Clinical utility gene card for: Hereditary thoracic aortic aneurysm and dissection including next-generation sequencing-based approaches

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Mine Arslan-Kirchner*[,1], Eloisa Arbustini[2], Catherine Boileau[3], Philippe Charron[4], Anne H Child[5], Gwenaelle Collod-Beroud[6], Julie De Backer[7], Anne De Paepe[7], Anna Dierking[1], Laurence Faivre[8], Sabine Hoffjan[9], Guillaume Jondeau[10], Britta Keyser[1], Bart Loeys[11], Karin Mayer[12], Peter N Robinson[13] and Jörg Schmidtke[1]

This CUGC intends to give guidance regarding molecular genetic testing in patients with thoracic aortic aneurysm and dissection (for definition see Loeys *et al.*[1]). It includes genes associated with non-syndromic and syndromic conditions. In cases of a strong clinical suspicion for a particular syndrome, it recommends testing the respective associated genes first. In cases without such suspicion, it recommends either a stepwise approach by Sanger sequencing or, if available, NGS-based procedures according to the ESHG recommendations (Matthijs *et al.*[2]) based on 'core genes' and 'additional genes'. The recommendations suggest that core gene lists are to be established by consensus among experts in the field. The 'list must result in a 'substantial contribution' to the quality of life of a patient, and hence the genes must be chosen with care; a two tier system would be acceptable, whereby some genes are scrutinized more in detail (in other words: with a more complete coverage) than others; the list must not inflict with the efficiency of a service, that is, overzealous testing is not helpful; the use of core gene panels must lead to better diagnosis of the group of disorders, if not it lacks clinical utility.'[2] Additional genes (those with a lower disease contribution) are optional components of the panel.

1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

See Table 1 column 1—'Disease'.

1.2 OMIM# of the disease

See Table 1 column 2—'OMIM# of Disease', and Table 2.

1.3 Name of the analysed genes or DNA/chromosome segments:

1.3.1 Core genes (irrespective if being tested by Sanger sequencing or next-generation sequencing)

Gene	OMIM# of associated gene(s)
ACTA2	102620
COL3A1	120180
FBN1	134797
FLNA	300017
MAT2A	601468
MFAP5	601103
MYH11	160745
MYLK	600922
NOTCH1	190198
PRKG1	176894
SMAD3	603109
TGFB2	190220
TGFB3	190230
TGFBR1	190181
TGFBR2	190182

1.3.2 Additional genes (if tested by next-generation sequencing, including whole-exome/genome sequencing and panel sequencing)

Gene	OMIM# of associated gene(s)
COL1A1	120150
COL4A5	303630
COL5A1	120215
COL5A2	120190
EFEMP2	604633
ELN	130160
FBN2	612570
GATA5	611496
PLOD1	153454
SKI	164780
SLC2A10	606145
SMAD4	600993

[1]Institute of Human Genetics, Hannover Medical School, Hannover, Germany; [2]IRCCS Fondazione Policlinico San Matteo, Pavia, Italy; [3]Department of Medical Genetics, Bichat University Hospital, APHP, Paris, France; [4]Centre de Référence Maladies Cardiaques Héréditaires; Université de Versailles-Saint Quentin, Hôpital Ambroise Paré, APHP, Boulogne-Billancourt, France; [5]Cardiovascular and Cell Sciences Research Institute, St George's University of London, London, UK; [6]Aix Marseille Université, INSERM, GMGF UMR_S 910, Marseille, France; [7]Center for Medical Genetics, Ghent University, Ghent, Belgium; [8]Centre de Génétique, Centre Hospitalier Universitaire de Dijon, Dijon, France; [9]Department of Human Genetics, Ruhr University Bochum, Bochum, Germany; [10]Centre National de Référence Pour le Syndrome de Marfan et Apparentés, CHU Hôpital Xavier Bichat—Claude Bernard, Paris, France; [11]Center for Medical Genetics, University of Antwerp/Antwerp University Hospital, Antwerp, Belgium; [12]Center for Human Genetics and Laboratory Diagnostics (AHC), Martinsried, Germany; [13]Institut für Medizinische Genetik und Humangenetik, Charité Universitätsmedizin Berlin, Berlin, Germany
*Correspondence: Dr M Arslan-Kirchner, Institute of Human Genetics, Hannover Medical School, Carl-Neuberg-Straße 1, Hannover 30625, Germany. Tel: +49 511 532 6532; Fax: +49 511 532 5865; E-mail: Arslan.Mine@mh-hannover.de

Table 1 Overview of diseases and genes associated with thoracic aortic aneurysm

Disease	OMIM# of disease	Orpha number of disease	Associated gene(s)	OMIM# of associated gene(s)
Aortic aneurysm, familial thoracic (AAT); Aneurysm, thoracic aortic; aortic dissection, familial; thoracic aortic aneurysm and dissection, familial	615436 (AAT8)	91387	*PRKG1*	176894
	613780 (AAT7)	91387	*MYLK*	600922
	611788 (AAT6)	91387	*ACTA2*	102620
	132900 (AAT4)	91387	*MYH11*	160745
Alport syndrome, X-linked (ATS[a])	301050	88917	*COL4A5*	303630
Aortic valve disease 1 (AOVD1); bicuspid aortic valve	109730	402075	*NOTCH1*	190198
Arterial tortuosity syndrome (ATS[a])	208050	3342	*SLC2A10*	606145
Contractural arachnodactyly, congenital, Beals syndrome (CCA)	121050	115	*FBN2*	612570
Cutis laxa, autosomal dominant 1 (ADCL1)	123700	90348	*ELN*	130160
Cutis laxa, autosomal recessive, type 1B (ARCL1B)	614437	90349	*EFEMP2*	604633
Ehlers–Danlos syndrome, classical type/type I (EDS I)	130000	90309	*COL1A1*	120150
			COL5A1	120215
			COL5A2	120190
Ehlers–Danlos syndrome, classical type/type II (EDS II)	130010	90318	*COL5A1*	120215
	130010	90318	*COL5A2*	120190
Ehlers–Danlos syndrome, vascular type/type IV (EDS IV)	130050	286	*COL3A1*	120180
Ehlers–Danlos syndrome, kyphoskoliotic type/type VI (EDS VI)	225400	1900	*PLOD1*	153454
Ehlers–Danlos syndrome, arthrochalasic type/type VIIA (EDS VIIA)	130060	1899	*COL1A1*	120150
Familial thoracic aortic aneurysm and aortic dissection		91387	*TGFB2*	190220
Heterotopia, periventricular, Ehlers–Danlos variant (PVNH4)	300537	82004	*FLNA*	300017
Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome (JPHT)	175050	2929	*SMAD4*	600993
Loeys–Dietz syndrome type 1, Furlong syndrome (LDS1/FS/AAT5)	609192	60030	*TGFBR1*	190181
Loeys–Dietz syndrome type 2, Marfan syndrome type 2 (LDS2/MFS2/AAT3)	610168	60030	*TGFBR2*	190182
Loeys–Dietz syndrome type 3, Aneurysm osteoarthritis syndrome (LDS3/AOS)	613795	284984	*SMAD3*	603109
Loeys–Dietz syndrome type 4 (LDS4)	614816		*TGFB2*	190220
Marfan syndrome (MFS)	154700	558	*FBN1*	134797
Moyamoya disease 5 (MYMY5)	614042	2573	*ACTA2*	102620
Shprintzen–Goldberg craniosynostosis syndrome (SGS)	182212	2462	*SKI*	164780

Note: the nomenclature of diseases associated with thoracic aortic aneurysm is in part controversial.[15,16] This CUGC does not attempt to resolve these controversies. In this table, both OMIM and Orpha numbers are given together with their alternative disease designations in the first column.
[a]ATS is used in OMIM as a symbol for both conditions.

1.4 Mutational spectrum:

(Please note the *EJHG* instructions for authors http://mts-ejhg.nature.com/cgi-bin/main.plex?form_type=display_auth_instructions regarding sequence variants and genetic databases.)

All types of variants have been reported[3] (missense, nonsense, splice site, small and large deletions/insertions). Many variants are listed in the Human Gene Mutation Database (http://www.hgmd.org/) and in ClinVar.[4] The 'Locus Specific Mutation Database' from HGVS gives an overview of gene-specific mutation databases, for example, *FBN1, FBN2, TGFBR1, TGFBR2, ACTA2, SMAD3, MYH11* and *MYLK* variants are also registered in The Universal Mutation Database (www.umd.be). In general, there are no frequent disease-causing mutations or hot spots for disease-causing mutations in the vast majority of the genes. Causative mutations are distributed throughout the genes. However, some trends are observed for disease-causing mutations in specific genes (for example, exons encoding intracellular domains of TGFBR1 and TGFBR2, and recurrent mutations in *PRKG1*).

SNPs or rare variants are listed in the dbSNP Database (http://www.ncbi.nlm.nih.gov/snp/), the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) and in the Exome Aggregation Consortium (http://exac.broadinstitute.org/). Please note that the above-mentioned databases include pathogenic mutations.

Table 2 Genes associated with thoracic aortic aneurysm not yet given an OMIM or Orpha number

Gene	OMIM# of gene
GATA5[17]	611496
MAT2A[18]	601468
MFAP5[19]	601103
TGFB3[20]	190230

1.5 Analytical validation

Sequencing of both DNA strands. Disease-causing mutations should be confirmed using genomic DNA from a new extraction. Causative mutations found with next-generation sequencing should be verified using Sanger sequencing or other specific molecular methods (eg, PCR digest); for further details, see the Eurogentest Guideline.[2]

1.6 Estimated frequency of the disease (Incidence at birth ('birth prevalence') or population prevalence)
If known to be variable between ethnic groups, please report):

Estimated population prevalence ranges between 1:5000 and 1:4 000 000 in adults depending on the occurrence of an isolated thoracic aortic aneurysm or as a symptom of a syndromic disorder, excluding non-genetic causes, for example, atherosclerosis.

1.7 Diagnostic setting:

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comment: panel diagnostic or WES/WGS filtering should be preferred if clinical signs of a specific syndrome are missing, for example, in young patients with an emerging syndrome. But also in older persons, a specific syndrome can have a widely variable expression.

Time constraints, for example, in pregnancy, is another reason to choose panel diagnostic, if there is a request for prenatal diagnosis (rarely) or if the modus of delivery is dependent on a specific condition of the child.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity
(proportion of positive tests if the genotype is present in the analyte)

2.1.1 if tested by conventional Sanger sequencing
Less than 100%.

The proportion is likely <100%, because primers may be localized on sequences containing SNPs or rare variants, which results in a preferential amplification of one allele (allele drop out). A supplementary deletion/duplication diagnostic test should be performed for genes with a known proportion of large genomic deletions/duplications.

2.1.2 if tested by next-generation sequencing
Less than 100%.

The proportion is likely <100%, because there might be disease-causing mutations in regions that could not be enriched and/or sequenced by NGS due to suboptimal coverage of some regions of interest with this technology but depending on NGS strategy. If amplicon-based enrichment strategies are being used, primers may be localized on SNPs or rare variants, which results in a preferential amplification of one allele. In patients with a highly suggestive phenotype in whom initial testing for specific gene alterations proves negative, a supplementary deletion/duplication diagnostic test should be performed for genes with a known proportion of large genomic deletions/duplications.

2.2 Analytical specificity
(proportion of negative tests if the genotype is not present)

2.2.1 if tested by conventional Sanger sequencing
Nearly 100%. False positives may at the most arise due to misinterpretation of rare polymorphic variants.

2.2.2 if tested by next-generation sequencing
Less than 100%. The risk of false positives due to misinterpretation of rare polymorphic variants may even be higher compared with Sanger sequencing because of the greater number of analysed genes.

2.3 Clinical sensitivity
(proportion of positive tests if the disease is present)

The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases, a general statement should be given, even if a quantification can only be made case by case.

2.3.1 if tested by conventional Sanger sequencing
In about 20% of patients presenting with familial AAT, a disease-causing mutation is found[5] (eg, *ACTA2*: 4–14%, *TGFBR2*: 4%, *SMAD3*: 2%, *TGFBR1*: 1%, *MYH11*: 1%, *MYLK*: 1%, *TGFB2*, *MAT2A*, *PRKG1*, *MFAP5*).

In syndromic forms of heritable thoracic aortic aneurysm clinical sensitivity is highly dependent on fulfillment of specific clinical criteria for a given entity.

2.3.2 if tested by next-generation sequencing
See 2.3.1.

2.4 Clinical specificity
(proportion of negative tests if the disease is not present)

The clinical specificity can be dependent on variable factors such as age or family history. In such cases, a general statement should be given, even if a quantification can only be made case by case.

2.4.1 if tested by conventional Sanger sequencing
Unknown.

2.4.2 if tested by Next-generation sequencing
See 2.4.1.

2.5 Positive clinical predictive value (life time risk to develop the disease if the test is positive)
Dependent on clinical subtype, typically >50%.

2.6 Negative clinical predictive value
(Probability not to develop the disease if the test is negative)
Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity must need to be considered.

Index case in that family had been tested and a causative mutation identified:

Nearly 100%. If the non-affected relative is not carrier of an identified disease-causing mutation, he or she has no increased risk, except a small risk related to the prevalence of the disease in the general population.

Index case in that family had not been tested or no causative mutation identified:

Up to 19% of patients with TAAD without a known genetic syndrome have a first-degree relative with TAAD.[6] In syndromic forms of heritable thoracic aortic aneurysm, the negative clinical predictive value corresponds to the detection rate in the known genes mutated in the different diseases.[7]

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected
(To be answered if in 1.8 'A' was marked)

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☐ (continue with 3.1.4)	
Yes	☒	
	Clinically	☒
	Imaging	☒
	Endoscopy	☐
	Biochemistry	☐
	Electrophysiology	☐
	Other (please describe)	Slit lamp examination

3.1.2 Describe the burden of alternative diagnostic methods to the patient
A clinically affected person has to be regularly examined by echocardiogram, CT or MR imaging.[8]

Alternative diagnostic methods might not capture early detection of none cardiovascular symptoms in syndromic cases.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?
No data available.

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☐	
Yes	☒	
	Therapy (please describe)	Earlier surgical therapy in young patients with disease-causing TGFBR1 and TGFBR2 mutations and clinical signs of LDS has been suggested by some authors[9] but not others[10]
	Prognosis (please describe)	Prophylactic surgery and pharmacological therapy lead to a better prognosis[11]
	Management (please describe)	Regular vascular examination and determination of the best time for surgery.[12,13] Depending on the disease-causing gene, it may be necessary to extend vascular imaging beyond the aorta. Furthermore, it is necessary to examine for specific syndromic complications, for example, hollow-organ rupture in Ehlers–Danlos syndrome type IV.

3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history
(To be answered if in 1.8 'B' was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?
If the test result is positive (please describe), see 3.1.4.

If the test result is negative (please describe), if the causative mutation is identified in the index case and not in the clinically unaffected proband, regular examinations are not necessary unless otherwise indicated.

Follow-up is recommended if the disease-causing mutation could not be identified. In contrast, follow-up is dispensable in a family member, if a familial mutation has been excluded.

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?
That person should avoid sport activity/professional activity with a high static burden, competitive sports and body contact sports.

3.3 Genetic risk assessment in family members of a diseased person
(To be answered if in 1.8 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?
Yes, if a causative mutation could be identified in the index patient.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
If a disease-causing mutation is identified in the index patient, family members can be tested (cascade screening). Test negative family members can be released from otherwise indicated diagnostic monitoring.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Yes.

3.4 Prenatal diagnosis
(To be answered if in 1.8 'D' was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?
Yes.

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe).

The genetic diagnostics contributes substantially to the classification of a heritable thoracic aortic aneurysm to a syndromic or non-syndromic entity.[14] Genetic testing gives insight to inheritance pattern and allows reasonable genetic counseling. If a causative mutation is identified in a gene also responsible for a syndromic form of TAAD, further clinical investigations regarding symptoms of this specific syndrome should be performed. In some cases it might be justified to start medical treatment at an earlier stage.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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