Published in final edited form as:
Hum Genet. 2016 May ; 135(5): 569–586. doi:10.1007/s00439-016-1655-9.

Pathogenetics of Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins

A full list of authors and affiliations appears at the end of the article.

Abstract

Alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV) is a lethal lung developmental disorder caused by heterozygous point mutations or genomic deletion copy-number variants (CNVs) of *FOXF1* or its upstream enhancer involving fetal lung-expressed long noncoding RNA genes *LINC01081* and *LINC01082*. Using custom-designed array comparative genomic hybridization, Sanger sequencing, whole exome sequencing (WES), and bioinformatic analyses, we studied 22 new unrelated families (20 postnatal and two prenatal) with clinically diagnosed ACDMPV. We describe novel deletion CNVs at the *FOXF1* locus in 13 unrelated ACDMPV patients. Together with the previously reported cases, all 31 genomic deletions in 16q24.1, pathogenic for ACDMPV, for which parental origin was determined, arose *de novo* with 30 of them occurring on the maternally inherited chromosome 16, strongly implicating genomic imprinting of the *FOXF1* locus in human lungs. Surprisingly, we have also identified four ACDMPV families with the pathogenic variants in the *FOXF1* locus that arose on paternal chromosome 16. Interestingly, a combination of the severe cardiac defects, including hypoplastic left heart, and single umbilical artery were observed only in children with deletion CNVs involving *FOXF1* and its upstream enhancer. Our data demonstrate that genomic imprinting at 16q24.1 plays an important role in variable ACDMPV manifestation likely through long-range regulation of *FOXF1* expression, and may be also responsible for key phenotypic features of maternal uniparental disomy 16. Moreover, in one family, WES revealed a *de novo* missense variant in *ESRP1,* potentially implicating FGF signaling in etiology of ACDMPV.

Keywords

Copy-number variants; genomic imprinting; hypoplastic left heart syndrome and single umbilical artery; transcriptional enhancer; uniparental disomy

INTRODUCTION

Alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV; MIM 265380) is a lethal neonatal lung disorder caused by abnormalities in air-blood barrier structure and function (Langston 1991; Bishop et al. 2011). Affected newborns typically present with severe respiratory failure and refractory pulmonary hypertension within a few

Correspondence to: Dr. Paweł Stankiewicz, Dept. of Molecular & Human Genetics, Baylor College of Medicine, One Baylor Plaza, Rm. R809, Houston, Texas 77030, USA, Tel: (713) 798-5370, Fax: (713) 798-7418, pawels@bcm.edu.

Conflict of interest No competing interest is declared.

hours after birth and die in the first month of life. Histopathologically, ACDMPV is characterized by decrease in number of capillaries adjacent to the alveolar epithelium, alveolar wall thickening, hypertrophy of the muscular layer of small pulmonary arteries with abnormal muscular extensions into intra-acinar vessels, and malposition of the small pulmonary veins. In addition, the majority of patients with ACDMPV manifest extra-pulmonary anomalies of the gastrointestinal, genitourinary, and cardiovascular systems (Sen et al. 2004; Bishop et al. 2011).

Heterozygous point mutations in *FOXF1* (OMIM 601089) and genomic deletion copy-number variants (CNVs) at chromosomal region 16q24.1 including *FOXF1* or its upstream regulatory region have been identified in the vast majority of patients with ACDMPV (Stankiewicz et al. 2009; Sen et al. 2013a, b; Szafranski et al. 2013a, b; Szafranski et al. 2014). FOXF1, expressed in lung mesenchyme and vascular endothelium, belongs to the forkhead family of transcription factors, and is a target of sonic hedgehog signaling (SHH) from epithelium.

Homozygous $Foxf1^{-/-}$ mice die by embryonic day 8.5 because of defects in the development of extraembryonic and lateral mesoderm-derived tissues (Mahlapuu et al. 2001), whereas heterozygous $Foxf1^{+/-}$ mice exhibit features resembling ACDMPV (Kalinichenko et al. 2001).

The *FOXF1* promoter overlaps a CpG island, does not contain a TATA-box, and requires enhancer function for its activity (Chang et al. 2001; Kim et al. 2005; Szafranski et al. 2013a,b). We have shown that the lung-specific enhancer region, mapping ~270 kb upstream of *FOXF1*, harbors genes for long non-coding RNAs (lncRNAs) that regulate *FOXF1* expression (Szafranski et al. 2013a; Szafranski et al. 2014). Further, we have also identified another *FOXF1* enhancer located within the *FOXF1* intron (Szafranski et al. 2013b).

Interestingly, all 17 reported pathogenic genomic deletions involving *FOXF1* or its upstream regulatory regions, for which parental origin was determined, arose *de novo* on the maternal chromosome 16, suggesting that the *FOXF1* locus is imprinted (Stankiewicz et al. 2009; Sen et al. 2013b; Szafranski et al. 2013a, b; Dharmadhikari et al. 2015). Segregation analysis of a missense mutation in *FOXF1* (c.416G>T; p. Arg139Leu) in a familial case of ACDMPV provided additional support for imprinting of *FOXF1* in humans (Sen et al. 2013a). Furthermore, previous bioinformatics studies, aimed at identification of imprinted genes, indicated that the *FOXF1* locus may be imprinted (Luedi et al. 2007).

We present 13 novel ACDMPV-causing *de novo* deletion CNVs in 16q24.1, 12 of which arose on maternal chromosome 16, providing statistically significant support for imprinting of the *FOXF1* locus. Surprisingly, we also identified one small pathogenic genomic deletion in the upstream regulatory region and three causative variants involving *FOXF1* that all arose on paternal chromosome 16, highlighting the complexity of genomic and epigenetic regulation of *FOXF1* expression that underlies etiology of ACDMPV. Lastly, we describe the results of whole exome sequencing (WES) in three unrelated ACDMPV families negative for both *FOXF1* point mutations and deletion CNVs. Our results demonstrate complexity of

genomic and epigenetic regulation of the *FOXF1* gene in 16q24.1 and implicate the role of other genes in ACDMPV.

METHODS

Subject recruitment

After informed consent, using protocols approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine (BCM) (H8712), histopathological specimens and DNA samples (peripheral blood or lung) from probands with ACDMPV: 20 postnatal (pts 114.3, 115.3, 117.3, 119.3, 120.3 (Decipher 285653), 121.3, 122.3, 123.3, 124.3, 125.3, 126.3, 127.3, 128.3, 130.3, 133.3, 134.3, 136.3, 138.5, 139.3, and 141.3) and two prenatal (135.3 and 140.3) and their family members (blood) were obtained.

Histopathological studies

Histopathological evaluations of all cases suspected of ACDMPV were performed in formalin-fixed paraffin-embedded (FFPE) specimens from lung biopsies or autopsies stained with hematoxylin and eosin.

Molecular biology

DNA isolation and sequencing, RNA isolation, RT-qPCR, cloning of the 16q24.1 region deleted in patient 122.3 and of the *FOXF1* promoter, and transcriptional activity assay were performed as described in Supplemental Methods.

Array CGH analyses

Genomic CNVs in the *FOXF1* locus were analyzed using customized 16q24.1 region-specific (1 Mb region flanking *FOXF1*) high-resolution 4×180K microarrays, manufactured by Agilent Technologies (Santa Clara, CA, USA), as previously described (Szafranski et al. 2013a) (pts 114.3, 115.3, 117.3, 119.3, 120.3 (Decipher 285653), 121.3, 122.3, 125.3, 126.3, 127.3, 128.3, 133.3, 135.3, 136.3, 139.3, and 140.3).

Characterization of deletion breakpoints

Deletion junction fragments were amplified using long-range PCR with LA Taq DNA polymerase (TaKaRa Bio, Madison, WI, USA) and primers designed by Primer3 software (http://frodo.wi.mit.edu/primer3) as described (Szafranski et al. 2013a, b). The sequence of a complex genomic rearrangement in exon 1 of *FOXF1* has been deposited in the GenBank database (http://ncbi.nlm.nih.gov/genbank) under accession number KT963011.

Parental origin of deletions and point mutations

Parental origin of the deletions was determined using informative microsatellites or single nucleotide polymorphisms (SNPs) mapping to the deleted genomic interval. For point mutations, PCR product containing the pathogenic variants and the neighboring SNPs were cloned into pGEM-T vector (Promega, Madison, WI, USA) and 10 clones were used for plasmid isolation and sequencing.

Bioinformatic analysis of the distant upstream *FOXF1* enhancer region

Reference DNA sequences, chromatin modification, location of CpG islands, and ChIP-seq data for the selected transcription regulators were accessed using the UCSC Genome Browser (http://genome.ucsc.edu, GRCh37/hg19). High-throughput chromosome conformation capture (Hi-C) analyses were performed as described in Supplemental Methods.

Whole exome sequencing

Three family trios with sporadic ACDMPV, negative for *FOXF1* mutations and deletions (using Sanger sequencing and custom-designed region-specific high-resolution array CGH) (pts 114.3, 121.3, and 128.3), were analyzed using whole exome sequencing (WES) as described in Supplemental Methods.

RESULTS

Clinical characterization

Histopathological examination of lung specimens revealed the characteristic pathognomonic features of ACDMPV in all cases analyzed. Identified anomalies involving other organs are listed in Table 1 and Supplemental Table S1.

Genomic deletions

We identified and characterized novel different-sized genomic deletions at 16q24.1 in 13 unrelated patients with ACDMPV (pts 115.3, 117.3, 119.3, 120.3 (Decipher 285653), 122.3, 125.3, 126.3, 127.3, 133.3, 135.3, 136.3, 139.3, and 140.3) (Fig. 1 and Supplemental Table S1). Twelve out of 13 deletions (pts 115.3, 117.3, 119.3, 120.3, 125.3, 126.3, 127.3, 133.3, 135.3, 136.3, 139.3 and 140.3), for which the parental origin could have been determined, arose *de novo* on the maternal chromosome 16 (Supplemental Table S1). In one of those cases (pt 115.3), the CNV deletion involved only *FOXF1*, in five cases (pts 118.3, 120.3, 125.3, 133.3, 135.3, and 140.3), deletions encompassed *FOXF1* and its upstream enhancer region, and in seven cases (pts 117.3, 119.3, 122.3, 126.3, 127.3, 136.3, and 139.3) the deletion CNVs harbored only the upstream enhancer, leaving *FOXF1* intact. We did not find any evidence of somatic mosaicism in the parental DNA samples from peripheral blood using PCR with primers specific for the patients' deletion junction fragment. Recently, we showed that new mutations that occur in the maternal allele are more likely to be recurrently transmitted to offspring (Campbell et al. 2014). Given that all published and all but one described here new deletions in the 16q24.1 *FOXF1* region arose *de novo* on the maternal chromosome 16, the recurrence risk for ACDMPV may potentially be elevated in comparison to that observed for other sporadic diseases.

In patient 122.3, we identified a small ~ 4.1 kb *de novo* deletion in the centromeric portion of the upstream *FOXF1* enhancer region, mapping ~ 9.1 kb upstream to *LINC01082* (Fig. 2). Surprisingly, three informative SNPs, mapping to the deleted region, consistently showed that the deletion arose *de novo* on the paternal chromosome 16 (Supplemental Table S1).

Bioinformatic analyses of the *FOXF1* locus on 16q24.1

Hi-C analysis of chromatin interactions around the *FOXF1* gene revealed that *FOXF1* and its upstream enhancer reside within the same topologically associated domain (TAD), spanning ~400 kb upstream of *FOXF1* (Fig. 3). Intriguingly, *FOXF1* maps at the distal edge of the TAD (Fig. 3), consistently with previous suggestion by Parris et al. (2013) based on distribution of the CTCF binding sites. This domain exhibits stronger or more frequent chromatin interactions within its boundaries in fetal lung fibroblasts IMR-90 compared to non-lung cell lines: GM12878, HUVEC, NHEK, and HMEC. The strength of these interactions positively correlated with *FOXF1* expression, which was stronger in IMR-90 cells than in, e.g., HUVEC, and was around the threshold level in peripheral blood lymphocytes (GM12878).

In silico analysis of the *FOXF1* upstream enhancer region identified an 880 bp-long CpG island (71% GC-rich) located 0.3 kb from the known transcription start site of *LINC01081* (Supplemental Fig. S1). We have previously shown that *LINC01081* positively regulates *FOXF1* expression (Szafranski et al. 2014). The methylation status of this CpG island is unknown. However, if this region is differentially methylated, it might contribute to the proposed epigenetic regulation of *LINC01081* and thus also *FOXF1* expression.

We also found that the ~4.1 kb region of the *FOXF1* upstream enhancer, adjacent upstream to another lncRNA gene, *LINC01082* and deleted in patient 122.3, contains the TATA-box (TTATAAATAGGAATT; chr16:86,220,297-86,220,311) and the binding sites for several transcription factors including myocyte enhancer factor-2 (MEF2), RING finger protein LUN1, hepatic leukemia factor (HLF), and myeloblastosis proto-oncogene protein (MYB) (Fig. 2a). Importantly, LUN1 and MEF2 are relatively highly expressed in the human lungs (Brand et al. 1997; Chu et al. 2001), and might be involved in long distance interaction with the *FOXF1* promoter.

Reporter assay

Because the 4.1 kb region, deleted in patient 122.3, contains the TATA-box located close to the lncRNA gene, *LINC01082*, we tested whether this region exhibits promoter activity. We found that the deleted fragment did not exhibit any promoter activity, even when compared with the residual activity of the *FOXF1* promoter (Supplemental Fig. S2), suggesting instead its function as a scaffold for LUN1, MEF2 and TATA-binding TFIID.

Molecular mechanisms of formation of 16q24.1 deletions

To infer the molecular mechanism of formation of the identified deletions and to better assess their recurrence risk, we mapped and sequenced 18 breakpoint junctions of nine 16q24.1 deletion CNVs (Fig. 1, Supplemental Table S1). Twelve breakpoints map to *Alu* repeats and three within LINE1 elements. In total, 53% (16 out of 30) of all characterized 16q24 deletions were *Alu-Alu*-mediated. Interestingly, in three cases (pts 57.3, 119.3, 127.3), the deletion breakpoints map to the same LINE element L1PA2 (chr16:86,266,902-86,272,916, GRCh37/hg19) within *LINC01081*, indicating that it is a genomic recombination hotspot (Fig. 1 and Supplemental Table S1). Microhomologies between 7 and 41 bp were found in six out of 10 cases analyzed, suggesting that those six

deletions might have arisen by a template switching replicative mechanism such as fork stalling and template switching (FoSTeS), or microhomology-mediated break-induced replication (MMBIR) (Hastings et al. 2009).

Familial cases of parentally transmitted *FOXF1* mutations

We have identified three ACDMPV families with novel pathogenic variants in *FOXF1* transmitted from the reportedly healthy carrier fathers (Fig. 4). Sequencing of *FOXF1* and its flanking regions in patient 124.3 (Fig. 4a) revealed a complex genomic rearrangement within a noncoding portion of the *FOXF1* 1st exon, mapping six base pairs upstream of the *FOXF1* ATG codon (Supplemental Fig. S3a). A copy of a portion of the 1st intron of the lncRNA *FENDRR* (chr16:86,540,260/271-86,540,607/610), encoded upstream and opposite to *FOXF1*, was inserted into the untranslated portion of the *FOXF1* 1st exon, replacing 5 to 15 bp in the position chr16:86,544,155/165-86,544,169. In addition, 265 bp of this inserted fragment (chr16:86,540,342-86,540,607/610) was inverted. This small insertion occurred within the centromeric portion of $(CGG)_n$ simple repeat, which is normally divided by a unique sequence containing the *FOXF1* AUG codon (Supplemental Fig. S3b). Importantly, it also introduced five alternative AUG initiation codons into the 5′ untranslated part of the *FOXF1* 1st exon, none of which is in frame with *FOXF1*, generating potential translation start sites for five novel peptides. This complex rearrangement within *FOXF1* was inherited from the reportedly healthy father, in whom it arose *de novo* on the grandmaternal chr16. No evidence of somatic mosaicism of this rearrangement was detected in grandmaternal peripheral blood DNA.

In family 130 (Fig. 4b) with two children affected by ACDMPV, we found a deletion of five nucleotides in the coding region of the *FOXF1* 1st exon, resulting in a translational frameshift (c.90_96del; p.Ser31fs). This pathogenic variant occurred *de novo* in the father who was found to be approximately 20% mosaic for the mutation in the peripheral blood nucleated cells.

We found a missense *FOXF1* mutation c.C231A, (p.Phe77Leu) in family 138 (Fig. 4c) with four children, one of them affected by ACDMPV, that was inherited from the healthy father who was approximately 70% mosaic for the mutation in the peripheral blood nucleated cells (Reiter et al. 2016).

In family 134 (pedigree not shown), the affected newborn had an in-frame duplication c. 54_59dup (p.Gly22_Gly23dup) in the 1st exon of *FOXF1*. The variant was located within a stretch of 11 Gly residues. There are few known in-frame deletions and duplications within this Gly repeat (e.g., p.Gly19del, p.Gly13_Gly17del, p.Gly17dup), listed in the ExAC database (http://exac.broadinstitute.org), interpreted as non-pathogenic. Moreover, the number of Gly in this repeat varies between species (e.g., there are six residues in rabbit, 12 in macaque). The healthy father is heterozygous for this variant. Therefore, we concluded that this Gly duplication is unlikely to be pathogenic for ACDMPV. Thus, the causative factor for the disease in this case remains unknown.

In addition, in family 123 (Fig. 4d), two siblings died of ACDMPV six years apart. Sequencing of *FOXF1* from one of the siblings revealed the presence of a deleterious frame-

shift mutation c.849_850del (p.I285fs) in the 1st exon. This variant was the cause of ACDMPV also in the other sibling, and was transmitted from the healthy mother who is likely germline mosaic or low-level somatic mosaic.

Whole exome sequencing

Three ACDMPV cases (families 114, 121, and 128), negative for *FOXF1* mutations and CNVs in *FOXF1* or its upstream enhancer region, were analyzed by WES to identify additional to *FOXF1* ACDMPV genes. From the list of variants obtained for each proband, we filtered out synonymous or non-exonic SNVs/indels and variants with minor allele frequency > 1% in 1000 Genomes, Exome Variant Server, and in-house exome databases, which left 762 (pt 114.3), 681 (pt 121.3), and 709 (pt 128.3) variants. Analysis of WES data in family 114 revealed one *de novo* missense mutation c.1564T>C (p.Trp522Arg) in *ZMYND11* validated by Sanger sequencing (Table 2). In family 121, we identified four *de novo* variants confirmed by Sanger sequencing: missense mutations c.463C>T (p.Arg155Cys) in *SLC50A1* and c.881A>G (p.Tyr294Cys) in *ESRP1,* a non-frameshift deletion c.533_542delinsC (p.Ser179_Ser173del) in *MPRIP*, and a frameshift insertion c. 2819_2820insT (p.Gly941fs) in *DOCK8* (Table 2). None of those *de novo* variants were present in ESP or 1000 Genomes databases. By exploring ExAC database we found that the variant in *SLC50A1* was reported in two healthy individuals (of Latino and European ancestry) and the variant in *MPRIP* was present in 152 individuals (MAF=0.0015). However, given the complexity of the *FOXF1* locus and its epigenetic regulation, none of these variants should be definitively excluded as a potential contributor to ACDMPV. In family 128, we did not find any *de novo* variants.

Moreover, in families 121 and 128, we identified inherited missense variants c.631C>G (p.Leu211Val) in pt 121.3 and c.3256G>A (p.Ala1086Thr in pt 128.3 in *PLXNB2* encoding plexin B2 (Table 2). Further, we identified homozygous and compound heterozygous variants in all affected probands from 114, 121 and 128 families. In patient 114.3, we found 66 homozygous variants in 63 genes and 27 compound heterozygous in 10 genes, in patient 121.3, we identified 56 homozygous and 24 compound heterozygous variants in 49 and 7 genes, respectively, and in patient 128.3, we identified five homozygous variants in five genes and five compound heterozygous variants in two genes.

CNV analyses of WES data did not reveal any non-polymorphic deletion or duplication in probands 114.3 and 121.3 and no genomic imbalances were identified by whole-genome array CGH in pt 128.3. By analyzing B-allele frequency in WES data in probands 114.3, 121.3, and 128.3, we did not identify any AOH region larger than 5 Mb.

DISCUSSION

To date, approximately 150 imprinted genes clustering in 16 genomic loci have been described in mice (Barlow et al. 2014). In humans, disease related genomic imprinting has been well defined only for a few loci: 15q11.2 in Prader-Willi (PWS, OMIM 176270) and Angelman (OMIM 105830) syndromes, 11p15 in Beckwith-Wiedemann syndrome (BWS, OMIM 130650) and Silver-Russell syndrome (OMIM 180860), 14q32 in Kagami-Ogata syndrome (OMIM 608149), 20q13.32 in McCune-Albright syndrome (OMIM 174800), and

6q25.3 in transient neonatal diabetes mellitus 1 (OMIM 601410) (Bartolomei and Ferguson-Smith 2011). However, recent analyses have shown that the occurrence of non-canonical imprinting, with a biased allele-specific gene expression as opposed to complete allele silencing, is likely underestimated (Gregg 2014). These partial allelic imbalances may result, e.g., from incomplete methylation of imprinting control regions (ICRs) or from an ICR being epigenetically modified only in a subpopulation of cells (Gregg 2014).

We have accumulated the largest collection of ACDMPV samples worldwide (N=141 families), in which we have identified 86 pathogenic variants in the *FOXF1 locus*: 38 deletion CNVs, a complex rearrangement and 47 point mutations. In the vast majority of the remaining 55 families, DNA was not of sufficient quality for genetic testing. We have previously reported pathogenic genomic deletions involving *FOXF1* or its upstream regulatory region in 17 patients with histopathologically-verified ACDMPV that arose *de novo* on the maternal chromosome 16. Based on these results, we proposed that the *FOXF1* locus in chromosome 16q24.1 is imprinted in the human lungs (Stankiewicz et al. 2009; Sen et al. 2013a, b; Szafranski et al. 2013a, b; Dharmadhikari et al. 2015). We previously suggested that this imprinting is incomplete (~35% expression from one parental allele vs ~65% expression from the other allele) (Szafranski et al. 2013a). Together with 13 novel deletions reported here, 30 out of 31 CNV deletions involving *FOXF1* or its upstream enhancer in patients with ACDMPV arose *de novo* on the maternal chr16, now providing statistically significant evidence for genomic imprinting at this locus ($p<4E-06$). However, we have now also identified an upstream deletion CNV (pt 122.3) and three non-deletion variants in *FOXF1* (pts 124.3, 130.3, 138.3), pathogenic for ACDMPV, that arose on paternal chromosome 16q24.1, indicating complex genomic and epigenetic regulation of the *FOXF1* locus.

The 4.1 kb *de novo* deletion within the upstream *FOXF1* enhancer on paternal chromosome 16 in pt 122.3 removed the binding sites for a number of transcription factors, including MEF2 and LUN1 that exhibit increased expression in the lungs. MEF2 regulates cell proliferation as a target of several growth factor signaling pathways and has been shown to play an important role in myogenesis, including morphogenesis of visceral muscles (Brand 1997; Black and Olson 1998). We hypothesize that loss of MEF2 binding in patient 122.3 might have contributed to the abnormal development of lung vasculature, a feature typical for ACDMPV. LUN1, which is highly expressed in alveolar epithelium (Chu et al. 2001) has binding sites located also in regulatory region of genes for E-cadherin and talin, which regulate cell motility (Oyanagi et al. 2004). Gene expression profiling in ACDMPV lungs and $Foxf1^{+/-}$ mouse lungs showed that E-cadherin is one of the Foxf1 targets (Sen et al. 2014). Thus LUN1 might control cell motility during alveolar development, targeting E-cadherin expression directly and indirectly through FOXF1. The deleted interval in pt 122.3 also includes the TATA-box, present in 25% of eukaryotic promoters; however, we found no promoter activity within this region. Since the upstream regulatory region becomes juxtaposed with the *FOXF1* promoter, we propose that the identified transcription factor binding sites, in particular those for MEF2, LUN1, and TBP (TFIID), similarly as the relatively closely located GLI2-binding sites (Szafranski et al. 2013a), may interact with the *FOXF1* promoter following chromatin looping to directly up-regulate *FOXF1* promoter activity.

In patient 124.3, we found a complex genomic rearrangement within the noncoding portion of *FOXF1* exon 1, potentially interfering with initiation of transcription and translation. The most plausible explanation of the absence of ACDMPV phenotype in the father is that he could be a mosaic for this rearrangement, as shown for the fathers of pts 130.3 and 138.3.

The other two identified paternally transmitted pathogenic variants in families 130 and 138, mapping within the coding portions of *FOXF1*, were inherited from the reportedly healthy fathers who are mosaics for the mutations in the blood. These two cases exemplify incomplete penetrance of ACDMPV likely due to somatic mosaicism for a pathogenic variant manifesting as non-mosaic in their affected children. The clinical relevance of somatic mosaicism has become more evident only recently (Campbell et al. 2014). Family 138 also illustrates variable expressivity of ACDMPV with two siblings presenting with severe pulmonary hypertension after birth, one of whom had hypoxemia, but survived beyond infancy, and the other had partial anomalous pulmonary venous return. The third sibling died in the neonatal period from ACDMPV (Reiter et al. 2016).

Given that all but one ACDMPV variants that arose on paternal chromosome 16 mapped within the *FOXF1* gene, whereas all but one deletions that included the upstream enhancer arose on the maternal chromosome, we propose that the *FOXF1* locus is imprinted through epigenetic modification of its distant lung-specific enhancer. In support of this notion, we have found that whereas the *FOXF1* promoter is not methylated, the CpG island overlapping cluster of GLI-binding sites within the upstream enhancer is differentially methylated, and this methylation reduces regulatory function of the enhancer (Szafranski et al. 2013a). Moreover, the promoter region of the lncRNA gene, *LINC01081*, that positively regulates *FOXF1* expression, located within the enhancer, overlaps with another CpG island, suggesting possibility of the regulation of the expression of *LINC01081* by allele-specific differential methylation of its promoter. Most recently, Dello Russo et al. (2015) reported a *de novo* ~ 2.6 Mb deletion (chr16:83,676,990-86,292,585) at maternal chromosome 16q23.3q24.1, encompassing *LINC01082* and disrupting *LINC01081*, in a patient with a rare developmental lung disease pulmonary capillary hemangiomatosis, providing further evidence for genomic imprinting and demonstrating allelic affinity of this genomic locus.

Whether epigenetic modification of the upstream enhancer occurs on paternal or on maternal chromosome 16 is currently unclear and requires further studies. Unlike ICRs of maternally imprinted genes that are typically located at the imprinted gene promoter, the intergenic localization of the *FOXF1* locus ICR suggests paternal imprinting (Ferguson-Smith 2011). Also the location of all but one pathogenic deletion on maternal chromosome suggests paternal imprinting. In the paternal imprinting model (Fig. 5A), deletion of the strong enhancer on maternal chromosome 16 leads to ACDMPV, whereas deletion of the weak enhancer on paternal chromosome 16 is benign. In the maternal imprinting model (Fig. 5B), deletion of the weak enhancer on maternal chromosome 16 slightly decreases *FOXF1* expression, resulting in ACDMPV whereas deletion of the strong enhancer on paternal chromosome 16 reduces expression of *FOXF1* more dramatically and is embryonic lethal.

Interestingly, multiple congenital malformations, including pulmonary hypoplasia, heart defects, tracheoesophageal fistula, gut malrotation, absent gall bladder, renal agenesis,

hydronephrosis, imperforate anus, and single umbilical artery (SUA), seen in the vast majority of children with ACDMPV, are also observed in patients with maternal uniparental disomy 16, UPD(16). In stark contrast, a relatively normal phenotype was reported in few patients with paternal UPD(16) (Kohlhase et al. 2000; Hamvas et al. 2009), and the presence of imprinted gene(s) on chromosome 16 was suggested as causative for maternal UPD(16) phenotype (Yong et al. 2002). Differences in the clinical features observed in maternal UPD(16) cases compared to paternal UPD(16) cases and similarities between ACDMPV and maternal UPD(16) phenotypes, indicate that the identified genomic imprinting at the *FOXF1* locus may be responsible for some phenotypic features of maternal UPD(16) (Dharmadhikari et al. 2015). The underlying mechanism could be similar to that in patients with maternal (but not paternal) duplication/triplication of the imprinted PWS/AS region in 15q11.2, e.g., due to inv dup(15). In the paternal imprinting model (Fig. 5A), in maternal UPD(16), *FOXF1* expression is increased, manifesting typical features of UPD(16) including organs involved also in patients with ACDMPV, whereas in paternal UPD(16), *FOXF1* expression only slightly decreases and is benign. In the maternal imprinting model (Fig. 5B), the level of *FOXF1* in maternal UPD(16) is reduced, whereas paternal UPD(16) increases *FOXF1* expression and is benign. However, given that there are only two reports of apparently benign paternal UPD16 (isodisomy) cases vs. high prevalence of maternal UPD16 (heterodisomy) cases due to common trisomy 16 (>1% of all pregnancies), it is also possible that paternal UPD(16) is early embryonically lethal.

The observed enhancer-dependent regulation and proposed genomic imprinting in the *FOXF1* locus are likely mediated by genomic insulator sites binding CTCF as was shown for the BWS region on chromosome 11p15. Supporting this model, *in silico* Hi-C analyses of the chromosome 16q24.1 genomic structure showed that this region is organized into ~ 400 kb TADs with *FOXF1* being located at the TAD boundary (Fig. 3). TAD boundaries exhibit conservation across species and remain largely constant across multiple cell types (Dixon et al. 2012), suggesting that variation in intra-domain interactions, such as chromatin looping, may be crucial for dynamic regulation of gene expression in a cell type-specific fashion. Using a chromosome conformation capture-on-chip (4C) analysis, we have previously shown that the region upstream of *FOXF1*, including its promoter, comes in contact with the upstream enhancer sequences in a time and tissue specific manner (Szafranski et al. 2013a); these interactions are stronger or more frequent in fetal lung fibroblasts than in cells of tissues other than lungs (Fig. 3).

Recently, disruption of TADs, resulting in “enhancer adoption”, has been shown as a novel disease-causing mechanism in patients with limb anomalies (Lupiáñez et al. 2015). We suggest that deletion CNVs or balanced paracentric inversions (Parris et al. 2013) removing or replacing, respectively, the *FOXF1* TAD boundary with CTCF binding sites (Guo et al. 2015), would expose genes neighboring TAD to a non-physiological environment deregulating their expression. This mechanism could explain our observation that in contrast to *FOXF1* point mutations (Sen et al. 2013b) and upstream deletion CNVs (Stankiewicz et al. 2009; Szafranski et al. 2013a; Szafranski et al. 2014), genomic deletions of *FOXF1* at its TAD boundary, and the flanking genes were associated with severe congenital heart defects, including hypoplastic left heart syndrome (HLHS) and SUA (Table 1 and Supplemental Table S1). Co-existence of HLHS and SUA has been well documented (Tasha et al. 2014;

Araujo et al. 2015). We have previously suggested that HLHS may result from variants in the neighboring *FOXC2* and *FOXL1* genes; however, screening for mutations in patients with HLHS revealed no pathogenic variants in those genes (Iascone et al. 2012). Alternatively, disruption of lncRNA *FENDRR* that maps 1.7 kb upstream of *FOXF1* in the opposite orientation, and likely utilizes the same bi-directional promoter as *FOXF1* could lead to HLHS and SUA. Corroboratively, Grote et al. (2013) reported that homozygous loss of *Fendrr* in mice led to hypoplasia of the myocardium affecting ventricular walls and the interventricular septum and ventral body wall (omphalocele), likely due to *in trans* deregulation of the cardiac master transcriptional regulators *Gata6* and *Nkx2-5.* Further, Sauvageau et al. (2013) and Lai et al. (2015) independently demonstrated defects in lungs and heart in the *Fendrr*$^{-/-}$ mouse neonates.

Interestingly, most of deletion CNVs in 16q24.1 were flanked by retrotransposons with greater than 50% being *Alu*-mediated and many representing different *Alu* families (Szafranski et al. 2013a). Recently, *Alu*-mediated genomic rearrangements were shown to be products of replication and not recombination errors (Gu et al. 2015). Of note, Jacques et al. (2015) demonstrated that transposable elements have contributed hundreds of thousands of novel regulatory elements to the primate lineage and reshaped the human transcriptional landscape. In vertebrates, transposable elements occur in more than two-thirds of mature lncRNAs, whereas they seldom occur in protein-coding transcripts. Moreover, transposable elements were found in biased positions and orientations within lncRNAs, particularly at their transcription start sites, which suggests a role in the regulation of lncRNA transcription (Fatica and Bozzoni 2014). We suggest that the high rate of the retrotransposon-mediated CNVs in 16q24.1 may result from replication-transcription collisions due to their residual transcriptional activity.

Besides variants in the *FOXF1* locus, CNVs or SNVs in other genes involved in SHH or other signaling pathways essential for lung development might be also causative for ACDMPV. Our WES analyses in three unrelated ACDMPV families (114, 121, and 128) revealed *de novo* variants in five genes: *DOCK8, ESRP1, MPRIP, SLC50A1,* and *ZMYND11.* All these genes are involved in cell signaling or transcription regulation in general and their variants may contribute to development of ACDMPV. *ESRP1* (pt 121.3) is particularly interesting in this context. It encodes endothelial splicing regulatory protein 1 (ESRP1) functioning as an epithelium-specific regulator of FGFR2 splicing into FGFR2-IIIb isoform] (Warzecha et al. 2009). Various isoforms of FGFR2 are involved in epithelial-mesenchymal crosstalk during embryonic development, and they also play a role in epithelial-mesenchyme transitions during lung and heart development. Thus, loss of ESRP1 or its function might contribute to ACDMPV by affecting FGF signaling.

In families 121 and 128, we identified two inherited missense variants in *PLXNB2* encoding plexin B2. Plexins function as receptors of semaphorins and were shown to play a crucial role in lung branching morphogenesis (Kagoshima et al. 2001). Interestingly, loss of class 3 semaphorins (SEMA3) was attributed to dysmorphic vascularization during mouse lung development, resembling features of ACDMPV (Joza et al. 2012). Since both variants were inherited from healthy carrier father, they may function as modifiers of the pathway(s)

contributing to ACDMPV. Nevertheless, other variants in *PLXNB2* might still be causative for ACDMPV.

In aggregate, our data highlight complexity of genomic architecture of the *FOXF1* locus at chromosome 16q24.1 and regulation of *FOXF1* expression through epigenetic modification of its upstream enhancer. Unlike the 31 identified CNVs that all arose *de novo* and, with one exception, on maternally inherited chromosome 16, *FOXF1* SNVs can be inherited from either parent, who may be a mosaic carrier. We propose that genomic imprinting of the *FOXF1* locus is due to parent- and tissue-specific activity of the *FOXF1* enhancer regulated by lncRNAs *LINC01081* and *LINC01082* and chromatin folding within a defined TAD, with *FOXF1* being located at its boundary. The *FOXF1* promoter is presumably activated by the enhancer-bound transcription factors (e.g., GLI2, MEF2, LUN1, and TFIID). This complex gene regulation in 16q24.1, in particular a non-canonical mode of *FOXF1* locus imprinting, likely contributes to variable expressivity and incomplete penetrance of ACDMPV. We also suggest that variants in two other genes could be causative (*ESRP1*) or function as modifiers (*PLXNB2*) of the ACDMPV phenotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Authors

Przemyslaw Szafranski,
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

Tomasz Gambin,
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

Avinash V. Dharmadhikari,
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX, USA

Kadir Caner Akdemir,
Genomic Medicine Department, MD Anderson Cancer Center, Houston, TX, USA

Shalini N. Jhangiani,
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA

Jennifer Schuette,
Division of Critical Care Medicine, Children’s National Health System, Washington, DC, USA

Nihal Godiwala,

Division of Critical Care Medicine, Children's National Health System, Washington, DC, USA

Svetlana A. Yatsenko,
Department of Obstetrics, Gynecology, and Reproductive Sciences, Center for Medical Genetics and Genomics, Magee-Womens Hospital of UPMC, Pittsburgh, PA, USA. Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Jessica Sebastian,
Department of Medical Genetics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, USA

Suneeta Madan-Khetarpal,
Department of Medical Genetics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, USA

Urvashi Surti,
Department of Obstetrics, Gynecology, and Reproductive Sciences, Center for Medical Genetics and Genomics, Magee-Womens Hospital of UPMC, Pittsburgh, PA, USA. Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, PA, USA

Rosanna G. Abellar,
Department of Pathology, Columbia University Medical Center, New York, NY, USA

David A. Bateman,
Department of Pediatrics, Columbia University Medical Center, New York, NY, USA

Ashley L. Wilson,
Children's Hospital of New York-Presbyterian, New York, NY, USA

Melinda H. Markham,
Division of Neonatology, Division of Maternal Fetal Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

Jill Slamon,
Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

Fernando Santos-Simarro,
INGEMM, Instituto de Genética Médica y Molecular, IdiPAZ, Madrid, Spain. CIBERER, ISCIII, Madrid, Spain

María Palomares,
INGEMM, Instituto de Genética Médica y Molecular, IdiPAZ, Madrid, Spain. CIBERER, ISCIII, Madrid, Spain

Julián Nevado,
INGEMM, Instituto de Genética Médica y Molecular, IdiPAZ, Madrid, Spain. CIBERER, ISCIII, Madrid, Spain

Pablo Lapunzina,
INGEMM, Instituto de Genética Médica y Molecular, IdiPAZ, Madrid, Spain. CIBERER, ISCIII, Madrid, Spain

Brian Chung Hon-Yin,
Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, China. Department of Obstetrics and Gynaecology, and Centre for Genomic Sciences, The University of Hong Kong, Hong Kong, China

Wong Wai-Lap,
Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, China

Yoyo Wing Yiu Chu,
Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, China

Gary Tsz Kin Mok,
Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, China

Kerem Eitan,
Pediatric Pulmonary Unit, Department of Pediatrics, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Joel Reiter,
Pediatric Pulmonary Unit, Department of Pediatrics, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Namasivayam Ambalavanan,
Departments of Pediatrics, University of Alabama at Birmingham, Alabama, USA. Cell Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL, USA

Scott A. Anderson,
Department of Surgery, Division of Pediatric Surgery, University of Alabama at Birmingham and Children's of Alabama, Birmingham, AL, USA

David R. Kelly,
Department of Pathology, University of Alabama at Birmingham and Pathology and Laboratory Medicine Service, Children's of Alabama, Birmingham, AL, USA

Joseph Shieh,
Division of Medical Genetics, Department of Pediatrics, and Institute for Human Genetics, University of California San Francisco, San Francisco, CA, USA

Taryn C. Rosenthal,
Genetics Department, Kaiser Permanente San Jose Medical Center, San Jose, CA, USA

Kristin Scheible,
Department of Pediatrics, University of Rochester, Rochester, NY, USA

Laurie Steiner,
Division of Neonatology, University of Rochester, Rochester, NY, USA

M. Anwar Iqbal,
Pathology and Laboratory Medicine, Rochester, Rochester, NY, USA

Margaret McKinnon,
Department of Medical Genetics, University of British Columbia, Vancouver, Canada

Sara Jane Hamilton,
Department of Medical Genetics, University of British Columbia, Vancouver, Canada

Kamilla Schlade-Bartusiak,
Department of Medical Genetics, University of British Columbia, Vancouver, Canada

Dawn English,
Department of Medical Genetics, University of British Columbia, Vancouver, Canada

Glenda Hendson,
Department of Pathology, University of British Columbia, Vancouver, Canada

Elizabeth R. Roeder,
Department of Pediatrics, Baylor College of Medicine, San Antonio, TX, USA.
Department of Molecular and Human Genetics, Baylor College of Medicine, San Antonio, TX, USA

Thomas S. DeNapoli,
Department of Pathology, Children's Hospital of San Antonio, San Antonio, TX, USA

Rebecca Okashah Littlejohn,
Department of Pediatrics, Baylor College of Medicine, San Antonio, TX, USA

Daynna J. Wolff,
Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC, USA

Carol L. Wagner,
Department of Pediatrics, Medical University of South Carolina, Charleston, SC, USA

Alison Yeung,
Victorian Clinical Genetics Services, Murdoch Childrens Research Institute, Parkville, SA, Australia

David Francis,
Victorian Clinical Genetics Services, Murdoch Childrens Research Institute, Parkville, SA, Australia

Elizabeth K. Fiorino,
Division of Pediatric Pulmonary Medicine, The Children's Heart Center Steven and Alexandra Cohen Children's Medical Center of New York, New York, NY, USA

Morris Edelman,

Division of Pediatric Pathology, The Children's Heart Center Steven and Alexandra Cohen Children's Medical Center of New York, New York, NY, USA

Joyce Fox,
Division of Medical Genetics, The Children's Heart Center Steven and Alexandra Cohen Children's Medical Center of New York, New York, NY, USA

Denise A. Hayes,
Pediatric Cardiology, The Children's Heart Center Steven and Alexandra Cohen Children's Medical Center of New York, New York, NY, USA

Sandra Janssens,
Centre for Medical Genetics Ghent, University Hospital Ghent, Ghent, Belgium

Elfride De Baere,
Centre for Medical Genetics Ghent, University Hospital Ghent, Ghent, Belgium

Bjorn Menten,
Centre for Medical Genetics Ghent, University Hospital Ghent, Ghent, Belgium

Anne Loccufier,
Department of Obstetrics, Gynaecology, and Fertility, AZ St Jan Brugge, Brugge, Begium

Lieve Van Walleghem,
Department of Anatomopathology, AZ St Jan Brugge, Brugge, Begium

Philippe Moerman,
Department of Pathology UZ Leuven, Leuven, Belgium

Yves Sznajer,
Center for Human Genetics, Cliniques Universitaires St-Luc, Universite Catholique de Louvain, Brussels, Belgium

Amy S. Lay,
Division of Pediatric Cardiology, Children's Mercy Hospital, Kansas City, MS, USA

Jennifer L. Kussmann,
Division of Clinical Genetics, Children's Mercy Hospital, Kansas City, MS, USA

Jasneek Chawla,
Division of Paediatric Respiratory & Sleep Medicine, Lady Cilento Children's Hospital, Children's Health Queensland Hospital and Health Service, Brisbane, QLD, Australia. The University of Queensland, Brisbane, QLD, Australia

Diane J. Payton,
Division of Anatomical Pathology, Lady Cilento Children's Hospital, Children's Health Queensland Hospital and Health Service, Brisbane, QLD, Australia. Pathology Queensland, Brisbane, QLD, Australia

Gael E. Phillips,

Division of Anatomical Pathology, Lady Cilento Children's Hospital, Children's Health Queensland Hospital and Health Service, Brisbane, QLD, Australia. Pathology Queensland, Brisbane, QLD, Australia

Erwin Brosens,
Clinical Genetics Department, Erasmus MC-Sophia, Rotterdam, Netherlands. Paediatric Surgery, Erasmus MC-Sophia, Rotterdam, Netherlands

Dick Tibboel,
Paediatric Surgery, Erasmus MC-Sophia, Rotterdam, Netherlands

Annelies de Klein,
Clinical Genetics Department, Erasmus MC-Sophia, Rotterdam, Netherlands

Isabelle Maystadt,
Institut de Pathologie et de Genetique, Gosselies, Belgium

Richard Fisher,
James Cook University Hospital, Middlesborough, UK

Neil Sebire,
Department of Paediatric Histopathology, Great Ormond Street Hospital for Children and UCL Institute of Child Health, London, UK

Alison Male,
Clinical Genetics Unit, Great Ormond Street Hospital for Children and UCL Institute of Child Health, London, UK

Maya Chopra,
Department of Molecular and Clinical Genetics, Royal Prince Alfred Hospital, Sydney, NSW, Australia

Jason Pinner,
Obstetrics, Gynaecology and Neonatology, Royal Prince Alfred Hospital, Westmead, NSW, Australia

Girvan Malcolm,
Obstetrics, Gynaecology and Neonatology, Royal Prince Alfred Hospital, Westmead, NSW, Australia

Gregory Peters,
Cytogenetics Department, The Children's Hospital at Westmead, Westmead, NSW, Australia

Susan Arbuckle,
Histopathology Department, The Children's Hospital at Westmead, Westmead, NSW, Australia

Melissa Lees,
Clinical Genetics Unit, Great Ormond Street Hospital for Children NHS Trust, London, UK

Zoe Mead,

Department of Histopathology, Addenbrooke's NHS Trust Pathology Department, Addenbrooke's Hospital, Cambridge, UK

Oliver Quarrell,
Dept of Clinical Genetics, Sheffield Children's Hospital, Sheffield, UK

Richard Sayers,
Dept of Clinical Genetics, Sheffield Children's Hospital, Sheffield, UK

Martina Owens,
Molecular Genetics Department, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK

Charles Shaw-Smith,
Molecular Genetics Department, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK

Janet Lioy,
Division of Neonatology, The Children's Hospital of Philadelphia, The University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, USA

Eileen McKay,
Department of Pathology, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

Nicole de Leeuw,
Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands

Ilse Feenstra,
Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands

Liesbeth Spruijt,
Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands

Frances Elmslie,
South West Thames Regional Genetics Service, St George's University Hospital, London, UK

Timothy Thiruchelvam,
Critical Care and Cardiorespiratory Unit, Great Ormond Street Hospital NHS Trust, London, UK

Carlos A. Bacino,
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. Texas Children's Hospital, Houston, TX, USA

Claire Langston,
Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA

James R. Lupski,
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA. Texas Children's Hospital, Houston, TX, USA. Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA

Partha Sen,
Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA

Edwina Popek, and
Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA

Paweł Stankiewicz
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX, USA

Affiliations

Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX, USA

Genomic Medicine Department, MD Anderson Cancer Center, Houston, TX, USA

Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA

Division of Critical Care Medicine, Children's National Health System, Washington, DC, USA

Division of Critical Care Medicine, Children's National Health System, Washington, DC, USA

Department of Obstetrics, Gynecology, and Reproductive Sciences, Center for Medical Genetics and Genomics, Magee-Womens Hospital of UPMC, Pittsburgh, PA, USA. Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Department of Medical Genetics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, USA

Department of Medical Genetics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, USA

Department of Obstetrics, Gynecology, and Reproductive Sciences, Center for Medical Genetics and Genomics, Magee-Womens Hospital of UPMC, Pittsburgh, PA, USA. Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, PA, USA

Department of Pathology, Columbia University Medical Center, New York, NY, USA

Department of Pediatrics, Columbia University Medical Center, New York, NY, USA

Children's Hospital of New York-Presbyterian, New York, NY, USA

Division of Neonatology, Division of Maternal Fetal Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

INGEMM, Instituto de Genética Médica y Molecular, IdiPAZ, Madrid, Spain. CIBERER, ISCIII, Madrid, Spain

INGEMM, Instituto de Genética Médica y Molecular, IdiPAZ, Madrid, Spain. CIBERER, ISCIII, Madrid, Spain

INGEMM, Instituto de Genética Médica y Molecular, IdiPAZ, Madrid, Spain. CIBERER, ISCIII, Madrid, Spain

INGEMM, Instituto de Genética Médica y Molecular, IdiPAZ, Madrid, Spain. CIBERER, ISCIII, Madrid, Spain

Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, China. Department of Obstetrics and Gynaecology, and Centre for Genomic Sciences, The University of Hong Kong, Hong Kong, China

Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, China

Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, China

Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, China

Pediatric Pulmonary Unit, Department of Pediatrics, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Pediatric Pulmonary Unit, Department of Pediatrics, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Departments of Pediatrics, University of Alabama at Birmingham, Alabama, USA. Cell Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL, USA

Department of Surgery, Division of Pediatric Surgery, University of Alabama at Birmingham and Children's of Alabama, Birmingham, AL, USA

Department of Pathology, University of Alabama at Birmingham and Pathology and Laboratory Medicine Service, Children's of Alabama, Birmingham, AL, USA

Division of Medical Genetics, Department of Pediatrics, and Institute for Human Genetics, University of California San Francisco, San Francisco, CA, USA

Genetics Department, Kaiser Permanente San Jose Medical Center, San Jose, CA, USA

Department of Pediatrics, University of Rochester, Rochester, NY, USA

Division of Neonatology, University of Rochester, Rochester, NY, USA

Pathology and Laboratory Medicine, Rochester, Rochester, NY, USA

Department of Medical Genetics, University of British Columbia, Vancouver, Canada

Department of Medical Genetics, University of British Columbia, Vancouver, Canada

Department of Medical Genetics, University of British Columbia, Vancouver, Canada

Department of Medical Genetics, University of British Columbia, Vancouver, Canada

Department of Pathology, University of British Columbia, Vancouver, Canada

Department of Pediatrics, Baylor College of Medicine, San Antonio, TX, USA. Department of Molecular and Human Genetics, Baylor College of Medicine, San Antonio, TX, USA

Department of Pathology, Children's Hospital of San Antonio, San Antonio, TX, USA

Department of Pediatrics, Baylor College of Medicine, San Antonio, TX, USA

Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC, USA

Department of Pediatrics, Medical University of South Carolina, Charleston, SC, USA

Victorian Clinical Genetics Services, Murdoch Childrens Research Institute, Parkville, SA, Australia

Victorian Clinical Genetics Services, Murdoch Childrens Research Institute, Parkville, SA, Australia

Division of Pediatric Pulmonary Medicine, The Children's Heart Center Steven and Alexandra Cohen Children's Medical Center of New York, New York, NY, USA

Division of Pediatric Pathology, The Children's Heart Center Steven and Alexandra Cohen Children's Medical Center of New York, New York, NY, USA

Division of Medical Genetics, The Children's Heart Center Steven and Alexandra Cohen Children's Medical Center of New York, New York, NY, USA

Pediatric Cardiology, The Children's Heart Center Steven and Alexandra Cohen Children's Medical Center of New York, New York, NY, USA

Centre for Medical Genetics Ghent, University Hospital Ghent, Ghent, Belgium

Centre for Medical Genetics Ghent, University Hospital Ghent, Ghent, Belgium

Centre for Medical Genetics Ghent, University Hospital Ghent, Ghent, Belgium

Department of Obstetrics, Gynaecology, and Fertility, AZ St Jan Brugge, Brugge, Begium

Department of Anatomopathology, AZ St Jan Brugge, Brugge, Begium

Department of Pathology UZ Leuven, Leuven, Belgium

Center for Human Genetics, Cliniques Universitaires St-Luc, Universite Catholique de Louvain, Brussels, Belgium

Division of Pediatric Cardiology, Children's Mercy Hospital, Kansas City, MS, USA

Division of Clinical Genetics, Children's Mercy Hospital, Kansas City, MS, USA

Division of Paediatric Respiratory & Sleep Medicine, Lady Cilento Children's Hospital, Children's Health Queensland Hospital and Health Service, Brisbane, QLD, Australia. The University of Queensland, Brisbane, QLD, Australia

Division of Anatomical Pathology, Lady Cilento Children's Hospital, Children's Health Queensland Hospital and Health Service, Brisbane, QLD, Australia. Pathology Queensland, Brisbane, QLD, Australia

Division of Anatomical Pathology, Lady Cilento Children's Hospital, Children's Health Queensland Hospital and Health Service, Brisbane, QLD, Australia. Pathology Queensland, Brisbane, QLD, Australia

Clinical Genetics Department, Erasmus MC-Sophia, Rotterdam, Netherlands. Paediatric Surgery, Erasmus MC-Sophia, Rotterdam, Netherlands

Paediatric Surgery, Erasmus MC-Sophia, Rotterdam, Netherlands

Clinical Genetics Department, Erasmus MC-Sophia, Rotterdam, Netherlands

Institut de Pathologie et de Genetique, Gosselies, Belgium

James Cook University Hospital, Middlesborough, UK

Department of Paediatric Histopathology, Great Ormond Street Hospital for Children and UCL Institute of Child Health, London, UK

Clinical Genetics Unit, Great Ormond Street Hospital for Children and UCL Institute of Child Health, London, UK

Department of Molecular and Clinical Genetics, Royal Prince Alfred Hospital, Sydney, NSW, Australia

Obstetrics, Gynaecology and Neonatology, Royal Prince Alfred Hospital, Westmead, NSW, Australia

Obstetrics, Gynaecology and Neonatology, Royal Prince Alfred Hospital, Westmead, NSW, Australia

Cytogenetics Department, The Children's Hospital at Westmead, Westmead, NSW, Australia

Histopathology Department, The Children's Hospital at Westmead, Westmead, NSW, Australia

Clinical Genetics Unit, Great Ormond Street Hospital for Children NHS Trust, London, UK

Department of Histopathology, Addenbrooke's NHS Trust Pathology Department, Addenbrooke's Hospital, Cambridge, UK

Dept of Clinical Genetics, Sheffield Children's Hospital, Sheffield, UK

Dept of Clinical Genetics, Sheffield Children's Hospital, Sheffield, UK

Molecular Genetics Department, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK

Molecular Genetics Department, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK

Division of Neonatology, The Children's Hospital of Philadelphia, The University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, USA

Department of Pathology, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands

Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands

Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands

South West Thames Regional Genetics Service, St George's University Hospital, London, UK

Critical Care and Cardiorespiratory Unit, Great Ormond Street Hospital NHS Trust, London, UK

Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. Texas Children's Hospital, Houston, TX, USA

Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA

Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA. Texas Children's Hospital, Houston, TX, USA. Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA

Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA

Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA

Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX, USA

Acknowledgments

We thank Drs. K. Aagaard, A.L. Beaudet, J.W. Belmont, A.K. Groves, B. Lee, J.R. Neilson, S.E. Plon, I.B. van den Veyver, and H.Y. Zoghbi for helpful discussion and J.A. Rosenfeld-Mokry for critically reading the manuscript. This work was supported by grants awarded by the US National Heart, Lung, and Blood Institute (NHLBI) grant RO1HL101975 to PSt, NORD grants to PSz, US National Human Genome Research Institute (NHGRI)/NHLBI grant HG006542 to the Baylor-Hopkins Center for Mendelian Genomics, and National Institute of Neurological Disorders and Stroke (NINDS) grant NS058529 to JRL.

References

Araujo E Júnior, Palma-Dias R, Martins WP, Reidy K, da Silva Costa F. Congenital heart disease and adverse perinatal outcome in fetuses with confirmed isolated single functioning umbilical artery. J Obstet Gynaecol. 2015; 35:85–87. [PubMed: 25020205]

Barlow DP, Bartolomei MS. Genomic imprinting in mammals. Cold Spring Harb Perspect Biol. 2014; 6:a018382. [PubMed: 24492710]

Bartolomei MS, Ferguson-Smith AC. Mammalian genomic imprinting. Cold Spring Harb Perspect Biol. 2011; 3:a002592. [PubMed: 21576252]

Bishop NB, Stankiewicz P, Steinhorn RH. Alveolar capillary dysplasia. Am J Respir Crit Care Med. 2011; 184:172–179. [PubMed: 21471096]

Black BL, Olson EN. Transcriptional control of muscle development by myocyte enhancer factor-2 (MEF2) proteins. Annu Rev Cell Dev Biol. 1998; 14:167–196. [PubMed: 9891782]

Brand NJ. Myocyte enhancer factor 2 (MEF2). Int J Biochem Cell Biol. 1997; 29:1467–1470. [PubMed: 9570140]

Campbell IM, Shaw CA, Stankiewicz P, Lupski JR. Somatic mosaicism: implications for disease and transmission genetics. Trends Genet. 2015; 31(7):382–392. [PubMed: 25910407]

Campbell IM, Yuan B, Robberecht C, Pfundt R, Szafranski P, McEntagart ME, Nagamani SC, Erez A, Bartnik M, Wiśniowiecka-Kowalnik B, Plunkett KS, Pursley AN, Kang SH, Bi W, Lalani SR, Bacino CA, Vast M, Marks K, Patton M, Olofsson P, Patel A, Veltman JA, Cheung SW, Shaw CA, Vissers LE, Vermeesch JR, Lupski JR, Stankiewicz P. Parental somatic mosaicism is underrecognized and influences recurrence risk of genomic disorders. Am J Hum Genet. 2014; 95:173–182. [PubMed: 25087610]

Chang VW, Ho Y. Structural characterization of the mouse Foxf1a gene. Gene. 2001; 267:201–211. [PubMed: 11313147]

Chu D, Kakazu N, Gorrin-Rivas MJ, Lu HP, Kawata M, Abe T, Ueda K, Adachi Y. Cloning and characterization of LUN, a novel ring finger protein that is highly expressed in lung and specifically binds to a palindromic sequence. J Biol Chem. 2001; 276:14004–14013. [PubMed: 11278651]

Dello Russo P, Franzoni A, Baldan F, Puppin C, De Maglio G, Pittini C, Cattarossi L, Pizzolitto S, Damante G. A 16q deletion involving *FOXF1* enhancer is associated to pulmonary capillary hemangiomatosis. BMC Med Genet. 2015; 16:94. [PubMed: 26462560]

Dharmadhikari AV, Szafranski P, Kalinichenko VV, Stankiewicz P. Genomic and Epigenetic Complexity of the *FOXF1* Locus in 16q24.1: Implications for Development and Disease. Curr Genomics. 2015; 16:107–116. [PubMed: 26085809]

Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature. 2012; 485:376–380. [PubMed: 22495300]

Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet. 2014; 15:7–21. [PubMed: 24296535]

Ferguson-Smith AC. Genomic imprinting: the emergence of an epigenetic paradigm. Nat Rev Genet. 2011; 12:565–575. [PubMed: 21765458]

Galambos C, Sims-Lucas S, Ali N, Gien J, Dishop MK, Abman SH. Intrapulmonary vascular shunt pathways in alveolar capillary dysplasia with misalignment of pulmonary veins. Thorax. 2015; 70:84–85. [PubMed: 25052575]

Garabedian MJ, Wallerstein D, Medina N, Byrne J, Wallerstein RJ. Prenatal diagnosis of cystic hygroma related to a deletion of 16q24.1 with haploinsufficiency of *FOXF1* and *FOXC2* genes. Case Rep Genet. 2012; 2012:490408. [PubMed: 23074687]

Gregg C. Known unknowns for allele-specific expression and genomic imprinting effects. F1000Prime Rep. 2014; 6:75. [PubMed: 25343032]

Grote P, Wittler L, Hendrix D, Koch F, Währisch S, Beisaw A, Macura K, Bläss G, Kellis M, Werber M, Herrmann BG. The tissue-specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. Dev Cell. 2013; 24:206–214. [PubMed: 23369715]

Gu S, Yuan B, Campbell IM, Beck CR, Carvalho CM, Nagamani SC, Erez A, Patel A, Bacino CA, Shaw CA, Stankiewicz P, Cheung SW, Bi W, Lupski JR. *Alu*-mediated diverse and complex pathogenic copy-number variants within human chromosome 17 at p13.3. Hum Mol Genet. 2015; 24:4061–4077. [PubMed: 25908615]

Guo Y, Xu Q, Canzio D, Shou J, Li J, Gorkin DU, Jung I, Wu H, Zhai Y, Tang Y, Lu Y, Wu Y, Jia Z, Li W, Zhang MQ, Ren B, Krainer AR, Maniatis T, Wu Q. CRISPR Inversion of CTCF Sites Alters Genome Topology and Enhancer/Promoter Function. Cell. 2015; 162:900–910. [PubMed: 26276636]

Hamvas A, Nogee LM, Wegner DJ, Depass K, Christodoulou J, Bennetts B, McQuade LR, Gray PH, Deterding RR, Carroll TR, Kammesheidt A, Kasch LM, Kulkarni S, Cole FS. Inherited surfactant deficiency caused by uniparental disomy of rare mutations in the surfactant protein-B and ATP binding cassette, subfamily a, member 3 genes. J Pediatr. 2009; 155:854–859. [PubMed: 19647838]

Handrigan GR, Chitayat D, Lionel AC, Pinsk M, Vaags AK, Marshall CR, Dyack S, Escobar LF, Fernandez BA, Stegman JC, Rosenfeld JA, Shaffer LG, Goodenberger M, Hodge JC, Cain JE, Babul-Hirji R, Stavropoulos DJ, Yiu V, Scherer SW, Rosenblum ND. Deletions in 16q24.2 are associated with autism spectrum disorder, intellectual disability and congenital renal malformation. J Med Genet. 2013; 50:163–173. [PubMed: 23335808]

Hastings PJ, Lupski JR, Rosenberg SM, Ira G. Mechanisms of change in gene copy number. Nat Rev Genet. 2009; 10:551–564. [PubMed: 19597530]

Iascone M, Ciccone R, Galletti L, Marchetti D, Seddio F, Lincesso AR, Pezzoli L, Vetro A, Barachetti D, Boni L, Federici D, Soto AM, Comas JV, Ferrazzi P, Zuffardi O. Identification of de novo mutations and rare variants in hypoplastic left heart syndrome. Clin Genet. 2012; 81:542–554. [PubMed: 21457232]

Jacques PÉ, Jeyakani J, Bourque G. The majority of primate-specific regulatory sequences are derived from transposable elements. PLoS Genet. 2015; 9:e1003504.

Joza S, Wang J, Fox E, Hillman V, Ackerley C, Post M. Loss of semaphorin-neuropilin-1 signaling causes dysmorphic vascularization reminiscent of alveolar capillary dysplasia. Am J Pathol. 2012; 181:2003–2017. [PubMed: 23063659]

Kagoshima M, Ito T, Kitamura H, Goshima Y. Diverse gene expression and function of semaphorins in developing lung: positive and negative regulatory roles of semaphorins in lung branching morphogenesis. Genes to Cells. 2001; 6:559–571. [PubMed: 11442635]

Kalinichenko VV, Lim L, Shin B, Costa RH. Differential expression of forkhead box transcription factors following butylated hydroxytoluene lung injury. Am J Physiol Lung Cell Mol Physiol. 2001; 280:L695–L704. [PubMed: 11238010]

Kim IM, Zhou Y, Ramakrishna S, Hughes DE, Solway J, Costa RH, Kalinichenko VV. Functional characterization of evolutionarily conserved DNA regions in Forkhead box f1 gene locus. J Biol Chem. 2005; 280:37908–37916. [PubMed: 16144835]

Kohlhase J, Janssen B, Weidenauer K, Harms K, Bartels I. First confirmed case with paternal uniparental disomy of chromosome 16. Am J Med Genet. 2000; 91:190–191. [PubMed: 10756340]

Lai KM, Gong G, Atanasio A, Rojas J, Quispe J, Posca J, White D, Huang M, Fedorova D, Grant C, Miloscio L, Droguett G1, Poueymirou WT, Auerbach W, Yancopoulos GD, Frendewey D, Rinn J, Valenzuela DM. Diverse Phenotypes and specific transcription patterns in twenty mouse lines with ablated lincRNAs. PLoS One. 2015; 10:e0125522. [PubMed: 25909911]

Langston C. Misalignment of pulmonary veins and alveolar capillary dysplasia. Pediatr Pathol. 1991; 11:163–70. [PubMed: 2014189]

Luedi PP, Dietrich FS, Weidman JR, Bosko JM, Jirtle RL, Hartemink AJ. Computational and experimental identification of novel human imprinted genes. Genome Res. 2007; 17:1723–1730. [PubMed: 18055845]

Lupiáñez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, Horn D, Kayserili H, Opitz JM, Laxova R, Santos-Simarro F, Gilbert-Dussardier B, Wittler L, Borschiwer M1, Haas SA, Osterwalder M, Franke M, Timmermann B, Hecht J, Spielmann M, Visel A, Mundlos S. Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. Cell. 2015; 161:1012–1025. [PubMed: 25959774]

Mahlapuu M, Enerback S, Carlsson P. Haploinsufficiency of the forkhead gene Foxf1, a target for sonic hedgehog signalling, causes lung and foregut malformations. Development. 2001; 128:2397–2406. [PubMed: 11493558]

Oyanagi H, Takenaka K, Ishikawa S, Kawano Y, Adachi Y, Ueda K, Wada H, Tanaka F. Expression of LUN gene that encodes a novel RING finger protein is correlated with development and progression of non-small cell lung cancer. Lung Cancer. 2004; 46:21–28. [PubMed: 15364129]

Parris T, Nik AM, Kotecha S, Langston C, Helou K, Platt C, Carlsson P. Inversion upstream of *FOXF1* in a case of lethal alveolar capillary dysplasia with misalignment of pulmonary veins. Am J Med Genet A. 2013; 161A:764–770. [PubMed: 23444129]

Prothro SL, Plosa E, Markham M, Szafranski P, Stankiewicz P, Killen SA. Prenatal Diagnosis of Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins. J Pediatr. 2016; 170:317–8. [PubMed: 26703872]

Reiter J, Szafranski P, Breuer O, Perles Z, Dagan T, Stankiewicz P, Karem E. Variable Phenotypic Presentation of a Novel *FOXF1* Missense Mutation in a Single Family. Pediatr Pulmonol. 2016 in press.

Sauvageau M, Goff LA, Lodato S, Bonev B, Groff AF, Gerhardinger C, Sanchez-Gomez DB, Hacisuleyman E, Li E, Spence M, Liapis SC, Mallard W, Morse M, Swerdel MR, D'Ecclessis MF, Moore JC, Lai V, Gong G, Yancopoulos GD, Frendewey D, Kellis M, Hart RP, Valenzuela DM, Arlotta P, Rinn JL. Multiple knockout mouse models reveal lincRNAs are required for life and brain development. Elife. 2013; 2:e01749. [PubMed: 24381249]

Sen P, Dharmadhikari AV, Majewski T, Mohammad MA, Kalin TV, Zabielska J, Ren X, Bray M, Brown HM, Welty S, Thevananther S, Langston C, Szafranski P, Justice MJ, Kalinichenko VV, Gambin A, Belmont J, Stankiewicz P. Comparative analyses of lung transcriptomes in patients with alveolar capillary dysplasia with misalignment of pulmonary veins and in foxf1 heterozygous knockout mice. PLoS One. 2014; 9:e94390. [PubMed: 24722050]

Sen P, Gerychova R, Janku P, Jezova M, Valaskova I, Navarro C, Silva I, Langston C, Welty S, Belmont J, Stankiewicz P. A familial case of alveolar capillary dysplasia with misalignment of pulmonary veins supports paternal imprinting of *FOXF1* in human. Eur J Hum Genet. 2013a; 21:474–477. [PubMed: 22990143]

Sen P, Thakur N, Stockton DW, Langston C, Bejjani BA. Expanding the phenotype of alveolar capillary dysplasia (ACD). J Pediatr. 2004; 145:646–651. [PubMed: 15520767]

Sen P, Yang Y, Navarro C, Silva I, Szafranski P, Kolodziejska KE, Dharmadhikari AV, Mostafa H, Kozakewich H, Kearney D, Cahill JB, Whitt M, Bilic M, Margraf L, Charles A, Goldblatt J, Gibson K, Lantz PE, Garvin AJ, Petty J, Kiblawi Z, Zuppan C, McConkie-Rosell A, McDonald MT, Peterson-Carmichael SL, Gaede JT, Shivanna B, Schady D, Friedlich PS, Hays SR, Palafoll IV, Siebers-Renelt U, Bohring A, Finn LS, Siebert JR, Galambos C, Nguyen L, Riley M, Chassaing N, Vigouroux A, Rocha G, Fernandes S, Brumbaugh J, Roberts K, Ho-Ming L, Lo IF, Lam S, Gerychova R, Jezova M, Valaskova I, Fellmann F, Afshar K, Giannoni E, Muhlethaler V,

Liang J, Beckmann JS, Lioy J, Deshmukh H, Srinivasan L, Swarr DT, Sloman M, Shaw-Smith C, van Loon RL, Hagman C, Sznajer Y, Barrea C, Galant C, Detaille T, Wambach JA, Cole FS, Hamvas A, Prince LS, Diderich KE, Brooks AS, Verdijk RM, Ravindranathan H, Sugo E, Mowat D, Baker ML, Langston C, Welty S, Stankiewicz P. Novel *FOXF1* mutations in sporadic and familial cases of alveolar capillary dysplasia with misaligned pulmonary veins imply a role for its DNA binding domain. Hum Mutat. 2013b; 34:801–811. [PubMed: 23505205]

Stankiewicz P, Sen P, Bhatt SS, Storer M, Xia Z, Bejjani BA, Ou Z, Wiszniewska J, Driscoll DJ, Maisenbacher MK, Bolivar J, Bauer M, Zackai EH, McDonald-McGinn D, Nowaczyk MM, Murray M, Hustead V, Mascotti K, Schultz R, Hallam L, McRae D, Nicholson AG, Newbury R, Durham-O'Donnell J, Knight G, Kini U, Shaikh TH, Martin V, Tyreman M, Simonic I, Willatt L, Paterson J, Mehta S, Rajan D, Fitzgerald T, Gribble S, Prigmore E, Patel A, Shaffer LG, Carter NP, Cheung SW, Langston C, Shaw-Smith C. Genomic and genic deletions of the FOX gene cluster on 16q24.1 and inactivating mutations of *FOXF1* cause alveolar capillary dysplasia and other malformations. Am J Hum Genet. 2009; 84:780–791. [PubMed: 19500772]

Szafranski P, Dharmadhikari AV, Brosens E, Gurha P, Kolodziejska KE, Zhishuo O, Dittwald P, Majewski T, Mohan KN, Chen B, Person RE, Tibboel D, de Klein A, Pinner J, Chopra M, Malcolm G, Peters G, Arbuckle S, Guiang SF 3rd, Hustead VA, Jessurun J, Hirsch R, Witte DP, Maystadt I, Sebire N, Fisher R, Langston C, Sen P, Stankiewicz P. Small noncoding differentially methylated copy-number variants, including lncRNA genes, cause a lethal lung developmental disorder. Genome Res. 2013a; 23:23–33. [PubMed: 23034409]

Szafranski P, Dharmadhikari AV, Wambach JA, Towe CT, White FV, Grady RM, Eghtesady P, Cole FS, Deutsch G, Sen P, Stankiewicz P. Two deletions overlapping a distant FOXF1 enhancer unravel the role of lncRNA LINC01081 in etiology of alveolar capillary dysplasia with misalignment of pulmonary veins. Am J Med Genet A. 2014; 164A:2013–2019. [PubMed: 24842713]

Szafranski P, Yang Y, Nelson MU, Bizzarro MJ, Morotti RA, Langston C, Stankiewicz P. Novel *FOXF1* deep intronic deletion causes lethal lung developmental disorder, alveolar capillary dysplasia with misalignment of pulmonary veins. Hum Mutat. 2013b; 34:1467–1471. [PubMed: 23943206]

Tasha I, Brook R, Frasure H, Lazebnik N. Prenatal detection of cardiac anomalies in fetuses with single umbilical artery: diagnostic accuracy comparison of maternal-fetal-medicine and pediatric cardiologist. J Pregnancy. 2014; 2014:265421. [PubMed: 24719766]

Warzecha CC, Sato TK, Nabet B, Hogenesch JB, Carstens RP. ESRP1 and ESRP2 are epithelial cell-type-specific regulators of FGFR2 splicing. Mol Cell. 2009; 33:591–601. [PubMed: 19285943]

Yong PJ, Marion SA, Barrett IJ, Kalousek DK, Robinson WP. Evidence for imprinting on chromosome 16: the effect of uniparental disomy on the outcome of mosaic trisomy 16 pregnancies. Am J Med Genet. 2002; 112:123–132. [PubMed: 12244544]

Yu S, Shao L, Kilbride H, Zwick DL. Haploinsufficiencies of *FOXF1* and *FOXC2* genes associated with lethal alveolar capillary dysplasia and congenital heart disease. Am J Med Genet A. 2010; 152A:1257–1262. [PubMed: 20425831]

Zufferey F, Martinet D, Osterheld MC, Niel-Bütschi F, Giannoni E, Schmutz NB, Xia Z, Beckmann JS, Shaw-Smith C, Stankiewicz P, Langston C, Fellmann F. 16q24.1 microdeletion in a premature newborn: usefulness of array-based comparative genomic hybridization in persistent pulmonary hypertension of the newborn. Pediatr Crit Care Med. 2011; 12:e427–432. [PubMed: 21572369]

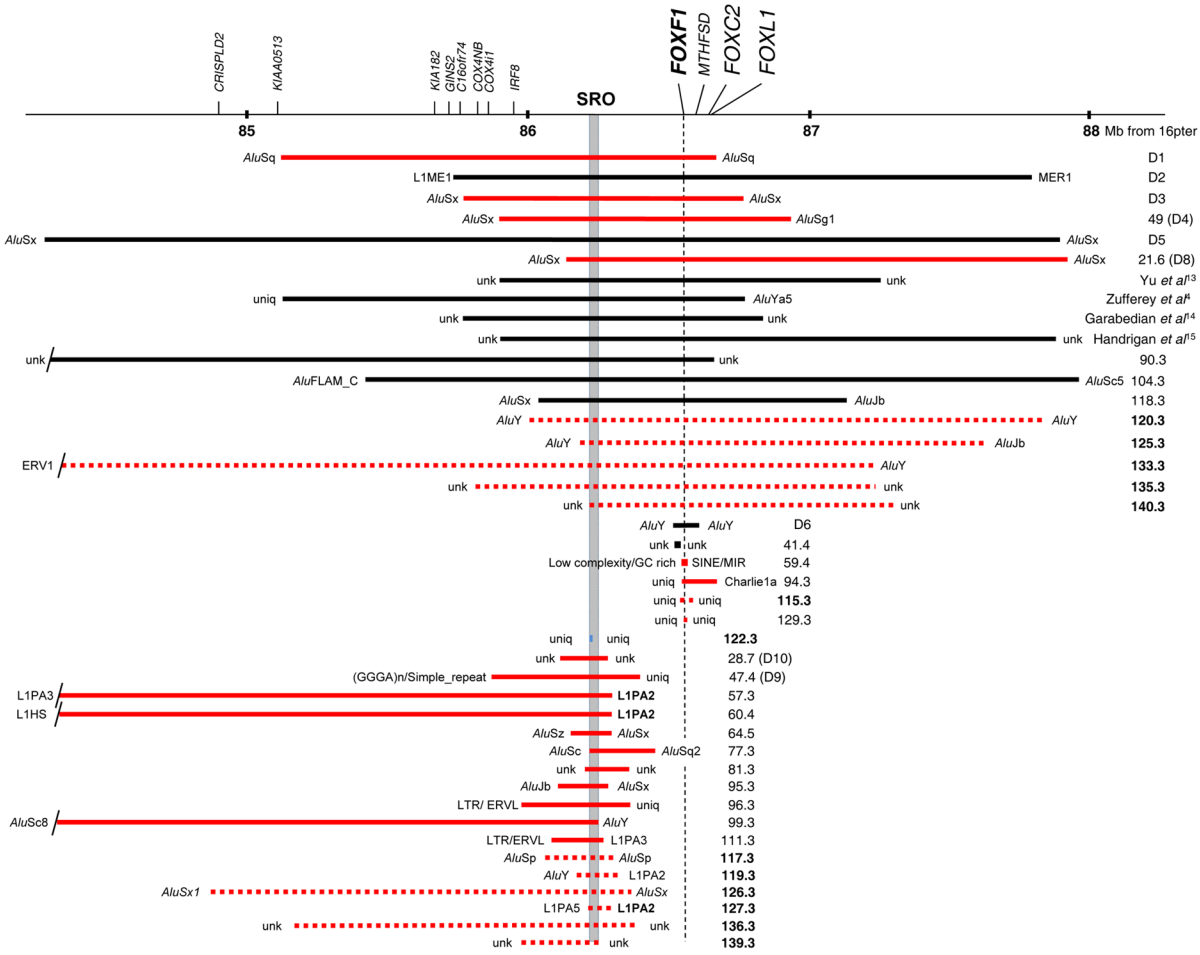

Fig. 1.

A compilation of chromosome 16q24.1 deletions pathogenic for ACDMPV. Deletions which occurred on maternal chromosome 16, are shown in red, the deletion on paternal chromosome is shown in blue, and deletions, for which parental origin could not be determined, are shown in black. Numbers refer to ACDMPV cases. Locations of deletion breakpoints (BPs) are indicated by names of flanking repetitive elements. Abbreviations: SRO, (smallest deletion overlap - delineating upstream enhancer region); unk, unknown sequence; uniq, unique sequence. Note that most of the deletions are flanked by retrotransposons, mainly by *Alu* repetitive segments. The LIPA2 element (chr16:86,266,902-86,272,916, GRCh37/hg19) within *LINC01081* at three distal deletion breakpoints in patients 57.3, 60.4, and 127.3 is bolded (Supplemental Table S1). Thirteen novel deletions are dashed and their number bolded to distinguished from the published cases.

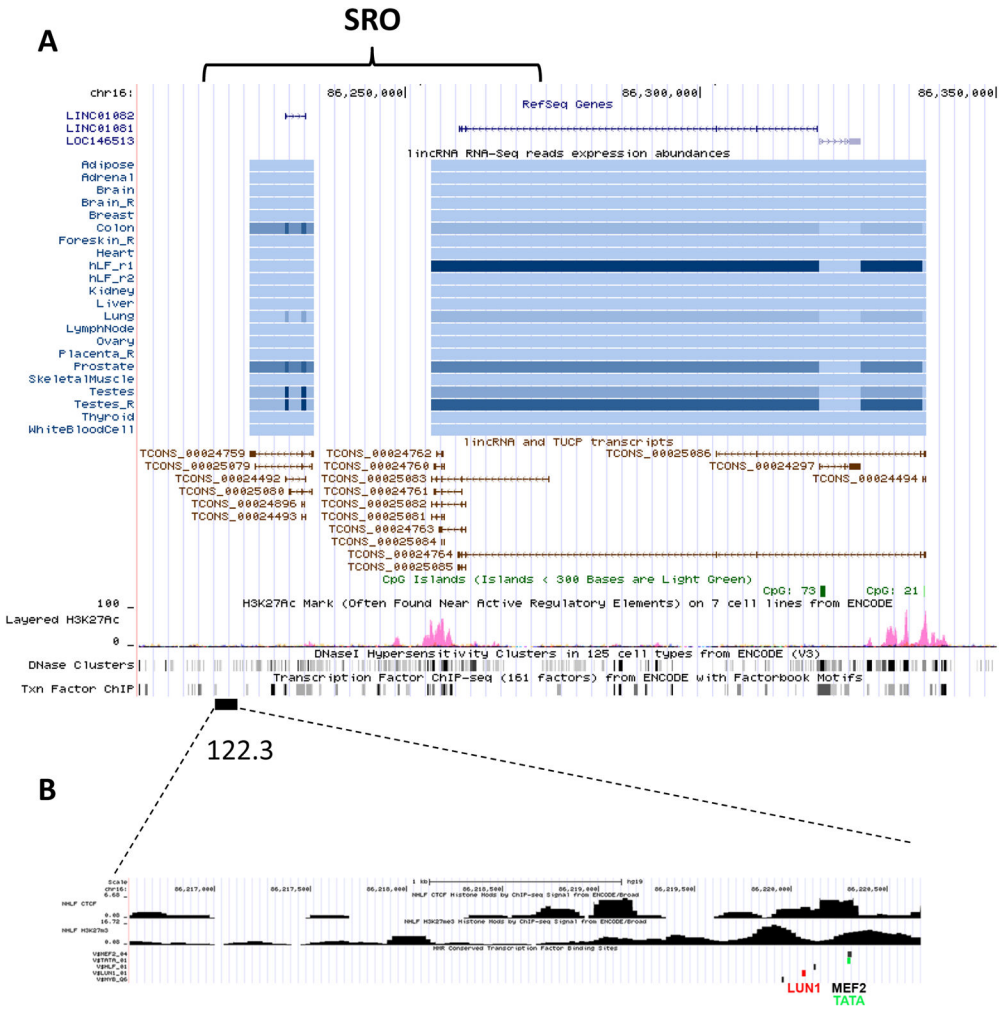

Fig. 2.

UCSC browser display of the enhancer region upstream to *FOXF1*. **a** An ~ 60 kb SRO showing overlapping *LINC01081* and *LINC01082,* CpG islands, H3K27Ac methylation, DNaseI hypersensitivity clusters, and transcription factor ChIP-Seq data. **b** The ~ 4.1 kb deletion CNV identified in pt 122.3 maps to the *FOXF1* upstream enhancer region. Location of the transcription factor and CTCF binding sites, and a diagram of histone modification in the deleted region is shown.

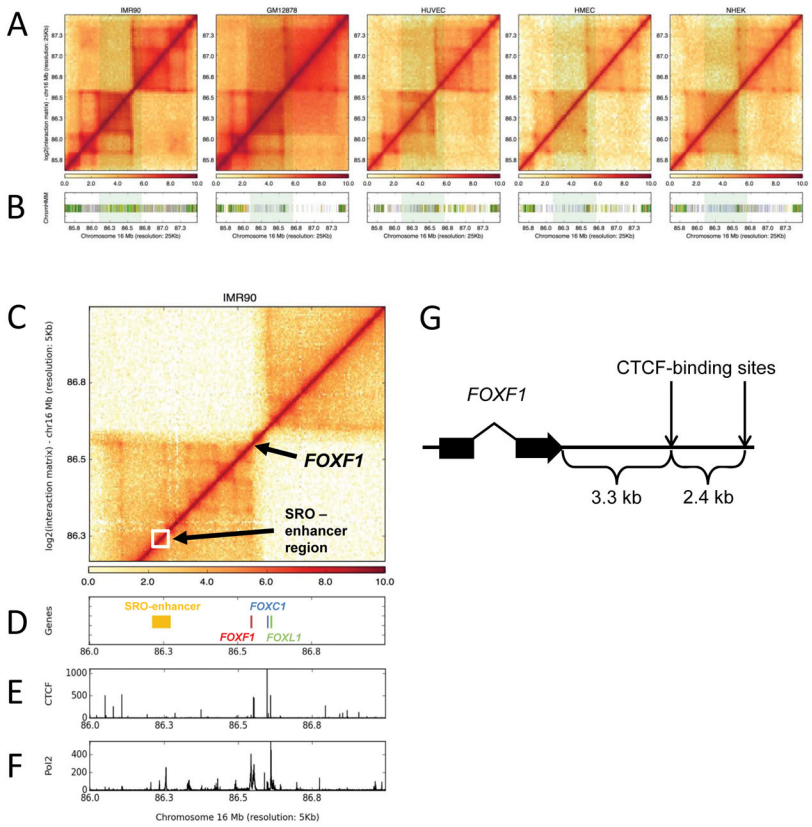

Fig. 3.

Genomic *in cis* interactions at chromosome 16q24.1. **a** Hi-C contact maps are visualized as heatmaps for IMR-90, GM12878, HUVEC, NHEK, and HMEC cell lines. **b** Tiles mark active TSS (red), transcribed (green), enhancer (yellow), low (gray) and heterochromatin (purple) state calls with ChromHMM within the represented locus. Shaded region highlights the active chromatin region in IMR-90 cells whereas the same locus in not enriched for active chromatin states in other cell types, especially on the left-side of TAD boundary. **c** Zoomed-in view of Hi-C contact map around the *FOXF1* locus in IMR-90 cells at 5 kb resolution. **d** Tiles mark genomic locations of SRO-enhancer region (yellow) and *FOXF1* (red), *FOXC1* (blue) and *FOXL1* (green) genes. **e** Histogram represents CTCF ChIP-seq enrichment levels within the visualized locus. **f** Histogram represents RNA PolII ChIP-Seq enrichment levels. **g** Positions of two strong CTCF-binding sites (chr16:86,551,417–528 and 86,553,812–944; hg19) at the TAD boundary, 3.3 and 5.7 kb downstream of the 3′ end of *FOXF1* (chr16: 86,548,070; hg19).

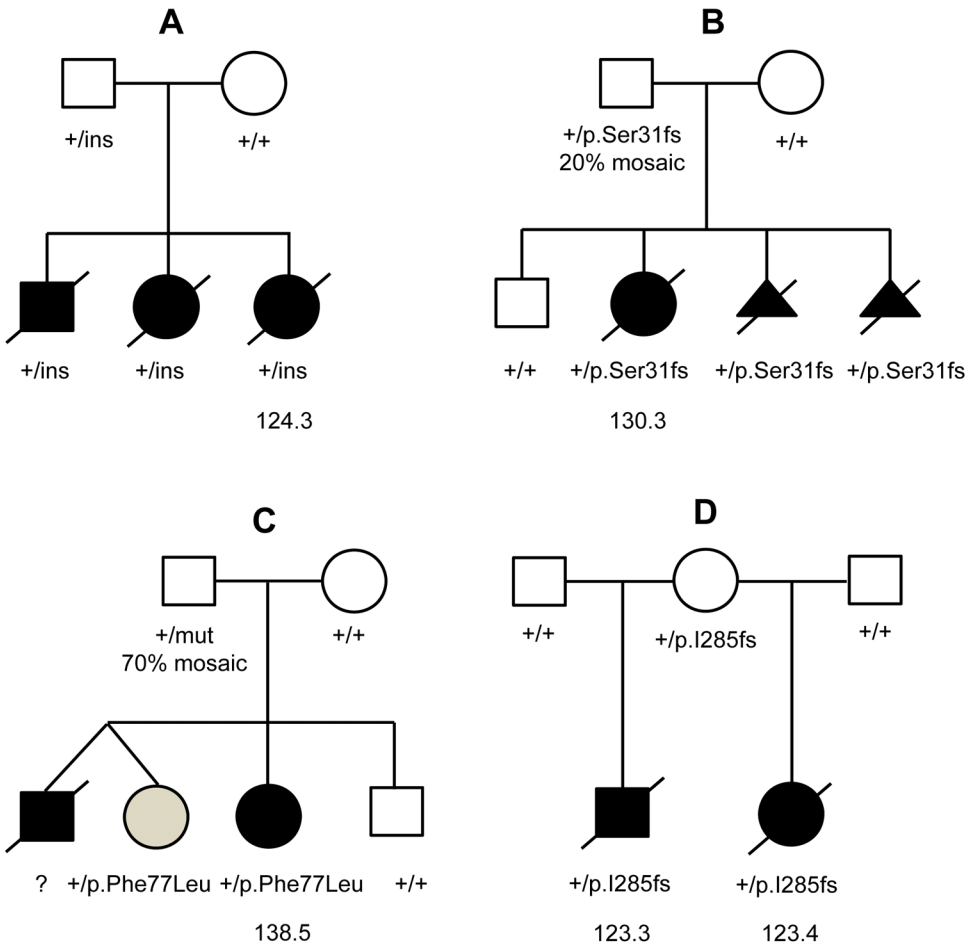

Fig. 4.

Unusual inheritance and manifestation of pathogenic variants in the *FOXF1* locus. Pedigrees of ACDMPV families with reportedly healthy parent transmitting *FOXF1* pathogenic variant to their affected children. **a** Family 124 with the complex genomic insertion rearrangement (ins) within the 5′ non-coding part of the *FOXF1* 1st exon. **b** Family 130 with the frameshift mutation within the *FOXF1* 1st exon. **c** Family 138 with the pathogenic missense mutation in the *FOXF1* 1st exon transmitted by healthy father, 70% mosaic in peripheral blood. A twin sister (shaded symbol) of the deceased ACDMPV patient has partial anomalous venous return (Reiter et al. (2016). **d** Family 123 with the frameshift mutation transmitted by healthy mother, likely germline mosaic for the mutation.

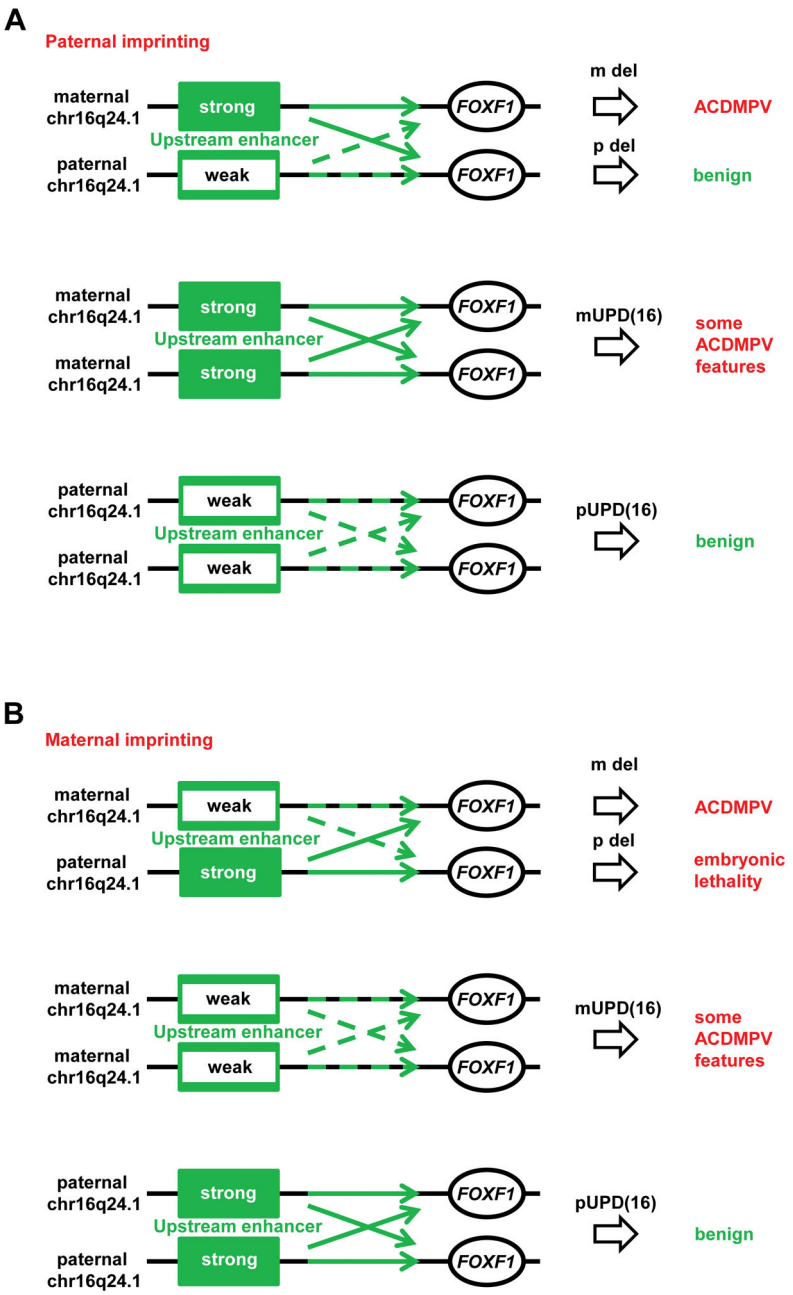

Fig. 5.

Proposed model of ACDMPV and UPD(16) etiologies due to (**a**) paternal or (**b**) maternal imprinting of the *FOXF1* upstream enhancer on chromosome 16q24.1. Epigenetic modification of the enhancer reduces its ability to stimulate *FOXF1* transcription. In paternal imprinting model (**a**), the majority of *FOXF1* transcription depends on the strong enhancer located on the maternal chromosome 16. Thus deletion of the enhancer on this chromosome would significantly reduce *FOXF1* expression and cause ACDMPV whereas the deletion on the paternal chromosome 16q24.1 would reduce *FOXF1* expression less with no clinical consequences. Consequently, maternal UPD(16) would increase *FOXF1* expression level

(pathogenic) whereas paternal UPD(16) would slightly decrease *FOXF1* expression level (benign). In the maternal imprinting model (**b**), the upstream deletion of the weak enhancer on the maternal chromosome 16 would reduce *FOXF1* expression, resulting in ACDMPV. The deletion of the strong enhancer on the paternal chromosome would reduce *FOXF1* expression would be embryonic lethal. According to this model, in maternal UPD(16), the level of *FOXF1* would be insufficient to prevent development of some ACDMPV features and other anomalies typical for maternal UPD(16). In contrast, point mutations in *FOXF1* are found on both parental alleles, suggesting *in-trans* function of the *FOXF1* enhancer, likely using lncRNAs (Szafranski et al. 2013a).

Table 1

Additional non-lung clinical features in 37 ACDMPV patients with the reported and novel pathogenic deletions involving the *FOXF1* locus in chromosome 16q24.1. Features present in greater than 10% of patients are bolded.

Cardiac	**Frequency**	**Gastrointestinal**	**Frequency**	**Genitourinary**	**Frequency**	**Other**	**Frequency**
Patent ductus arteriosus	17 (46%)	Intestinal malrotation	8 (22%)	Hydronephrosis	9 (24%)	**Single umbilical artery**	7 (19%)
Atrioventricular septal defect	6 (16%)	Annular pancreas	4 (11%)	Renal pelviectasis	5 (14%)	Broad flat nasal bridge	2 (5%)
Hypoplastic left heart syndrome	5 (14%)	Imperforate anus	3 (8%)	Hydroureter	4 (11%)	Deep-set eyes	2 (5%)
Ventricular septal defect	5 (14%)	Duodenal atresia	2 (5%)	Hypospadias	2 (5%)	Butterfly vertebra	2 (5%)
Atrial septal defect	4 (11%)	Duodenal dilation proximal to the pancreas	1 (3%)	Bicornuate uterus with cervical duplication/Uterus didelphys	2 (5%)	Cystic hygroma	2 (5%)
Patent foramen ovale	3 (8%)	Absent spleen	1 (3%)	Tortuous dilated ureters and thickened urinary bladder wall,	1 (3%)	Fetal hydrops	2 (5%)
Aortic coarctation	3 (8%)	Abnormal placement of anus	1 (3%)	Right renal cyst extending into the abdomen	1 (3%)	Segmental abnormality of T10 vertebral body	1 (3%)
Bicuspid aortic valve	3 (8%)	Esophageal atresia	1 (3%)			Posterior rib fusions	1 (3%)
Persistent left superior vena cava	3 (8%)	Tracheoesophageal fistula	1 (3%)			Brachycephaly	1 (3%)
Tetralogy of Fallot	2 (5%)	Adhesions between bowel loops, second part of duodenum and gallbladder	1 (3%)			Cleft lip	1 (3%)
Significant R/L shunt	2 (5%)	Stomach and initial part of intestines malformed and tubular in nature	1 (3%)			Cleft palate	1 (3%)
Hypoplasia of mitral valve	2 (5%)	Suspected bowel obstruction	1 (3%)			Pleural effusion	1 (3%)
Pulmonary artery stenosis	2 (5%)	Shortened mesenteric root with kinking and luminal narrowing of the small intestine	1 (3%)			Polyhydramnios	1 (3%)
Hypoplastic aortic arch	2 (5%)	Omphalocele with a diameter of 6 cm only intestines present	1 (3%)			Hypertelorism	1 (3%)
Right ventricular dilatation and hypertrophy	2 (5%)					Bilateral choroid plexus cysts	1 (3%)
Hypoplasia of left ventricle	2 (5%)					Mild retrognathia	1 (3%)
Interrupted aortic arch	1 (3%)					Thickened nuchal fold	1 (3%)
Dilated pulmonary artery	1 (3%)					Bilateral cerebellar heterotopia	1 (3%)

Cardiac	Frequency	Gastrointestinal	Frequency	Genitourinary	Frequency	Other	Frequency
Parachute configuration of mitral valve with mild dilation of the left ventricle	1 (3%)					Hypoglycaemia	1 (3%)
Dysplastic tricuspid valve and two mitral valve leaflets	1 (3%)					Hypotonia	1 (3%)
Pulmonary valve atresia	1 (3%)						
Right ventricular outflow tract obstruction							

Table 2

WES-detected SNVs identified in three families with ACDMPV patients negative for *FOXF1* mutations and CNVs on chromosome 16q24.1.

Gene	Protein	Phenotype	Variant			SIFT	Polyphen2	MutationTaster	RadialSVM	Inheritance	Family
ZMYND11	Transcriptional repressor	Mental retardation, autosomal dominant 30 (OMIM 616083)	g.10:298327_T>C	c.1564T>C	p.Trp522Arg	D	D	D	D	*de novo*	114
SLC50A1	Sugar transporter	-	g.1:155110692_C>T	c.463C>T	p.Arg155Cys	D	B	D	T	*de novo*	121
ESRP1	Epithelial RNA splicing regulator	-	g.8:95677280_A>G	c.881A>G	p.Tyr294Cys	D	D	D	T	*de novo*	121
MPRIP	Myosin phosphatase Rho-interacting protein	-	g.17:17039561_CCA GCAGCAG>C	c.533_542delinsC	p.Ser179_Ser173del	-	-	-	-	*de novo*	121
DOCK8	Dedicator of cytokinesis	Hyper-IgE recurrent infection syndrome, autosomal recessive (OMIM 243700)	g.9:386371_C>CT	c.2819_2820insT	p.Gly941TrpfsTer943	-	-	-	-	*de novo*	121
PLXNB2	Semaphorin transmembrane receptor	-	g.22:50728383_G>C	c.631C>G	p.Leu211Val	T	B	D	T	paternal	121
			g.22:50720372_C>T	c.3256G>A	p.Ala1086Thr	T	B	N	T	paternal	128

D, deleterious; B, benign; T, tolerant; N, neutral