JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
VOL. 72, NO. 6, 2018

PUBLISHED BY ELSEVIER

EDITORIAL COMMENT

When Genes, More Than Phenotype, Identify Different Diseases

The Case of Nonsyndromic HTAA/D*

Eloisa Arbustini, MD, Lorenzo Giuliani, MS, Alessandro Di Toro, MD

The diagnosis of heritable thoracic aortic aneurysms and dissections (HTAA/D) applies to both families with more affected members and families with a unique affected member who carries a pathological mutation that is causally linked with the TAA (1). HTAA/D manifests as either isolated trait (nonsyndromic) or in syndromes (syndromic) (2). Patients with syndromic HTAA/D demonstrate the involvement of skeletal, ocular, respiratory, neuromuscular, and cutaneous/integumentary systems in variable combinations and characteristics: well-known examples are Marfan syndrome and Ehlers-Danlos syndrome. Specific clinical nosologies (3,4) guide the phenotype-based diagnosis of syndromic HTAA/D; the genetic test plays a confirmatory role and allows preclinical, prenatal, and preimplantation diagnosis when feasible/indicated. Vice versa, nonsyndromic HTAA/Ds encompass phenotypically similar but genetically heterogeneous conditions. Family history and imaging-based family screening provide the evidence of familial versus sporadic disease (2). In nonsyndromic HTAA/D, the genetic test in the proband does not have a diagnostic role for the aneurysm itself, but precisely defines its specific cause that may influence the therapeutic decisions, especially timing of pre-emptive surgery. A gene-based classification is emerging for nonsyndromic HTAA/D (1) that, for instance, can be allelic at the same loci of syndromic HTAA/D. Alternatively, nonsyndromic HTAA/D can be grouped based on the pathways in which disease-genes are involved (transforming growth factor (TGF)-β, vascular smooth muscle cells, collagens, and so on). From a clinical point of view, a nosology orientation is useful, especially when similar phenotypes with different causes demonstrate different levels of risk and call for tailored clinical decisions. Therefore, the clinical diagnosis of familial HTAA requires the echocardiographic family screening while the precise diagnosis of nonsyndromic HTAA/D requires the identification of the genetic cause.

NONSYNDROMIC HTAA/D: DISEASE GENES AND PATHOLOGICAL MUTATIONS

The current fast next-generation sequencing-based tools for gene/genome analysis offer fast strategies for identification of novel disease genes (5). In the last 10 years, the list of candidate and disease genes has been rapidly expanding. In 2015, the National Institutes of Health-funded Clinical Genome Resource (ClinGen) created a new open-access resource to define clinically relevant genes and variants, based on a standardized assessment of the levels of evidence (6). Expert curators have the role of assessing the associations of the disease genes with the phenotype on the basis of pre-defined criteria (7). In this issue of the *Journal*, Renard et al. (8), curators for HTAA/D genes, analyzed 53 candidate genes with variable levels of evidence

SEE PAGE 605

(from strong to no evidence). The levels of evidence of the causative role of mutations in candidate genes are established on the basis of functional data and segregation studies. A clinically oriented

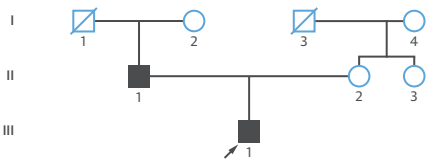
*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of *JACC* or the American College of Cardiology.

From the Centre for Inherited Cardiovascular Diseases, IRCCS Foundation, University Hospital Policlinico San Matteo, Pavia, Italy. Research on heritable connective tissue diseases is supported by grants Ricerca Corrente, Ministry of Health, to the IRCCS Foundation, Policlinico San Matteo, Italy. The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ISSN 0735-1097/$36.00
https://doi.org/10.1016/j.jacc.2018.03.547

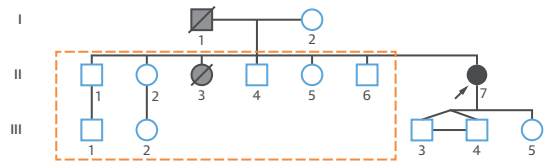
FIGURE 1 Genetics in HTAA/D

A Complex genetics



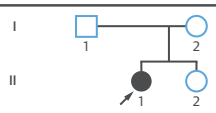
I:1, 65 years; cause of death described as due to "vascular disease"; no autopsy. No genetic test
I:2, 62 years; healthy; *TGFBR2:* negative
I:3, 61 years; DM with vascular complications; no genetic test
I:4, 72 years; Hypertension; no genetic test
II:1, 42 years; Phenotype: TAA, Aortic root Z-score= 2.92; myopia; arterial tortuosity of the neck and brain vessels; craniofacial score=2; *TGFBR2* positive; *COL5A1* negative
II:2 40 years; Clinical screening negative; *TGFBR2* negative; *COL5A1* negative
II:3 38 years; Clinical screening negative
III:1 → 7 years; Z-score= 2.41 - Phenotype: S/H Ratio =1.068; arachnodactyly; wrist and thumb signs: positive; pes planus; mild pectus deformity; prominent joint laxity; light blue sclerae; velvety, translucent skin; craniofacial score=3; arterial tortuosity of the brain vessels. *TGFBR2* p.(His507Arg) + *de novo COL5A1* p.(Gln1825GlufsTer5)

B Pathologic mutation in a definite HTAA/D gene. The mother (1:2) and relatives in the squared area did not undergo clinical screening and genetic testing.



I:1, 50 years; sudden death, vascular (?)
I:2, 65 years; II:1, 51 years; II:2, 50 years; II:4, 47 years; II:5, 45 years; II:6, 43 years → all described as healthy
II:3, 37 years; unexplained cause of death
II:7,→ proband, age at the event (Type B dissection): 41 years; height 169 cm; weight 82 kg; myopia, no iris flocculi; *ACTA2*, p.(Arg149Cys)
III:1, 22 years and III:2, 25 years: both described as healthy
III:3 and III:4, 7 years; clinically healthy, 2DTTE negative
III:5, 3 years, clinically healthy, 2DTTE negative

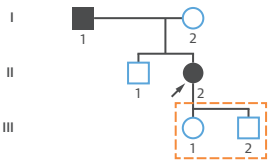
C De novo mutations support the pathogenicity of the mutation identified in the proband



I:1, 54 years and 1:2, 49 years: clinical and genetic screening negative
II:1, → 18 years;
- TAA aortic root (Z-score = 4,24) and mitral valve disease with moderate regurgitation: flat feet; myopia; short stature.
- Genetic testing: *de novo TGFB2* p.(Arg330Cys)

II:2, 16 years; clinical and genetic screening negative

D Novel variants of uncertain/unknown significance in HTAA/D definite genes



I:1 67 years; acute type A dissection at 48 years; three aortic surgeries: Bentall, arch and descending thoracic aorta; flat feet; astigmatism. Carrier of the variant identified in the proband (II:2)
I:2, 65 years; clinically healthy
II:1, 40 years; clinically and genetically healthy
II:2 → 36 years; TAA (Z-score 3.62), bicuspid aortic valve, flat feet, astigmatism, arched palate. Carrier of the *MYH11* variant c.748-5G>A
III:1, 7 years and III: 2, 4 years. Both are clinically healthy; genetic testing not performed because of the uncertain role of the maternal variant

(A) Complex genetics. The proband (III:1) was addressed to clinical attention for aortic root dilation, incidentally identified in the context of a multidisciplinary evaluation for severe joint laxity. Family screening demonstrated mild aortic root dilation in both father and son. Genetic testing in III:1 identified 2 novel variants/mutations: *TGFBR2* p.(His507Arg) and *COL5A1* p.(Gln1825GlufsTer5). The former was inherited from the father; the latter was de novo. The boy inherited the *TGFBR2* variant from the father, who showed aortic root dilation and arterial tortuosity of the neck and brain arteries. The *COL5A1* truncation predicting mutation was de novo in the proband and likely explains the severe joint laxity. **(B)** Validated pathological mutations in definite genes. Pedigree and summary data of a Nigerian family in which the proband (II:7) experienced acute post-isthmic thoracic aorta dissection (type B) at the seventh month of her second pregnancy. Clinical family screening could not be performed. The pedigree was generated on the narrative of the proband. 2DTTE performed in the 3 children was negative; the genetic test has not yet been performed. Although missing the study of segregation in the family, a pathogenic role is assigned to the mutation because *ACTA2* is a definite HTAA/D gene and the mutation (p.(Arg149Cys)) is confirmed as pathogenic. **(C)** De novo mutations in definite disease genes support their pathological role. The young proband (II:1) demonstrated both aortic and valve involvement and minor, nonspecific, noncardiovascular traits. The parents tested negative. The sister had a genetic test aimed at excluding germinal mosaic (requested by parents). **(D)** VUS and interpretation. The identification of a VUS in the proband, the small size of the family, and the uncertain role of the variant suggest caution before testing the children of the proband. Both are very young and their 2DTTE showed negative findings. 2DTTE = 2-dimensional transthoracic echocardiography; DM = diabetes mellitus; S/H Ratio = span/height ratio.

selection of patients to be addressed to testing of pre-specified genes is easy in syndromic but is difficult in nonsyndromic HTAA/D. In some probands, the association of HTAA/D with extravascular traits (e.g., *ACTA2*-HTAA/D and iris flocculi [9]; *MYH11*-HTAA/D and patent ductus arteriosus [10]) may suggest specific disease genes; however, these traits can be absent. The demonstration of familial aggregation, although increasing the probability of identifying the genetic cause, does not inform about the disease gene in the given family. The pattern of inheritance may further help; however, most nonsyndromic HTAA/Ds are autosomal dominant diseases (2). Therefore, the search for the genetic cause of nonsyndromic HTAA/D remains based on the screening of unselected known disease

genes; in this context, the levels of evidence of causal association between disease genes and nonsyndromic HTAA/D must be robust and make the curators' work valuable, who periodically monitor new data contributing to the validation of the associations. Renard et al. (8) applied the semiquantitative ClinGen framework to assess presumed gene-disease relationships between the selected 53 candidate genes and TAAD. Eleven genes demonstrated definite, strong levels of evidence. Some "recent" genes (e.g., *FOXE3, MFAP5,* and *MAT2A*) are robust candidates but need replicas and confirmation. Genes with moderate/limited evidence (n = 12) should be eventually included in multigene panels, but the corresponding phenotypes are expected to fall in the syndromic HTAA/D group. Genes with "no reported" (n = 23) evidence may be tested, but more in the research setting than in routine diagnostics for nonsyndromic HTAA/D (8).

ANALYSIS OF MULTIGENE PANELS: SIMPLE AND COMPLEX GENETIC DATA

The analysis of multigene panels, comprehensively including all known disease and candidate genes, can identify a unique causative mutation, give negative/nonconclusive results, or demonstrate >1 genetic variant/mutation in 1 or 2 genes. The interpretation of complex genetics (>1 pathological mutation) is supported by segregation studies in families: one may play a modifying role with respect to the deterministic role of the other one. Alternatively, both contribute to the phenotype that is therefore present, with possible phenotype differences, in the carrier of the 2 mutations, as well as in the affected family members who carry each single mutation (**Figure 1A**). Validated pathological mutations have a disease-specific diagnostic role even in families in which segregation studies are not feasible (**Figure 1B**). De novo mutations strongly argue in favor of a pathogenic role of the mutation (**Figure 1C**). However, clinicians should be aware that even testing all known disease genes might be inconclusive (**Figure 1D**). Genes of recent identification, novel missense variants, and nonexplorable functional effects of the variants are all factors that limit interpretation and increase the number of annotated variants of uncertain significance (VUS) (11). When a genetic test is negative or identifies a VUS, but the HTAA/D is present in >1 family member, clinical monitoring should be regularly performed irrespective of the negative/noninformative genetics.

NONSYNDROMIC HTAA/D: HOW CLINICIANS CAN CONTRIBUTE TO CONFIRM DISEASE GENES

Family screening strategies.

1. The patient is diagnosed with TAA/D and referred to genetic counseling and testing. The family screening comes later, only in the case of identification of a genetic mutation in the proband. This path subordinates family screening to the results of the genetic test in the proband.
2. The patient is diagnosed with TAA/D, and imaging-based family screening is performed independently of the genetic test. Cardiologists can identify clinically affected members. The availability of genetic testing does not condition the clinical family screening.

When a potential disease-causing mutation (known and validated or novel and worthy of further investigation) is identified in the proband, cascade genetic testing in relatives provides the tool for segregation studies (affected and mutated vs. nonaffected, nonmutated family members).

The interpretation of the pathogenicity of the mutation is critical for genetic diagnosis and is regulated by guidelines (11). Truncation-predicting mutations in disease genes are more commonly pathogenic. However, most mutations in HTAA genes are missense: for known and validated mutations, the interpretation is simple. For novel variants/mutations, the interpretation (pathogenic or nonpathogenic) must be supported by evidence of their role in the phenotype: when validation is not feasible, the variants remain classified as VUS.

Genetic tests are taking a key role in the precise diagnosis of the cause of nonsyndromic HTAA/D. However, the absence of genetic data or inconclusive genetic results should not be considered a limit to the benefits of clinical screening in HTAA/D families. The clinical value of early diagnoses in the relatives of probands is an essential prevention tool that cardiologists must not give up when a genetic diagnosis is not available. Nonsyndromic HTAA/D is a potentially life-threatening condition that should be timely diagnosed, monitored, and preventively treated before the occurrence of catastrophic events. Where possible, the 11 genes in definitive and strong groups proposed by Renard et al. (8) are priority genes to be tested in patients with nonsyndromic HTAA/D; novel variants in these genes deserve segregation studies. Screening of genes with moderate and limited levels of evidence is also advisable as current evidence can

rapidly change with the expansion of genetic studies in HTAA/D genes.

ACKNOWLEDGMENT The authors thank Valentina Favalli (MyWay Genetics, srl) for the critical revision of the text content, in particular for her patient-oriented vision.

ADDRESS FOR CORRESPONDENCE: Dr. Eloisa Arbustini, Centre for Inherited Cardiovascular Diseases, IRCCS Foundation, University Hospital Policlinico San Matteo, Piazzale Golgi 19, 27100 Pavia, Italy. E-mail: e.arbustini@smatteo.pv.it.

REFERENCES

1. Bradley TJ, Bowdin SC, Morel CF, Pyeritz RE. The expanding clinical spectrum of extracardiovascular and cardiovascular manifestations of heritable thoracic aortic aneurysm and dissection. Can J Cardiol 2016;32:86–99.

2. Arbustini E, Favalli V, Di Toro A, Giuliani L, Limongelli G. Common presentation of rare diseases: aortic aneurysms & valves. Int J Cardiol 2018;257:358–65.

3. Loeys BL, Dietz HC, Braverman AC, et al. The revised Ghent nosology for the Marfan syndrome. J Med Genet 2010;47:476–85.

4. Malfait F, Francomano C, Byers P, et al. The 2017 international classification of the Ehlers-Danlos syndromes. Am J Med Genet C Semin Med Genet 2017;175:8–26.

5. Kuang SQ, Medina-Martinez O, Guo DC, et al. FOXE3 mutations predispose to thoracic aortic aneurysms and dissections. J Clin Invest 2016;126:948–61.

6. Rehm HL, Berg JS, Brooks LD, et al. ClinGen—the Clinical Genome Resource. N Engl J Med 2015;372:2235–42.

7. Patel RY, Shah N, Jackson AR, et al., for the ClinGen Resource. ClinGen Pathogenicity Calculator: a configurable system for assessing pathogenicity of genetic variants. Genome Med 2017;9:3.

8. Renard M, Francis C, Chosh R, et al. Clinical validity of genes for heritable thoracic aortic aneurysm and dissection. J Am Coll Cardiol 2018;72:605–15.

9. Guo DC, Pannu H, Tran-Fadulu V, et al. Mutations in smooth muscle alpha-actin (ACTA2) lead to thoracic aortic aneurysms and dissections. Nat Gen 2007;39:1488–93.

10. Zhu L, Vranckx R, Khau Van Kien P, et al. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. Nat Genet 2006;38:343–9.

11. Richards S, Aziz N, Bale S, et al., for the ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–24.

KEY WORDS aortic aneurysm, aortic dissection, genetics