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Genomic and Epigenetic Complexity of the *FOXF1* Locus in 16q24.1: Implications for Development and Disease

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Abstract: The *FOXF1* (Forkhead box F1) gene, located on chromosome 16q24.1 encodes a member of the FOX family of transcription factors characterized by a distinct forkhead DNA binding domain. FOXF1 plays an important role in epithelium-mesenchyme signaling, as a downstream target of Sonic hedgehog pathway. Heterozygous point mutations and genomic deletions involving *FOXF1* have been reported in newborns with a lethal lung developmental disorder, Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV). In addition, genomic deletions upstream to *FOXF1* identified in ACDMPV patients have revealed that *FOXF1* expression is tightly regulated by distal tissue-specific enhancers. Interestingly, *FOXF1* has been found to be incompletely paternally imprinted in human lungs; characterized genomic deletions arose *de novo* exclusively on maternal chromosome 16, with most of them being *Alu-Alu* mediated. Regulation of *FOXF1* expression likely utilizes a combination of chromosomal looping, differential methylation of an upstream CpG island overlapping GLI transcription factor binding sites, and the function of lung-specific long non-coding RNAs (lncRNAs). *Foxf1* knock-out mouse models demonstrated its critical role in mesoderm differentiation and in the development of pulmonary vasculature. Additionally, epigenetic inactivation of *FOXF1* has been reported in breast and colorectal cancers, whereas overexpression of *FOXF1* has been associated with a number of other human cancers, e.g. medulloblastoma and rhabdomyosarcoma. Constitutional duplications of *FOXF1* have recently been reported in congenital intestinal malformations. Thus, understanding the genomic and epigenetic complexity at the *FOXF1* locus will improve diagnosis, prognosis, and treatment of ACDMPV and other human disorders associated with *FOXF1* alterations.

Keywords: ACDMPV, Gene regulation, Genomic-imprinting, Long non-coding RNA, Lung development, Pulmonary vasculature.

INTRODUCTION

The superfamily of Forkhead Box (FOX) transcription factors in mammals includes 50 members that share a common, evolutionary conserved winged helix DNA binding domain [1, 2]. To date, 19 subfamilies (A-S) have been identified in this superfamily [3]. The forkhead domain contains three N-terminal α-helices (H1–3), three β-strands, and two C-terminal region loops (W1–2) comprising the winged helix (forkhead) structure [4]. In the human genome, 52% (26/50) of the FOX genes are organized in nine clusters, e.g. *FOXE3-FOXD2* (1p33), *FOXQ1-FOXF2-FOXC1* (6p25.3), and *FOXF1-FOXC2-FOXL1* (16q24.1). The focus of this review is genomic and epigenetic complexity in the regulation of Forkhead Box F1 (*FOXF1)*, previously known as Forkhead RElated ACtivator (*FREAC-1)* or Hepatocyte nuclear factor 3/fork head homolog (*HFH-8*), as well as functional consequences of genetic variants involving *FOXF1* in human development and disease.

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Expression Pattern

Expression studies in humans have shown that *FOXF1* is mostly expressed in fetal and adult lungs, neonate lung mesenchymal stromal cells, placenta, and prostate tissue [5-7]. In mice, *Foxf1* expression initiates at embryonic day 6.5 (E6.5) in the extra-embryonic and lateral plate mesoderm [8]. Later in embryonic development, *Foxf1* expression is found in the septum transversum mesenchyme and splanchnic mesoderm, ultimately being expressed in the mesenchyme surrounding developing epithelium of the respiratory tract, oral cavity, and urinary and digestive systems [8-10]. In mouse embryonic lungs, *Foxf1* expression is localized in mesenchyme-derived cells, including endothelial cells and peribronchiolar smooth muscle cells [11, 12]. Additional sites of *Foxf1* expression include the mesenchyme of the brain, neural crest, cardiac cushion, as well as endothelial cells of the yolk sac, and embryonic regions of the placenta [12-14, 10]. In adult mice, *Foxf1* continues to be expressed in alveolar endothelial cells [12, 15], stellate cells of the liver [16], and visceral smooth muscle cells surrounding trachea, bronchi, stomach, small intestine, colon, and gallbladder [8-10, 12, 15, 16]. Additionally *Foxf1* is expressed in adult mice in the pituitary gland, eyes, and a subset of cortical and cerebellar astrocytes

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[13]. *FOXF1* has also been identified as a novel marker of nucleus pulposus (NP) cells and is used to determine the differentiation of mesenchymal stem cells (MSCs) to NP cells [17].

Role of *Foxf1* in Mouse Embryonic Development

To date, two different *Foxf1* knockout mouse lines have been described [11, 18, 19]. *Foxf1*$^{-/-}$ mice are embryonic lethal at E9.5 due to defects in mesodermal differentiation and cell adhesion [18]. The embryos fail to turn and exhibit extra-embryonic defects such as lack of vasculogenesis in the yolk sac and allantois and failure of chorioallantoic fusion. Haploinsufficiency of *Foxf1* in *Foxf1*$^{+/-}$ mice causes 90% perinatal lethality on a CD-1 mouse background [19]. The *Foxf1*$^{+/-}$ phenotype was associated with lung hypoplasia and various tracheal abnormalities such as esophageal atresia and tracheo-esophageal fistula. Mahlapuu *et al.* [19] also showed that *Foxf1* plays a role in epithelium-mesenchyme cross talk during lung development as a downstream target of sonic hedgehog (*Shh*) (Fig. **1**). This was demonstrated by the lack of *Foxf1* expression in lungs, foregut, and sclerotomes of *Shh*$^{-/-}$ embryos and the activation of *Foxf1* by exogenous SHH in lung organ explants. Additionally, SHH has been shown to activate expression of *Bmp4* during primary vascular tube formation via FOXF1 [20]. In the developing stomach and intestine, *Foxf1* along with another FOX transcription gene *Foxl1*, controls epithelial proliferation as a target of GLI2, which functions downstream of SHH [21]. Additionally, *Foxf1* was found to be upregulated in *Shh*$^{-/-}$; *Gli3*$^{-/-}$ lungs relative to Shh$^{-/-}$ lungs, suggesting that GLI3 is a potential repressor of *Foxf1*, independent of SHH [22].

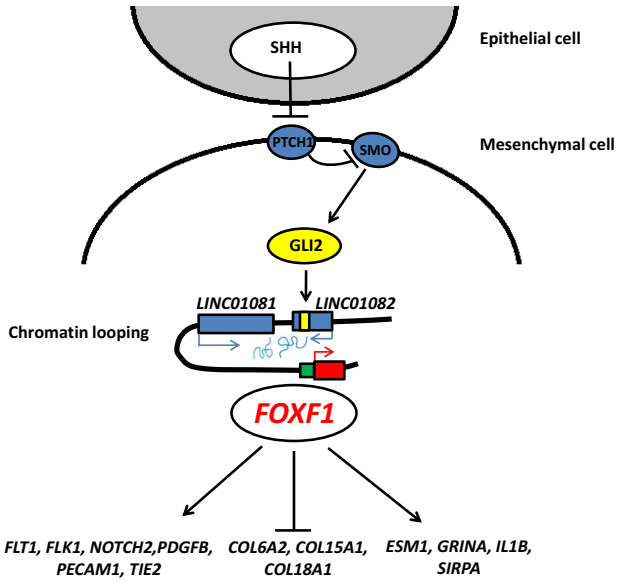

Fig. (1). Epithelial–mesenchymal interactions mediated by Sonic Hedgehog pathway in embryonic lung. *FOXF1* expression is regulated by SHH, GLI2, and lncRNAs. Downstream effectors of FOXF1 include notch, collagen and endothelial genes.

On a Swiss black background, 55% of *Foxf1*$^{+/-}$ mice die perinatally due to lung hemorrhages and respiratory insufficiency [11]. Additional pulmonary defects in *Foxf1*$^{+/-}$ embryos include fusion of lung lobes and vessels [23]. NOTCH2 and its downstream target HES1 are downregulated in *Foxf1*$^{+/-}$ mouse lungs, suggesting that FOXF1 acts upstream of Notch signaling associated with vascular stabilization [24]. *Foxf1*$^{+/-}$ mice that survived after birth exhibited pulmonary mastocytosis, enhanced pulmonary inflammation, and abnormal lung repair after chemically-induced or allergen-mediated lung injury [25, 26]. *Foxf1*$^{+/-}$ mice also display defects in gall bladder development [10]. Gall bladders in *Foxf1*$^{+/-}$ mice are smaller in size with severe structural abnormalities such as a deficient external smooth muscle cell layer. In addition, *Foxf1*$^{+/-}$ mice exhibit defective stellate cell activation and abnormal liver regeneration following CCl4 injury [16].

Tissue-specific knock out of *Foxf1* using *Tie2-Cre* transgene (endothelium and hematopoietic lineage specific) also leads to embryonic lethality in mice [12]. *Tie2-Cre Foxf1fl/fl* mice die around E13.5-E16.5 exhibiting growth retardation, polyhydramnios, cardiac ventricular hypoplasia, and vascular abnormalities in the lung, placenta, and yolk sac. Endothelial specific deletion of *Foxf1* (*Pdgfb-CreER*) at E9.5 was sufficient to cause polyhydramnios and reduced vascular branching in the placenta, yolk sac, and lung of E12.5 embryos. Ablation of *Foxf1* during the postnatal period (P0-P2) using *Pdgfb-CreER* impaired retinal angiogenesis [12]. Smooth muscle cell specific knockout of *Foxf1* (smMHC-Cre) causes neonatal lethality and the loss of differentiated smooth muscle layers in esophagus [27]. Most recently, *Foxf1* along with another forkhead gene, *Foxf2*, has been shown to regulate cardiac septation in mouse embryos. Atrioventricular septal defects were found in *Foxf1*$^{+/-}$; *Foxf2*$^{+/-}$ compound heterozygote embryos at E14.5 [28].

Interestingly, mice that overexpress *Foxf1* by knocking-in *Foxf1* at the ROSA26 locus also exhibit embryonic lethality. *ROSA26-Lox-Stop-Lox (LSL)-Foxf1* mice mated to *CMV-cre* mice to overexpress *Foxf1* in all tissues exhibit early embryonic lethality around E12.5. *ROSA26-LSL-Foxf1* mice mated to *Tie2-cre* mice to overexpress *Foxf1* in endothelial and hematopoietic cells, exhibit hemorrhages around E15.5 and die perinatally (Dharmadhikari *et al.* manuscript in preparation). Additional studies are needed to determine developmental defects caused by constitutive overexpression of *Foxf1*.

Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins

In 2009, heterozygous genomic deletions and point mutations in *FOXF1* were identified in patients with Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV; MIM# 265380), suggesting that haploinsufficiency of the gene causes this rare lethal developmental disorder of the lung [29-31]. ACDMPV is primarily diagnosed by a post-mortem lung autopsy or a lung biopsy. To date, over 100 cases have been described in the literature; however, the actual occurrence of ACDMPV is under estimated given the challenging diagnosis. The cardinal diagnostic features of ACDMPV include misalignment (malposition) of pulmonary veins, medial thickening of smooth muscles in pulmonary arteries, hyperplasia of alveolar epithelium, and drastically decreased number of capillaries and lobular underdevelopment [32]. Approximately one third of the pa-

tients also have lymphangiectasis. Recent reports using 3D-reconstruction of post-mortem ACDMPV lungs suggest that the misaligned pulmonary veins are in fact intrapulmonary shunt vessels [33, 34]. The disease usually presents within a few hours after birth although late presentations have been reported [35-37]. The first case of ACDMPV was described by McMahon in 1948 [38]; however, the seminal case of ACDMPV was described by Janney *et al.* in 1981 [39]. The majority of the patients with ACDMPV also have extra-pulmonary anomalies, including various defects in gastrointestinal, cardiovascular, and genitourinary systems [40, 41]. Infants with ACDMPV present with severe hypoxemia and pulmonary hypertension [42]. Almost all patients die within the first month of life although some prolonged survivals have been described [43].

Treatment, including high pressure oxygen, nitric oxide, extra corporeal membrane oxygenation (ECMO) [44-46], and Sildenafil [47] provide only temporary relief as the disease is uniformly lethal. Recent advances towards treatment include use of a paracorporeal lung assist device that led to a successful lung transplant in patients with ACDMPV [48, 49].

Thus far, 44 heterozygous point mutations [29, 31, 50-52] and 36 heterozygous genomic deletions involving *FOXF1* or upstream of *FOXF1* in 16q24.1 have been reported [29, 37, 53-57]. Additionally, a 1.1 Mb genomic deletion involving *FOXF1* was detected in a prenatal case with cystic hygroma [58].

Mouse Modeling of ACDMPV Lungs

The phenotype of *Foxf1*$^{+/-}$ mice partially resembles the symptoms seen in patients with ACDMPV [29]. Most *Foxf1*$^{+/-}$ mice (55-90%) die shortly after birth, exhibiting alveolar capillary dysplasia and additional cardiac and/or gastrointestinal defects. However, the characteristic misalignment of pulmonary veins has not been observed in the lungs of *Foxf1*$^{+/-}$ mice. Additional genetic mouse models have also been described with phenotypes resembling ACDMPV.

Of note, mesodermal inactivation of *Pten* in mice leads to an ACD-like phenotype with evidence of failure in blood oxygenation [59]. These mice also show decreased expression of *Foxf1*. Interestingly patients with ACDMPV also showed decreased *PTEN* expression [59]. Further, loss of semaphorin-neuropilin-1 signaling in mice causes dysmorphic vascularization reminiscent of ACDMPV [60]. These mice also displayed misalignment of pulmonary veins which is absent in the *Foxf1*-deficient and *Pten*-deficient mouse models. Endothelial NO synthase (*eNOS*)-deficient mice also exhibit defective lung vasculature development and fatal respiratory distress similar to ACDMPV patients [61]. These findings suggest that *Foxf1, Pten, Sema3c-Nrp1,* and *eNOS* might all be involved in the same signaling network regulating development of pulmonary vasculature.

Upstream Gene Regulation

In mice, *Foxf1* has a ~ 400 bp conserved downstream regulatory element located 1 kb 3' to *Foxf1*, that is essential for the tissue-specific regulation of the *Foxf1* promoter during mouse embryogenesis [62]. About 7.5 kb upstream of *Foxf1*, an ~ 100 bp conserved region was identified as crucial for GLI-mediated transcriptional activation of *Foxf1* and *Foxl1* in the murine gut [21]. An additional 48 bp regulatory element located 90 kb upstream of *Foxf1* was recently described that mediates GLI1, GLI3, and TBX5 regulation of *Foxf1* expression during cardiac septation in the mouse embryo [28].

In addition to genomic deletions encompassing *FOXF1*, a comparable number of overlapping copy-number deletions upstream of *FOXF1* and leaving the gene intact have been found in ACDMPV patients [29, 37, 56]. These deletions enabled to define an ~ 60 kb noncoding, evolutionarily-conserved, and differentially-methylated *cis*-regulatory enhancer region that maps ~ 272 kb upstream of *FOXF1* and harbors lung-specific long non-coding RNA (lncRNA) genes [29, 56]. This enhancer region physically interacts with the *FOXF1* promoter, and a lncRNA *LINC01081*, encoded in this region, has been recently shown to positively regulate *FOXF1* expression [37]. The enhancer region also includes GLI2 binding sites overlapping with a differentially methylated CpG island, located within the intronic region of another lncRNA *LINC01082*. These findings further support conclusions from mouse models that showed *Foxf1* acting downstream of SHH and GLI transcription factors. Additionally, a deep intronic deletion in *FOXF1* in a patient with ACDMPV enabled to identify an intronic transcriptional enhancer region at the *FOXF1* locus [63]. This deletion reduces *FOXF1* expression in the peripheral lung tissue by 40%, causing fully manifested ACDMPV.

Interestingly, a substantial fraction of these deletions is mediated by *Alu* repetitive elements, suggesting that an *Alu*-rich genomic architecture at chromosome 16q24.1 may predispose to microhomology-mediated DNA replication errors [64]. *Alu-Alu* mediated copy-number changes have been reported previously at various genomic regions, e.g. the *SPAST* locus on 2p22.3 [65]. Additionally, transposable elements have been attributed to be major players in the origin and regulation of lncRNAs [66]. Thus, the presence of *Alu* repetitive elements at chromosome 16q24.1 may also explain the abundance of multiple lncRNA genes at this locus. Moreover, it is possible that some patients with ACDMPV that are *FOXF1* mutation and deletion negative, may carry submicroscopic retrotransposon (*e.g.* LINE-LINE)-mediated balanced paracentric inversions [67, 68] that separate *FOXF1* from its long-range upstream regulatory elements [69]. Such rearrangements are challenging for detection using currently available diagnostic technologies.

The bidirectional lncRNA gene *FENDRR*, encoded 1.67 kb upstream of *FOXF1*, has been shown to interact with the chromatin-modifying complex (PRC) 2 to regulate gene expression [70]. Homozygous loss of *Fendrr* in mice has been demonstrated to be either embryonic lethal due to heart and body wall defects [71] or perinatal lethal due to multiple defects in lung, heart, or gastrointestinal tract [72]. Interestingly, lncRNAs have been also shown to play an important role in lung development, often by regulating the expression of transcription factors like *Nkx2.1, Gata6, Foxa2,* and *Foxf1*

[73] and by linking epigenetic control mechanisms to gene regulatory networks [74].

An additional potential upstream regulator of *Foxf1* expression is HOXA13. In the mouse placenta *Foxf1* has been shown to be a target of HOXA13, which is essential for placental vascular patterning and labyrinth endothelial specification [75]. *Foxf1* expression has been found to be decreased in the yolk sacs of keratin (-/-) embryos [76] and in lungs of epithelial-specific *Gpr177* knockout embryos [77], both mouse lines exhibiting impaired embryonic vascular development.

Genomic Imprinting of the *FOXF1* Locus

In patients with ACDMPV for whom the parental origin of deletions involving the *FOXF1* locus could be determined, all 24 studied arose *de novo* on the maternal chromosome 16, suggesting that *FOXF1* is paternally imprinted in the human lungs. The 60 kb *cis*-regulatory enhancer region of *FOXF1* has been found to harbor a differentially methylated CpG island, located within the intronic region of the lncRNA *LINC01082* and differential allelic expression of *FOXF1* was detected in newborn human lungs [56], further suggesting that *FOXF1* is likely paternally imprinted in the human lungs, although incompletely. Furthermore, segregation analysis of a missense mutation in *FOXF1* (c.416G>T; p. Arg139Leu) in a familial case of ACDMPV provided additional support for paternal imprinting of *FOXF1* in humans [78].

Trisomy 16, typically resulting from maternal meiosis I nondisjunction, is the most common trisomy observed prenatally and lethal postnatally [79]. In a third of cases, trisomy rescue leads to maternal uniparental disomy 16 [UPD(16)], which is the most common UPD reported other than UPD(15), and often accompanied by confined placental mosaicism with trisomy 16 cell line [80]. Maternal UPD(16) has been associated with intrauterine growth restriction (IUGR), congenital heart defects, and pulmonary hypoplasia [81]. In contrast, a relatively normal phenotype with only prenatal and postnatal growth retardation is associated with a very rarely reported paternal UPD(16) [82], suggesting the presence of paternally imprinted gene(s) on chromosome 16 [81] and further confirming the incomplete paternal imprinting of *FOXF1* in the human lungs. We propose that paternal imprinting of *FOXF1* could explain key phenotypic differences between maternal vs. paternal UPD(16).

In contrast to humans, *Foxf1* has been found not to be imprinted in mice, with no difference in its expression between parental alleles in E15.5, E18.5, and P0.5 lungs from reciprocal crosses. Additionally, biallelic expression of *Foxf1* has been identified in E15.5 placentas and P21 lungs from reciprocal C57 and PWD strain of mice (unpublished data). The perinatal mortality in *Foxf1*$^{+/-}$ mice also does not show a parent-of-origin inheritance pattern when investigated on the CD-1 [69] and C57BL/6J backgrounds (unpublished data). Surviving *Foxf1*$^{+/-}$ Swiss Black pups upregulated the level of *Foxf1* to wild type levels and showed only mild abnormalities in alveolar septation without obvious vascular defects [11]. This compensation phenomenon described by Kalinichenko *et al*. [11] could be specific to Swiss Black background or may reflect the influence of stochastic methylation in the β-galactosidase (β-gal) construct used to knock-out the *Foxf1* gene. The presence of modifiers of *Foxf1* expression in different mouse strains might explain the differences in phenotypes observed.

Future studies will be directed towards deciphering the entire landscape of lncRNAs involved in the epigenetic regulation and imprinting of *FOXF1*. Novel treatment strategies for ACDMPV could involve using anti-sense oligos (ASOs) to manipulate lncRNAs to modify *FOXF1* expression.

Downstream Expression Effects

FOXF1 has been demonstrated to activate expression of *P-selectin* in response to cytokines such as IL-6 [8] as well as expression of the growth hormone variant (*GHV*) gene in placental BeWo choriocarcinoma cells [83].

FOXF1 has been shown to be essential for the migration of mesenchymal cells and to directly induce integrin-beta3 expression in mouse embryonic lungs [84], and to regulate expression of the *Flk1*, *Flt1*, *Pdgfb*, *Pecam1*, and *Tie2* genes critical for VEGF, PDGF, and Ang/Tie2 signaling [11, 12].

Additionally, FOXF1 regulates cell adhesion, migration, and mesenchymal cell differentiation in the gall bladder by decreased expression of vascular cell adhesion molecule-1 (*Vcam-1*), alpha(5) integrin, platelet-derived growth factor receptor alpha (*Pdgfr*α), and hepatocyte growth factor (*Hgf*) genes [10]. In visceral smooth muscle cells, FOXF1 regulates gene transcription by binding to myocardin, serum response factor (*Srf*), and myocardin–related transcription factors (MRTFs) [27].

Comparative analyses of lung transcriptomes in patients with ACDMPV and in *Foxf1*$^{+/-}$ newborn mice show similar pathways deregulated [85]. Several genes and pathways involved in lung development, angiogenesis, and in pulmonary hypertension development, were found to be deregulated. Expression changes in 14 genes, *COL15A1*, *COL18A1*, *COL6A2*, *ESM1*, *FSCN1*, *GRINA*, *IGFBP3*, *IL1B*, *MALL*, *NOS3*, *RASL11B*, *MATN2*, *PRKCDBP*, and *SIRPA*, overlapped in ACDMPV and *Foxf1*$^{+/-}$ lungs. Down-regulation of Notch pathway genes as previously described in *Foxf1*$^{+/-}$ lungs [24] was identified. Additionally, down-regulation of *Sema3c* was found, further suggesting a cross-talk between *Foxf1* and semaphorin-neuropilin signaling during development of pulmonary vasculature. Mast cell chymases, tryptases, and the chemokine CXCL-12 essential for mast cell migration and chemotaxis were significantly up-regulated as previously described in *Foxf1*$^{+/-}$ lungs [25]. Numerous members of collagen genes were up-regulated in lungs of both ACDMPV patients and *Foxf1*$^{+/-}$ mice, suggesting that loss of *FOXF1* may stimulate endothelial-mesenchymal transition leading to pulmonary fibrosis and lung dysfunction. However, this hypothesis requires further experimentation with endothelial-specific and fibroblast-specific *Foxf1* knockout mice. Of note, differential expression of *FOXF1* has been detected in cases of usual and nonspecific interstitial pneumonia, idiopathic pulmonary fibrosis, and in fibrotic lesions in human lung allografts [86-88].

Role of *FOXF1* in Cancer

While there have been various reports of *FOXF1* levels being deregulated in cancer, the role of *FOXF1* in carcinogenesis is still controversial. In fact, several studies proposed that *FOXF1* functions as a tumor suppressor. *FOXF1* has been reported to be epigenetically inactivated by hypermethylation of its promoter in breast cancer cell lines and invasive ductal carcinomas [89]. *FOXF1* was also found to be included in a panel of genes methylated with high frequency in colorectal cancer but showing very low methylation in peripheral blood [90]. Due to this differential methylation pattern, *FOXF1* was proposed as a suitable diagnostic marker for colorectal cancers. *FOXF1* was also shown to be a target of vitamin D3 in human colon cancer cells [91] and was found deregulated in hepatitis C-related hepatocellular carcinoma cells [92]. In addition, *FOXF1* was identified as a target gene of tumor suppressor p53 and along with p53 forms a transcriptional network that regulates cancer cell migration and invasiveness [93]. In prostate cancer, genomic deletions involving *FOXF1* have been identified and *FOXF1* expression has been found to be decreased in prostate cancer samples [93, 94]. Finally, *FOXF1* has also been identified as a reprogramming mediator contributing to mesenchymal stem cell fusion-induced reprogramming of lung cancer cells [95].

On the other hand, several studies have shown that *FOXF1* may function as an oncogene. Overexpression of *FOXF1* promotes invasion and metastasis of breast carcinomas [96]. In lung cancer, *FOXF1* enhances the tumor-promoting properties of cancer-associated fibroblasts [97]. *FOXF1* may contribute to hedgehog-associated tumorigenesis [98] because its levels are up-regulated in patched-associated tumors like basal cell carcinoma (BCC), medulloblastoma (MB), rhabdomyosarcoma (RMS), and non-small cell lung cancer (NSCLC) [99-101]. FOXF1 target genes *Bmi1* and *Notch2* were up-regulated in PTCH1-associated BCC and MB, further confirming its key role in hedgehog-associated tumorigenesis. FOXF1 overexpression in NSCLC correlated with lymph node metastasis and over expression of SHH associated genes *PTCH1*, *GL1* and its target gene *BMI1*. Common variants mapping on chromosome 16q24.1 close to *FOXF1* have also been associated with susceptibility to Barrett's esophagus and esophageal carcinoma (rs9936833) [102, 103], and breast cancer (rs1728400) [104] in genome-wide association studies. These SNPs are located approximately 141 kb and 109 kb upstream of *FOXF1*, respectively. Further analysis of the genomic region close to the SNP rs9936833, led to the identification of additional SNPs associated with susceptibility to esophageal carcinoma [105].

These contrasting findings in different cancer types suggest that the role of *FOXF1* in tumorigenesis can be context-dependent and epigenetically regulated. Since the majority of published studies utilized either cultured tumor cell lines or transplantation of tumor cells into immunocompromised mice, transgenic mouse models are needed to identify molecular mechanisms regulated by *Foxf1* during carcinogenesis.

Constitutional *FOXF1* Duplications

A patient harboring a complex de novo duplication-triplication rearrangement in 16q24.1-q24.3 involving *FOXF1*, presented with severe psychomotor disability, numerous dysmorphic features, and congenital malformations, including gut malrotation and gall bladder agenesis [106]. Recently, 16q24.1 duplications involving *FOXF1* were reported in four unrelated families 1-4 [107]. In families 1 and 2, 16q24.1 duplications that included *FOXF1* but not its upstream regulatory enhancer region were found. Both patients did not exhibit any pulmonary abnormalities. In families 3 and 4, 16q24.1 duplications involved *FOXF1* as well as its upstream regulatory region. Whereas patient 3 presented with pyloric stenosis, mesenterium commune, and aplasia of the appendix, patient 4 did not manifest any pulmonary or intestinal abnormalities.

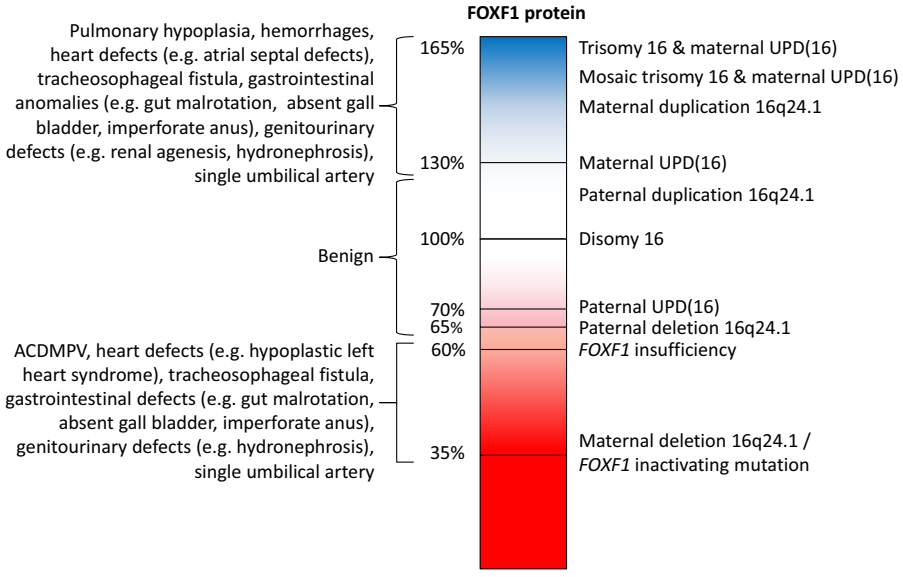

Fig. (2). Correlation of predicted FOXF1 deficiency and overexpression levels and associated ACDMPV, 16q24.1 duplication, and UPD16 phenotypes. Predicted FOXF1 levels are shown in a gradient pattern to depict decrease in FOXF1 levels due to deletions or mutations and increase in FOXF1 levels as a result of duplications, UPD(16), and trisomy 16.

A summary of the phenotypes associated with predicted levels of FOXF1 deficiency and overexpression is shown in (Fig. **2**).

CONCLUSION

In aggregate, *FOXF1* is a transcription factor involved in hedgehog-regulated developmental processes. Disruptions or amplifications in *FOXF1* cause severe human disorders. The identification of *FOXF1* as a causative gene for ACDMPV has enabled prenatal genetic testing and estimation of recurrence risks for parents of infants with ACDMPV. Consistent with previous empirical observations for mutations in some genes located on the X chromosome [108, 109], recent mathematical analyses of the sexual dimorphisms of gametogenesis suggest that new mutations that occur on the maternal allele are more likely to be recurrently transmitted to offspring [110, 111]. Thus, given that all hitherto analyzed deletions of the *FOXF1* locus arose de novo on the maternal chromosome 16q24.1, the recurrence risk for ACDMPV may potentially be elevated in comparison to that observed for other sporadic diseases.

Discerning the effects of *FOXF1* over- and/or ectopic expression is of primary importance for any future work toward *FOXF1*-based gene therapies for ACDMPV and other disorders caused by *FOXF1* abnormal dosage. Future studies will involve designing novel therapeutic strategies to treat ACDMPV by manipulation of the epigenetic lncRNA regulation of *FOXF1*, using antisense oligos (ASOs). Generation of novel mouse models with conditional inactivation or overexpression of *Foxf1* in different cell types will help elucidate molecular mechanisms regulated by *Foxf1* during embryonic development and various human diseases. Due to phenotype similarities in haploinsufficient mice and humans, *Foxf1*$^{+/-}$ mouse line can be used as a preclinical model to develop novel therapeutic strategies to treat ACDMPV.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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