



## Case Report

# Human *SMAD4* Genomic Variants Identified in Individuals with Heritable and Early-Onset Thoracic Aortic Disease

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**Abstract:** Thoracic aortic aneurysms (TAAs) that progress to acute thoracic aortic dissections (TADs) are life-threatening vascular events that have been associated with altered transforming growth factor (TGF)  $\beta$  signaling. In addition to TAA, multiple genetic vascular disorders, including hereditary hemorrhagic telangiectasia (HHT), involve altered TGF $\beta$  signaling and vascular malformations. Due to the importance of TGF $\beta$ , genomic variant databases have been curated for activin receptor-like kinase 1 (*ALK1*) and endoglin (*ENG*). This case report details seven variants in *SMAD4* that are associated with either heritable or early-onset aortic dissections and compares them to pathogenic exon variants in gnomAD v2.1.1. The TAA and TAD variants were identified through whole exome sequencing of 346 families with unrelated heritable thoracic aortic disease (HTAD) and 355 individuals with early-onset (age  $\leq$  56 years old) thoracic aortic dissection (ESTAD). An allele frequency filter of less than 0.05% was applied in the Genome Aggregation Database (gnomAD exome v2.1.1) with a combined annotation-dependent depletion score (CADD) greater than 20. These seven variants also have a higher REVEL score ( $>0.2$ ), indicating pathogenic potential. Further in vivo and in vitro analysis is needed to evaluate how these variants affect *SMAD4* mRNA stability and protein activity in association with thoracic aortic disease.

**Keywords:** genomic variants; hereditary hemorrhagic telangiectasia; thoracic aortic aneurysm; vascular malformations; *SMAD4*

## 1. Introduction

Transforming growth factor  $\beta$  (TGF $\beta$ ) plays a critical role in vascular development. Many vascular disorders, such as hereditary hemorrhagic telangiectasia (HHT), Marfan syndrome, and thoracic aortic aneurysm and dissection (TAA/TAD) have been associated with disruption of the TGF  $\beta$  signaling axis. Mutations in many proteins that are involved in this pathway have been identified. For example, pathogenic variants that underlie HHT are found in multiple genes, including endoglin (*ENG*) associated with HHT1, activin receptor-like kinase 1 (*ALK1*) associated with HHT2, mothers against decapentaplegic homolog 4 (*SMAD4*) associated with HHT3, and bone morphogenetic protein 9 (*BMP9*) associated with HHT5. The majority of HHT cases result from variants in *ENG* or *ALK1*, and genomic variant databases have been established and maintained that elegantly catalogue numerous *ENG* and *ALK1* genetic variants [1]. Such databases are critical for curating

genetic variants associated with a disorder and can assist early clinical diagnoses. To date, no such resource exists for *SMAD4* or *BMP9* variants. Herein, we report seven confirmed *SMAD4* variants associated with heritable or early-onset TAA.

Aortic aneurysms are enlargements of the aorta. Thoracic aortic aneurysms are typically asymptomatic and may lead to sudden death due to acute aortic dissections. TAAs are less prevalent and occur in younger patients when compared to abdominal aortic aneurysms. TAAs can result from single-gene pathogenic variants that confer a high risk of TAA, termed heritable thoracic aortic disease (HTAD) [2,3]. Early detection and clinical management of TAAs are critical to prevent deaths due to TAD. *SMAD4* protein is a central molecule in TGF $\beta$  signal transduction through the canonical arm of the TGF $\beta$  signaling pathway. The recognition of the *SMAD4* variants reported herein was facilitated by GeneMatcher and MyGene2, nodes of the MatchMaker Exchange platform which serves to connect investigators with an overlapping interest in a registered gene, providing an interface to clinicians and scientists that supports the discovery of genes underlying rare diseases [4]. Herein, we report *SMAD4* variants that were identified through a screen of exome sequencing data from individuals with HTAD or TAD performed in the Milewicz lab which employed whole exome sequencing [5,6]. This report increases the accessibility of the identified *SMAD4* variants and may be utilized in future efforts that aim to build a *SMAD4* variant database for genetic disorders.

## 2. Methods

### 2.1. Study Population

Whole exome sequencing data were obtained for affected probands and family members from 346 unrelated heritable thoracic aortic disease families (HTAD) and 355 individuals with early-onset (age  $\leq$  56 years old) thoracic aortic dissection (ESTAD) from 2000 to 2019. Blood or saliva samples were collected after obtaining approval from the Institutional Review Board at the University of Texas Health Science Center at Houston. Informed consent was obtained from all participants.

### 2.2. Whole Exome Sequencing

Exome sequences were captured by SeqCap EZ Exome probes version 2.0 (Roche) and recovered according to the manufacturer's directions. Enriched libraries were then sequenced on an Illumina GAIIx using manufacturer protocols. Reads were mapped to the reference human genome (UCSC hg19) with BWA (Burrows–Wheeler Aligner), and variant detection and genotyping were performed using the UnifiedGenotyper (UG) tool from GATK. Annotation of variants was performed using the SeattleSeq server (<http://gvs.gs.washington.edu/SeattleSeqAnnotation>, SeattleSeq v.151, accessed on 17 August 2018) and Annovar variant annotation (<https://annovar.openbioinformatics.org/> accessed on 17 August 2018). Sanger DNA sequencing assay was performed to validate *SMAD4* variants identified by exome sequencing.

To identify potential pathogenic or likely pathogenic *SMAD4* (NM\_005359.6) variants, whole exome sequencing data were filtered based on three criteria: (1) variants that altered amino acids, including nonsynonymous, stop-loss, stop-gain, coding indel, frameshift, and splice site variants; (2) variants with a minor allele frequency less than 0.05% in the Genome Aggregation Database (gnomAD exome v2.1.1) and a combined annotation-dependent depletion score (CADD) that was larger than 20, and (3) variants that segregated with thoracic aortic disease in HTAD families. The Sorting Intolerant from Tolerant for Genome (SIFT4G) database was used to predict possible damaging variants.

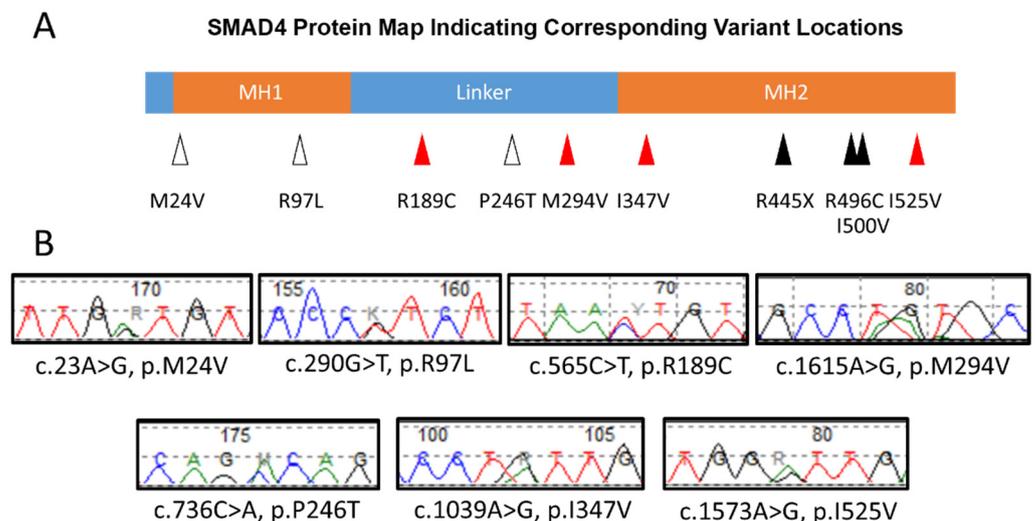
### 2.3. Mapping of Genomic Variants

*SMAD4* variants in the gnomAD structural variant (SV) v2.1 were examined and compared to TTA-associated genes. The gnomAD is based on more than 141,000 exomes and genomes from unrelated individuals sequenced as part of various disease-specific and population genetic studies and is aligned against the GRCh37 reference. The referenced

data are derived from a whole exome sequencing database described by Karczewski et al. and Collins et al. [7,8].

### 3. Results

Exome sequencing analysis identified seven rare variants in *SMAD4* with a CADD score of more than 20 that are predicted to result in amino acid substitutions. *SMAD4* protein is comprised of two functional domains, named MH1 and MH2, which are held together by a linker. The MH1 domain complexes with DNA [9–11], while the MH2 domain located at the C-terminal interacts with other proteins, including other SMAD proteins. For these variants, two are located in each MH1 and MH2 domain, while the remaining three are located in the linker domain (Figure 1A). The variants M24V, R97L, and P246V, indicated in the Figure by white arrows, have been previously reported [5]. The R97L mutation in the MH1 domain had the highest CADD score (32) and a Rare Exome Variant Ensemble Learner (REVEL) score (0.938), suggesting a more severe phenotype. Further analysis of these variants using the SIFT4G database identified possible damaging (D) or tolerant (T) variants (Table 1). All variants were validated by Sanger sequencing (Figure 1B). Subsequent analysis showed that R97L is characterized by decreased *SMAD4* stability and reduced TGF $\beta$  signaling [5]. Two other variants, M24V and P246T, were also associated with TAD [5]. R97L and I525V were identified in unrelated HTAD families; R97L segregated with disease, and I525V was shared by two affected cousins. With the exception of I525V, which was found in two unrelated ESTAD families, the remaining variants were identified in only one ESTAD family. The GenomeAD v2.1.1 database also identified three more variants, viz., R445X, R496C, and I500V (black arrows). Taken together, we report seven novel variants of *SMAD4* identified in individuals with either earl- onset or familial TAA.



**Figure 1.** *SMAD4* protein map and locations of the identified variants. (A) *SMAD4* protein map indicating domains that harbor the identified variants; MH domains are indicated in orange. Arrows indicate the approximate location of variants identified by exon sequencing which were reported in Duan et al. 2019 (white), were novel (red), or identified as pathogenic or likely pathogenic variants in gnomAD v2.1.1 (black). (B) Representative Sanger sequencing results of seven variants that were identified by whole exome sequencing of TAA patient samples.

**Table 1.** Human *SMAD4* Genomic Variants Identified by Thoracic Aortic Aneurysm Screening. A list of variants with  $MAF \leq 0.001$  and CADD score  $\geq 20$  are included. The ESTAD and HTAD columns denote the number of unrelated cases for each variant. Abbreviations: Alternate amino acid (Aa alt), Rare Exome Variant Ensemble Learner (REVEL), Combined annotation-dependent depletion score (CADD), Sorting Intolerant from Tolerant for Genome (SIFT4G) prediction, Damaging (D), Tolerant (T), Clinical significance of the Variant (ClinVar), Genome Aggregation Database allele frequency (gnomAD exome v2.1.1), early-onset thoracic aortic dissection (ESTAD), and unrelated heritable thoracic aortic disease (HTAD).

Gene.	Aa alt	REVEL	CADD	SIFT4G	Clin Var		gnomAD v2.1.1	
					Clinical Significance	MAF	Allele Count	Homozygote Count
SMAD4	M24V	0.579	26.3	D	Uncertain significance	$3.19 \times 10^{-5}$	1	0
SMAD4	R97L	0.925	32	D	Conflicting interpretations			
SMAD4	R189C	0.24	23.5	T	Conflicting interpretations	$3.64 \times 10^{-4}$	103	1
SMAD4	P246T	0.214	22.5	D	Uncertain significance	$7.95 \times 10^{-6}$	2	0
SMAD4	M294V	0.35	22.2	T	Conflicting interpretations	$1.74 \times 10^{-4}$	49	0
SMAD4	I347V	0.387	20.4	T	Uncertain significance	$7.95 \times 10^{-6}$	2	0
SMAD4	R445X				Pathogenic	$3.98 \times 10^{-6}$	1	0
SMAD4	R496C				Pathogenic/Likely pathogenic	$7.95 \times 10^{-6}$	2	0
SMAD4	I500V				Pathogenic	$3.98 \times 10^{-6}$	1	0

#### 4. Discussion

Many TAA are asymptomatic and only diagnosed when a life-threatening TAD occurs. Several parameters are used for early diagnosis of TAA, so that monitoring and clinical interventions can be pursued to prevent TAD. These include a family history of TAA or TAD, genetic testing, and familial screening. There are over 20 genes with evidence that variants within the gene predispose to TAA [12], and sufficient evidence of TAA disease-causing for 11 of these genes [13]. Genetic variants that predispose to TAA lead to decreased smooth muscle cell (SMC) contraction and survival, altered extracellular matrix (ECM) integrity, and decreased canonical TGF $\beta$  signaling [2,14–16]. Thus, variants in genes involved in TGF $\beta$  signaling, that regulate many processes associated with vascular development and repair, are associated with TAA [12,17,18].

*SMAD4* protein plays a critical role in TGF $\beta$  signal transduction [12,17,18]. It binds to phosphorylated (i.e., activated) *SMAD2/3*, and in turn this complex translocates to the nucleus, where it alters target gene expression in concert with other transcription factors [19]. We report rare, predicted damaging *SMAD4* variants identified through exome sequencing of a large cohort of patients with TAA. Exome sequencing identified seven variants distinct from the three variants reported in gnomAD v2.1.1 with potential pathogenic or likely pathogenic consequences. These variants are located within regions of *SMAD4* that encode the MH1, MH2, and linker domains of *SMAD4* protein. It remains unclear whether specific *SMAD4* variants associate with AVM formations as seen in HHT, underscoring the need for a *SMAD4* variant database. Further analysis of individual variants is necessary to establish disease specificity. Together with available data, the identification of these variants contributes to our understanding of how *SMAD4* may protect against vascular disorders.

Research with the global *Smad4* knockout mouse model has revealed critical roles in embryogenesis and in the development of cancer [20–22]. *Smad4* plays a key role in blood vessel angiogenesis, and establishing its importance in vascular development remains a very active area of research. To understand the importance of *Smad4* in embryonic blood vessel development and disease, elegant studies have been conducted with animal models of inducible, tissue-specific *Smad4* loss. Total loss of *Smad4* in mice is embryonically lethal due to the inability of the embryo to complete gastrulation, supporting a critical role for *Smad4* in embryonic development [20]. Endothelial cell-specific deletion is also embryonically lethal due to an angiogenesis failure and impaired recruitment of smooth muscle cells [21]. In adult and neonatal mice, inducible *Smad4* conditional knockout models have been used to understand the role of *Smad4* in adult tissues. Inducible endothelial cell-specific *Smad4* knockout mice develop AVMs and vascular defects following tamoxifen

injection at postnatal day 1 (PN1), recapitulating, in part, the AVMs that form in patients with HHT [23]. Importantly, endothelial cell-specific inducible deletion of *Smad4* has uncovered that AVM formation is associated with reduced signaling through TEK due to increased expression of its antagonistic ligand angiopoietin-2 [24]. This phenotype can be rescued through inhibition of angiopoietin 2, supporting the development of new therapeutic approaches to treat AVMs [24]. While complete loss of *Smad4* has been shown to result in early embryonic lethality, the variants identified here were sufficient to support human development but are associated with early onset of familial TAA. These data raise the possibility that specific *SMAD4* variants may partially disrupt vascular development and/or exacerbate angiogenic mechanisms that contribute to thoracic aortic disease risk.

In addition to the critical roles of *Smad4* in embryonic development in mice, human *SMAD4* variants have been identified and are associated with several human diseases. Variants in the *SMAD4* gene have been associated with juvenile polyposis syndrome (JPS) and also in association with HHT and Myhre syndrome. More specifically, variants that encode the MH2 domain associate with JPS and HHT and result in decreased activity of SMAD4 protein due to increased ubiquitination-mediated degradation [25]. Additional variants in the MH2 domain that increase SMAD4 activity are also associated with a different disorder, Myhre syndrome [26]. Vascular malformations are a common factor amongst HHT, Myhre syndrome, and TAA, yet only a limited number of specific TAA/TAD-associated *SMAD4* variants have been identified. A report by Lifei Wu suggests that an S271N mutation in non-MH regions of the protein may not be directly causal to TAA but may contribute to TAA in combination with other risk factors [27]. More recently, an R97L mutation in *SMAD4* has also been associated with TAA in the absence of HHT or JPS [5]. Herein, we report results from exome sequencing of a cohort of thoracic aortic disease patients without HHT or JPS and the identification of novel *SMAD4* rare and damaging variants, in addition to the previously reported R97L [5]. Together with available data, the knowledge of these variants contributes to our understanding of how *SMAD4* variants may contribute to vascular diseases.

With the exception of R97L [5], further investigation and mechanistic validation are needed to determine the physiological relevance of each variant and to examine the mechanistic relationship between *SMAD4* and vascular diseases, including thoracic aortic disease, HHT, and Myhre syndrome. Additional research is needed to pursue how these variants affect *SMAD4* expression and activity, such as a reduction of *SMAD4* mRNA levels, altered protein structure, or allosteric inhibition of the endogenous, normal SMAD4 protein. Finally, the comparison of variants identified through exon sequencing of TAA tissue with potential pathogenic and likely pathogenic variants in gnomAD strengthens the significance of exon sequences encoding the MH2 domain. Taken together, the *SMAD4* variants identified in ESTAD and HTAD patients in this report may have utility in future work aimed at generating a detailed database for human *SMAD4* variants with possible pathogenic potential. Future investigation in vivo and in vitro of the reported variants is now needed to pursue their molecular functions and advance our understanding of the interaction between the reported *SMAD4* variants and vascular health.

**Author Contributions:** D.G. and D.M.M. planned and executed all the experiments. M.J.B. and D.A.N. executed the exome sequencing. D.G. and M.C.W. facilitated the use of GeneMatcher and MatchMaker Exchange. S.A.B. and D.G. contributed the majority of the original writing. All authors contributed to data analysis and interpretation and writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** This study was approved by the Institutional Review Board at the University of Texas Health Science Center at Houston (Protocol code HSC-MS-01-251 approved on 20th October 2020).

**Informed Consent Statement:** Informed consent was obtained from all the participants involved in the study.

**Data Availability Statement:** The availability of exome data is depended on individuals' consent. For individuals who signed agreement to deposit their exome data on the NCBI database of Genotypes and Phenotypes (dbGaP), the exome data are available on the dbGaP phs000693.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Sánchez-Martínez, R.; Iriarte, A.; Mora-Luján, J.M.; Patier, J.L.; López-Wolf, D.; Ojeda, A. For the RiHHTa investigators of the rare diseases working group from the Spanish society of internal medicine. Current HHT genetic overview in Spain and its phenotypic correlation: Data from RiHHTa registry. *Orphanet J. Rare Dis.* **2020**, *15*, 138. [[CrossRef](#)]
2. Isselbacher, E.M.; Cardenas, C.L.L.; Lindsay, M.E. Hereditary Influence in thoracic aortic aneurysm and dissection. *Circulation* **2016**, *133*, 2516–2528. [[CrossRef](#)]
3. Isselbacher, E.M. Thoracic and abdominal aortic aneurysms. *Circulation* **2005**, *111*, 816–828. [[CrossRef](#)]
4. Philippakis, A.A.; Azzariti, D.R.; Beltran, S.; Brookes, A.J.; Brownstein, C.A.; Brudno, M.; Rehm, H.L. The matchmaker exchange: A platform for rare disease gene discovery. *Hum. Mutat.* **2015**, *36*, 915–921. [[CrossRef](#)] [[PubMed](#)]
5. Duan, X.-Y.; Guo, D.; Regalado, E.S.; Shen, H.; Coselli, J.S.; Estrera, A.L.; Milewicz, D.M. SMAD4 rare variants in individuals and families with thoracic aortic aneurysms and dissections. *Eur. J. Hum. Genet.* **2019**, *27*, 1054–1060. [[CrossRef](#)] [[PubMed](#)]
6. Milewicz, D.M.; Regalado, E.; Shendure, J.; Nickerson, D.A.; Guo, D. Successes and challenges of using whole exome sequencing to identify novel genes underlying an inherited predisposition for thoracic aortic aneurysms and acute aortic dissections. *Trends Cardiovasc. Med.* **2014**, *24*, 53–60. [[CrossRef](#)] [[PubMed](#)]
7. Karczewski, K.J.; Francioli, L.C.; Tiao, G.; Cummings, B.B.; Alföldi, J.; Wang, Q.; MacArthur, D.G. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* **2020**, *581*, 434–443. [[CrossRef](#)] [[PubMed](#)]
8. Collins, R.L.; Brand, H.; Karczewski, K.J.; Zhao, X.; Alföldi, J.; Francioli, L.C.; Talkowski, M.E. A structural variation reference for medical and population genetics. *Nature* **2020**, *581*, 444–451. [[CrossRef](#)]
9. Baburajendran, N.; Jauch, R.; Tan, C.Y.Z.; Narasimhan, K.; Kolatkar, P.R. Structural basis for the cooperative DNA recognition by Smad4 MH1 dimers. *Nucleic Acids Res.* **2011**, *39*, 8213–8222. [[CrossRef](#)]
10. Baburajendran, N.; Palasingam, P.; Narasimhan, K.; Sun, W.; Prabhakar, S.; Jauch, R.; Kolatkar, P.R. Structure of Smad1 MH1/DNA complex reveals distinctive rearrangements of BMP and TGF- $\beta$  effectors. *Nucleic Acids Res.* **2010**, *38*, 3477–3488. [[CrossRef](#)]
11. Chai, N.; Li, W.-X.; Wang, J.; Wang, Z.-X.; Yang, S.-M.; Wu, J.-W. Structural basis for the Smad5 MH1 domain to recognize different DNA sequences. *Nucleic Acids Res.* **2015**, *43*, 9051–9064. [[CrossRef](#)]
12. Pinard, A.; Jones, G.T.; Milewicz, D.M. Genetics of thoracic and abdominal aortic diseases. *Circ. Res.* **2019**, *124*, 588–606. [[CrossRef](#)] [[PubMed](#)]
13. Renard, M.; Francis, C.; Ghosh, R.; Scott, A.F.; Witmer, P.D.; Adès, L.C.; Backer, J.D. Clinical validity of genes for heritable thoracic aortic aneurysm and dissection. *J. Am. Coll. Cardiol.* **2018**, *72*, 605–615. [[CrossRef](#)]
14. Boileau, C.; Guo, D.-C.; Hanna, N.; Regalado, E.S.; Daint, D.; Gong, L.; Milewicz, D.M. TGF $\beta$ 2 mutations cause familial thoracic aortic aneurysms and dissections associated with mild systemic features of Marfan syndrome. *Nat. Genet.* **2012**, *44*, 916–921. [[CrossRef](#)]
15. Guo, D.-C.; Pannu, H.; Tran-Fadulu, V.; Papke, C.L.; Yu, R.K.; Avidan, N.; Milewicz, D.M. Mutations in smooth muscle alpha-actin (ACTA2) lead to thoracic aortic aneurysms and dissections. *Nat. Genet.* **2007**, *39*, 1488–1493. [[CrossRef](#)] [[PubMed](#)]
16. LeMaire, S.A.; McDonald, M.-L.N.; Guo, D.-C.; Russell, L.; Miller, C.C.; Johnson, R.J.; Milewicz, D.M. Genome-wide association study identifies a susceptibility locus for thoracic aortic aneurysms and aortic dissections spanning FBN1 at 15q21.1. *Nat. Genet.* **2011**, *43*, 996–1000. [[CrossRef](#)] [[PubMed](#)]
17. Milewicz, D.M.; Trybus, K.M.; Guo, D.-C.; Sweeney, H.L.; Regalado, E.; Kamm, K.; Stull, J.T. Altered smooth muscle cell force generation as a driver of thoracic aortic aneurysms and dissections. *Arterioscler. Thromb. Vasc. Biol.* **2017**, *37*, 26–34. [[CrossRef](#)]
18. Milewicz, D.M.; Prakash, S.K.; Ramirez, F. Therapeutics targeting drivers of thoracic aortic aneurysms and acute aortic dissections: Insights from predisposing genes and mouse models. *Annu. Rev. Med.* **2017**, *68*, 51–67. [[CrossRef](#)]
19. Zhao, M.; Mishra, L.; Deng, C.-X. The role of TGF- $\beta$ /SMAD4 signaling in cancer. *Int. J. Biol. Sci.* **2018**, *14*, 111–123. [[CrossRef](#)]
20. Chu, G.C.; Dunn, N.R.; Anderson, D.C.; Oxburgh, L.; Robertson, E.J. Differential requirements for Smad4 in TGF $\beta$ -dependent patterning of the early mouse embryo. *Development* **2004**, *131*, 3501–3512. [[CrossRef](#)]
21. Lan, Y.; Liu, B.; Yao, H.; Li, F.; Weng, T.; Yang, G.; Yang, X. Essential role of endothelial smad4 in vascular remodeling and integrity. *Mol. Cell. Biol.* **2007**, *27*, 7683–7692. [[CrossRef](#)]

22. Takaku, K.; Miyoshi, H.; Matsunaga, A.; Oshima, M.; Sasaki, N.; Taketo, M.M. Gastric and duodenal polyps in Smad4 (Dpc4) knockout mice. *Cancer Res.* **1999**, *59*, 6113–6117. [[PubMed](#)]
23. Crist, A.M.; Lee, A.R.; Patel, N.R.; Westhoff, D.E.; Meadows, S.M. Vascular deficiency of Smad4 causes arteriovenous malformations: A mouse model of hereditary hemorrhagic telangiectasia. *Angiogenesis* **2018**, *21*, 363–380. [[CrossRef](#)] [[PubMed](#)]
24. Crist, A.M.; Zhou, X.; Garai, J.; Lee, A.R.; Thoele, J.; Ullmer, C.; Meadows, S.M. Angiopoietin-2 Inhibition rescues arteriovenous malformation in a Smad4 hereditary hemorrhagic telangiectasia mouse model. *Circulation* **2019**, *139*, 2049–2063. [[CrossRef](#)]
25. Gallione, C.; Aylsworth, A.S.; Beis, J.; Berk, T.; Bernhardt, B.; Clark, R.D.; Marchuk, D.A. Overlapping spectra of SMAD4 mutations in juvenile polyposis (JP) and JP-HHT syndrome. *Am. J. Med. Genet. Part A* **2010**, *152*, 333–339. [[CrossRef](#)] [[PubMed](#)]
26. Le Goff, C.; Mahaut, C.; Abhyankar, A.; Le Goff, W.; Serre, V.; Afenjar, A.; Cormier-Daire, V. Mutations at a single codon in Mad homology 2 domain of SMAD4 cause Myhre syndrome. *Nat. Genet.* **2012**, *44*, 85–88. [[CrossRef](#)]
27. Wu, L. Functional characteristics of a novel SMAD4 mutation from thoracic aortic aneurysms (TAA). *Gene* **2017**, *628*, 129–133. [[CrossRef](#)] [[PubMed](#)]