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 epitheliun-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.

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.
[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 ex-thal at E9.5 due to defects in mesodermal differentiation and lungs relative to Shh−/− lungs, suggesting that GLI3 is a potential repressor of SHH, GLI2, and lncRNAs. Downstream effectors of Sonic Hedgehog pathway in embryonic lung.

On a Swiss black background, 55% of Foxf1−/− 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. 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-

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**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 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 (endothelial 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].
patients 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 extrapulmonary 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 Foxll 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.
Genomic Imprinting of the FOXFI Locus

In patients with ACDMPV for whom the parental origin of deletions involving the FOXFI locus could be determined, all 24 studied arose de novo on the maternal chromosome 16, suggesting that FOXFI is paternally imprinted in the human lungs. The 60 kb cis-regulatory enhancer region of FOXFI has been found to harbor a differentially methylated Cpg island, located within the intronic region of the lncRNA LINCO1082 and differential allelic expression of FOXFI was detected in newborn human lungs [56], further suggesting that FOXFI is likely paternally imprinted in the human lungs, although incompletely. Furthermore, segregation analysis of a nonsense mutation in FOXFI (c.416G>T; p. Arg139Leu) in a familial case of ACDMPV provided additional support for paternal imprinting of FOXFI in humans [78].

Trisomy 16, typically resulting from maternal meiosis I nondisjunction, is the most common trisomy observed prenatally and lethally 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 FOXFI in the human lungs. We propose that paternal imprinting of FOXFI 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 CD1 [69] and C57BL/6j backgrounds (unpublished data). Surviving Foxf1"+/-" Swiss Black pups up-regulated 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 FOXFI. Novel treatment strategies for ACDMPV could involve using anti-sense oligos (ASOs) to manipulate lncRNAs to modify FOXFI expression.

Downstream Expression Effects

FOXFI 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].

FOXFI 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, FOXFI regulates cell adhesion, migration, and mesenchymal cell differentiation in the gall bladder by decreased expression of vascular cell adhesion molecule-1 (Vcam-1), alpha5 integrin, platelet-derived growth factor receptor alpha (Pdgfra), and hepatocyte growth factor (Hgf) genes [10]. In visceral smooth muscle cells, FOXFI 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, JGBP3, 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 FOXFI 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 FOXFI 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, GLI, 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 IncRNA 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<sup>M<sup>−/−</sup></sup> mouse 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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REFERENCES

[1] Benayoun, B.A.; Caburet, S.; Verića, R.A. Forkhead transcription factors: key players in health and disease. Trends Genet., 2011, 27, 224-232.
[2] Katoh, M.; Igarashi, M.; Fukuda, H.; Nakagama, H.; Katoh, M. Cancer genetics and genomics of human FOX family genes. Cancer Lett., 2013, 328, 198-206.
[3] Hannenhalli, S.; Kaestner, K.H. The evolution of Fox genes and their role in development and disease. Nat. Rev. Genet., 2009, 10, 233-240.
[4] Clark, K.L.; Halay, E.D.; Lai, E.; Burley, S. K. Co-crystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. Nature, 1993, 364, 412-420.
[5] Hellqvist, M.; Mahlapuu, M.; Samuelsson, L.; Enerbäck, S.; Carlsson, P. Differential activation of lung-specific genes by two forkhead proteins, FREC-1 and FREC-2. J. Biol. Chem., 1996, 271, 4482-4490.
[6] Bozyk, P.D.; Popova, A.P.; Bentley, J.K.; Goldsmith, A.M.; Linn, M.J.; Weiss, D.J.; Hershenson, M.B. Mesenchymal stromal cells from neonatal tracheal aspirates demonstrate a pattern of lung-specific gene expression. Stem Cells Dev., 2011, 20, 1995-2007.
[7] Van der Heul-Nieuwenhuijzen, L.; Dits, N.F.; Jenster, G. Gene expression of forkhead transcription factors in the normal and disease human prostate. BJU Int., 2009, 103, 1574-1580.
[8] Peterson, R. S.; Lim, L.; Ye, H.; Zhou, H.; Overdier, D. G.; Costa, R. H. The winged helix transcriptional activator HHF-8 is expressed in the mesoderm of the primitive streak stage of mouse embryos and its cellular derivatives. Mech. Dev., 1997, 69, 53-69.
[9] Mahlapuu, M.; Pei-to-Huikko, M.; Aitola, M.; Enerbäck, S.; Carlsson, P. FREC-1 contains a cell-type-specific transcriptional activation domain and is expressed in epithelial-mesenchymal interfaces. Dev. Biol., 1998, 202, 183-195.
[10] Kalinichenko, V. V.; Zhou, Y.; Bhattacharyya, D.; Kim, W.; Shin, B.; Bambal, K.; Costa, R. H. Haploinsufficiency of the mouse Forkhead Box f1 gene causes defects in gall bladder development. J. Biol. Chem., 2002, 277, 12369-12374.
[11] Kalinichenko, V. V.; Lim, L.; Stolz, D. B.; Shin, B.; Rausa, F. M.; Clark, J.; Whitsett, J. A.; Watkins, S. C.; Costa, R. H. Defects in pulmonary vasculature and perinatal lung hemorrhage in mice heterozygous for the Forkhead Box f1 transcription factor. Dev. Biol., 2001, 235, 489-506.
[12] Ren, X.; Ustiyan, V.; Pradhan, A.; Cai, Y.; Haurilak, J. A.; Bolte, C. S.; Shannon, J. M.; Kalin, T. V.; Kalinichenko, V. V. FOXF1 transcription factor is required for formation of embryonic vasculature by regulating VEGF signaling in endothelial cells. Circ. Res., 2014, 115, 709-720.
[13] Kalinichenko, V. V.; Gusarova, G. A.; Shin, B.; Costa, R. H. The forkhead box f1 transcription factor is expressed in brain and head mesenchyme during mouse embryonic development. Gene Expr. Patterns, 2003, 3, 153-158.
[14] Jeong, J.; Mao, J.; Tenzen, T.; Kottmann, A. H.; McMahon, A. P. Hedgehog signaling in the neural crest cells regulates the patterning and growth of facial primordia. Genes Dev., 2004, 18, 937-951.
[15] Kalinichenko, V.V.; Lim, L.; Shin, B.; Costa, R.H. Differential expression of forkhead box transcription factors following butylated hydroxytoluene lung injury. Am. J. Physiol. Lung Cell. Mol. Physiol., 2001, 280, L695-L704.
[16] Kalinichenko, V.V.; Bhattacharyya, D.; Zhou, Y.; Gusarova, G.A.; Kim, W.; Shin, B.; Costa, R. H. Foxf1<sup>−/−</sup> mice exhibit defective stellate cell activation and abnormal liver regeneration following CCl<sub>4</sub> injury. Hepatology, 2003, 37, 107-117.
[17] Minogue, B.M.; Richardson, S.M.; Zeef, L.A.H.; Freemont, A. J.; Hoyland, J. A. Characterization of the human nucleus pulposus cell phenotype and evaluation of novel marker gene expression to define adult stem cell differentiation. Arthritis Rheum., 2010, 62, 3695-3705.
[18] Mahlapuu, M.; Ormestad, M.; Enerbäck, S.; Carlsson, P. The forkhead transcription factor Foxf1 is required for differentiation of extraembryonic and lateral plate mesoderm. Development, 2001, 128, 155-166.
[19] Mahlapuu, M.; Enerbäck, S.; Carlsson, P. Haploinsufficiency of the forkhead gene Foxf1, a target for sonic hedgehog signaling, causes lung and foregut malformations. Development, 2001, 128, 2397-2406.
[20] Astorga, J.; Carlsson, P. Hedgehog induction of murine vasculogenesis is mediated by Foxf1 and Bmp4. Development, 2007, 134, 3753-3761.
[21] Madison, B. B.; McKenna, L. B.; Dolson, D.; Epstein, D. J.; Kaestner, K. H. Foxf1 and Foxl1 link hedgehog signaling and the control of epithelial proliferation in the developing stomach and intestine. J. Biol. Chem., 2009, 284, 5936-5944.
[22] Li, Y.; Zhang, H.; Choi, S.C.; Litingtung, Y.; Chiang, C. Sonic hedgehog signaling regulates Gli3 processing, mesenchymal proliferation, and differentiation during mouse lung organogenesis. Dev. Biol., 2004, 270, 214-231.
[23] Lim, L.; Kalinichenko, V. V.; Whitsett, J. A.; Costa, R. H. Fusion of lung lobes and vessels in mouse embryos heterozygous for the forkhead box f1 targeted allele. Am. J. Physiol. Lung Cell. Mol. Physiol., 2002, 282, L1012-L1022.
Role of FOXF1 in Development and Disease

[24] Kalinichenko, V. V.; Gussarov, G. A.; Kim, I.-M.; Shin, B.; Yoder, H. M.; Clark, J.; Sapozhnikov, A. M.; Whitsett, J. A.; Costa, R. H. FOXF1 haploinsufficiency reduces Notch-2 signaling during mouse lung development. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, 2004, 286, L521-L530.

[25] Kalinichenko, V. V.; Zhou, Y. A. N.; Shin, B.; Stolz, D. B.; Watkins, S. C.; Whitsett, J. A.; Costa, R. H. Wild-type levels of the mouse Forkhead Box f1 gene are essential for lung repair. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, 2002, 1, 1253-1265.

[26] Kalinichenko, V. V.; Zhou, Y. A. N.; Shin, B.; Zolt, D. B.; Watkins, S. C.; Whitsett, J. A.; Costa, R. H. Late presentation of misalignment of lung vessels with alveolar capillary dysplasia. *Langston, C.* Misalignment of pulmonary veins and alveolar capillary shunt vessels in alveolar capillary dysplasia. *Galambos, C.* Three-dimensional reconstruction identifies misaligned pulmonary veins as intrapulmonary shunt vessels in alveolar capillary dysplasia. *Am. J. Physiol. Lung Cell. Mol. Biol.*, 2008, 39, 390-399.

[27] Hoffman, A. D.; Yang, X. H.; Burnicka-Turek, O.; Bosman, J. D.; Ren, X.; Steimle, J. D.; Vokes, S. A.; McMahon, A. P.; Kalinichenko, V. V.; Moskovitz, I. P. FOXF1 genes integrate tbx5 and hedgehog pathways in the second heart field for cardiac septation. *PLoS Genet.*, 2014, 10, e1004066.

[28] Stankiewicz, P.; Sen, P.; Bhatt, S. S.; Storer, M.; Xia, Z.; Beijer, B. A.; Ou, Z.; Wiszniewska, J.; Driscoll, D. J.; Maisenbacher, B. K.; Bolivar, J.; Bauer, M.; Zackai, E. H.; McDonald-McGinn, D.; Nowaczyk, M. J. J.; Murray, M.; Huestad, V.; Mascott, K.; Schultz, R.; Hallam, L.; MaCae, D.; Nicholson, A. G.; Newbury, R.; Durham-O’Donnell, J.; Knight, G.; Kini, U.; Shaikh, T. H.; Martin, V.; Tyerman, M.; Simonic, I.; Willott, L.; Paterson, J.; Mchta, S.; Rajan, D.; Fitzgerald, T.; Gribble, S.; Prigmore, E.; Patel, A.; Shaffer, L. G.; Carter, N. P.; Cheung, S. W.; Langston, C.; Shaw-Smith, C. Genomic and genic deletions of the FOXE 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.

[30] Bishop, N. B.; Stankiewicz, P.; Steinhorn, R. H. Alveolar capillary dysplasia. *Am. J. Respir. Crit. Care Med.*, 2011, 184, 172-179.

[31] Sen, P.; Yang, Y.; Navarro, C.; Silva, I.; Szafisz, P.; Kolodiejska, K. E.; Duchamhakiri, A. V.; Mostafa, H.; Kozakewich, H.; Kearney, D.; Caihill, J. B.; Whitt, M.; Blici, M.; Margraf, L.; Charles, A.; Goldblatt, J.; Gibson, K.; Lantz, P. E.; Garvin, A. J.; Petty, J.; Kiblawi, Z.; Zuppan, C.; McConkie-Rosell, A.; McDonald, M. T.; Peterson-Carmichael, S. L.; Gaede, J. T.; Shivanna, B.; Schady, D.; Friedlich, P. S.; Hays, S. R.; Palafoll, I. V.; Sanders-Panchal, U.; Bebling, A.; Finin, L. S.; Sieber, J. R.; Galambos, C.; Ngue, 185-461.

[32] Riley, M.; Chassing, N.; Vigouroux, A.; Rakza, T.; Riou, Y.; Manouvrier, S.; Lequien, P.; Storme, L. Alveolar capillary dysplasia: a cause of persistent pulmonary hypertension. *Arch. Pediatr.*, 2007, 14, e1004604.

[33] Al-Hathloul, K.; Phillips, S.; Seshia, M.M.K.; Casiro, O.; Alvaro, R. E.; Rigatto, H. Alveolar capillary dysplasia. Report of a case of prolonged life without extracorporeal membrane oxygenation (ECMO) and review of the literature. *Early Hum. Dev.*, 2000, 57, 85-94.

[34] Alameh, J.; Bachiri, A.; Deviseme, L.; Truffert, P.; Rakza, T.; Riou, Y.; Manouvrier, S.; Lequien, P.; Storme, L. Alveolar capillary dysplasia: a cause of persistent pulmonary hypertension of the newborn. *Pediatr. J.*, 2002, 161, 262-266.

[35] Plat, G.; Rouquette, I.; Marcoux, M.O.; Bloom, M.C.; Acar, P.; Dulac, Y. Alveolar capillary dysplasia and persistent pulmonary hypertension of the newborn. *Arch. Mal. Coeur Vaiss.*, 2007, 100, 420-424.

[36] Hoganson, D. M.; Gazit, A. Z.; Boston, U. S.; Fehr, J.; Gazit, A. Z.; Eghtesady, P. Paracorporeal lung assist device: an innovative surgical strategy for bridging to lung transplant in severe pulmonary hypertension caused by alveolar capillary dysplasia. *J. Thorac. Cardiovasc. Surg.*, 2013, 146, e42-e43.

[37] Castilla-Fernandez, Y.; Copons-Fernandez, C.; Jordan-Lucas, R.; Linde-Sillo, A.; Valenzuela-Palafoll, I.; Ferreres Piñas, J. C.; Moreno-Galdó, A.; Castillo-Salinas, F. Alveolar capillary dysplasia with malalignment of pulmonary veins: concordance between pathologic and molecular diagnosis. *J. Pediatr.*, 2013, 33, 401-403.

[38] Miranda, J.; Rocha, G.; Soares, P.; Morgado, B.; Baptista, M.J.; Azevedo, I.; Fernandes, S.; Brandão, O.; Sen, P.; Guimaraes, A. A novel mutation in FOXF1 gene associated with alveolar capillary dysplasia with malalignment of pulmonary veins, intestinal malformation and anular pancreas. *Neonatology*, 2013, 103, 241-245.

[39] Nguyen, L.; Riley, M. M.; Sen, P.; Galambos, C. Alveolar capillary dysplasia with malalignment of pulmonary veins with a wide spectrum of extrapulmonary manifestations. *Pediatr. Int.*, 2013, 63, 519-521.

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

[54] Zufferey, F.; Martinet, D.; Osterheld, M.; Niel-bütschi, F.; Becquet, S. N.; Beckmann, J. S.; Xia, Z.; 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.

[55] Handrigan, G. R.; Chitayat, D.; Lionel, A. C.; Pinski, M.; Vaags, A. K.; Marshall, C. R.; Dyack, S.; Escobar, L. F.; Fernandez, B. A.; Stegman, J. C.; Rosenfeld, J. A.; Shaffer, L. G.; Goodenberger, M. D.; Hodge, J. C.; Cain, J. E.; Babul-Hirji, R.; Stavropoulos, D. J.; Yiu, V.; Scherer, S. W.; Rosenblum, N. D. Deletions in 16q24.22 are associated with autism spectrum disorder, intellectual disability and congenital malformations. Genome Res., 2013, 23, 23-33.

[56] Bellamkonda-Athraman, V.; Sulman, C. G.; Basel, D. G.; Southern, J.; Konduri, G. G.; Basir, M. A. Alveolar capillary dysplasia with multiple congenital anomalies and bronchopulmonary airway abnormalities. J. Perinatol., 2014, 34, 326-328.

[57] Garabedian, M. J.; Wallerstein, D.; Medina, N.; Byrne, J.; Wallerstein, R. J. 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.

[58] Tizzio, C.; Carraro, G.; Al Alam, D.; Baptista, S.; Danopolos, S.; Li, A.; Lavareda-Pearce, M.; Li, C.; De Langhe, S.; Chan, B.; Borok, Z.; Bellusi, S.; Minoo, P. Mesodermal Pten inactivation leads to alveolar capillary dysplasia-like phenotype. J. Clin. Invest., 2012, 122, 3862-3872.

[59] 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.

[60] Han, R. N. N.; Babaei, S.; Robb, M.; Lee, T.; Ridsdale, R.; Ackerley, C.; Post, M.; Stewart, D. J. Defective lung vascular development and fatal respiratory distress in endothelial NO synthase-2 knockouts. Hum. Mol. Genet., 2013, 22, 2375-2386.

[61] Hatsing, P. J.; Ira, G.; Mangelsdorf, J. D.; Mendelsohn, F. J. Microarray analysis of transcription factor expression in human placental tissue from a case of lethal alveolar capillary dysplasia with misalignment of pulmonary veins and maternal imprinting of semaphorin-neuropilin-1. Hum. Molecular Genet., 2013, 22, 3577-3587.

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

[63] Sauvageau, M.; Goff, L. A.; Lodato, S.; Bonev, B.; Groff, A. F.; Gerhardinger, C.; Sanchez-Gomez, D. B.; Hacisuleyman, E.; Li, E.; Spence, M.; Liapis, S. C.; Haldal, M.; Morse, M.; Saez-Rodriguez, J. C. J. C.; Lai, V.; Gonzalez, G. D.; Kendrew, D.; Kellis, M.; Hart, R. P.; Valenzuela, D. M.; Arlotta, P.; Rinn, J. L. Multiple knockout mouse models reveal lncRNAs are required for life and brain development. Elife, 2013, 2, e01749.

[64] Herring, M. J.; Swart, D. T.; Morley, M. P.; Rathi, K. S.; Peng, T.; Stewart, K. M. Morrisey, E. E. Long noncoding RNAs are spatially correlated with transcription factors and regulate lung development. Genes Dev., 2014, 28, 1363-1379.

[65] Grote, P.; Herrmann, B. G. The long non-coding RNA Fendrr links epigenetic control mechanisms to gene regulatory networks in mammalian embryogenesis. RNA Biol., 2013, 10, 1579-1585.

[66] Shaut, C. A. E.; Keene, D. R.; Sorensen, L. K.; Li, D. Y.; Stadler, H. S. HOXA13 Is essential for placental vascular patterning and labyrinth endothelial specification. PLoS Genet., 2008, 4, e1000073.

[67] Vijayaraj, P.; Kroeger, C.; Reuter, U.; Hartmann, D.; Magin, T. M. Keratin regulate yolk sac hematopoiesis and vasculogenesis through reduced BMP-4 signaling. Eur. J. Cell Biol., 2010, 89, 299-306.

[68] Jiang, M.; Ku, W.; Fu, J.; Offermanns, S.; Hsu, W.; Que, J. Gpr17 regulates pulmonary vasculature development. Development, 2013, 140, 3589-3594.

[69] Sen, P.; Gerychova, R.; Janku, P.; Jezova, M.; Valaskova, I.; Hristova, K. E.; Zhishuo, O.; Dittrich, P.; Majewski, T.; Mohammad, M. A.; Szafranski, P.; Gambin, T.; Campbell, I. M.; Zhou, Y.; Ramakrishna, S.; Hughes, D. E.; Solway, J.; Stankiewicz, P.; Carlsson, P.; C.; Carlsson, P.; Stankiewicz, P.; Eggermann, T., Curtis, M., Zerres, K., Hughes H. E. Maternal imprinting of semaphorin-neuropilin-1 signaling causes dysmorphic vascularization reminiscent of alveolar capillary dysplasia. Am. J. Pathol., 2012, 181, 2003-2017.

[70] Reines, W.; Smith, S.; Herring, M.; Lai, V.; Saez-Rodriguez, J. C.; Lai, V.; Hanson, C.; Lai, V.; Goll, A. F. The role of semaphorin-neuropilin-1 in alveolar capillary dysplasia. Mol. Cell. Biol., 2013, 50, 163-173.

[71] Startek, M.; Szafranski, P.; Gambin, T.; Campbell, I. M.; Hixson, P.; Shaw, C. A.; Stankiewicz, P.; Gambin, A. Genome-wide analyses of LINE-L1-mediated nonallelic homologous recombination. Nucleic Acids Res., 2015, pii: gku1394.

[72] Parris, T.; Nik, A. M.; 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.

[73] Khalil, A. M.; Guttman, M.; Huarte, M.; Garber, R.; Raj, A.; Rivea Morales, D.; Thomas, K.; Presser, A.; Bernstein, B. E.; van Oudenaarden, A.; Regev, A.; Lander, E. S.; Rinn, J. L. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc. Natl. Acad. Sci. U. S. A., 2009, 106, 11667-11672.

[74] Lomenick, J. P.; Hubert, M. A.; Handwerger, S. Transcription factor FOXP1 regulates growth hormone variant gene expression. Am. J. Physiol. Endocrinol. Metab., 2006, 291, E947-E951.

[75] Reines, W.; Smith, S.; Herring, M.; Lai, V.; Saez-Rodriguez, J. C.; Lai, V.; Hanson, C.; Lai, V.; Goll, A. F. The role of semaphorin-neuropilin-1 in alveolar capillary dysplasia. Mol. Cell. Biol., 2007, 27, 2486-2498.

[76] Sen, P.; Dharmadhikari, A. V.; Majewski, T.; Mohammad, M. A.;
Kalin, T. V.; Zabielska, J.; Ren, X.; Bray, M.; Brown, H. M.; Welty, S.; Thevananthar, S.; Langston, C.; Szafrański, P.; Justice, M. J.; Kalinichenko, V. V.; 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. Proc Natl Acad Sci U S A. 2014, 111, 9439-9444.

Coon, D. R.; Roberts, D. J.; Lescartales, M.; Krudin, R. Differential epithelial expression of SHH and FOXL1 in usual and nonspecific interstitial pneumonia. Exp Mol Pathol. 2006, 80, 119-123.

Melboucy-Belkhir, S.; Pradère, P.; Tadibbi, S.; Habib, S.; Bacrot, A.; Brayer, S.; Mari, B.; Besnard, V.; Mailleux, A. A.; Guenther, A.; Castier, Y.; Mal, H.; Crebasti, C.; Plantier, L. Forkhead Box F1 represses cell growth and inhibits COL1 and ARPC2 expression in lung fibroblasts in vitro. Am J Respir Cell Mol Biol. 2014, 50, 1838-847.

Walker, N.; Badri, L.; Wettlaufer, S.; Flint, A.; Sajjan, U.; Krebsbach, P. H.; Keshamouni, V. G.; Peters-Golden, M.; Lama, V. N. Resident tissue-specific mesenchymal progenitor cells contribute to fibrogenesis in human lung allografts. Am J Pathol. 2011, 178, 2461-2469.

Lo, F.-K.; Lee, J.; Liang, X.; Han, L.; Mori, T.; Fackler, M. J.; Kalin, T. V.; Zhang, R.; Winter, H.; Corley, D. A.; Panter, S.; Risch, H. A.; Reid, B. J.; Sargeant, I.; Gammon, M. D.; Smart, H.; Dhar, A.; McMurtry, H.; Ali, H.; Liu, G.; Casson, A. G.; Chow, W.-H.; Rutter, M.; Tawil, A.; Morris, D.; Nwokolo, C.; Isaacs, P.; Rodgers, C.; Ragnauth, K.; MacDonald, C.; Haigh, M.; Monk, D.; Davies, G.; Wajen, S.; Johnston, D.; Gibbons, M.; Cullen, S.; Church, N.; Langley, R.; Griffin, M.; Allison, D.; Deloukas, P.; Huston, S. E.; Ray, G.; Eron, S.; Potter, V. C.; Thakshakoon-Ghanabaria, A.; Anderson, M.; Brooks, P. J.; Blackwell, J.; Bramon, E.; Brown, M.; Casas, J. P.; Corvin, A.; Duncanson, A.; Markus, H. S.; Mathew, C. G.; Palmer, C. N. A.; Plomin, R.; Rautanen, A.; Sawyer, S. J.; Trehub, R. C.; Viswanathan, A. A.; Wood, N.; Trynka, G.; Wijmenga, C.; Cazier, J.-B.; Atherfold, P.; Nicholson, A. M.; Gellatly, N. L.; Glancy, D.; Cooper, S. C.; Cunningham, D.; Lind, T.; Hapeshi, J.; Ferry, D.; Rathbone, B.; Brown, J.; Love, S.; Attwood, S.; MacGregor, S.; Watson, P.; Sanders, S.; Ek, W.; Harrison, R. F.; Moayyedi, P.; de Caestecker, J.; Barr, H.; Stupka, E.; Vaughan, T. L.; Peltonen, L.; Spencer, C. C. A.; Tomlinson, I.; Donnelly, P.; Jankowski, J. A. Z. Common variants at the MHC locus and at chromosome 16q24.1 predispose to Barrett's esophagus. Nat Genet. 2012, 44, 1131-1136.

Dura, P.; van Veen, E. M.; Salomon, J. ; H. M.; Roeheofs, H. M. J.; Kristinsson, J. O.; Wobbes, T.; Witteman, B. J.; van Thriel, A. C.; Lubberink, M.; Peters, Peter; H. M. Barrett associated MHC and FOXF1 variants also increase esophageal carcinoma risk. Int J Cancer. 2013, 133, 1751-1755.

Rafiq, S.; Khan, S.; Tapper, W.; Collins, A.; Upstill-Goddard, R.; Gerty, S.; Blomqvist, C.; Aittomaki, K.; Cush, J. F.; Liu, J.; Nevanlinna, H.; Eccles, D. A genome wide meta-analysis study for identification of common variation associated with breast cancer prognosis. PLoS One. 2014, 9, e101488.

Levine, D. M.; Ek, W. E.; Zhang, R.; Liu, X.; Onstad, L.; Sather, C.; Lao-Serieix, P.; Gammon, M. D.; Corley, D. A.; Shaeen, N. J.; Bird, N. C.; Hardie, L. J.; Murray, L. J.; Reid, B. J.; Chow, W.-H.; Risch, H. A.; Nyren, O.; Ye, W.; Liu, G.; Romero, Y.; Bernstein, L.; Wu, A. H.; Casson, A. G.; Chau and, S. J.; Harrington, P.; Caldas, I.; Debiaram-Beecham, I.; Caldas, C.; Hayward, N. K.; Pharaoh, P. D.; Fitzgerald, R. C.; Macgregor, S.; White, C. G.; Vaughan, T. L. A genome-wide association study identifies new susceptibility loci for esophageal adenocarcinoma and Barrett's esophagus. Nat Genet. 2015, 47, 1487-1493.

Kucharczyk, M.; Kochański, A.; Jezela-Stanek, A.; Kugaudo, M.; Sielska-Robucka, D.; Gikutowska, A.; Krajewska-Walasek, M. The first case of a patient with de novo partial distal 16q tetrasomy and a data's review. Am J Med Genet A. 2014, 164, 2541-2550.

Khalili, C.; A. M.; Ginn, D. F.; Qin, Z.; Brackenbury, J.; Golinovski, K.; Gambin, A.; George-ki, A.; Jezela-Stanek, A.; Kugaudo, M.; Andaya, A.; Chinnayain, A.; van Der Heeren, E. H.; Ginzing, D.; Haqq, C.; James, K.; Kamkar, S.; Kowbel, D.; Pinck, D.; Schmitt, L.; Simko, J. P.; Volik, S.; Weinberg, V. K.; Paris, P. L.; Collins, C. Integration of high-resolution array comparative genomic hybridization analysis of chromosome 16q with expression array data refines common regions of loss at 16q23-qter and identifies underlying candidate tumor suppressor genes in prostate cancer. Oncogene. 2014, 23, 3487-3494.

Wei, H.J.; Nickoloff, J. A.; Chen, W.; Liu, H.; Chang, Y.; Yang, P.; Wu, C.; Williams, D. F.; Gelovani, G.; Deng, W. FOXF1 mediates mesenchymal stem cell fusion-induced reprogramming of lung cancer cells. Oncotarget. 2014, 5, 9514-9529.

Nilsson, J.; Helou, K.; Kovács, A.; Bendahl, P.-O.; Bjursell, G.; Fernö, M.; Carlsson, P.; Kinnon-Jansos, J. Nuclear Janus-activated kinase 2/nuclear factor I-C2 suppresses tumorigenesis and epithelial-to-mesenchymal transition by repressing Forkhead box F1. Cancer Res. 2010, 70, 2020-2029.

Kato, H.; Kato, M. Hedgehog target genes: mechanisms of carcinogenesis induced by aberrant hedgehog signaling activation. Curr Med Chem. 2009, 16, 873-886.

Wendling, D. S.; Luck, C.; von Schweinitz, D.; Kaplan, R. Characteristic overexpression of the forkhead box transcription factor FOXF1 in Patched-associated tumors. Int J Mol Med. 2008, 22, 787-792.
Leuer, M., Oldenburg, J., Lavergne, J.M., Ludwig, M., Fregin, A., Eigel, A., Ljung, R., Goodeve, A., Peake, I., Olek, K. Somatic mosaicism in hemophilia A: a fairly common event. *Am. J. Hum. Genet.,* 2001, 69, 75-87.

Helderman-van den Enden, A.T.; de Jong, R., den Dunnen, J.T., Houwing-Duistermaat, J.J., Kneppers, A.L., Ginjar, H.B., Breuning, M.H., Bakker, E. Recurrence risk due to germ line mosaicism: Duchenne and Becker muscular dystrophy. *Clin. Genet.,* 2009, 75, 465-472.

Campbell, I.M.; Yuan, B.; Robberecht, C.; Pfundt, R.; Szafranski, P.; McEntagart, M.E.; Nagamani, S.C.; Erez, A.; Bartnik, M.; Wiśniowiecka-Kowalnik, B.; Plunkett, K.S.; Pursley, A.N.; Kang, S.H.; Bi, W.; Lalani, S.R.; Bacino, C.A.; Vast, M.; Marks, K.; Patton, M.; Olofsson, P.; Patel, A.; Veltman, J.A.; Cheung, S.W.; Shaw, C.A.; Vissers, L.E.; Vermeesch, J.R.; Lupski, J.R.; Stankiewicz, P. Parental somatic mosaicism is underrecognized and influences recurrence risk of genomic disorders. *Am. J. Hum. Genet.,* 2014, 95, 173-182.

Campbell, I.M.; Stewart, J.R.; James, R.A.; Lupski, J.R.; Stankiewicz, P.; Olofsson, P.; Shaw, C.A. Parent of origin, mosaicism, and recurrence risk: probabilistic modeling explains the broken symmetry of transmission genetics. *Am. J. Hum. Genet.,* 2014, 95, 345-359.