MAT2A Mutations Predispose Individuals to Thoracic Aortic Aneurysms

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Up to 20% of individuals who have thoracic aortic aneurysms or acute aortic dissections but who do not have syndromic features have a family history of thoracic aortic disease. Significant genetic heterogeneity is established for this familial condition. Whole-genome linkage analysis and exome sequencing of distant relatives from a large family with autosomal-dominant inheritance of thoracic aortic aneurysms variably associated with the bicuspid aortic valve was used for identification of additional genes predisposing individuals to this condition. A rare variant, c.1031A>C (p.Glu344Ala), was identified in *MAT2A*, which encodes methionine adenosyltransferase II alpha (MAT IIα). This variant segregated with disease in the family, and Sanger sequencing of DNA from affected probands from unrelated families with thoracic aortic disease identified another *MAT2A* rare variant, c.1067G>A (p.Arg356His). Evidence that these variants predispose individuals to thoracic aortic aneurysms and dissections includes the following: there is a paucity of rare variants in *MAT2A* in the population; amino acids Glu344 and Arg356 are conserved from humans to zebrafish; and substitutions of these amino acids in MAT Iα are found in individuals with hypermethioninemia. Structural analysis suggested that p.Glu344Ala and p.Arg356His disrupt MAT IIα enzyme function. Knockdown of *mat2aa* in zebrafish via morpholino oligomers disrupted cardiovascular development. Co-transfected wild-type human *MAT2A* mRNA rescued defects of zebrafish cardiovascular development at significantly higher levels than mRNA edited to express either the Glu344 or Arg356 mutants, providing further evidence that the p.Glu344Ala and p.Arg356His substitutions impair MAT IIα function. The data presented here support the conclusion that rare genetic variants in *MAT2A* predispose individuals to thoracic aortic disease.

Aneurysms or enlargements of the thoracic aorta above the heart, which involve the aortic root or ascending thoracic aortic or both, can progressively enlarge over time and predispose individuals to acute aortic dissection and rupture, events that are associated with a high degree of mortality, morbidity, and medical expenditure. Prophylactic repair of an ascending aortic aneurysm is recommended to prevent a life-threatening aortic dissection or rupture. Family studies indicate that up to 20% of individuals who have thoracic aortic aneurysms and dissections (TAAD) but no syndrome (e.g., Marfan syndrome [MIM 154700]) have a family history of TAAD, termed familial TAAD (FTAAD).[1,2] Mutations in several genes, including *FBN1* (fibrillin-1 [MIM 134797]), *TGFBR1* (transforming growth factor β receptor 1 [MIM 190181]), *TGFBR2* (transforming growth factor β receptor II [MIM 190182]), *TGFB2* (transforming growth factor β2 [MIM 190220]), *SMAD3* (SMAD family member 3 [MIM 603109]), *MYH11* (smooth muscle myosin heavy chain [MIM 160745]), *ACTA2* (smooth muscle α actin [MIM 102620]), *MYLK* (myosin light chain kinase [MIM 600922]), and *PRKG1* (cGMP-dependent protein kinase type I [MIM 176894]) have been identified as causing FTAAD in approximately 25% of families. These genes encode proteins involved in either smooth muscle cell (SMC) contraction or the TGF-β signaling pathway.[3–10]

FTAAD is primarily inherited in an autosomal-dominant manner with decreased penetrance and variable expression. The expression of TAAD in families is also variable in terms of TAAD-associated clinical features, such as patent ductus arteriosus (PDA [MIM 607411]), early-onset coronary artery disease, or intracranial aneurysms.[5,9,11] A bicuspid aortic valve (BAV) is another cardiovascular feature that can be inherited in families affected by TAAD. Interestingly, BAV is a common congenital heart defect, found in 1%–2% of the general population.[12] It is estimated that up to 20% of individuals with BAV will go on to develop ascending thoracic aortic aneurysms. Thus, a strong association between BAV and TAAD risk has been observed.[13,14] Although the risk for BAV might be slightly increased in individuals with *TGFBR2* and *ACTA2* mutations,[11,15] to date no genes have been identified as

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causing TAAD-associated BAV in multiple members of a family.[16]

A large family, TAA059, with autosomal-dominant inheritance of TAAD with decreased penetrance underwent sequencing of the genes in which mutations have been identified as causing FTAAD and no mutations were identified in these genes (Figure 1A). Eight individuals in this family have dilatation of the aortic root and ascending aorta with or without BAV. To identify the gene responsible for thoracic aortic disease in this family, we collected blood or saliva samples from both affected and unaffected family members after obtaining approval from the institutional review board at the University of Texas Health Science Center at Houston and informed consent from the participants. We conducted genome-wide linkage analysis on DNA from eight family members, and we used the Affymetrix 50K SNP array to map the mutant locus. Under the assumption of age-dependent penetrance with reduced penetrance in women, parametric multipoint LOD score analyses obtained a score of approximately 2.0 at 2p21–p16.2, 2p12–q11.2, 3q28–q29, and 7p21.1–p15.2 (Figure 1B). To assess whether rare copy-number variants (CNVs) contributed to TAAD in this family, we assayed DNA from the proband of TAA059 (III:17) on an Illumina Human 660W-Quad BeadChip and used PennCNV and CNV Partition software to identify unique CNVs in the proband's genome by comparison with 6809 Illumina genotyped subjects obtained from the Database of Genotypes and Phenotypes (dbGAP) by previously described methods.[17] No unique CNVs were identified in the proband either within the loci delineated by whole-genome linkage analysis peaks or outside these peaks.

DNA from two affected family members (coefficient of relationship = 1/8) was used for whole-exome sequencing (Figure 1A). Using previously described filtering strategies, we identified 25 variants that were shared between the two affected relatives, resulted in altered amino acid sequences, and had minor-allele frequencies (MAFs) less than 0.05% in the NHLBI Exome Sequencing Project and 1000 Genomes Project.[18] Two rare variants fell under a linkage peak (both under a major peak, spanning 78.3 Mb to 113.6 Mb on chromosome 2) and disrupted the coding sequence of *MAT2A* (methionine adenosyltransferase II, alpha [MIM 601468]; RefSeq accession number NM_005911.5) by introducing the mutation c.1031A>C (p.Glu344Ala) and of *PROM2* (prominin 2; RefSeq NM_144707.2) by introducing the mutation c.1381A>G (p.Ser461Gly) (Figure 1B). The PROM2 p.Ser461Gly alteration is present with a MAF of 0.047 in the European Americans in the NHLBI Exome Sequencing Project (ESP) database, and Ser461 is not conserved (it is glycine in the mouse and cat genomes). In contrast, MAT IIα p.Glu344Ala is not in any exome databases, and the variant disrupts a highly conserved amino acid (Figure 1C). *MAT2A*, with 91% identity at the amino acid level between humans and zebrafish, is highly conserved through evolution, and 13 rare variants in the ESP database alter amino acids. One of these, a nonsense variant in the last exon, is not predicted to lead to nonsense-mediated decay (Figure 1D).Furthermore, MAT IIα p.Glu344Ala is predicted to be damaging by six bioinformatics tools (PolyPhen-2 [both HVAR and HDIV scores], PROVEAN, SIFT, MutationTaster, MutationAssessor, likelihood-ratio test [LRT], and Functional Analysis Through Hidden Markov Models [FATHMM]) and has a C score of 24.[19] Linkage analysis of thoracic aortic disease with the *MAT2A* variant (c.1031A>C) in TAA059 generated a two-point LOD score of 2.31.

The proband, III:17, was diagnosed with aortic root and ascending aortic dilatation and BAV at age 37 years and underwent surgical repair of a 4.9 cm ascending aortic aneurysm at age 43 years. Her first cousin, III:9, was diagnosed with a 5.2 cm aortic root aneurysm with a normal aortic valve at age 45 years and underwent a valve-sparing aortic root replacement. There was no reported history of aortic dissection, but an obligate carrier (II:5) died suddenly from unknown causes at age 32 years. A total of 18 individuals with the *MAT2A* rare variant underwent evaluation for thoracic aortic disease and bicuspid aortic valve. Eight (44%) of these individuals were diagnosed with dilatation of the ascending aorta and/or aortic root at a median age of 50 years (range 37–56 years), and four individuals (24%) were diagnosed with bicuspid aortic valves. Ten individuals (56%), whose median age was 30 years (range 16–50 years) at last follow-up, did not have aortic disease. None of the individuals with the *MAT2A* variant had other cardiovascular disease. Four individuals were evaluated by a geneticist, and no systemic features of Marfan or Loeys-Dietz syndrome were observed. Aortic tissue excised during aortic aneurysm repair of III:9 and III:17 showed mild medial degeneration in the aortic media, characterized by focal areas of increased proteoglycan deposition and fragmentation of elastic fibers, but minimal loss of SMCs (Figure 2).

To confirm that *MAT2A* mutations predispose individuals to FTAAD, we analyzed exome data from 78 FTAAD probands and Sanger-sequencing data of all *MAT2A* exons and flanking introns from an additional 447 FTAAD probands in whom no variants responsible for the disease have been identified. DNA samples were obtained from affected individuals and other family members after informed consent and approval from all participating institutions, including the Cleveland Clinic Center for Personalized Genetic Healthcare and the Centre de Reference pour les Syndromes de Marfan et Apparente's in France, were obtained. One *MAT2A* rare variant, c.1067G>A (p.Arg356His), was identified in family TAA450. MAT IIα p.Arg356His is predicted to be damaging by six bioinformatics tools, and this variant is not reported by the NHLBI ESP database in 13,006 chromosomes. However, the proband from TAA450 also has an *ACTA2* (RefSeq NM_001613.2) rare variant, c.143G>T (p.Gly48Val), predicted to be probably damaging by PolyPhen-2 and absent in the ESP database; this variant has not been identified

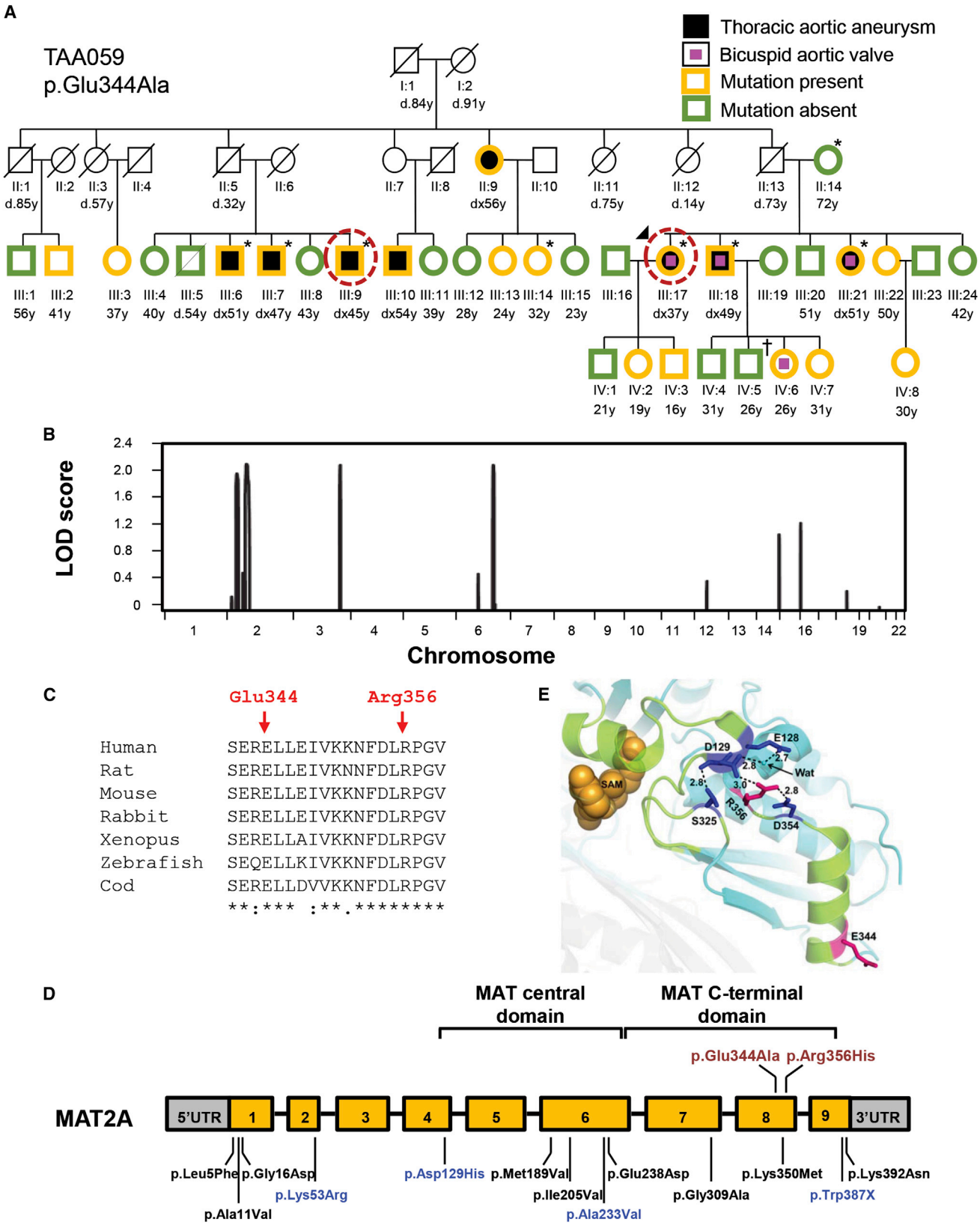

Figure 1. Identification and Characterization of *MAT2A* Rare Variants in Families Affected by Thoracic Aortic Disease
(A) TAA059 family pedigree. The legend indicates the designations for disease and mutation status of family members. The age at diagnosis of aortic aneurysm (dx), age at death (d), or age at last aortic imaging are shown in years. Individuals with aortic dilatation measuring ≥4.2 cm or *Z* scores of ≥2 were marked as affected. A diagonal line across a symbol indicates that the individual is deceased, an arrow indicates the proband, a single asterisk indicates an individual whose DNA was used for whole-genome linkage analysis, and a

(legend continued on next page)

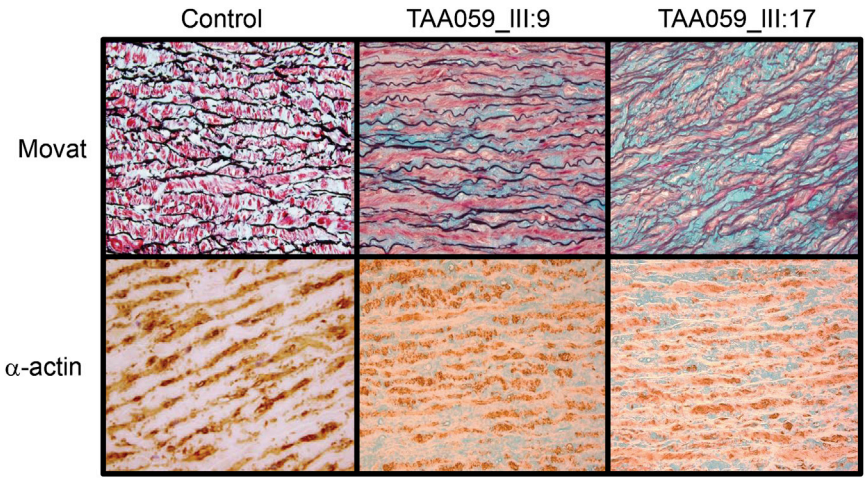

Figure 2. Aortic Pathology Associated with Aneurysms in Individuals with *MAT2A* Variants

Compared with the control aorta, aortas from affected individuals showed medial degeneration upon Movat staining, which showed increased proteoglycan deposition (blue), focal mild fragmentation of elastic fibers (black), and a decreased number of cells (red). Immunostaining for α-actin confirmed the mild focal loss of SMCs.

as a cause of FTAAD, but disease-causing *ACTA2* variants have been identified in the adjacent amino acid (p.Met49Val).[20,21] No additional samples were available for testing the segregation of these variants with disease in the family.

MAT2A encodes the enzyme MAT IIα, which catalyzes the transfer of the adenosyl moiety from ATP to L-methionine to synthesize S-adenosylmethionine (SAM). SAM serves as the methyl-group donor for methylation reactions involving DNA, RNA, and protein.[22] After donating its methyl group, SAM is converted to S-adenosylhomocysteine (SAH), which is a competitive inhibitor of methyltransferases and is rapidly hydrolyzed to homocysteine.[23] In mammals, methionine adenyltranferases are encoded by two genes, *MAT1A* (methionine adenosyltransferase I, alpha [MIM 610550]) and *MAT2A*.[24] *MAT1A* expression is limited to the adult liver, whereas *MAT2A* is expressed in all tissues and at a high level in aortic SMCs.[25] The activity of MAT IIα is regulated by a β subunit (MAT IIβ), which is encoded by a separate gene, *MAT2B* (methionine adenosyltransferase II, beta [MIM 605527]).[26] Exome data from 88 affected FTAAD probands did not identify any rare variants in either *MAT1A* or *MAT2B*. The amino acid sequences of human MAT Iα and MAT IIα are 84% identical, and the structure of both of these enzymes has been determined. *MAT1A* encodes the catalytic subunit (α1) that organizes into dimers (in MAT III) and tetramers (in MAT I). Recessive mutations in *MAT1A* cause hypermethioninemia, and both p.Glu344Ala and p.Arg356His have been reported as disease causing.[27,28] Arg356 is located close to the SAM binding pocket in the protein and is part of a hydrogen-bonding network involving residues Glu128, Asp129, Ser325, and Asp354 and a water molecule (Figure 1E). Altering Arg356 to His356 would be expected to destabilize the SAM binding pocket and lead to a loss of enzymatic activity. This prediction is consistent with a ~90% loss of MAT I/III activity in the p.Arg356Gln substitution and a ~97% loss of activity in the p.Arg356Typ substitution.[27,28] Although Glu344 is farther away from the SAM binding site, loss of activity in the Glu344 substitution suggests that its strategic position at the end of the helix in relation to Arg356 is most likely required for electrostatic interaction with either another molecule of MAT IIα or the interaction partner MAT IIβ. Alternatively, an undesired electrostatic interaction involving Arg356 might also lead to alteration of the helix position and thereby adversely affect the "cantilever" (green, Figure 1E) leading into the SAM binding site and thus SAM binding.

The zebrafish genome encodes two MAT IIα paralogs: Mat2aa (RefSeq NP_001277009) has 395 amino acids with 91% identity (96% similarity) with human MAT IIα (RefSeq NP_005902), and Mat2ab (RefSeq NP_001014318) has 363 amino acids with 89% identity (96% similarity) to MAT IIα but lacks the last 32 amino acids of the C-terminal region of MAT IIα. Previous studies have shown that expression of *mat2aa* in zebrafish is

red circle indicates the individuals whose DNA was used for exome sequencing. Individual IV:5, marked by symbol †, has had stable aortic-root measurements around the upper limit of normal for 6 years; the ascending aorta is normal.
(B) Profile of the parametric multipoint LOD score for segregation of TAAD with SNPs across the human genome in family TAA059.
(C) Amino acid alignment of MAT2A sequences containing the rare variants identified in this study.
(D) Schematic representation of *MAT2A*. The boxes represent exons 1–9, and the UTRs and the open reading frame are designated. The *MAT2A* rare variants identified in this study are above the gene diagram, and the rare variants identified in the ESP database are below. Blue letters designate variants predicted to be possibly or probably damaging by PolyPhen-2 analysis, and black letters designate variants predicted to be benign.
(E) X-ray crystallographic structure of MAT IIα (PDB identifier 2P02) shows positions of Glu344 (E344) and Arg356 (R356, designated in pink) relative to the SAM binding site. Analysis of hydrogen bonds and non-bonded contacts of the respective residues and others in the vicinity was performed with PYMOL. Arg356 is located near the SAM pocket and is part of a hydrogen-bonding network involving residues Glu128 (E128), Asp129 (D129), Ser325 (S325), and Asp354 (D354, all designated in blue) and a water molecule (Wat). Structural elements that we propose as part of a "cantilever system" are shown in green and blue and include Glu344 and Arg356; the rest of the monomer is in cyan. Part of a second monomer is also seen (pale gray).

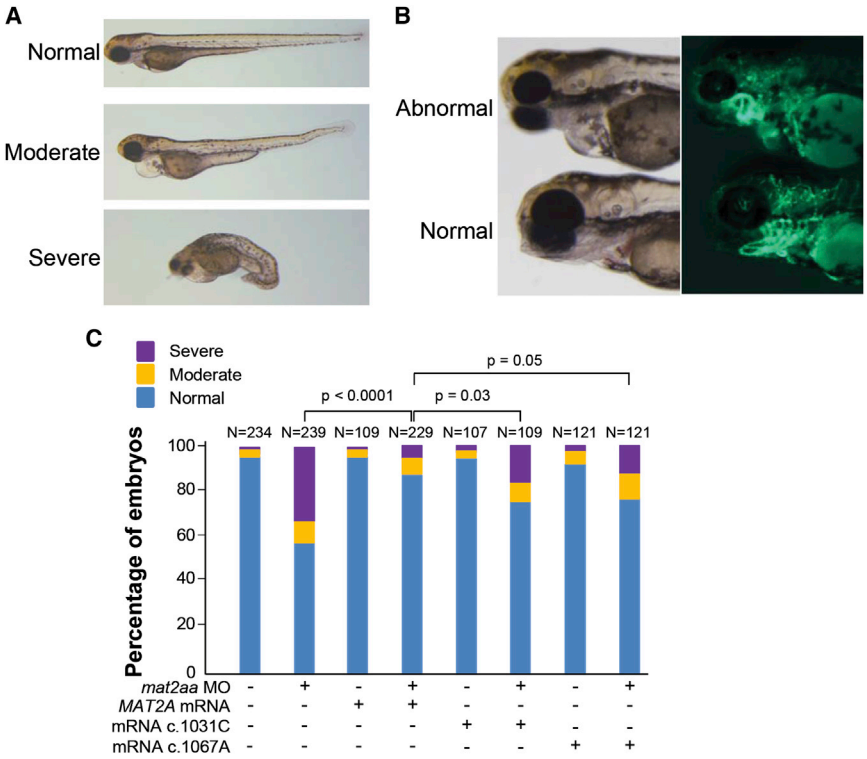

Figure 3. Phenotypic Spectrum after *mat2aa* MO Injection and mRNA Rescue
Zebrafish were phenotyped with light microscopy 72 dpf after *mat2aa* MO injection in the zebrafish *Tg(flk1:EGFP.)* Representative images are shown.
(A) Normal phenotype of control-MO-injected zebrafish showing minimal pericardial effusion and no tail defects; a moderately affected morphant with a large pericardial effusion and small eyes; and a severely affected morphant with a large pericardial effusion, small eyes, and a curly tail or severe tail curvature.
(B) At 3 dpf after *mat2aa* MO injection, significant defects in the development of the aortic arches were observed.
(C) Zebrafish *mat2aa* MO injection resulted in significant defects on embryonic development. Co-injection of wild-type (WT) *MAT2A* mRNA, in comparison with the substitution mRNA, significantly reduced defects of cardiovascular development.

restricted to the pharyngeal arch, and embryonic heart and transposon knockout of *mat2aa* expression results in a pericardial edema phenotype at 3 days postfertilization (dpf) and death at 8 dpf.[29] To investigate whether the p.Glu344Ala substitution disrupts MAT IIα function, we used a morpholino (MO) oligomer to disrupt expression of *mat2aa* in *Tg(flk1:EGFP)* zebrafish, which express enhanced GFP in the entire vasculature under the control of the *flk1* promoter and thus enable the visualization of vascular defects in live zebrafish embryos.[30] Injection of 4 ng of the *mat2aa* MO and assessment of the morphant zebrafish at 3 dpf showed the expected pericardial edema phenotype, along with other embryonic-development defects that were classified as moderate or severe (Figure 3A). Zebrafish were classified as phenotypically normal if they were indistinguishable from control-MO-injected fish or had minimal pericardial effusion and no tail defects; moderately affected if they had a large pericardial effusion and mild tail defects; and severely affected if they had widespread edema, malformed eyes, and either a very short malformed tail or no tail at all. Zebrafish classified with moderate or severe defects in development also had disruption of the development of the aortic arches (Figure 3B). To determine whether the human wild-type and p.Gly344Ala and p.Arg356His mutant *MAT2A* mRNA rescued these embryonic defects, we co-injected *mat2aa* MO with either wild-type or mutant *MAT2A* mRNA. Co-injection of either wild-type or mutant *MAT2A* mRNA partially rescued the moderate and severe defects of zebrafish embryonic development by *mat2aa* MO ($p < 0.0001$). However, wild-type *MAT2A* mRNA rescued the developmental defects in the zebrafish at a significantly higher frequency than did the mutant *MAT2A* mRNAs encoding either the p.Gly344Ala variant ($p = 0.03$) or the p.Arg356His variant ($p = 0.05$; Figure 3C).

Thus, accumulating evidence indicates that *MAT2A* mutations predispose individuals to thoracic aortic disease. The *MAT2A* rare variant is located within one of the linkage peaks identified by whole-genome linkage analysis in TAA059 and is not present in exome databases. An additional *MAT2A* rare variant, which is not in the databases, was identified in a FTAAD proband. Exome sequencing databases indicate that variants in *MAT2A* are rare in the population. Protein-structure analysis indicates that p.Glu344Ala and p.Arg356His are loss-of-function alterations that significantly reduce catalytic activity of MAT IIα. The substitution of either p.Glu344Ala or p.Arg356Trp in MAT I/III reduces the enzymatic activity and leads to hypermethioninemia. Finally, human wild-type *MAT2A* mRNA was significantly more efficient in rescuing *mat2aa*-MO-knockout-induced defects of zebrafish cardiovascular development than *MAT2A* mRNA altered to express either p.Glu344Ala or p.Arg356Trp.

Therefore, *MAT2A* mutations are a rare cause of FTAAD, and the rarity of these mutations could be because disease-causing variants fall in or near the active site and disrupt the activity of the enzyme. Alternatively, the decreased penetrance of the thoracic aortic aneurysms and low risk for acute aortic dissections in families affected by *MAT2A* mutations might prevent clinical recognition of families with variants in this gene. The penetrance of TAAD in TAA059 is low in comparison to that in families affected by Marfan syndrome with *FBN1* mutations that demonstrate nearly complete penetrance of aortic disease. The

youngest age of a family member diagnosed with a thoracic aortic aneurysm in this family was 37 years old, whereas *FBN1* mutations typically lead to aortic dilatation in childhood.[31] Of the 15 individuals who have the *MAT2A* variant and are over the age of 30 years, seven have been diagnosed with TAAD. It is also possible that the development of TAAD in individuals with *MAT2A* loss-of-function variants might need an additional genetic or environmental "hit" to develop thoracic aortic disease. In TAA059, one arm of the family is affected by BAV, which potentially could increase the risk of TAAD. Additionally, the proband in TAA450 has a *MAT2A* rare variant but also has an *ACTA2* variant that has not been previously identified in families with FTAAD.

Methionine adenosyltransferases (MATs) catalyze the synthesis of SAM, an enzyme that plays a critical role in cellular metabolism. Mutations in *MAT1A* primarily affect the MAT I/III C-terminal domain and have been identified in individuals with autosomal-dominant or -recessive hypermethioninemia; aortic disease has not been reported in these individuals.[32] These mutations lead to a significant reduction or loss of MAT I/III activity, increased levels of plasma methionine, and normal or reduced levels of SAM.[27,28] It is interesting that MAT I/III p.Glu344Ala and p.Arg356Trp substitutions result in autosomal-recessive inheritance of hypermethioninemia, whereas MAT IIα p.Glu344Ala and p.Arg356His cause autosomal-dominant inheritance of TAAD.[27,28] One possible explanation is that MAT IIα activity in the liver increases in individuals with *MAT1A* mutations and that this compensation cannot occur with loss of MAT IIα activity in aortic SMCs. Supporting this hypothesis is the observation that ectopic expression of *Mat2a* has been reported in the *Mat1a*$^{-/-}$ mouse.[33] Additionally, an individual with hypermethioninemia due to a homozygous *MAT1A* mutation leading to a premature stop codon (p.Thr185*) and slightly decreased SAM levels has been reported, and one proposed explanation for this observation is that ectopic expression of *MAT2A* in the liver might maintain SAM levels.[34]

The rare variants in *MAT2A* in FTAAD families are predicted to decrease MAT IIα function and reduce cellular SAM levels, which could lead to aortic disease through a number of potential pathways. The SAM/SAH ratio is considered to be an indicator of cellular methylation potential, and a decrease in the SAM/SAH ratio is predicted to reduce methylation capacity.[35] In SMCs, global hypomethylation has been shown to occur with phenotypic modulation and proliferation (reviewed by Liu et al.[36]). More recently, ten-eleven translocation-2 (TET2), which oxidizes 5-methylcytosine to generate 5-hydroxymethylcytosine, and subsequently unmethylated cytosine, has been identified as an epigenetic regulator of SMC differentiation.[37] Thus, disrupting the methylation potential of SMCs has the potential to alter the phenotype of these cells. Alternatively, decreased cellular SAM activity has the potential to decrease glutathione (GSH) activity and increase oxidative stress in the aortic SMCs. This mechanism is supported by the observation that the *Mat1a*$^{-/-}$ mouse has a marked decrease in hepatic GSH and an increase in serum lipid peroxides, indicating that *Mat1a* deficiency triggers hepatic oxidative stress. *MAT2A* loss-of-function mutations have the potential to similarly decrease GSH and increase oxidative stress in aortic SMCs. Increased oxidative stress has been previously shown to increase the sensitivity of SMCs to angiotensin II (Ang II).[38] Because Ang II infusion leads to aortic aneurysms and dissections in mice,[39] increased and chronic oxidative stress might also lead to aortic disease via increased signaling through the Ang II pathway. A third possibility is that loss of MAT IIα activity limits intracellular cysteine pools. Fibrillin-1, the protein that is altered in individuals with Marfan syndrome, is a cysteine-rich extracellular matrix protein. When SMCs are cultured under conditions of cysteine deficiency, fibrillin-1 deposition into the matrix is greatly diminished. Therefore, the pathology leading to aortic disease with loss of MAT IIα activity might overlap with that of *FBN1* mutations leading to Marfan syndrome. Finally, pharmacologic inhibition of MAT IIα induces apoptosis in T leukemic cells, and apoptosis of aortic SMCs has been observed in thoracic aortic aneurysms and might contribute to disease progression.[40]

In summary, this study provides evidence that *MAT2A* loss-of-function variants predispose individuals to FTAAD. The identification of additional families affected by *MAT2A* disease-causing variants is needed before these results can be translated to clinical testing to identify individuals at risk for thoracic aortic disease. Further studies are also needed to address whether *MAT2A* variants require a second "hit" to cause thoracic aortic disease and to identify the pathway by which decreased enzymatic activity of MAT IIα leads to thoracic aortic aneurysms.

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Web Resources

The URLs for data presented herein are as follows:

1000 Genomes, http://browser.1000genomes.org
dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/
Ensembl Genome Browser, http://www.ensembl.org/index.html
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
PolyPhen-2, http://www.genetics.bwh.harvard.edu/pph2/
RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq
UCSC Genome Browser, http://genome.ucsc.edu

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