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ARTICLE

Incomplete segregation of *MYH11* variants with thoracic aortic aneurysms and dissections and patent ductus arteriosus

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Thoracic aortic aneurysms and dissections (TAAD) is a serious condition with high morbidity and mortality. It is estimated that 20% of non-syndromic TAAD cases are inherited in an autosomal-dominant pattern with variable expression and reduced penetrance. Mutations in myosin heavy chain 11 *(MYH11)*, one of several identified *TAAD* genes, were shown to simultaneously cause TAAD and patent ductus arteriosus (PDA). We identified two large Dutch families with TAAD/PDA and detected two different novel heterozygote *MYH11* variants in the probands. These variants, a heterozygote missense variant and a heterozygote in-frame deletion, were predicted to have damaging effects on protein structure and function. However, these novel alterations did not segregate with the TAAD/PDA in 3 out of 11 cases in family TAAD01 and in 2 out of 6 cases of family TAAD02. No mutation was detected in other known *TAAD* genes. Thus, it is expected that within these families other genetic factors contribute to the disease either by themselves or by interacting with the *MYH11* variants. Such an oligogenic model for TAAD would explain the variable onset and progression of the disorder and its reduced penetrance in general. We conclude that in familial TAAD/PDA with an *MYH11* variant in the index case caution should be exercised upon counseling family members. Specialized surveillance should still be offered to the non-carriers to prevent catastrophic aortic dissections or ruptures. Furthermore, our study underscores that segregation analysis remains very important in clinical genetics. Prediction programs and mutation evaluation algorithms need to be interpreted with caution.
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Keywords: thoracic aortic aneurysm and dissection (TAAD); patent ductus arteriosus (PDA); myosin heavy chain 11 (*MYH11*); segregation analysis; oligogenic model

INTRODUCTION

Thoracic aortic aneurysms and dissections (TAAD) is a serious condition with high morbidity and mortality.[1,2] Aortic medial degeneration, histopathologically characterized by loss of elastin fibers and smooth muscle cells and increased proteoglycan deposition, is thought to precede TAAD.[3] TAAD can be classified as sporadic, syndromic and familial non-syndromic.[4] Sporadic forms of TAAD tend to develop at an older age and their main risk factor is hypertension.[5] The syndromic forms of TAAD include the connective tissue diseases Marfan syndrome, Loeys-Dietz syndrome, the vascular type of Ehlers-Danlos syndrome, and the newly described aneurysms osteoarthritis syndrome (AOS), which are caused by mutations in the *FBN1*, *TGFBR1*/*TGFBR2*, *COL3A1* and *SMAD3* genes, respectively.[4,6,7]

In the non-syndromic TAAD cases, 20% is estimated to be familial with an autosomal-dominant inheritance pattern (FTAAD).[8] FTAAD is a heterogeneous and complex condition with variable expression and reduced penetrance.[9] Up until now, seven loci have been linked to familial TAAD and from these loci four genes have been identified; *TGFBR1*, *TGFBR2*, *MYH11* (myosin heavy chain 11) and *ACTA2*.[4,7] Recently, two other *TAAD* genes have been identified. *MYLK* was discovered by a candidate gene sequencing approach.[10] Mutations in *SMAD3* were first identified by traditional linkage analysis followed by candidate gene sequencing in patients with syndromic TAAD.[7] Later on *SMAD3* mutations were also discovered by whole-exome sequencing in familial non-syndromic TAAD.[11] All six genes can be analyzed in diagnostic settings when familial TAAD is suspected. Identification of a causal mutation in the index case will have great

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implications. First, it will enable the genetic screening of family members, which will allow non-carriers to be excluded from further clinical monitoring. Second, for mutation carriers, the identification of a causal mutation will influence pharmaceutical treatment and decision-making concerning surgical treatment.[12]

MYH11 and *ACTA2* encode for specific vascular smooth muscle cell (VSMC) components. In aortic tissue, in which these genes were disrupted, VSMC loss, disorganization and hyperplasia were observed.[13–16] Additionally, carriers of *MYH11* and *ACTA2* mutations may present with a patent ductus arteriosus (PDA),[13,14] possibly as a result of an impaired ability of the VSMC to proliferate, migrate and/or contract.[17] *MYH11* mutations are thought to account for only 1% of the familial TAAD cases and until now they have only been described in a few families, which had both TAAD and PDA cases and not in families with TAAD as the only vascular phenotype.[15]

Here, we describe two large TAAD/PDA families in which *MYH11* variants do not fully segregate with the vascular phenotype and discuss its clinical implications.

MATERIALS AND METHODS

Patients

Parents of the probands were referred to our Department of Clinical Genetics by their specialist. Initial pedigrees were constructed. Patients were examined for associated skeletal and skin features of connective tissue disorders. First- and second-degree family members of persons with TAAD/PDA were referred to a cardiologist for monitoring according to standard recommendations.[12] Upon consent, all relevant medical information was retrieved. The diagnosis of TAAD/PDA was determined by post mortem section, ultrasound, CT or MRI imaging and the aortic diameters were measured in the sinus of Valsalva or in the ascending aorta (Table 1). Individuals were marked as affected if they had a ruptured thoracic aortic aneurysm, a dissection type A or B and/or a PDA. As no complete information on body surface area was available, individuals in whom the sinus of Valsalva or the ascending aorta had a diameter >42 mm were marked as affected. Persons with mild dilatations, of whom there could be doubt about affected status, are not expected to be classified as affected with this wide cutoff.[18] Individuals at risk for inheriting the condition with normal or unknown aortic diameters were considered as 'unknown' because of the variable expression and reduced penetrance of the condition. After obtaining informed consent, blood was collected and genomic DNA was isolated according to standard procedures.

Genetic testing and segregation analysis

Candidate *TAAD* genes were sequenced in the proband of family TAAD01 and/or his affected brother, in the mother of the proband of family TAAD02 and in individuals without the *MYH11* mutations in a diagnostic setting (VUMC, The Netherlands). *MYH11* mutations were subsequently sequenced in all available family members (TAAD01: 24 individuals, TAAD02: 16 individuals) as well as in control chromosomes by standard Sanger sequencing. Primer information is available upon request.

As rare copy nuber variants have recently been associated with susceptibility to TAAD development,[19,20] array-CGH (comparative genome hybridization) analysis was performed in individual VI:1 of family TAAD01 and individual III:4

Table 1 Clinical information of the affected family members

Individual	Age at diagnosis (TAAD)	Age at diagnosis (PDA)	Gender	Deceased[a]	Aneurysm	AM (mm) (location)	Dissection type A	Dissection type B	Rupture	PDA	Method	DNA available	MYH11 mutation	Used for NPL
TAAD01														
V:3	65		F	−	+	44 (SV)	−	−	−	[b]	U	+	+	+
V:5	28		M	+	+		+	−	−	[b]	PM[c]	−		−
V:6	58		M	−	+	44 (SV)	−	−	−	[b]	U	+	+	+
V:8	70		M	+			+	−	−	[b]	PM	+	+	+
V:9	62		M	−	+	65 (Asc)	+	−	−	[b]	CT	+	+	+
V:11	70		F	−			+	−	−	[b]	CT	+	+	+
V:15	56		M	−	+	47 (Asc)	−	−	−	[b]	MRI	+	−	+
V:18	52		F	+					+	[b]	PM	−		−
V:19	59		F	−	+	45 (SV)	+	−	−	[b]	U	+	+	+
V:20	58		M	+	−		−	+	−	[b]	CT	+	−	+
VI:1	24		M	−	+	80 (Asc)	+	−	−	[b]	U	+	+	+
VI:2, P	25		M	+	+		+	−	−	[b]	U	+	+	+
VI:8	47		M	+			−	+	−	[b]	PM[e]	−		−
VII:1		35	F	−	−	31 (SV)	−	−	−	+	U	+	−	−
TAAD02														
III:4	49		F	−	+	48 (SV)[d]	−	−	−	−	MRI	+	−	+
III:7		5	F	−	−	27 (SV)[d]	−	−	−	+	MRI	+	+	+
III:10		57	F	−	−	35 (SV)	−	−	−	+	MRI	+	+	+
III:11		54	M	−	−	34 (SV)	−	−	−	+	MRI	+	+	+
IV:3, P	18		M	+	+	44 (SV)	+	−	+	−	PM[f]	+	+	+
V:1		3 months	F	−					−	+	U	+	−	+

Abbreviations: AM, aortic measurement; Asc, ascending aorta; CT, computed tomography; F, female; M, male; MRI, magnetic resonance imaging; MYH11, myosin heavy chain 11; NPL, non-parametric multipoint linkage; P, proband; PDA, patent ductus arteriosus; PM, post mortem; SV, sinus of valsalva; TAAD, thoracic aortic aneurysms and dissections; U, ultrasound; +, present; −, absent.
Blank places denote unknown data.
[a]All deceased individuals died as a result of (complications of) TAAD, except for V:20, who died probably of a cerebrovascular accident (no autopsy was performed).
[b]PDA not systematically examined, no clinical evidence for hemodynamically significant PDA.
[c]Large dissection type A until a. iliaca communis, extended media degeneration.
[d]Markedly decreased distensibility of ascending aorta.
[e]Dissection type B until a. renalis ruptured to the left pleura wall, minimal atherosclerosis, no evident media degeneration.
[f]Ruptured aneurysm of aortic root, cardiac tamponade and minimal coartation (16 mm >14 mm) at level of the closed ductus arteriosus.

of family TAAD02 using 105K microarray slides from Agilent Technologies (Santa Clara, CA, USA) following manufacturer's protocols. Data analysis was performed using DNA analytics 4.76 software from Agilent technologies using the ADM-2 algorithm. Interpretation of CNV-data was performed as described in the guidelines of Vermeesch *et al.*[21] The resolution was ~50 kb.

The presence of intragenic deletions or duplications in *SMAD3* in individual III:4 of family TAAD02 was tested by QPCR on a lightcycler LC480 384 well (Roche, Basel, Switzerland), using primers that amplify single exons of *SMAD3* and *FBN1* (sequences available on request). Melting curve analysis was used to confirm presence of a single PCR product in each well. The quantification of *SMAD3* exons was compared with exons of *FBN1* in which deletions/duplications had been excluded by MLPA testing. A standard curve for quantification was created by amplification of dilutions of a standardized DNA solution of 20 ng/μl with PCR fragments of *FBN1* and *SMAD3*. The QPCR was validated by confirming deletions in *FBN1* or *SMAD3* that had been found in other patients by MLPA or microarray.

Genome-wide genotyping of SNP markers and non-parametric linkage analysis

DNA of twenty-four family members of family TAAD01 was genotyped on Illumina's SNP-based linkage panel V (Illumina Inc., San Diego, CA, USA), including 6056 informative SNP markers distributed evenly across the human genome with a median genetic distance of 0.38 cM and a physical distance of 341 kb, using the BeadArray technology on an Illumina BeadStation following the manufacturer's protocol (www.illumina.com). DNA of 16 family members of family TAAD02 was genotyped on Illumina's HumanCytoSNP-12v2_A Genotyping BeadChip SNP array and analyses were performed according to the protocol of the manufacturer (Illumina Inc.). All SNPs were examined for their genotyping quality and those that had a low signal or were poorly clustered or were not polymorphic were excluded before analysis.

Data for family TAAD02 were pruned to 13 156 autosomal polymorphic markers with median distance of 0.13 cM or 116 kb.

Non-parametric multipoint linkage (NPL) analysis was carried out with Merlin (v.1.1.2)[22] on a reduced pedigree to accommodate limitations of the software and DNA availability (Figure 1 and Table 1). Related individuals with normal or unknown aortic diameters were marked as 'unknown' phenotypes. Spouses were marked as 'unaffected'. Results report NPL LOD-scores. Linkage results at candidate genes/loci are reported as the highest NPL LOD-score (interpolated) within the boundaries of the gene/locus.

RESULTS

Clinical evaluations

Family TAAD01. Family TAAD01 was a seven-generation Dutch family, in which TAAD had been diagnosed in two generations

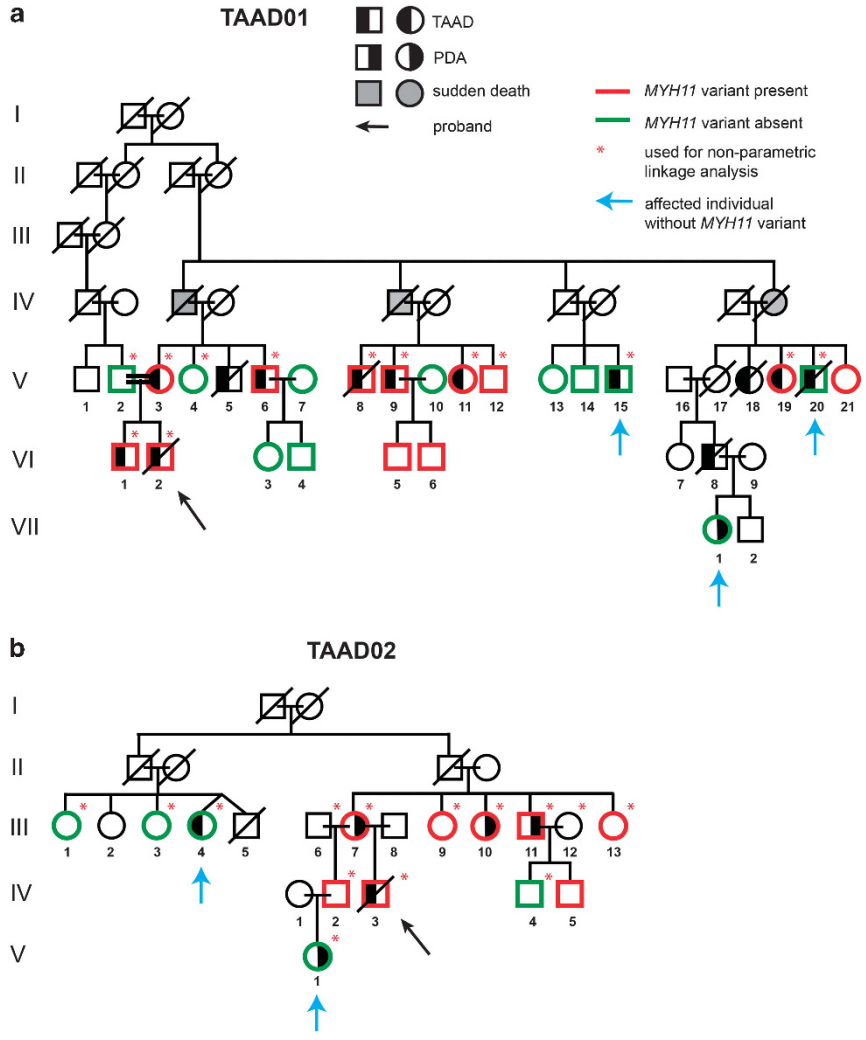

Figure 1 Pedigrees of family TAAD01 (**a**) and family TAAD02 (**b**) that participated in the study demonstrating an autosomal-dominant inheritance with reduced penetrance. Affected family members in whom the *MYH11* variant was not present are indicated with a light blue arrow, red asterisks indicate individuals used in NPL analysis.

(V and VI) and a PDA in one generation (VII) (Figure 1a and Table 1). Sudden death occurred frequently in generation IV between the ages of 40 and 70 years, but no autopsies were performed to explore the causes of these deaths. The proband (VI:2) died of complications of an emergency TAA repair at the age of 25. Subsequently, his brother (VI:1) was diagnosed with a TAA >80 mm and a dissection type A at the age of 24, which was successfully repaired. The parents of the proband were related with a kinship coefficient of 1/128. In the family of father (V:2) no aortic disease was documented. In the family of mother (V:3) there were several additional confirmed cases of TAAD and one case of PDA. Her brother (V:5) died from an acute dissection type A at 28 years of age. Her cousin (V:9) underwent TAAD repair at the age of 70 and his brother (V:8) died of a dissection type A at the age of 70. Their sister (V:11) had emergency repair for a type A dissection at the age of 70. In another cousin of the mother of the proband (V:20) a chronic dissection type B was diagnosed at the age of 58, which was conservatively treated. He died at the age of 63, possibly of a cerebrovascular accident. No autopsy was performed. His sister (V:19) underwent surgery for an acute dissection type A at the age of 59. Another sister (V:18) died from a ruptured TAA at the age of 52. Their nephew (VI:8) died of a dissection type B at the age of 47. His daughter (VII:1) was recently diagnosed with a PDA at the age of 35.

By cardiological assessment of first- and second-degree family members of TAAD patients, thoracic aortic aneurysms were discovered in the mother of the proband V:3, (44 mm), her brother V:6 (44 mm) and another cousin V:15 (47 mm). The latter had no history of hypertension and recently underwent prophylactic surgical repair of his TAA (Bentall procedure). None of the clinically evaluated family members exhibited skin or skeletal signs of a connective tissue disease.

Family TAAD02. Family TAAD02 was a five-generation Dutch family, in which both TAAD (III and IV) and PDA (III and V) had been diagnosed in two generations (Figure 1b and Table 1). Unfortunately, no medical data or information about causes of deaths of generations I and II could be retrieved. The proband (IV:3) died of an acute type A dissection at the age of 18. His mother (III:7) was diagnosed with a PDA at the age of 5. One of her three sisters (III:10) had a ductal diverticulum on a MRI, which we considered as an abortive form of PDA, and her only brother (III:11) also had a PDA. The daughter of the half-brother of the proband (V:1), who was born at term, was also diagnosed with a PDA and recently underwent a reparative surgery. Her father (IV:2) was diagnosed with a mild pulmonary stenosis. A cousin of the mother of the proband (III:4) had pathological dilated ascending aorta that was repaired when it exceeded 50 mm in diameter at the age of 50. Histological examination revealed extensive medial degradation with mucoid accumulation. This patient was not known to be hypertensive.

None of the clinically evaluated family members exhibited skin or skeletal signs of a connective tissue disease.

Sequencing and segregation analysis of TAAD candidate genes

Family TAAD01. No mutation in the *TGFBR1*, *TGFBR2*, *FBN1*, *COL3A1*, *ACTA2*, *MYLK* and *SMAD3* genes was discovered in the proband or his affected brother. In *MYH11* a heterozygous missense variant was found: a nucleotide substitution 232A>G in an AAG triplet encoding the basic lysine leading to a GAG triplet encoding the acidic glutamic acid (c. 232A>G, p. K78E). The variant is localized in a highly conserved block, just one amino acid before the start of the ATP-binding motor domain of the myosin head (Figure 2a). The Genomic Evolutionary Rate Profiling (GERP) scores were 3.38 for the 19-way GERP score and 3.7 for the 34-way GERP score (www.ensembl.org), which indicates a high degree of revolutionary conservation across several species down to *C. elegans*. PolyPhen2 and SIFT analysis of this alteration predicted a probably damaging and damaging effect, respectively. Segregation analysis revealed that the *MYH11* variant was present in most TAAD cases in this family ($n=8$). However, it was not identified in three affecteds: an individual with a TAA (V:15), an individual with a chronic dissection type B (V:20) and an individual with a PDA (VII:1). The 232A>G variant was not present in 200 control alleles and has not been reported in dbSNP131, the 1000 Genome Project (www.1000genomes.org) and our in-house whole-exome sequencing database, consisting of 60 whole-exomes of Dutch origin(MH, IJN, EC). The variant was also not present within the Exome Variant Server database (http://snp.gs.washington.edu/EVS).

In the three individuals without the *MYH11* variant, *TGFBR1*, *TGFBR2*, *ACTA2*, *MYLK* and *SMAD3* were sequenced. No mutation was identified.

Array-CGH analysis in individual VI:1 showed no rare deletion or duplication.

Family TAAD02. No mutation was detected in the *ACTA2* gene in the mother of the proband (III:7). However, in *MYH11* a heterozygous deletion of an AAG triplet from a conserved stretch of four lysines 1256K localizing in the prefoldin domain (part of the chaperone system) of myosin tail was discovered (Figure 2b). Conservation scores for the three deleted nucleotides are 1.45, 2.59 and 2.59 for the 19-way GERP score and 0.98, 3.45 and 2.36 for the 34-way GERP score indicate a high evolutionary conservation. PolyPhen2 and SIFT analysis of this alteration is not possible as these programs do not predict the effect of (in-frame) deletions. Segregation analysis revealed that the *MYH11* variant was also present in the proband (IV:3) and in two cases of PDA (III:10 and III:11). However, it was not identified in the individual with the pathological aortic dilatation (III:4) and neither in another PDA case (V:1). The 3766-68delAAG variant was not present in 800 control alleles and has not been reported in the 1000 Genome Project (www.1000genomes.org) and our in-house whole-exome sequencing database. Additionally, in case III:4 *ACTA2*, *FBN1*, *TGFBR1*, *TGFBR2*, *MYLK* and *SMAD3* were analyzed, but no mutation was identified.

Array-CGH analysis in individual III:4 showed no rare deletion of duplication.

Non-parametric linkage analysis in TAAD candidate genes

Non-parametric LOD-scores (NPL) were checked for loci and genes that have previously been shown to be involved in TAAD. None of these loci showed NPL-scores near the maximum obtainable NPL of 1.84 for family TAAD01 (Table 2). As informativeness was very high, this suggested there was no complete sharing of these regions between all affected family members. Reconstruction of the most likely haplotypes in these regions supported the assumption that not all affected individuals shared the same haplotype in any of these regions (data not shown), including the *MYH11* locus (Supplementary Figure). The NPL LOD-score of this locus was 1.0, which is consistent with partial sharing of a haplotype between affected family members. Segregation analysis of p. K78E had already revealed incomplete sharing of this variant between affected family members. In accordance, haplotype reconstruction of the *MYH11* locus indeed indicated incomplete sharing of the full *MYH11* gene between affected family members (Supplementary Figure). It is therefore not expected that

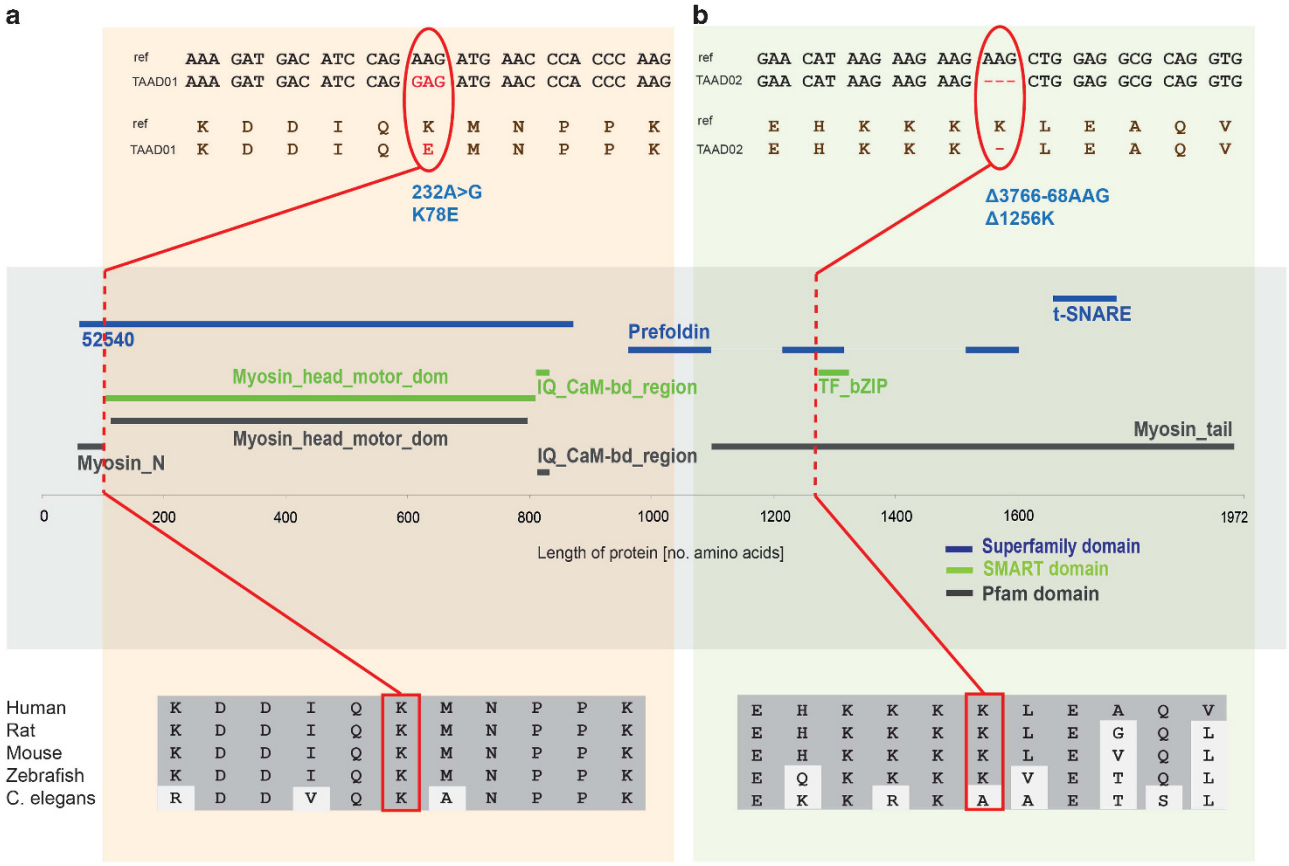

Figure 2 Representation of the *MYH11* variants identified in family TAAD01 (**a**) and TAAD02 (**b**), including protein domain localization and high evolutionary conservation.

Table 2 Non-parametric linkage analysis of known *TAAD* genes and loci in families TAAD01 and TAAD02

Candidate gene	*Location (GRCh37/hg19)*	*TAAD01 LOD-NPL*[a]	*TAAD02 LOD-NPL*[b]
ACTA2	Chr10:90 694 831–90 751 147	0.00	−0.04
FBN1	Chr15:48 700 505–48 937 918	0.00	0.40
SMAD3	Chr15:67 358 183–67 487 533	0.00	0.90
MYH11	Chr16:15 796 992–15 950 890	1.00	−0.01
COL3A1	Chr2:189 839 046–189 877 472	0.40	−0.04
MYLK	Chr3:123 328 896–123 603 178	0.00	−0.06
TGFBR2	Chr3:30 647 994–30 735 634	0.00	−0.02
TGFBR1	Chr9:101 867 412–101 916 585	0.00	−0.05
Candidate linkage locus	*Marker (locus start–end, GRCs37/hg19)*		
FAA1: 11q23.3–q24	D11S1341-AFMB031WC9 (chr11:118 121 702–120 131 962)	0.00	−0.01
TAAD1: 5q13–q14	D5S806–D5S641 (chr5: 81 493 301–82 003 227)	0.00	0.11
TAAD5: 12q13–14	D12S1669–D12S335 (chr12:19 538 394–68 129 880)	0.07	0.43

Abbreviations: NPL, non-parametric multipoint linkage; TAAD, thoracic aortic aneurysms and dissections.
[a]Maximal LOD-NLP = 1.84.
[b]Maximal LOD-NLP = 0.91.

another cryptic mutation in the *MYH11* gene, shared by all TAAD patients within this family, is responsible for the NPL LOD-score of 1.0 in this region.

In family TAAD02, NPL analysis showed complete sharing at a 19-Mb locus on chromosome 15 (NPL $Z = 5.9$, NPL LOD = 0.9), encompassing the *SMAD3* gene, but many other genes as well. Though sharing was estimated to be complete, supported by best guess haplotypes, this was not statistically significant, owing to the small family size. The *SMAD3* gene was sequenced in individual III:4, but no mutation was identified. Array-CGH analysis in the same

patient revealed no large deletion or duplication within this region. Similarly, intragenic deletion and duplication were excluded by QPCR.

DISCUSSION

We have clinically characterized two independent large Dutch families with TAAD and PDA. In routine diagnostic testing a *MYH11* variant was found in both families. However, segregation analysis revealed that some individuals with a vascular phenotype did not carry the variant.

TAAD is usually an asymptomatic condition until a life-threatening event with high mortality occurs (ie, aortic dissection or rupture). Therefore, genetic counseling and specialized monitoring of families with inherited forms of TAAD/PDA is crucial. Identification of a causal gene in the family will have implications for screening and pharmaceutical or surgical treatment.[9] However, assigning a pathogenic potential to a given variant can be challenging. When functional assays are not readily available, segregation analysis in additional family members is usually the only way to claim causality of a variant predicted to be pathogenic by current mutation evaluation algorithms. However, families may not be large enough to solidly perform this type of analyses. In addition, the variable expression and reduced penetrance of TAAD/PDA complicates segregation patterns as mutation carriers may not (yet) have developed clinical phenotypes. Furthermore, even though standard values, stratified for gender and body surface area, of the diameter of different thoracic aortic segments are available there is still a gray area in which a person with a mildly dilated aorta can be considered as affected or not.

Circumstantial evidence for pathogenicity of a mutation can be obtained from conservation scores and bioinformatic predictions of the effect of the variant on protein structure. The variants discovered in our study were either resulting in substitution or loss of a lysine in highly conserved regions, with the missense variant of family TAAD01 affecting the head and the in-frame deletion of family TAAD02 the tail of myosin. The families reported here were thoroughly clinically examined and large enough to conclude that the identified *MYH11* variants could not fully explain the vascular phenotypes observed. However, in general practice substantial clinical information and multiple (affected) family members may not be available and therefore incorrect conclusions about causality of variants may be drawn. As an example, we experienced an unexpected diagnostic turn in family TAAD02 when vascular disease in the two non-carriers III:4 and V:1 was discovered after the *MYH11* variant originally was present in all affected family members that were used for segregation analysis in the early stage. Luckily, an aortic dilatation was detected in individual III:4 and prophylactic surgery was performed before she was released from cardiological follow-up.

There are three possible explanations for our findings. First, the *MYH11* variants are not pathogenic and do not contribute to the initiation of the disease in the carriers at all. If this is the case, taking into account the mode of inheritance in the pedigrees, there is most likely another monogenic factor that is responsible for the TAAD/PDA. However, because of the pathogenic prediction of the alterations by mutation evaluation programs and/or conservation scores, it is tempting to assume that there is an effect of the alterations. Moreover, it would be very coincidental to find novel alterations in *MYH11* within these TAAD/PDA families that do not contribute to the vascular phenotype at all. A second explanation of our findings is that the *MYH11* variants are fully responsible for the vascular disease in the carriers and that in affected non-carriers in the described families the phenotype is caused by other genetic or environmental factors. However, because the incidence of TAAD is low, especially at an age below 60 years, as is the prevalence of PDA in the adult population, this is not a very plausible explanation.[5,23] Furthermore, mutations in the known *TAAD* genes were excluded in the non-carriers by mutation analysis.

A third possibility is that the *MYH11* variants are responsible for part of the phenotype and that there are additional genetic factors that can lead to the disease either by themselves or by interaction with the *MYH11* mutation, so operating in a di- or even multigenic model. The theory of an oligogenic disease would also explain the generally variable onset and severity as well as the reduced penetrance between and within TAAD families.[7,8] We therefore consider this third explanation as the most likely one. Further research of the described families including whole- exome sequencing may reveal the explicit genetic causes of TAAD/PDA. In family TAAD02, NPL analysis showed complete sharing of a region of 19 Mb on chromosome 15 encompassing the *SMAD3* gene. Subsequent mutation analysis and QPCR of *SMAD3* did not reveal a mutation or an intragenic deletion/duplication, suggesting that *SMAD3* is probably not the gene causing TAAD within this family. Other genes within this 19 Mb region may contribute to the vascular phenotype, but the power is too small to reach significant NPL results because of the limited number of affected family members.

In summary, we present two examples of large families with incomplete segregation of independent *MYH11* variants with TAAD/PDA. We hypothesize that within these families additional genetic factors are involved in the etiology of the disease, acting as an oligogenic model. Such a model would also explain the variability of the onset and progression of the disorder and its reduced penetrance in general. Recently upcoming high-throughput sequencing technologies such as (whole) exome or even whole-genome sequencing may help to uncover the genetic basis in the families described in this report, as well as in other TAAD families that have remained unresolved. For now, our main message is that caution should be exercised when family members who do not carry a family-specific *MYH11* variant are counseled, as they cannot be reassured and should still participate in specialized surveillance programs. Furthermore, our study underscores that segregation analysis remains very important in clinical genetics. Prediction programs and mutation evaluation algorithms should be interpreted with caution.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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