Atrioventricular canal defect and genetic syndromes: The unifying role of Sonic Hedgehog

M.C. Digilio,[1] F. Pugnaloni,[2] A. De Luca,[3] G. Calcagni,[1] A. Baban,[1] M.L. Dentici,[1] P. Versacci,[2] B. Dallapiccola,[1] M. Tartaglia,[1] B. Marino[2]

[1] Medical Genetics, Pediatric Cardiology, Genetics and Rare Diseases Research Division, Bambino Gesù Pediatric Hospital, Rome, Italy

[2] Department of Pediatrics, Sapienza University, Rome, Italy

[3] Laboratory of Molecular Biology, CSS-Mendel Institute, Rome, Italy

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Corresponding author: M.Cristina Digilio, MD, Medical Genetics, Bambino Gesù Paediatric Hospital, IRCCS, Piazza S. Onofrio 4, 00165 Rome, Italy. Tel: +39-06-68592743; E-mail: digilio@opbg.net

The atrioventricular canal defect (AVCD) is a congenital heart defect (CHD) frequently associated with

extracardiac anomalies (75%). Previous observations from a personal series of patients with AVCD and "polydactyly syndromes" demonstrated that the distinct morphology and combination of AVCD features in some of these syndromes is reminiscent of the cardiac phenotype found in heterotaxy, a malformation complex previously associated with functional cilia abnormalities and aberrant Hedgehog (Hh) signaling. Hh signaling coordinates multiple aspects of left-right lateralization and cardiovascular growth. Being active at the venous pole of the Secondary Heart Field (SHF), is essential for normal development of dorsal-mesenchymal protrusion and AVCD formation and septation. Experimental data show that perturbations of different components of the Hh pathway can lead to developmental errors presenting with partially overlapping manifestations and AVCD as a common denominator. We review the potential role of Hh signaling in the pathogenesis of AVCD in different genetic disorders. AVCD can be viewed as part of a "developmental field", according to the concept that malformations can be due to defects in signal transduction cascades or pathways, as morphogenetic units which may be altereted by Mendelian mutations, aneuploidies, environmental causes.

Key words: atrioventricular septal defect – heterotaxy - sonic hedgehog – cilium - secondary heart field - syndrome

1 INTRODUCTION

This article reviews the potential role of Hedgehog signaling in the pathogenesis of atrioventricular

canal defect (AVCD) in different genetic disorders, based on recent experimental findings which show that dorsal mesocardial protrusion (DMP) development requires cilia-based Hedgehog (Hh) signaling, and that cilia are structurally present in the secondary heart field (SHF).[1,2] Previous observations from a personal series of patients with AVCD and "polydactyly syndromes" demonstrated that the distinct morphology and combination of AVCD features in some of these syndromes is reminiscent of the cardiac phenotype found in heterotaxy, a malformation complex previously associated with functional cilia abnormalities and aberrant Hedgehog (Hh) signaling. Recent experimental data provide new insights into the relationship between Hh signaling and AVCD in other genetic conditions, including Noonan and Down syndromes.

2.1 Atrioventricular canal defect

AVCD, also known as endocardial cushion defect or atrioventricular septal defect, is a common congenital heart defect (CHD), affecting 3.5/10.000 live births, and accounting for 7.4% of all CHDs.[3] AVCD is characterized by a spectrum of anomalies of the atrioventricular valves, and atrial and ventricular septa. In the complete form, a single common atrioventricular valve occurs with an atrial septal defect (ostium primum), and a confluent posterior ventricular septal defect. In the partial form, two separate right and left atrioventricular valves are found with a *cleft* of the mitral valve, an atrial septal defect (ostium primum), and no ventricular septal communication.[4]

From an embryological point of view, AVCD, with absent or incomplete fusion of ventral (antero-superior) and dorsal (postero-inferior) atrioventricular cushions, was traditionally considered caused by a primary intracardiac mechanism consisting in the maldevelopment of endocardial cushions in relation to defects of extracellular matrix.[5-7] Some recent studies, however, have shown that perturbation of extracardiac tissue, known as DMP, represents a major determinant of AVCD.[8-10] This population of extracardiac mesenchymal cells was reported in 1880 by Wilhelm His as *spina vestibuli.*

These cells arise from the posterior segment of the SHF in the splanchnic mesoderm, grow towards the atrial surface of the primitive AVCD, in particular, towards the inferior dorsal endocardial cushion, to close the primary atrial foramen and form the AV junction.[11,12]

AVCD is a CHD frequently associated with extracardiac anomalies (75%),[3] familiar as the distinctive CHD in Down syndrome.[13] Indeed, in large series of individuals with AVCD, extracardiac anomalies were present in about 75% of cases,[3,14] of whom, 45% had Down syndrome. Many patients with AVCD occur in syndromes of known etiologies (chromosome, gene). The second specific association is heterotaxy, occurring in 15% of patients with AVCD. Heterotaxy is also known as Ivemark syndrome or atrial isomerism or situs ambiguous with splenic anomalies.[4] The 15% of the cases of AVCD are affected by other different genetic syndromes. Non-syndromic conditions are observed in the remaining 25% of AVCD individuals.[14] The morphology and combination of CHDs in several syndromes with AVCD as reported in the 1990s in personal observations is similar to what seen in heterotaxy.[15-19]

2.2 Ciliary function and the Hedgehog signaling pathway

The cilium is a multifunctional organelle which projects from the cell surface of many different cells. Cilia are conserved in a wide variety of eukaryotic species throughout evolution. Studies in the last 15 years have shown that dysfunctional cilia can lead to several human genetic diseases with overlapping phenotypes, the so called "ciliopathies".[20,21] The affected tissues are as diverse as the different cell types that carry cilia. The ciliary membranes harbor receptors for crucial signaling cascades, including Hh signaling.[22,23] Disruption of cilium function in a single tissue may result in a tissue-restricted phenotype, while disruption of cilium function at early embryonic stages, and perturbation of proper Hh signaling, is generally associated to more severe disorders, and may not be compatible with embryonic development.

Molecular advances have shown that several disease genes implicated in syndromes with AVCD encode proteins that participate in the ciliary function, and that dysfunction of the nodal cilium results in left-right axis defects in vertebrates.[24,25] Ciliary dysfunction and abnormal localization and/or function of proteins participating in Hh signaling have been recognized in syndromes with AVCD, which is not surprising since the relevant role of Hh signaling in coordinating multiple aspects of left-right lateralization and cardiovascular growth. In addition, Sonic Hedgehog knock-out mice show CHDs in the setting of heterotaxy and left pulmonary isomerism.[26-28]

Genetic defects of ciliary function are known as ciliopathies, and include short rib-polydactyly, Joubert, Oral-Facial.-Digital, Bardet-Biedl, and Meckel syndromes. Hh signaling is an essential developmental pathway coordinated at the primary cilium.[29] In vertebrates, three Hh signaling molecules, Sonic hedgehog (SHH), Indian hedgehog (IHH), and Desert hedgehog (DHH). Their effects are mediated by three different transcription factors, glioma-associated oncogenes 1 to 3 (GLI1-3). The binding of Hh molecules to their receptors, Patched 1 and 2 (PTCH1-2), results in the internalization and lysosomal degradation of the PTCH1/SHH complexes and in the release of the inhibition on smoothened (SMOH). SMOH translocates to cilia and functions as the activator of the pathway promoting transcriptional activation of the GLI proteins.[30-33]

Experimental studies performed in mice have shown that cilia are required for both left/right body axis determination and SHF Hh signaling.[2] Dnah11 mutants, affecting ciliary motility, do not disrupt SHF Hh signaling, but cause AVCD, concurrently with heterotaxy. Differently, Mks1 mutants create dysfunction in the structure of primary cilium, disrupt SHF Hh signaling, and cause AVCD without heterotaxy. In summary, normal structure of primary cilia is required for SHF Hh signaling: both cilia structure and motility can generate distinct cardiac phenotypes.

It has been found that perturbations of the different components of SHH pathway are associated with different developmental errors in patients manifesting partially overlapping features.[19,34] In the

following paragraphs, we will review the role of Hh pathway and ciliary dysfunction in the development of AVCD in some genetic disorders.

2.3 Heterotaxy

Heterotaxy is characterized by the combination of the abnormal arrangement of the abdominal and thoracic organs, usually with complex CHDs, including AVCD, common atrium, anomalous systemic and pulmonary venous drainage, persistent left superior vena cava with unroofed coronary sinus, and conotruncal defects.[4,35,36] Heterotaxy includes patients with situs inversus and situs ambigous with "asplenia" (right isomerism phenotype) and "polysplenia" (left isomerism phenotype). Patients with asplenia or polysplenia may be associated with cardiac and extracardiac malformations different from each other.[37-43] Patients with the "asplenia phenotype" (right isomerism of lungs and atrial appendages) display right pulmonary and right atrial appendage isomerism and more severe cardiac defects,[37,39] such as total anomalous pulmonary venous drainage,[38,40,44] complete AVCD,[38,40,45] and pulmonary stenosis or atresia.[38,40,46] In contrast, the "polysplenia phenotype" is characterized by left isomerism of lungs and atrial appendages, in general with less severe CHD,[38,40] such as partial AVCD, interruption of the inferior vena cava,[37,38,40,41,44] and systemic outflow tract obstruction,[47-50] which are rare in asplenia.[51]

2.4 Ciliopathies with postaxial polydactyly

This group of disorders includes several genetically heterogeneous conditions sharing AVCD and postaxial polydactyly. AVCD, particularly in association with common atrium, has been reported in several syndromes with postaxial polydactyly, including Ellis-van Creveld syndrome and other "short rib-polydactlyly" (SRP) disorders, oral-facial-digital syndromes, Bardet-Biedl syndrome, and Smith-Lemli-Opitz syndrome.[15,34] The original clinical observation of cardiac anatomical similarities between patients with polydactyly syndromes and heterotaxy and polysplenia[15] has been corroborated by

experimental studies in transgenic mice. The suggested involvement of the SHH pathway in syndromes with postaxial polydactyly and heterotaxy has been confirmed by the detection of ciliary dysfunction in several disorders with polydactyly. Ciliary dysfunction through abnormal processing of the Hh proteins has been documented in Ellis-van Creveld and other short-rib polydactyly, Smith-Lemli-Opitz, and oral-facial-digital type IV syndromes,[19,52,53] while ciliary function directly is involved in Bardet-Biedl, oral-facial-digital I and VI syndromes.[54-57]

2.4.1 *Ellis-van Creveld syndromes*

The **Ellis-van Creveld syndrome** is a chondroectodermal dysplasia, characterized by skeletal and craniofacial abnormalities associated with polydacyly, dysplastic teeth and nails.[58] Approximately two thirds of the affected individuals also have CHD, more commonly AVCD associated with common atrium and/or systemic and pulmonary venous abnormalities.[14,15,17,18,59-61] AVCD is rarely associated with common atrium in the non-syndromic patients, but relatively common in Ellis-van Creveld syndrome[15] and in heterotaxy.[38]

Ellis-van Creveld syndrome is due to mutations in *EVC* and *EVC2* genes, and both genes are required for normal transcriptional activation of IHH signalling.[52,58] Specifically, *EVC* and *EVC2* act as positive modulators of IHH signaling, located at the proximal end of the primary cilium and expressing normally in the growth plates and chondrocyte cilia.[62] In the growth plates of long bones, IHH is secreted by the prehypertrophic chondrocytes, generating a gradient of signal that coordinates chondrocyte differentiation, chondrocyte proliferation and perichondrial development.[63] The bulk of the Ellis-van Creveld complex localizes to the proximal end of cilia, just above the transition zone, and physically interacts with the main activator of the pathway, SMOH, retaining this protein to that section of the cilium.[64] Experiments in Evc knock-out mice have shown that loss of Evc and Evc2 function affect Ihh signaling, and expression levels of Ihh downstream targets (*Ptch1*, *Gli1*, and *Pthrp*). As a result, the cilia-mediated response to Hh ligands is diminished.[65] Recently, a proportion of patients with

clinical diagnosis of Ellis-van Creveld syndrome has been linked to mutations in *WDR35*, which encodes a retrograde intraflagellar transport (IFT) protein that is required for the recruitment of the EVC-EVC2-SMOH complex to the cilium.[66] Experiments in WDR35$^{-/-}$ fibroblasts have shown that the mutations associated with Ellis-van Creveld syndrome lead to Hh signaling defects similar to what observed in cells with EVC or EVC2 loss of function.[67] In addition, mouse models for the *WDR35* gene mutations result in congenital abnormalities usually associated with defects in the Hh signaling pathway.[67] More recently, biallelic *DYNC2LI1* variants have been identified as a novel molecular event underlying Ellis-van Creveld syndrome in small number of families.[68,69] *DYNC2LI1* codes for a component of the intraflaggelar transport-related dynein-2 complex, a machinery mediating retrograde traffic along the cilium, and whose function is required for cilium assembly and function, including signal transduction, and is functionally related to *WDR35*.

2.4.2 *Oral-Facial-Digital syndromes*

The **oral-facial-digital (OFD) syndromes** include a group of conditions (13 clinical subtypes), in association with malformations of the face, oral cavity, and digits (polysyndatyly).[70] CHD is not common in this group, but AVCD has been detected in patients with OFD syndrome type II[71] and type VI,[72] and common atrium in OFD syndrome type I.[73]

OFD I can be differentiate from other subtypes by X-linked dominant inheritance and mutations of *OFD1* gene, encoding for a centrosomal protein involved in ciliary function.[74] Interestingly, it has been demonstrated that knockout male mouse embryos lacking this gene (*Ofd1*) have failure of left-right axis specification with abnormal cardiac tube retaining a midline position or reversal of the heart loop.[55] Ultrastructural analysis has shown lack of cilia in the embryonic node, supporting a specific role for Ofd1 protein in cilium assembly through basal body dysfunction. OFD type II has been recently linked to the planar cell polarity ciliogenesis *WDPCP* gene,[75] and truncating *TCTN3* mutations, a gene implicated in transduction of SHH signalling, have been found in patients with atypical forms of OFD

type IV.[53]

2.4.3 *Joubert syndrome*

Joubert syndrome is a group of genetically heterogeneous conditions classified among ciliopathies, characterized by multiorgan involvement (retinal, renal, hepatic and skeletal) and the pathognomonic neuroradiological "molar tooth sign". Joubert syndromes can be associated also with CHDs, including left ventricular obstructions, alone or associated with AVCD.[15,76]

2.4.4 *Smith-Lemli-Opitz syndrome*

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive syndrome characterized by developmental delay, growth retardation, microcephaly, distinct facial anomalies, cleft palate, hypospadias, postaxial polydactyly, and CHD.[77] SLOS is due to an inborn error of cholesterol metabolism with deficiency of the 7-dehydrocholesterol-7 reductase (DHCR7) activity resulting in reduced plasma and tissue cholesterol levels and elevated 7-dehydrocholesterol concentrations due to inactivating mutations in the *DHCR7* gene.[78]

CHD occurs in half of the patients with RSH/SLOS.[79] Septal defects and AVCD are the most common CHDs in SLOS, and AVCD is often associated with anomalous pulmonary venous return, the latter is also a cardiac manifestation of heterotaxy with asplenia.[79]

Cholesterol plays a critical role in formation of the normally active hedgehog proteins.[80] Abnormal processing of Hedgehog proteins secondary to abnormal cholesterol levels seems to have a role in the development of SLO syndrome malformations.[19,80]

2.5 Ciliopathies without polydactyly

2.5.1 VACTERL Association

VACTERL is a non-random association of congenital anomalies, including vertebral defects (V), anal atresia (A), esophageal atresia (TE), radial and renal dysplasia (R) and limb anomalies (L).[81] This

spectrum was broadened to include diverse CHDs which are noted in 50-80% of patients, and include septaland and conotruncal defects.[82] In addition, laterality defects have been observed, and include dextrocardia, heterotaxy, AVCD, and transposition of the great arteries.[14,83]

The causal mechanism underlying VACTERL association has not been established. Clinical observations and molecular studies focused on Shh signaling in mice strongly suggest that the VACTERL phenotype observed in humans could be caused by a defective SHH signaling.[84] In fact, Gli mutant mice display a spectrum of defects resembling the human VACTERL association. As anticipated, *GLI* genes encode transcription factors mediating SHH signal transduction. In addition, VACTERL with hydrocephalus (VACTERL-H) has been associated to a hypomorphic mutation of the intraflagellar transport protein 172 (Ift172), in mice.[85] Ift172 is required for ciliogenesis and Hh signaling. Thus, the IFT defect could impair the localization of Hh signaling components to the distal ciliary axoneme. CHDs in avc1 mutant mice include AVCD with common atrium.[85] Some studies have implicated the *FOX* transcription factor gene cluster in VACTERL association.[86] Haploinsufficiency of *Foxf1* gene in mice can be associated with a variable phenotype that includes lung immaturity and hypoplasia, fusion of right lung lobes, narrowing of esophagus and trachea, esophageal atresia and tracheo-esophageal fistula.[87] The above mechanism does involve the Hh signaling pathway, since it has been demonstrated that exogenous Shh activates transcription of *Foxf1* in the developing lung.[87]

2.5.2 Alveolar Capillary Dysplasia with Misaligned Pulmonary Veins

In humans, *FOXF1* gene mutations cause alveolar capillary dysplasia with misaligned pulmonary veins.[88] **Alveolar capillary dysplasia**, usually associated with misalignment of the pulmonary vessels, is a congenital pulmonary vascular abnormality characterized histologically by a lack of formation and ingrowth of alveolar capillaries leading to a failing air–blood barrier. In about 10% of cases, alveolar capillary dysplasia is associated with CHD, in particular partial or complete AVCD and various degrees of left heart obstruction.[89] AVCD in these cases may consist in a small left ventricle with or

without aortic coarctation.

The *FOXF1* gene is activated by Shh signaling and, on a specific genetic background, its haploinsufficiency results in lung and foregut malformations similar to those observed in *Shh* and *Gli* mutants.[87]

2.5.3 Noonan syndrome and related disorders (RASopathies)

The term **RASopathies,** also known as the Noonan Syndrome-spectrum disorder (i.e., LEOPARD syndrome or "Noonan syndrome-Multiple Lentigines", cardio-facio-cutaneous syndrome, Costello syndrome, Mazzanti syndrome and others), are characterized by distinctive facial features, CHD, growth retardation, hematological, ectodermal and skeletal defects, and variable neuropsychologic impairment.[90] CHD occurs in about 65-85% of cases, depending on the mutated genes. AVCD, usually a partial form, may occur in Noonan syndrome and in LEOPARD syndrome/Noonan syndrome-Multiple Lentigines,[91-93] and may be associated with systemic obstructions including subaortic stenosis or aortic coarctation.[91,93] Structural abnormalities causing congenital subaortic stenosis include accessory fibrous tissue and/or anomalous insertion of mitral valve and anomalous papillary muscle of left ventricle.[94]

Noonan syndrome and related disorders are caused by mutations in genes encoding proteins with a role in the RAS/MAP kinase (MAPK), signaling pathway.[90,95] *PTPN11* gene mutations, which underlie Noonan and LEOPARD/Noonan syndrome –Multiple Lentigines syndromes, have been detected in patients with AVCD associated with RASopathies.[93] It has been established that germline mildly activating mutations in *PTPN11* cause Noonan syndrome, whereas more activating mutations in the same gene transmitted as somatic events cause childhood myeloproliferative disease and leukemias.[96]

Strikingly, heterozygous inactivating mutations of *PTPN11* gene have been associated in patients with metachondromatosis, a rare autosomal dominant tumor syndrome characterized by the

predisposition of forming multiple exostoses and enchondromas, joint destruction and bony deformities.[97,98] Of note, chondroid neoplasms result from decreased ERK pathway activation, increased Indian hedgehog (IHH) and parathyroid hormone-related protein (PTHRP), and excessive proliferation. Normal SHP2/PTPN11 function seems to act as IHH suppressor, and experiments in mice have documented decreased IHH levels in Noonan syndrome caused by germline activating mutations in *PTPN11* gene.[99]

2.5.4 Down syndrome

Down syndrome, which is caused in most cases by complete trisomy of 21, is characterized by developmental delay, facial anomalies, and gastrointestinal malformations. CHDs occur in about 40-50% of these patients, typically complete AVCD.[100] Children with Down syndrome show a "simple type" of AVCD, usually complete, rarely associated with other CHDs, with the notable exception of tetralogy of Fallot.[101] In fact, associated CHDs, in particular left-sided obstructions, are significantly more rare in patients with Down syndrome and AVCD in comparison of patients with AVCD and normal chromosomes.[13,49,100,102]

The molecular relationship between AVCD and Down syndrome is still unclear. A number of genes located in the CHD critical region on chromosome 21 have been causally related to AVCD, including *DSCAM*, *COL6A1*, *COL6A2*, and *DSCR1*,[103-104] so as variants in genes mapping on other chromosomes including *CRELD1*, *FBLN2*, *FRZB*, and *GATA5*.[105] Experimental studies, using mouse models of Down syndrome, consisting in crossing loss-of-function alleles of *Creld1* or *Hey2* genes onto the trisomic background, showed a significant increased frequency of CHD, suggesting an interaction between modifiers and trisomic genes.[106]

Some recent observations in mouse models have pointed to a role of the Shh signaling pathway also in Down syndrome. Cerebral, skin, liver and intestine mice trisomic cells have shown a defective

mitogenic Shh activity with cell proliferation impairment due to a higher expression of *Ptch1*, a receptor normally repressing the Shh pathway.[107] This suggests that PTCH-dependent inhibition of SHH signaling may underlie proliferation impairment in trisomic peripheral tissues leading to defective neuronal production in the Down syndrome brain.[108] In addition, subcutaneous administration of the Shh pathway agonist SAG to trisomic Ts65Dn mice at birth resulted in an increased proliferation of granule cell precusors in the cerebellum.[109]

2.5.5 Non-syndromic AVCD

Non-syndromic AVCD can be a sporadic or display familial clustering. In this latter case, familial recurrence follows an autosomal dominant pattern of inheritance. Little is known about molecular basis of non-syndromic AVCD. The first chromosomal locus for isolated ACVD was assigned to chromosome 1p31-p21, and a second locus was mapped within the 3p25 critical region, with *CRELD1* as a putative candidate gene.[110] Based on the association between AVCD and 8p deletion, *GATA4* was also considered as a possible relevant gene, but thus far a pathogenic mutation was found only in a single patient.

The gene most frequently associated with AVCD is *CRELD1*, and heterozygous mutations occur in 6% of non-syndromic partial AVCDs.[110] In addition, some *CRELD1* gene mutations have been implicated in AVCD present in Down syndrome patients.[111] Gene interaction has been found also in the Ts65Dn model of Down syndrome mice, where the introduction of a null allele of *Creld1* in theDs65Dn mouse was observed to increase the penetrance of CHDs. It was also found that overexpression of *Jam2* gene was a necessary potentiator of the disomic genetic modifier *Creld1*.[112] Incomplete penetrance has been documented in families with AVCD, arguing that deleterious *CRELD1* mutations function as risk factors for AVCD, while can occur as benign variants in the general population. One *CRELD1* mutation, c.985C>T (p.Arg329Cys has been reported as a recurrent

variant.[111] A pathogenic link with ciliary dysfunction has been suspected also for *CRELD1* gene.[113] Based on the model analysed by Burnicka-Turek et al.[2] *CRELD1* is not required for left/right determination, since *Creld1* null mice do not manifest L/R abnormalities,[114] suggesting that *CRELD1* mutations contribute to the cause of AVCD through disruption of SHF Hh signaling rather than cilia motility. In the same study, it was suggested that hypomorphic mutations of the genes contributing to primary cilia structure and signaling could contribute also to the development of non-syndromic AVCDs.

2.6 Pathogenetic mechanism of AVCD: the role of Sonic Hedgehog

Nonsyndromic AVCD is a CHD with genetic heterogeneity. It involves a progenitor and secondary fields, and is homologous to AVCD in related species who share corresponding structure due to descent with modification from a common ancestor.[115,116] Tissue of extracardiac origin, from the posterior segment of the SHF (the so-called dorsal mesocardial protrusion (DMP) is recognized as a fundamental component of atrial and atrioventricular septation of the heart.[8-10,117] Cells of the DMP growing towards the primary atrial septum fuse with the posteroinferior and anterosuperior atrioventricular cushions to close the primary atrial foramen and to form two separate atrioventricular valves.[8,10-12]

In mice, defects of Shh signaling cause developmental anomalies of the DMP resulting in AVCD.[1,12,118] Abnormal expression of Shh signaling in the DMP disrupts the proliferation of this structure, preventing the fusion of the atrioventricular cushions with the inferior margin of the septum primum.[1,12] Additional experimental studies have shown that *Shh* null mouse heart can have AVCD.[119,120] Nevertheless, the primary AVCD signal transduction cascade involves Hh parsimoniously since Hh is involved in the induction of other developmental fields, as neural and ocular holoprosencephaly, anterior-posterior limb axis and ventral somites.

Therefore, perturbation of the SHH pathway and its effect on the DMP, represent unifying pathogenic patterns, causing AVCD in many genetic disorders, including Down and Noonan syndromes.

3 CONCLUSIONS

Hh signaling coordinates multiple aspects of left-right lateralization and cardiovascular growth, and being active at venous pole of the SHF, it is essential for normal development of dorsal-mesenchymal protrusion and AVC formation and septation. Molecular studies have demonstrated that several genes responsible for syndromes with AVCD are causally involved in ciliary function and/or abnormal processing of proteins with role in Hh signaling. Perturbations of different components of the Hh pathway lead to several developmental errors presenting with partially overlapping manifestations and AVCD as a common denominator (Figure 1, Table 1).

Similarly to that occurring for conotruncal heart defects in deletion 22q11.2 syndrome and branchial arch anomalies, AVCD should be considered as the cardiac manifestation linking ciliopathy syndromes. AVCD could be part of a "developmental field", accordingly to the concept that malformations can be due to defects in signal transduction cascades or pathways in developmental field defects.[115,116] The single morphogenetic unit may be altereted by Mendelian mutations, aneuploidies, and environmental causes.

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Legenda figure

FIGURE 1. Different mechanisms altering Hedgeghog signaling pathway in syndromes associated with AVCD

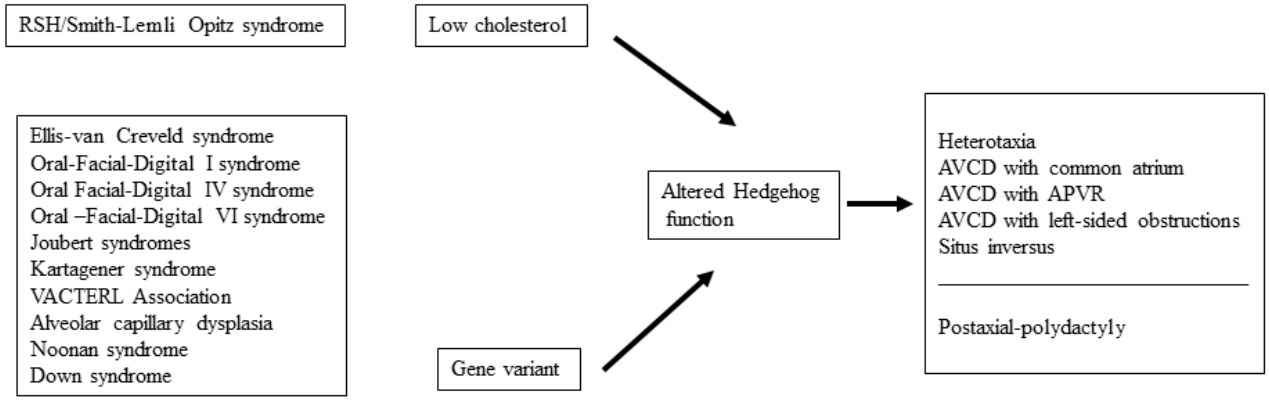

TABLE 1. Review of genes, mutation effects in Hedgehog pathway, cilia involvement, and cardiac defects in ciliopathies with AVCD

Disease	**Gene**	**Mutation effect on Hedgehog pathway**	**Cilia involvement**	**AVCD and laterality defects**	**Left-sided obstructions**	**Pulmonary venous abnormalities**	**Conotruncal defects**	**Septal defects, other**
Ellis-van Creveld syndrome	*EVC* *EVC2* *WDR35* *DYNC2LI1*	Decrease of Ihh	Proximal end of the primary cilium	AVCD (partial) with common atrium	HLH, Ao Co, AS			ASD (ostium secundum)
Oral-Facial-Digital syndromes								
- OFD type I	*Ofd1*	-	Basal body of the primary cilia	Situs inversus Common atrium	-			
- OFD type II	*WDPCP*	Involvement of Hedgehog pathway	Basal body of the primary cilia	AVCD (partial) with common atrium	HLH, Ao Co, AS			
- OFD type VI	*TMEM216*	-	Basal body of the primary cilia	AVCD (partial)	Ao Co, AS			
Joubert syndromes	*KIF7* *TMEM216*	Increased in activity of Shh pathway, through regulation of Gli	Microtubule dynamics Basal body of the primary cilia	AVCD (partial)	AS, BAV			
Smith Lemli-Opitz syndrome	*DHCR7*	Abnormal processing of the Hedgehog proteins secondary to low cholesterol	-	AVCD (complete) with APVR		APVR		VSD, ASD (ostium secundum)

Kartagener syndrome	Dyneins	Not required for SHF Hedgehog signaling.	Dynein arms, linking microtubules Cilia motility	Situs inversus	-			
VACTERL Association	*GLI3*, *FOXF1*, *IFT172*	Defective Sonic and Indian Hedgehog signaling	Distal ciliary axoneme	AVCD (partial) Dextrocardia Heterotaxy		APVR	TOF, DORV	VSD (subaortic), ASD (ostium secundum)
Alveolar capillary dysplasia	*FOXF1*	Activated by Sonic Hedgehog signalling	-	AVCD (partial) with left-sided obstructions	-			
Noonan / RASopathies	*PTPN11*	Decreased Indian Hedgehog levels	-	AVCD (partial) with Ao Co	Ao Co			PVS, ASD (ostium secondum)
Down syndrome	Trisomy 21	Defective mitogenic Sonic Hedgehog activity	-	AVCD (complete) with/without TOF			TOF	VSD (inlet), ASD (ostium secundum)
Non-syndromic AVCD	CRELD1	Disruption of Hedgehog signaling	Primary cilium structure	AVCD (partial, complete)	-			

Abbreviations : Ao Co, aortic coarctation; APVR, anomalous pulmonary venous return; AS, aortic stenosis; ASD, atrial septal defect; AVCD, atrioventricular canal defect ; BAV, bicuspid aortic valve; DORV, double outlet right ventricle; HLH, hypoplastic left heart; Ihh : Indian Hedgehog; OFD, Oral-Facial-Digital, PVS, pulmonary valve stenosis; TOF, tetralogy of Fallot; VSD, ventricular septal defect.