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Peer review file

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Reviewer A

Chen Z-R et al tried to report possible genetic mutations of the known pathogenic genes and clinical characteristics in patients with sporadic Stanford type A aortic dissection (STAAD). They identified 60 suspicious pathogenic mutations (56 novel and 4 reported) from 19 genes in 50% of patients, and 14 patients had more than 1 mutation. They found that patients with FBN1 mutations were younger than those without 6 FBN1 mutations. They also observed an increased risk of in-hospital mortality in mutation carriers. Based on these results, they concluded that half of Chinese patients with a sporadic form of STAAD may carry mutations on known pathogenic genes and may present distinct clinical features and poor clinical outcomes.

Major comments

Comment 1: They described the results of genetic mutations and clinical outcomes in relatively small number of patients with sporadic STAAD. They described the criteria to define as a pathogenic variant, which should be more stringent with the ClinVar evaluation and allele frequency evaluation in control individuals.

Reply 1: As has been shown in method part (page 5, line5), ***"Variants were considered to be pathological if they met one of the following criteria: (1) previously reported to be pathological."*** Actually, we did use the ClinVar database to verify every variant that supposed to be potentially pathological, and 4 mutations had been previously reported from Clinvar database (FBN1 p.R565X, ACTA2 p.R149H, ACTA2 p.R212Q and TGFBR p.D522N, and detailed information has been shown in **Table 3**). We have clarified these sentence in the manuscript.

As to the allele frequency in control individuals, we reviewed the 60 potentially disease-causing mutations in the 568 controls, and all the allelic frequency lower than 0.01, same as in the 1000 Genomes Project. (As shown in the method part Page 5 Line123, "***Polymorphic variants were excluded if their allelic frequency > 0.01 in the 1000 Genomes Project or in the 568 healthy controls.***") The detailed information for allelic frequency in healthy control has been shown in **Supplement material Table Ⅲ**.

Changes in the text:

Variants were considered to be pathological if they met one of the following criteria:

(pISSN: 2072-1439; eISSN: 2077-6624)
JOURNAL of THORACIC DISEASE

(1) previously reported to be pathological from NCBI ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/).

Comment 2: Clinical features should be described based on the current diagnostic nosology for Marfan, Loeys-Dietz and vascular Ehlers-Danlos.

Reply 1: Thanks for the reviewer's advice. Hereditary diseases of connective tissues (including Marfan, Loeys-Dietz and vascular Ehlers-Danlos syndrome) are often associated with syndromic AD which means the abnormalities are not limited to the cardiovascular system and showed clinical features like facial features and skeletal abnormalities. Moreover, most of these patients with significant clinical feature and severe phenotype often showed autosomal dominant disorder usually caused by severe pathogenic mutations in those certain genes, and often had family history.

In order to discover novel candidate disease causing genes and relative genetic variants, thus we firstly exclude those patients with clear family history of aortic disease, and the excluded cases often showed facial and skeletal features but not those included in the study. (As has been shown in the Method part, **page 3 line 97** "***All the included patients reported the absence of a first-degree relative with aortic aneurysm or dissection by detailed medical history inquiry***.") We have carefully checked our database and medical records again, and no enrolled patient showed these syndromic clinical features like facial features and skeletal abnormalities except extended aortic diameter. But according to the reviewer's advice, we have added these features in the **Table 2**

Changes in the text:

Table 2. Clinical characteristics of tested patients with Stanford type A AAD

Clinical characteristics	Total (n=100)
Age at onset, years	52.7±12.3
Male, n (%)	62 (62.0)
Family history, n (%)	0 (0.0)
Phenotype	
DeBakey type I, n (%)	84 (84.0)
Comorbidities and risk Factors	
Hypertension, n (%)	65 (65.0)
Diabetes mellitus, n (%)	4 (4.0)
Coronary artery disease, n (%)	4 (4.0)
Smoke, n (%)	34 (34.0)

(pISSN: 2072-1439; eISSN: 2077-6624)
JOURNAL of THORACIC DISEASE

Alcohol history, n (%)	13 (13.0)
Clinical features	
BMI, kg/m^2	25.2±3.7
Ascending aorta diameter, mm	46.4±12.1
Facial features	0 (0.0)
Skeletal abnormalities	0 (0.0)

Comment 3: Minor comments

FBN2 variation may be pathogenic for Beals syndrome but individuals with those variants show phenotypes without aortic events.

Reply 1: We agree with the reviewer's point. In our study, we included those genes that has been reported to genetically affect aorta. People with Beals syndrome have many of the skeletal and aortic enlargement problems as people with Marfan syndrome, and treatments for these problems are similar (https://www.marfan.org/). Consequently, we decided to include FBN2 in this panel due to its relationship with the aortic and connective tissue disorder, and we have corrected the Marfan-like syndrome as Beals syndrome in the text.

Changes in the text:

(line 73-75) most of them show autosomal dominant disorder caused by mutations in certain genes, such as FBN1 (Marfan syndrome (MFS)), FBN2 (Beals syndrome)

Table 1. Panel of the 26 tested genes

No.	Type	Gene	OMIM No.	Clinical manifestation
1	ECM proteins	FBN1	154700	Marfan's syndrome
2	ECM proteins	FBN2	612570	Beals syndrome; Contractural arachnodactyly
3	ECM proteins	MFAP5	616166	Aortic aneurysm, familial thoracic 9
4	ECM proteins	COL1A1	130000	Ehlers-Danlos syndrome, classic type
5	ECM proteins	COL1A2	130060	Ehlers-Danlos syndrome, procollagen proteinase deficient
6	ECM proteins	COL3A1	130050	Ehlers-Danlos syndrome, vascular type
7	ECM proteins	COL5A1	130000	Ehlers-Danlos syndrome, classic type

(pISSN: 2072-1439; eISSN: 2077-6624)

JOURNAL of THORACIC DISEASE

8	ECM proteins	COL5A2	130000	Ehlers-Danlos syndrome, classic type
9	ECM proteins	ADAMTS2	225041	Ehlers-Danlos syndrome type 7
10	ECM proteins	ADAMTS10	277600	Weill-Marchesani syndrome 1
11	ECM proteins	PLOD1	225400	Ehlers-Danlos syndrome, hydroxylysine-deficient
12	ECM proteins	PLOD3	612394	Bone fragility with contractures, arterial rupture, and deafness
13	ECM proteins	ELN	123700	Williams syndrome,Supravalvar aortic stenosis
14	ECM proteins	EFEMP2	614437	Cutis laxa autosomal recessive IIA
15	TGF-β pathway	TGFBR1	609192	Loeys-Dietz syndrome 1
16	TGF-β pathway	TGFBR2	190182	Loeys-Dietz syndrome 2
17	TGF-β pathway	SMAD3	613795	Loeys-Dietz syndrome 3
18	TGF-β pathway	TGFB2	190220	Loeys-Dietz syndrome 4
19	TGF-β pathway	TGFB3	615582	Loeys-Dietz syndrome 5
20	Cytoskeletal/ smooth muscle contraction apparatus proteins	MYH11	132900	Aortic aneurysm, familial thoracic 4
21	Cytoskeletal/ smooth muscle contraction apparatus proteins	ACTA2	611788	Aortic aneurysm, familial thoracic 6
22	Cytoskeletal/ smooth muscle contraction apparatus proteins	MYLK	613780	Aortic aneurysm, familial thoracic 7
23	Cytoskeletal/ smooth muscle	PRKG1	615436	Aortic aneurysm, familial thoracic 8

(pISSN: 2072-1439; eISSN: 2077-6624)

JOURNAL of THORACIC DISEASE

	contraction apparatus proteins			
24	Cytoskeletal/ smooth muscle contraction apparatus proteins	FLNA	300375	Heterotopia, periventricular, Ehlers-Danlos variant
25	Neural crest migration	NOTCH1	109730	Familial thoracic aortic aneurysm with Bicuspid Aortic Valve
26	Facilitative glucose transporter	SLC2A10	208050	Arterial tortuosity syndrome

Comment 4: Minor comments. They need to analyze more cases with more vigorous evaluation of variants as well as clinical features.

Reply 1: We agree with the reviewer's point and the current small sample size did not allow extended analysis of subgroups and corrections for the clinical features. We now doing further confirmation in a larger validation cohort on other potentially disease-causing genetic variants such as CNV or SNV on new potential genes found by the WGS data, as well as the clinical features.

Changes in the text: None.

Reviewer B

Comment 1. line 193: please clarify in which pts were you refereed about in-hospital mortality 12% (12/100)? In operated or not-operared pts?

Reply 1: The in-hospital mortality 12% (12/100) is for all the enrolled patients, both the patients treated with operated and non-operared meatures.

Changes in the text: The overall in-hospital mortality was 12.0% (12/100) in all the enrolled patients with STAAD.

Comment 2. line 201-201: please clarify in which pts were you refereed about in-hospital mortality, in operated or not operated pts?

Reply 1: According to the reviewer's advice, we have added such information in the text (line 202-204), and provided the detailed information for in-hospital mortality in Supplementary Table VI.

(pISSN: 2072-1439; eISSN: 2077-6624)
JOURNAL of THORACIC DISEASE

Changes in the text:

(Main text, line 202-204)The in-hospital mortality was 27.0% (10/37) and 3.2% (2/63) in patients with conservative and a surgical treatment, respectively.

Supplementary Table VI. Reasons account for in-hospital mortality in type A AAD

No.	Age, Gender	Cause of death	Treatment	No. of mutation	Mutation involved genes
1	49, male	aortic rupture	conservative	1	COL5A2
2	58, male	aortic rupture	conservative	0	
3	20, female	aortic rupture	conservative	3	FBN1, COL1A2, TGFBR2
4	32, male	cardiac death	conservative	0	
5	49, female	cardiac death	surgical	0	
6	67, male	aortic rupture	surgical	1	MYH11
7	66, male	aortic rupture	conservative	1	COL5A1
8	63, male	aortic rupture	conservative	1	MYH11
9	53, female	aortic rupture	conservative	1	FLNA
10	76, female	cardiac death	conservative	1	ACTA2
11	59, female	cardiac death	conservative	2	FBN1, MYH11
12	45, male	aortic rupture	conservative	1	FBN1

Comment 3. line 204-206: as I understood 37 pts with STAAD underwent conservative treatment . Can you explain me why these 37 pts did not operated for STAAD? These patients were older? The patients did not give consent for operation? Please clarify.

Reply 1: The rationale and strategy of surgical intervention were determined according to the guidelines on the diagnosis and treatment of aortic diseases. Once

(pISSN: 2072-1439; eISSN: 2077-6624)
JOURNAL of THORACIC DISEASE

diagnosis of Stanford type A AAD is confirmed, an open surgery repair should be performed as soon as possible after initial risk assessment. But the condition of patients may discharge without surgery treatment due to various reasons such as economic reasons, death caused by complications before operation and rejection for surgery due to personal reasons.
Changes in the text: None.

Comment 4. line 208-209: Obviously, the patients with aortic dissection without operation have worse outcomes with compared the pts with operation for aortic dissection repair. It is old knowledge.
Reply 1: We agree with the reviewer's point. In this part, we want to analyze the relationship between genetic variants and clinical outcome. And because of the significant impact of operation on prognosis, we subdivided the patients according to different treatments as subgroup analysis.
Changes in the text: None.

Comment 5. line 209-211: In which groups with mutations the in-hospital mortality was similar (operated or not operated)
Reply 1: In all the enrolled patients including those with or without surgical treatment (Figure 3A, in this part patients has not been subdivided into surgical and non-surgical yet.) We have divided the text into 2 paragraphs in order to avoid confusion.

Changes in the text:
(line 200-201): The in-hospital death rate was comparable despite the number or type of the mutation patients carried (All $P > 0.05$, Figure 3A).
Because of the significant impact of surgical treatment on in-hospital death from STAAD, patients were then subdivided into a conservative treatment group and a surgical intervention group.

Comment 6. I confused about the groups of pts who operated and not operated and mutations and not mutations groups. Please clarify it and please revise the section: "in hospital outcomes"=line 196-211
Reply 1: The sub-group analysis of in-hospital mortality were performed in conservative and a surgical treatment, respectively. Also according to with or without mutation, single or multiple mutations, and ECM related mutation or not, as has been analyzed in the whole cohort. And we have clarified the sentence in order to avoid confusion.
Changes in the text:

(pISSN: 2072-1439; eISSN: 2077-6624)
JOURNAL of THORACIC DISEASE

Because of the significant impact of surgical treatment on in-hospital death from STAAD, patients were then subdivided into a conservative treatment group and a surgical intervention group. The in-hospital mortality was 27.0% (10/37) and 3.2% (2/63) in patients with conservative and a surgical treatment, respectively. The sub-group analysis of in-hospital mortality were also performed in different treatments group according to the presence, number and type of the mutation.As shown in Figure 3B, in-hospital mortality was comparable between patients with and without mutations for those who received surgical treatment (3.1% vs. 3.2%, P=1.000). However, increased in-hospital mortality in mutation carriers was observed only in patients receiving conservative treatment (44.4% vs. 10.5%, P=0.029). When the in-hospital mortality was compared between the mutation number and mutations on different genes groups, no statistically significant differences were observed (all P >0.05, in Figure 3C and 3D).

Comment 7. line 319-322: While the patients without operation for aortic dissection have worse outcomes (mortality rate is 70-80% during 48 hours), I don't understand how the gene mutation affects on outcomes in patients. On the other hand you did not observe differences of in hospital mortality in patients who operated and with or without mutation (line 206-207). Since the patients is not operated the gene mutation does not any role in patients outcomes. Please revise your in the text and in abstract. I think that the central message of your job is that the patients should be operated early or superstitious if the gene mutations was identified in patients with high risk factors or diagnosed aortic aneurysm in relatives.

Reply 1: Thanks for the reviewer's question, but the authors want to share some different opinions.

First, it is widely known that the mortality rate is extremely high in patient with aortic dissection if not receiving surgical treatment, especially those with Stanford type A AD. However, it is just the ones without surgical treatment that are closer to the natural progress of the disease. As we have illustrated in discussion part (page line 273-302), those mutations on genes encoding some key components of ECM, smooth muscle cells could weaken aortic integrality and stiffness. These protein defects can lead to aortic dilatation before the severe aortic event onset such as Marfan's syndrome and other hereditary connective tissue diseases. So why could not them lead to aortic rupture in a more severe condition like artery dissection? The outcome did not show statistic difference in this relative small sample size and short in-hospital periods. But this observation is not conclusive that the gene mutation does not play any role in patients' outcomes with or without surgical treatment.

Second, whether an immediate operation is appropriate should be evaluated under

(pISSN: 2072-1439; eISSN: 2077-6624)
JOURNAL of THORACIC DISEASE

different circumstances. According to the guideline in management of aortic disease (ESC 2014 and AHA 2010), patients with Ehlers-Danlos syndrome (mutations on collagen encoding genes) show a high risk of surgical intervention due to the fragility of aortic tissues and poor wound healing, thus open surgery are only considered in patients with potentially fatal complications, and prolonged post-operative monitoring are often needed.

Consequently, we want to emphasize the importance of genetically personalized care and subsequent precision treatment in aortic disease (discussion part, line 292-302). As has been explained above, we hope to reserve the current interpretation.

Changes in the text: None

Comment 8. Did you observe differences about post-op neurological complications and acute kidney injury rate between patients who underwent operation with and without gene mutations.

Reply 1: Most of the genes we analyzed mainly refer to the extracellular matrix, smooth muscle component, which may play an important role in homeostasis of aorta, but may not in nervous system and urinary system. As a genetic study, we admitted that the clinical information was collected relatively limited due to our original design. On the other hand, from Supplementary Table VI (Reasons account for in-hospital mortality in type A AAD, which has been shown above in Comment 3), aortic rupture is the most common cause of short-term death. Thus we think the influence of genetic variants on neurological complications and acute kidney injury may not play a role in in-hospital death of those patients as important as we imaged.

Changes in the text: None.