manuscript and edit the final version. MA designed the study, read and approved the manuscript. KBB read the draft, revised the manuscript. CB designed the study, performed the critical revision and approved the final version.

## Conflict of interest

None to declare.

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