Human Genetics of Ventricular Septal Defect

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Abstract

Ventricular septal defects (VSDs) are recognized as one of the commonest congenital heart diseases (CHD), accounting for up to 40 % of all cardiac malformations, and occur as isolated CHDs as well as together with other cardiac and extracardiac congenital malformations in individual patients and families. The genetic etiology of VSD is complex and extraordinarily heterogenous. Chromosomal abnormalities such as aneuploidy and structural variations as well as rare point mutations in various genes have been reported to be associated with this cardiac defect. This includes both well-defined syndromes with known

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genetic cause (e.g., DiGeorge syndrome and Holt–Oram syndrome) and so far undefined syndromic forms characterized by unspecific symptoms. Mutations in genes encoding cardiac transcription factors (e.g., *NKX2-5* and *GATA4*) and signaling molecules (e.g., *CFC1*) have been most frequently found in VSD cases. Moreover, new high-resolution methods such as comparative genomic hybridization enabled the discovery of a high number of different copy number variations, leading to gain or loss of chromosomal regions often containing multiple genes, in patients with VSD. In this chapter, we will describe the broad genetic heterogeneity observed in VSD patients considering recent advances in this field.

23.1 Introduction

Ventricular septal defects are recognized as one of the commonest congenital heart defects, accounting for up to 40 % of all cardiac malformations [1]. They can be classified according to their location, either within the muscular septum (muscular defects) or at its margins (perimembranous and supracristal defects) (see Chap. 22) [1]. Further, ventricular septal defect (VSD) is not only a common isolated congenital heart disease (CHD) but is often associated with other congenital cardiac defects, either in an individual or within a family. This heart defect also exists as an intrinsic component of several complex malformations, including tetralogy of Fallot (see Chap. 31) and univentricular heart (see Chap. 49) [1].

Epidemiologic studies strongly suggest that genetic factors play an important role in CHD etiology, although environmental exposures are also relevant (see Chap. 16) [2]. In a nationwide population-based study, Oyen et al. reported that CHDs in general show highly variable familial clustering in first degree relatives and indicated a threefold recurrence risk for isolated VSD [3]. In the following chapter, we will focus on the role of genetic factors associated with ventricular septal defects and describe their various genetic causes including chromosomal aberrations, structural variants, and single disease genes and mutations.

23.2 Isolated VSD

A number of studies have shown that isolated VSDs (without further cardiac and/or extracardiac congenital defects) can be associated with copy number variations as well as single gene mutations in patients and related families. Of note, the affected families often comprise individuals showing isolated VSDs as well as individuals with other CHDs (the latter will be presented in Sect. 23.3).

23.2.1 Copy Number Variation in Isolated VSD

A copy number variation (CNV) is a structural genomic variant that results in confined copy number changes in a specific chromosomal region that often contains multiple contiguous genes [4]. Commonly, CNVs are defined as any submicroscopic

chromosomal changes affecting more than 1000 bases [5]. Frequently, microdeletions and microduplications are identified by high-resolution comparative genomic hybridization (array CGH) as changes of DNA quantity (see Chap. 18) [4].

Studies analyzing CNVs commonly used cohorts of patients with a broad range of different CHDs [6–10]. To date, in five studies analyzing a range of CHDs including isolated VSDs, a total of eight affected loci have been identified with the chromosomal region 22q11.2 and 8p23.1 being most frequently affected. At the 22q11.2 locus, the *TBX1* gene (transcription factor T-box 1) known to be implicated in DiGeorge syndrome (see Sect. 23.4.2) was duplicated in one familial case [6], while the protein kinase *CRKL* gene (V-Crk avian sarcoma virus CT10 oncogene homolog-like) was affected in two independent patients [9, 10]. The locus 8p23.1 contains the candidate genes *GATA4* (transcription factor GATA binding protein 4), playing an important role in cardiac development (see Sect. 23.2.2.1), and *SOX7* (SRY (sex determining region Y)-box 7) [8, 9]. Rare copy number gains at the locus 11q25 were found in two patients but not associated with known risk genes [6, 7]. A summary of all CNVs found in isolated VSD cases is given in Table 23.1.

23.2.2 Single Gene Defects in Isolated VSD

In patients with isolated VSD, a number of different mutations have been found in genes encoding for transcription factors, signaling molecules, and proteins of other functions (see Table 23.2).

23.2.2.1 Transcription Factors

The development of the heart is orchestrated by transcription factor (TF) networks including members of the NK2 homeobox, T-box, and GATA binding families (see Chap. 12) [26]. GATA binding protein 4 (GATA4) is a transcriptional activator found to be affected in sporadic and familial cases of isolated VSD. Three different missense mutations in the *GATA4* gene (p.Pro407Val, p.Ser175Cys, and p.Ala411Val) have been reported in sporadic cases [12–14]. In addition, two families carrying *GATA4* missense mutations (p.Arg43Trp and p.Gly296Arg) were discovered in a follow-up analysis of index patients that participated in a screen of unrelated individuals (see also Sect. 23.3.2.1) [15, 16]. In the follow-up of affected family members, two and four additional cases of isolated VSD were found in the studies by Wang et al. [15] and Yang et al. [16], respectively.

So far, mutations affecting three members of the T-box family, namely, TBX1, TBX5, and TBX20, have been associated with isolated VSDs. Pan et al. screened 230 CHD cases and observed a heterozygous nonsense mutation (p.Gln277X) in the DNA-binding domain of *TBX1* in one patient with double outlet right ventricle who had one affected relative with isolated VSD carrying the same mutation (see also Sect. 23.3.2.1) [19]. A missense mutation (p.Ile152Met) in *TBX20* causing impaired DNA binding was found in a family affected by multiple septal defects including isolated VSD, atrial septal defect (ASD), and a large patent foramen ovale in different relatives [21]. In the case of *TBX5*, a non-coding variant in one of its enhancers was suggested to impact on the development of VSD [20]. The variant was found homozygous in a case of isolated VSD with unaffected heterozygous parents and

Table 23.1 Copy number variation in isolated VSD

Cytoband	CNV start (hg18)	CNV end (hg18)	Size	Copy number	Candidate genes	References
1q21.1 22q11.2	144723763 19389671		1574 kb 406 kb	Deletion Duplication	*ACP6, BCL9, CHD1L, FMO5, GJA5, PRKAB2 CRKL*	[9]
2p22.3	32.51 Mb	33.21 Mb	0.70 Mb	Duplication	*LTBP1*	[6]
3p14.2				Deletion		[8]
3p22.1				Deletion		[8]
3q25				Deletion		[8]
3q29				Duplication		[8]
5q31.3				Deletion		[8]
6p12.1	55356489	55493937	137 kb	Deletion		[10]
6q24.1	142187041	142290373	103 kb	Deletion		[10]
8p23.1				Deletion	*GATA4*	[8]
8p23.1				Duplication		[8]
8p23.1	8027361		4456 kb	Deletion	*GATA4, SOX7*	[9]
9p24.1	6770364	6953533	183 kb	Deletion		[10]
9q33.2	124774046	125024684	251 kb	Duplication		[10]
11p13	34458230[a]	34460862[a]	2.6 kb	Deletion		[7]
11p15.4				Deletion		[8]
11q25	134598043[a]	134617838[a]	19.8 kb	Duplication		[7]
11q25	132.23 Mb	132.76 Mb	0.53 Mb	Duplication		[6]
14q32.12	92475603	92709736	234 kb	Duplication		[10]
15q11.2	20384417		251468 bp	Deletion		[8]
15q13.3				Duplication		[8]
16p13.11				Duplication		[8]
17q12				Duplication		[8]
18q11.1-11.2	16795645		6118 kb	Duplication	*GATA6*	[9]
18q22.1				Duplication		[8]
18q23	75996798	75076224	921 kb	Duplication	*NFATC1*	[10]
20p12.3				Duplication		[8]
20q13.2				Duplication		[8]
22q11.2	17.39 Mb	19.74 Mb	2.35 Mb	Duplication	*TBX1, CRKL*	[6]
22q11.2				Deletion		[8]
22q11.2	19051034	19825156	774 kb	Deletion		[10]
Xq28	153436333	154895334	1.5 Mb	Deletion		[10]

Each row refers to one case
hg18 human reference genome (full sequence) version 18, *kb* kilobases, *Mb* megabases, *bp* basepairs
[a]Genomic coordinates refer to hg19

Table 23.2 Single gene defects in isolated VSD

Gene	Protein function	References
Transcription factors (TF)		
CITED2	Transcriptional coactivator	[11]
GATA4	GATA binding TF	[12–16]
IRX4	Iroquois homeobox TF	[17]
NKX2-6	Homeobox TF	[15]
PITX2	Homeodomain TF	[18]
TBX1	T-box TF	[19]
TBX5	T-box TF	[20]
TBX20	T-box TF	[21]
Signaling molecules		
CFC1	Ligand (TGFβ signaling)	[22]
GDF3	Ligand (TGFβ signaling)	[23]
TDGF1	Co-receptor (TGFβ signaling)	[24]
Other genes		
HAS2	Hyaluronan synthase	[25]

supported by functional evidence based on transgenic expression studies in mouse and zebrafish [20].

A study focusing on the analysis of *PITX2* (paired-like homeodomain 2) in a cohort of 170 unrelated neonates with CHD found two missense mutations (p.Arg91Gln and p.Thr129Ser) in two affected families [18]. Four mutation carriers presented with isolated VSD whereas two other relatives showed transposition of the great arteries (TGA) with VSD (see Sect. 23.3.2.1) [18].

Mutations have also been identified in the TF genes *NKX2-6*, *CITED2*, and *IRX4* [11, 17, 27]. Screening a CHD cohort including 66 isolated VSD cases, Wang et al. identified a missense mutation (p.Lys152Gln) in the homeodomain of *NKX2-6* (NK2 homeobox 6) [27]. Subsequent analysis identified the mutation in two further family members with isolated VSDs [27]. In a cohort of nearly 400 sporadic CHD cases, a nine amino acid deletion (p.Ser170_Gly178del) in *CITED2* (Cbp/P300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2), resulting in impaired activity, was detected by us in one patient with isolated perimembranous VSD [11]. For the ventricle-specific TF *IRX4* (Iroquois homeobox 4), two missense mutations (p.Asn85Tyr and p.Glu92Gly) were reported in two unrelated patients with isolated VSD by direct sequencing of the gene in a cohort of about 700 CHD patients [17]. The two mutations affected the interaction with retinoid X receptor alpha, a nuclear receptor of the vitamin A signaling pathway important in cardiac morphogenesis.

23.2.2.2 Signaling Molecules

Various cellular processes in both the embryonic and adult organism are regulated via transforming growth factor beta (TGFβ) signaling pathways. Important

developmental steps, such as the establishment of left–right asymmetry, are driven by the NODAL signaling pathway, which is named after the TGFβ superfamily member of the same name (see Chap. 7). Two genes encoding cofactors of the NODAL signaling pathway, *TDGF1* (teratocarcinoma-derived growth factor 1 also known as CRIPTO) and *CFC1* (Cripto, FRL-1, Cryptic family 1 also known as CRYPTIC), have been analyzed in a cohort of 500 CHD cases [22, 24]. Three missense mutations, p.Arg41Gly in *TDGF1* [24] as well as p.Leu219Phe and p.Gly169Val in *CFC1* [22], were identified in three patients with isolated VSD (see also Sect. 23.3.2.2). Another member of the NODAL signaling pathway, *GDF3* (growth differentiation factor 3), was analyzed by Xiao et al. [23]. Direct sequencing of *GDF3* in a cohort of 200 CHD patients led to the identification of a missense mutation (p.Ser212Leu) in a patient with isolated muscular VSD [23].

23.2.2.3 Other Genes

The gene *HAS2* encodes hyaluronan synthase 2, an enzyme that synthesizes hyaluronic acid (a major component of the extracellular matrix) during embryogenesis [25]. Among 100 non-syndromic VSD cases, Zhu et al. detected a *HAS2* missense mutation (p.Glu499Val) in one patient. The synthesis of hyaluronic acid was significantly impaired in the mutant enzyme as shown by *in vitro* assays [25].

23.3 Non-syndromic VSD

VSDs do not only occur as isolated malformations but most frequently are part of a more complex malformation. In the absence of extracardiac malformations, these VSDs are classified as "non-syndromic" (in contrast to syndromic VSD; see Sect. 23.4). Of note, VSD is an intrinsic component of complex malformations such as tetralogy of Fallot and double outlet right ventricle as well as univentricular heart (see Chaps. 31 and 49, respectively).

23.3.1 Copy Number Variation in Non-syndromic VSD

Two microduplications and two microdeletions were described in four cases of VSD with additional cardiac malformations (see Table 23.3) [6, 9]. Tomita-Mitchell et al. analyzed a cohort of several hundred CHD cases and identified among others one patient with VSD and pulmonary atresia who carried a duplication at the locus 1q21.1 comprising candidate genes such as *CHD1* (chromodomain helicase DNA binding protein 1) and *GJA5* (gap junction protein alpha 5, 40 kDa; also known as connexin 40) [9]. Screening a cohort of 105 CHD patients by array CGH, Erdogan et al. found a deletion at the 22q11.2 locus (including *TBX1*) in a case of VSD and aortic coarctation [6]. Further, they detected a duplication at chromosome 4q32.3 in one patient with VSD and PDA and a large 4 megabase deletion at chromosome 17p11.2 in a patient with VSD and ASD. No evident candidate genes have been identified in either of these regions [6].

Table 23.3 Copy number variation in non-syndromic VSD

Cytoband	CNV start (hg18)	CNV end (hg18)	Size	Copy number	Candidate genes	References
1q21.1	144812585		1480 kb	Duplication	*ACP6, BCL9, CHD1L, FMO5, GJA5, PRKAB2*	[9]
4q32.3	167.52 Mb	169.28 Mb	1.76 Mb	Duplication		[6]
17p11.2	16.47 Mb	16.47 Mb	3.98 Mb	Deletion		[6]
22q11.2	17.39 Mb	20.00 Mb	2.61 Mb	Deletion	*TBX1*	[6]

Each row refers to one case
hg18 human reference genome (full sequence) version 18, *kb* kilobases, *Mb* megabases

Table 23.4 Single gene defects in non-syndromic VSD

Gene	Protein function	References
Transcription factors (TF)		
GATA4	GATA binding TF	[15, 16, 28]
NKX2-5	Homeobox TF	[12, 29–32]
PITX2	Homeodomain TF	[18]
TBX1	T-box TF	[19]
ZIC3	Zink finger TF	[33]
Signaling molecules		
NF1	Negative regulator (RAS signaling)	[34]
Sarcomere genes		
MYH7	Thick filament	[35, 36]
TNNI3	Thin filament	[37]

23.3.2 Single Gene Defects in Non-syndromic VSD

Mutations have been reported in transcription factors, signaling molecules, and sarcomeric proteins in patients and families with non-syndromic VSD (see Table 23.4).

23.3.2.1 Transcription Factors

The evolutionary highly conserved homeobox factor NKX2-5 controls the expression of various cardiac genes during heart development [26]. Frameshift and missense mutations in the DNA-binding domain of *NKX2-5* have been described in six families mainly affected by ASD and atrioventricular conduction block, but also showing other CHDs [29–32]. In total, 11 affected mutation carriers from those families showed additional VSDs [29–32]. Moreover, screening of 135 sporadic CHD cases revealed an *NKX2-5* missense mutation (p.Pro283Gln) in a patient characterized by VSD, ASD, and PDA [12].

Direct interaction partners of NK2 homeobox 5 include the cardiac TFs T-box 5 and GATA binding protein 4. In a large pedigree with familial ASD, Garg et al. described a *GATA4* missense mutation (p.Gly296Ser) located between the nuclear localization sequence and one of two *GATA4* zinc fingers, which altered the interaction between *GATA4* and *TBX5* [28]. Three affected mutation carriers in that family presented with an additional VSD [28]. Furthermore, sequencing of *GATA4* in two VSD cohorts revealed two missense mutations (p.Arg43Trp and p.Gly296Arg) in two families [15, 16]. Besides isolated VSDs, affected family members presented VSDs in combination with ASD in three cases and with PDA in one case (see also Sect. 23.2.2.1) [15, 16].

Additional cardiac TF genes mutated in patients with non-syndromic VSD comprise *TBX1*, *ZIC3* (Zic family member 3), and *PITX2*. A nonsense mutation in *TBX1* (p.Gln277X) has been found in a family including relatives with isolated VSD as well as one individual with VSD and PDA (see Sect. 23.2.2.1) [19]. *ZIC3*, a zinc finger TF known for its association with laterality defects (see Chap. 38), was mutated in one sporadic heterotaxy case showing VSD in combination with ASD, pulmonary stenosis, and TGA [33]. Wei et al. identified two subjects with VSD and TGA from two affected families who carried *PITX2* missense mutations (see also Sect. 23.2.2.1) [18].

23.3.2.2 Signaling Molecules

As described before, various signaling pathways are active during cardiac development such as the NODAL signaling pathway (see Sect. 23.2.2.2). In a cohort of 362 severe CHD cases, the *NF1* gene encoding neurofibromin 1, a negative regulator of the RAS signaling pathway, was found to be mutated in one case with VSD accompanied by pulmonary atresia and multiple aorticopulmonary collaterals [34].

23.3.2.3 Sarcomere Genes

Contraction of the heart involves the shortening of sarcomeres by the ATP-dependent interaction between thin (actin) and thick (myosin) filaments (see Chap. 17). Mutations in genes encoding sarcomeric proteins have been well established as disease-causing for different forms of cardiomyopathy (see Chap. 59). For *MYH7* encoding cardiac specific β-myosin heavy chain, two mutations (p.Met362Arg and p.Glu1220del, respectively) in two families characterized by Ebstein's anomaly (EA), left ventricular noncompaction cardiomyopathy (LVNC), and VSD were reported [35, 36]. Two mutation carriers from each family showed the phenotype with the combination of EA, LVNC, and VSD [35, 36]. Troponin I (encoded by *TNNI3*) is a cardiac specific thin filament component important for calcium sensing during contraction of the heart muscle. Yang et al. detected a *de novo* missense mutation in *TNNI3* (p.Arg204His) in a patient first diagnosed with perimembranous VSD who then gradually developed restrictive cardiomyopathy [37].

23.4 Syndromic VSD

In the following section, we describe VSDs observed in patients showing additional congenital malformations in other organs. Those so-called syndromic forms include well-defined syndromes with known genetic cause (e.g., DiGeorge syndrome) as

well as so far undefined syndromic forms with unspecific symptoms such as mental retardation and dysmorphic features of unknown genetic etiology.

23.4.1 Aneuploidy Syndromes

Chromosomal aneuploidy is the presence of an abnormal number of chromosomes in the cell leading to various syndromic genetic disorders. Aneuploidy syndromes can occur with almost any cardiac malformation [38]. CHD occurs in about 45–50 % of patients with Down syndrome (trisomy 21), the most common aneuploidy syndrome [39, 40]. Källen et al. showed in a large epidemiologic study with more than 5000 patients with trisomy 21 that VSD was present in 28 % of individuals with cardiac malformation [41]. In the National Down Syndrome Project cohort, Freeman et al. found a VSD rate of 19 % regarding all registered infants (65 % were membranous and 35 % were muscular VSDs) [40]. A retrospective cohort study including about 4300 Down syndrome patients undergoing CHD surgery was performed by Fudge et al. to examine postoperative outcomes [42]. VSD closure of any type was the second most common procedure (19 %) performed for patients with Down syndrome [42].

Further, CHD occurs in about 35 % of cases of Patau syndrome (trisomy 13) and about 45 % of cases with Edwards syndrome (trisomy 18) as Pont et al. described in a large epidemiologic study of hospitalizations of live-born infants with chromosomal abnormalities [43]. VSD was the most common heart defect in trisomy 13 (18 %) and in trisomy 18 (31 %) [43]. Pallister–Killian syndrome, a sporadic multisystem developmental disorder, is typically caused by the presence of a supernumerary isochromosome composed of the short arms of chromosome 12 resulting in tetrasomy 12p [44]. Tilton et al. evaluated 30 patients with this syndrome and CHD and found a VSD in 10 % of those [44].

23.4.2 Copy Number Variation in Syndromic VSD

A number of studies have shown the importance of CNVs, mainly microdeletions and microduplications, in syndromic CHD [38, 45]. Those studies reported subjects with well-known syndromes as well as so far undefined syndromic forms.

In Table 23.5, clinically delineated microdeletion and microduplication syndromes are listed in which a specific association to VSD was described. Of note, the most frequent genomic disorder associated with CHD is DiGeorge syndrome (DGS; 22q11 deletion or velocardiofacial syndrome) (see, e.g., Chap. 38). Cardiovascular anomalies are present in about 80 % of neonates with DGS [54]. Ryan et al. evaluated a cohort of 545 DGS patients and could show that VSDs were observed in 14 % of these patients (among other heart defects) [55]. Momma described in his review of several studies of DGS cohorts (ranging from 100 up to 222 patients) quite similar VSD rates [54]. All other syndromes show lower VSD rates. For example, in Williams–Beuren syndrome (caused by a deletion of about 1.5 megabases in chromosome 7q11.23), VSDs, mainly muscular ones (75 %), are present in 4–9 % of all

Table 23.5 Copy number variation in well-defined syndromic VSD

Cytoband	Copy number	Syndrome	Candidate genes[a]	References
4p16.3	Deletion	Wolf–Hirschhorn	*WHSC1, FGFRL1*	[46]
5p15.2	Deletion	Cri-du-chat	*TERT*	[47]
5q35.2-q35.3	Deletion	Sotos	*NSD1*	[48]
7q11.23	Deletion	Williams–Beuren	*ELN*	[49]
9q34.3	Deletion	Kleefstra	*EHMT1*	[50]
11q23	Deletion	Jacobsen	not specified	[51]
17p11.2	Deletion	Smith–Magenis	*RAI1*	[52]
17p11.2	Duplication	Potocki–Lupski	*MAPK7*	[53]
22q11.2	Deletion	DiGeorge	*TBX1*	[54, 55]

Abbreviations: *WHSC1* Wolf–Hirschhorn syndrome candidate 1, *FGFRL1* fibroblast growth factor receptor-like 1, *TERT* telomerase reverse transcriptase, *NSD1* nuclear receptor binding SET domain protein 1, *ELN* elastin, *EHMT1* euchromatic histone-lysine *N*-methyltransferase 1, *RAI1* retinoic acid induced 1, *MAPK7* mitogen-activated protein kinase 7, *TBX1* T-box 1

[a]These include known disease genes (such as TBX1 and ELN) as well as genes causing heart defects when deleted in mice and/or by mutations in CHD patients

patients [49]. A similar VSD rate (8 %) was described by Jefferies et al. in patients with Potocki–Lupski syndrome whereby most individuals harbor a common 3.7 megabase duplication within chromosome 17q11.2 [53].

Besides well-known syndromes, there are a number of studies describing syndromic patients with VSD who present different unspecific symptoms (see Table 23.6). Using array-based CGH, Syrmou et al. screened a cohort of 55 syndromic CHD patients and detected CNVs in 37 of them [57]. They found five patients with VSD showing either one CNV or a combination of up to three different deletions and duplications (ranging from 0.023 to 6.6 megabases) [57]. Through a genome-wide survey of two independent cohorts of CHD subjects with extracardiac abnormalities (700 subjects in total), Lalani et al. identified 16 CNV regions, present in two or more cases and absent in about 3000 controls [60]. Interestingly, one of the most frequent CNVs they found was a *de novo* copy number loss of 16q24.3 (affecting *ANKRD11* encoding ankyrin repeat domain 11) in five subjects of whom four presented with VSD together with other CHDs (two with perimembranous and one each with muscular and conoventricular VSD) [60]. Screening a cohort of 60 syndromic CHD cases by array CGH, Thienpont et al. identified among others one patient with muscular VSD and various extracardiac manifestations showing a 3.8 megabase duplication at locus 19p13.12-13.11 [61]. Using the same detection method, a similar cohort of 90 patients was analyzed by Breckpot et al. [56]. They found two deletions at locus 1p36.33 (ranging from 3.5 to 5.9 megabases) in two patients with VSD and minor extracardiac malformations, one subject with additional hypertrophic cardiomyopathy and one with microcephaly [56]. Goldmuntz et al. analyzed 58 syndromic CHD cases and reported six different CNVs in six patients characterized by VSD and further congenital abnormalities and dysmorphic features such as cleft palate [59]. They detected copy number gains at loci 5q21.1-21.2 and 18p11.32, whereas losses were detected for the loci 9p23,

Table 23.6 Copy number variation in syndromic VSD with unspecific symptoms

Cytoband	CNV start (hg18)	CNV end (hg18)	Size	Copy number	Candidate genes	References
1p36.33	1 kb	4608–5866 kb	4.61–5.87 Mb	Deletion		[56]
1p36.33	1 kb	3514–3519 kb	3.51–3.52 Mb	Deletion		[56]
1q41 Xp11.3 Xq21.31			0.37 Mb 0.042 Mb 0.118 Mb	Duplication Deletion Deletion	*DISP1*	[57]
3p24.1-23	29,757 kb		1488 kb	Deletion		[58]
3q13.32 10q26.3 16p12.1			0.074 Mb 0.043 Mb 0.023 Mb	Duplication Deletion Duplication		[57]
4q34.1	185603346[b]	185638397[b]	35.1 kb	Deletion		[7]
5q21.1-21.2	100934417[a]	103557609[a]	2.6 Mb	Duplication		[59]
6p24.1-22.3			6.64 Mb	Deletion	*EDN1, DTNBP1, MYLIP*	[57]
6q24.2-25.1	143649–143708 kb	150253–150271 kb	6.62 Mb	Deletion		[56]
9p23	9163303[a]	9606645[a]	0.44 Mb	Deletion		[59]
10p12.1-11.21	28728683[a]	35905297[a]	7.1 Mb	Deletion	*NRP1*	[59]
15q26.1	86439405[a]	90753814[a]	4.3 Mb	Deletion	*NTRK3, MESP1*	[59]
16q24.3	86406011–86406037	87962518–87962533	1.58 Mb	Deletion	*ANKRD11*	[60]
16q24.3	86051611	88133224	2.07 Mb	Deletion	*ANKRD11*	[60]
16q24.3	87822867–87862929	88001859–88011936	139 kb	Deletion	*ANKRD11*	[60]
16q24.3			1.8 Mb	Deletion	*ANKRD11*	[60]
18p11.32	284494[a]	918189[a]	0.64 Mb	Duplication		[59]
19p13.12-13.11			3.86 Mb	Duplication		[61]
20p12.2 21q21.3			0.219 Mb 0.095 Mb	Duplication Duplication	*ADAMTS, ADAMTS5*	[57]
22q11.21	18351387[a]	20306802[a]	1.96 Mb	Deletion		[59]

Each row refers to one case
hg18 human reference genome (full sequence) version 18, *kb* kilobases, *Mb* megabases
[a]Genomic coordinates refer to hg17
[b]Genomic coordinates refer to hg19

10p12.1-11.21, 15q26.1, and 22q11.21 (ranging from 0.4 to 7.1 megabases in length) [59]. Analyzing several hundred CHD trios (non-syndromic and syndromic cases) by SNP arrays and whole exome sequencing, a paternally inherited deletion of 35 kilobases at chromosome 4q34.1 was detected in a patient with VSD, TGA, and extracardiac manifestations [7]. Fakhro et al. genotyped a cohort of more than

250 heterotaxy cases by SNP arrays: they identified a heterozygous 1.5 megabase deletion at locus 3p24.1-23 (affecting *TGFBR2* encoding TGFβ receptor II) in a patient characterized by VSD, ASD, partial anomalous pulmonary venous return, and situs inversus (see Chap. 38) [58].

23.4.3 Single Gene Defects in Syndromic VSD

In addition to structural genomic variations, syndromic CHD can be caused by point mutations influencing the dosage of genes functioning in developmental pathways that are broadly used in organogenesis and therefore affecting many organs. As patients covered in Sect. 23.4.2, the cases presented in the following section are also characterized by a variety of extracardiac manifestations. All described single gene syndromes are summarized in Table 23.7.

23.4.3.1 Transcription Factors

Mutations in the transcription factor *TBX5* (T-box 5) cause Holt–Oram syndrome (HOS), which is characterized by upper-extremity malformations and cardiac defects including VSD [106] (see Chap. 20). Septal defects (VSD and ASD) are the most common cardiac malformations observed in HOS as Al-Qattan et al. showed in their meta-analysis of 16 different studies [106]. In total, 13 different TBX5 mutations (mostly missense ones) were associated with VSDs as part of complex cardiac malformations [106]. Borozdin et al. described VSDs in five out of 23 HOS patients with *TBX5* mutations of which four were accompanied by ASD [75]. Another T-box gene, *TBX3*, is causative to ulnar–mammary syndrome characterized by limb malformations and sporadically associated with VSDs [73, 74].

Cardiac malformations, in particular VSDs, are described in about 14 % of Townes–Brocks syndrome patients, which are further characterized by hand and ear abnormalities and are caused by mutations in *SALL1* encoding spalt-like transcription factor, a zinc finger TF [70]. In contrast, mutations of *SALL4* (spalt-like transcription factor 4) cause Okihiro syndrome that is characterized by forearm and renal malformations (also known as Duane-radial ray syndrome) and rarely associated with VSD [71, 72].

VSDs are frequently seen in syndromes such as oculofaciocardiodental (OFCD), Ellis–van Creveld, Mowat–Wilson, and congenital diaphragmatic hernia with CHD. OFCD syndrome is an inherited X-linked dominant disorder caused by mutations in *BCOR* (BCL6 corepressor), a key transcriptional regulator during embryogenesis [62]. Ng et al. were the first to describe three OFCD patients who showed a VSD (one perimembranous, one subpulmonary, and one not further characterized VSD) [62]. In a later study, Hilton et al. presented six OFCD patients with VSD from 21 families showing that VSD was the second most common CHD in these patients [63].

Mutations in *EVC1* and *EVC2* (two leucine zipper transcription factors) lead to Ellis–van Creveld syndrome, which is mainly associated with atrioventricular septal defect and more sporadically with VSDs [64, 65]. Mowat–Wilson syndrome is

Table 23.7 Single gene defects in well-defined syndromic VSD

Gene	Protein function	Syndrome	References
Transcription factors			
BCOR	Transcriptional corepressor	Oculofaciocardiodental (OFCD) (X)	[62, 63]
EVC1, EVC2	Leucine zipper proteins	Ellis–van Creveld	[64, 65]
FOXL2	Forkhead TF	Blepharophimosis (AD)	[66, 67]
GATA6	GATA binding TF	Diaphragmatic hernia	[68, 69]
SALL1	Spalt-like TF	Townes–Brocks	[70]
SALL4	Spalt-like TF	Okihiro	[71, 72]
TBX3	T-box TF	Ulnar–mammary	[73, 74]
TBX5	T-box TF	Holt–Oram	[75]
TFAB2B	AP-2 TF	Char	[76]
ZEB2/ZFHX1B	Zinc finger homeobox TF	Mowat–Wilson	[77]
Signaling molecules			
FGFR3	Fibroblast growth factor	Muenke	[78]
HRAS	Ras/MAPK signaling	Costello	[79]
JAG1, NOTCH2	Notch ligand, Notch signaling	Alagille	[80]
KRAS	Ras/MAPK signaling	Noonan 3	[81]
NOTCH1	Notch signaling	Adams–Oliver 5	[82]
PTPN11	Protein tyrosine phosphatase	Noonan 1	[83, 84]
ROR2	Receptor protein tyrosine kinase	Robinow (AR)	[85]
SHOC2	Ras/MAPK signaling	Noonan-like	[86]
SOS1	Ras/MAPK signaling	Noonan 4	[87]
WNT5A, DVL1	Wnt signaling	Robinow (AD)	[88, 89]
Chromatin regulators			
CHD7	DNA helicase	CHARGE	[90]
MLL2	Methyltransferase	Kabuki	[91, 92]
NIPBL	Sister chromatid cohesin	Cornelia de Lange 1	[93, 94]
NSD1	Methyltransferase	Sotos (AD)	[95]
LBR	Chromatin binding	Pelger–Huet	[96]
Structural and cell adhesion molecules			
FBN1	Fibrillin	Marfan with hypophosphatemia (X)	[97]
GPC3	Proteoglycan	Simpson–Golabi–Behmel (X)	[98]
SH3PXD2B	Adapter protein	Frank–ter Haar (AR)	[99]
Enzymes for posttranslational modification			
B3GALTL	Glycosyltransferase	Peters plus	[100–102]
B3GAT3	Glucuronyltransferase	Larsen-like	[103]

(continued)

Table 23.7 (continued)

Gene	Protein function	Syndrome	References
Other			
DNAI1, DNAH5	Ciliary dynein arm proteins	Kartagener	[104]
MID1	RING finger ubiquitin ligase	Opitz (X)	[105]
MKRN2	E3 ubiquitin ligase	Heterotaxy	[34]

AD autosomal recessive, *AR* autosomal recessive, *X* X linked, *TF* transcription factor

caused by mutations in *ZEB2 (ZFHX1B)* encoding a zinc finger E-box binding factor and is frequently accompanied by cardiac malformations including VSD and ASD [77]. Mutations in *GATA6* have been found in familial cases of congenital diaphragmatic hernia accompanied by VSD [68, 69].

Char and blepharophimosis syndrome infrequently present with VSD. Char syndrome is caused by mutations in the gene *TFAP2B* (transcription factor AP-2 beta) and associates with patent ductus arteriosus (PDA) [76]. However, in one case Char syndrome was accompanied by VSD [76]. Blepharophimosis syndrome is an autosomal dominant disorder that is characterized by a malformation of the eyelids and is caused by loss-of-function mutations in the forkhead TF *FOXL2* (forkhead box L2) [66, 67]. VSDs are rare (about 1 %) in patients with this syndrome [66, 67].

23.4.3.2 Signaling Molecules

Inter- and intracellular communication via signaling molecules coordinates heart development, as has been shown, for example, for the Notch signaling pathway (see Chap. 11). Alagille syndrome, an autosomal dominant multisystem disorder, is associated with CHD in about 25 % of patients [80]. This syndrome is caused by mutations in either *JAG1* or *NOTCH2*, with Jagged 1 being a ligand of the membranous Notch 2 receptor. Emerick et al. showed in a cohort of 73 Alagille syndrome patients a frequent association with cardiac malformation of which 32 % showed VSDs [80]. A further NOTCH signaling driven syndrome is Adams–Oliver syndrome characterized by limb defects and less frequent cardiac malformations including VSDs [82]. A study based on 11 familiar cases identified truncating mutations in *NOTCH1* of which three cases showed VSDs [82].

Mutations of genes of the Ras/MAPK (Ras/mitogen-activated protein kinase) signaling pathway represent a frequent cause of Noonan syndrome, which is after Down syndrome the most common syndromic disorder involving cardiac malformations [83]. Noonan syndrome is genetically heterogenous and characterized by short stature, facial dysmorphism, and cardiac defects of different nature such as pulmonary stenosis, hypertrophic cardiomyopathy, ASD, and at a lower rate VSDs. *PTPN11* (protein tyrosine phosphatase non-receptor type 11) causing Noonan syndrome 1 was described being mutated in about 50 % of cases [83]. Snayer et al. reported an incidence of 7 % of VSDs in Noonan syndrome 1 patients [84]. Single case reports showed VSDs in the less frequent Noonan syndrome 3 caused by mutations in KRAS (Kirsten rat sarcoma viral oncogene homolog) [87] and Noonan

syndrome 4 caused by mutations in SOS1 (son of sevenless homolog 1) [81]. In 25 patients with a phenotype termed Noonan-like syndrome with anagen hair, the missense mutation p.Ser2Gly in *SHOC2* (Soc-2 suppressor of clear homolog), a leucine-rich repeat-containing protein, was detected by Cordeddu et al. [86]. While the majority of patients have cardiac malformations, VSDs were found only in two cases [86]. Mutations in another member of the Ras/MAPK pathway, *HRAS* (Harvey rat sarcoma viral oncogene homolog), cause Costello syndrome, a disorder phenotypically closely related to Noonan syndrome. For Costello syndrome, one case report with VSD has been published [79].

Robinow syndrome is a rare skeletal dysplasia, inherited either in an autosomal dominant or recessive manner. Autosomal dominant Robinow syndrome, either caused by pathogenic variants in *WNT5A* (wingless-type MMTV integration site family member 5A) or *DVL1* (dishevelled segment polarity protein 1), is frequently accompanied by VSDs [88, 89]. In contrast, VSDs are rarely present in autosomal recessive Robinow syndrome, caused by biallelic pathogenic variants in *ROR2* (receptor tyrosine kinase-like orphan receptor 2) [85]. A further rare case with VSD has been documented for Muenke syndrome, which is characterized by fusion of cranial bones and is caused by mutations of *FGFR3* encoding fibroblast growth factor receptor 3 [78].

23.4.3.3 Chromatin Regulators

In syndromes related to chromatin regulators, the VSD rates greatly vary ranging from about 40 % in Cornelia de Lange syndrome to about 4 % in Sotos syndrome. Cornelia de Lange syndrome 1 is caused by mutations in *NIPBL* encoding Nipped-B homolog with putative sister chromatid cohesion function [93, 94]. From a cohort of 24 typical Sotos cases, Cecconi et al. observed one individual with VSD [95]. Sotos syndrome is caused by haploinsufficiency of the *NSD1* gene encoding a histone methyltransferase [95]. Microdeletions involving this gene are the major cause of the syndrome in Japanese patients (see Sect. 23.3) [48], whereas intragenic mutations are more frequent in non-Japanese patients [95].

About two-thirds of patients with CHARGE syndrome show cardiac malformations including 12 % with VSDs [90]. Genetically CHARGE syndrome is caused by mutations in *CHD7* encoding a chromodomain helicase DNA binding protein. A far less frequent association of VSDs is given in Pelger–Huet anomaly, where one case was described in a cohort of 20 families harboring mutations in *LBR* encoding for the lamin B receptor, which mediates the interaction of chromatin and lamin B [96].

Finally, mutations in *MLL2* encoding a DNA methyltransferase have been found in cases of Kabuki syndrome [91]. Half of these patients present with VSDs often accompanied by an ASD. In a meta-analysis, Yuan examined the cardiac phenotype of 76 published Kabuki cases and showed that about 20 % presented a VSD either isolated or as part of a complex cardiac malformation [92].

23.4.3.4 Structural and Cell Molecules

The integrity of the cell is maintained by structural proteins while cell adhesion factors are crucial for cell–cell contacts in a respective tissue. There are three different syndromes associated with VSD caused by mutations in structural and cell adhesion proteins.

Mutations in *SH3PXD2B* (SH3 and PX domains 2B, involved in cell adhesion and migration of numerous cell types) cause Frank–ter Haar syndrome, an autosomal recessive skeletal dysplasia characterized among others by cardiovascular malformations [99]. VSD is commonly seen in patients with this syndrome (in 50 % of mutation carriers) as Iqbal et al. showed in their study [99]. Lin et al. reviewed 26 patients with genetically confirmed Simpson–Golabi–Behmel syndrome, a disorder characterized by high birth weight and length, which is caused by mutations in *GPC3* encoding glypican 3, a cell surface proteoglycan [98]. A VSD in association with other CHDs was present in five of them (19 %) [98]. One case with Marfan syndrome (with the typical cardiovascular feature of aortic aneurysm) combined with X-linked hypophosphatemia showing VSD and ASD was described by Sheng et al. [97]. They found a *de novo* missense mutation in *FBN1* encoding fibrillin 1, a large extracellular matrix glycoprotein, using whole exome sequencing [97].

23.4.3.5 Enzymes for Posttranslational Modification

Enzymes involved in posttranslational modifications of proteins such as glycosylation play a role in two syndromes associated with VSDs. Peters plus syndrome (named after the Peters anomaly, an eye-chamber defect) is caused by mutations in *B3GALTL* encoding a glycosyltransferase involved in addition of glycans to proteins and is associated with VSDs in about one-third of cases [100–102]. The rare Larsen-like syndrome is caused by mutations in *B3GAT3* coding for glucuronyltransferase-I and is characterized by joint dislocations, short stature, craniofacial dysmorphism, and heart defects including VSDs [103].

23.4.3.6 Other Genes

Defects in ciliary structure cause Kartagener syndrome that is characterized by primary ciliary dyskinesia leading to situs inversus, the mirror image arrangement of all internal organs. It is caused by mutations in a number of genes encoding dynein arm components (see more details in Chap. 38). Kennedy et al. examined 21 Kartagener patients and found VSDs among other CHDs in three individuals (14 %) [104]. Zaidi et al. searched for *de novo* mutations in 362 severe CHD cases [34]. In one heterotaxy patient with abdominal situs inversus and CHD including VSD, they identified a missense mutation (p.Ala251Val) in *MKRN2* (encoding makorin ring finger protein 2, a putative E3 ubiquitin ligase) [34]. The gene implicated in the X-linked form of Opitz syndrome, *MID1* (midline 1), encodes a RING finger protein and is involved in the formation of multiprotein structures acting as anchor points to microtubules [105]. In a meta-analysis of several studies, Fontanella et al. showed that about 22 % of cases have heart defects with a high incidence of VSDs and ASDs [105].

23.5 Associations with Common Variations

Genome-wide associations studies (GWAS) look for associations between DNA sequence variants and phenotypes of interest [107]. They do so by studying many hundreds of individuals (affecteds and non-affecteds) and determining their

genotype at the positions of hundreds of thousands of single nucleotide polymorphisms (SNPs) followed by statistical analysis and subsequent confirmation experiments in replication cohorts [107].

A small case–control association study genotyped 58 SNPs in the TBX5 region in 192 VSD cases and matched controls of Han Chinese origin and identified a significant association of SNP rs11067075 within intron eight of the TBX5 gene with VSD [108]. Hu et al. performed a large GWAS in 945 cases with septation defects (ASD, VSD, and ASD/VSD) and 1246 controls of Han Chinese ancestry followed by two-stage validation with further 2160 cases and 3866 controls [109]. They identified a highly significant association of two SNPs at chromosome 1p12 (SNP rs2474937 near *TBX15*) and 4q31.1 (SNP rs1531070 near *MAML3* encoding mastermind like three involved in Notch signaling) [109]. A GWAS with 1995 CHD cases (including VSD) and 5159 controls of European Caucasian origin was carried out by Cordell et al. and failed to identify any SNPs with genome-wide significance [110]. A further replication study focusing on a subgroup analysis of about 200 VSD cases also did not find any significant association [110].

Conclusion

VSDs, one of the most common cardiac malformations in the general population, are commonly associated with a high number of well-known genetic syndromes caused by chromosomal aberrations or by smaller deletions and duplications as well as by single point mutations. Moreover, so far undefined syndromic disorders are often accompanied by VSDs.

As shown by a number of studies, mutations in genes encoding cardiac transcription factors (e.g., *NKX2-5*, *GATA4*, and *TBX5*) and signaling molecules (e.g., *CFC1*) have been most frequently found in VSD cases and families. This holds true for isolated VSD as well as for syndromic and non-syndromic forms. Of note, there is a great overlap of genes associated with other CHDs such as ASD (see Chap. 20), atrioventricular septal defect (see Chap. 26), tetralogy of Fallot, and double outlet right ventricle (see Chap. 32), as well as situs defects (see Chap. 38).

Over the last decade, tremendous effort has been made in the area of genetic technologies such as calling of CNVs and next-generation sequencing of whole exomes and genomes [111]. As a consequence, we better understand the genetic basis of VSDs and other CHDs. However, future studies are still required to unravel the effects of altered gene dosage or loss of function on formation of VSD. In addition, the interpretation of complex patterns of inheritance and phenotypic heterogeneity remains a difficult obstacle in individual VSD cases and families [45]. Nevertheless, these new technologies hold the potential to improve patient care. Especially in VSD cases with a growing number of surviving adults after successful cardiac surgery or intervention, the urgency for a better characterization of the CHD-related morbidities and mortality occurring late after surgery is evident [112]. Recently, Menting et al. reported on the outcomes in about hundred late survivors up to 40 years after VSD closure and demonstrated the ongoing clinical problems in this patient cohort such as arrhythmias and heart

failure [113]. In fact, genetics may open new ways for identifying novel risk-stratifying factors in those patients and offer them earlier and better-tailored treatment.

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