Author's response to reviews

Title: Whole exome sequencing reveals a stop-gain mutation of PKD2 in an autosomal dominant polycystic kidney disease family complicated with aortic dissection

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Author's response to reviews:
Point-by-point Responses

Thank the editor and reviewers for these precious comments concerning my manuscript entitled "Whole exome sequencing reveals a stop-gain mutation of PKD2 in an autosomal dominant polycystic kidney disease family complicated with aortic dissection" (MGTC-D-17-00066R1). These comments are all valuable and very helpful for revising and improving my paper, as well as the important guiding significance to my researches. We have studied comments carefully and have made corrections which we hope meet with approval. Their original critiques are highlighted in bold and italic, followed by our response.

Referee 1
The manuscript entitled "Whole exome sequencing reveals a stopgain mutation of PKD2 in an autosomal dominant polycystic kidney disease family complicated with aortic dissection" by Zhang et al described a PKD2 mutation associated with ADPKD with aortic dissection. The aortic dissection in ADPKD is not a novel finding, neither the mutation found in the PKD2 locus.
The authors will be needed to rewrite the discussion and some of the sentences before the manuscript can be accepted for publication.

1. Define "stopgain" mutation.

As for the referee’s concern, we defined “stopgain” mutation in the revised discussion part. “The PKD2 mutation (R592X) identified in our study introduces a premature stop codon that leads to removal of the last 377 amino acids, altering both the transmembrane segments and the coiled-coil region located in the C-terminus (Fig. 3B). As a result, the mutation could impair protein function and hence was considered pathogenic.”

2. Through a study with 139 ADPKD patients and in 149 healthy family members was unable to establish the association of aortic aneurysms and ADPKD. It also suggests the uncontrolled hypertension might be a factor for development of aortic dissection (J Am Soc Nephrol. 1996; 7(11):2483.). In this case report, the affected patient has 10 year of uncontrolled hypertension which could be the reason for the presentation of aortic dissection. In the discussion, the authors should discuss the role of hypertension in development of aortic dissection and which group of ADPKD patients should be monitored for it, not all the ADPKD patients.

As for the referee’s concern, we have discussed the role of hypertension in the revised manuscript. “ADPKD is a heterogeneous monogenic disorder with considerable intra-and interfamily phenotypic diversity. Even with the same mutation, the presence of vascular phenotype was highly variable as illustrated in our study, suggesting modifier effects of additional genetic or environmental factors. Indeed, ADPKD patients are prone to develop hypertension via multiple mechanism, and hypertension in turn could cause tear in already vulnerable aortic walls. In accordance, hypertension has been reported to be more prevalent in ADPKD-associated aortic dissection patients. Long-term hypertension may therefore serve as superimposed risk factor that could modify the propensity toward dissection formation in ADPKD”.

With respect to monitoring, thoracic aortic dissection is a rare vascular complication of ADPKD. Reports concerning ADPKD complicated with aortic dissection are scarce. Therefore, genotype-phenotype correlation regarding ADPKD-associated aortic dissection could hardly be
established, making the identification of at-risk patients challenging. Silverio et al. summarized clinical features of ADPKD-associated aortic dissection patients and found that they were markedly younger than those in IRAD (International Registry of acute Aortic Dissection), despite a small sample number of patients. More relevant studies are warranted to decipher the precise mechanism by which specific PKD mutations predispose to a vascular phenotype. Up to now, there is no clear answer as to which group of ADPKD patients should be monitored.

3. Page 8, line 45, correct the sentence: "with the underlying the underlying genetic aberration is rarely explored"

As for the referee’s concern, we have corrected the mistake in the revised manuscript. “Case reports of dissection aneurysm in patients with ADPKD are currently limited, with the underlying genetic aberration rarely explored”.

Referee 2
Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a genetic hereditary systemic syndrome characterized by bilateral renal cysts, other potential cysts in several organs and extrarenal manifestations, including cardiovascular disorders. Case reports of aortic dissection (AD) in patients with ADPKD are currently limited and the underlying genetic aberration is rarely explored; of note, this article for the first time reports a case of ADPKD complicated with aortic dissection caused by PKD2 mutation, that is generally considered more "benign" compared to PKD1 mutation.

BMC Medical Genetics represents the appropriate journal for this study because it publishes papers which illustrate all aspects inherent genomics in relation to human health and disease.

Major Comments
1. Polycystins might be required to maintain vascular integrity but the precise mechanism by which specific PKD mutations predispose to a vascular phenotype remains unclear. Moreover, not all individuals from high-risk ADPKD families will experience vascular complications. Can you explain the possible potential genetic mechanism of aneurysm formation of this stop-gain mutation c.1774C>T, p.Arg592Ter detected in your case?
Our theory is as follows: this stop-gain mutation introduces a premature stop codon that leads to removal of the last 377 amino acids, altering both the transmembrane segments and the coiled-coil region located in the C-terminus. Loss of protein function is presumed and vascular integrity is hence influenced. Long-term hypertension serves as superimposed risk factor that increases the propensity toward aneurysm formation in ADPKD.

2. Despite congenital genetic aberration influence, hypertension represents the main risk factor for AD with a prevalence of about 75% in the overall AD. Can you better explain in the discussion the relationship hypertension-ADPK and the adding role of this classical cardiovascular risk factor in the development of dissection?

We have added the corresponding discussion part in the revised manuscript. “ADPKD is a heterogeneous monogenic disorder with considerable intra- and interfamilial phenotypic diversity. Even with the same mutation, the presence of vascular phenotype was highly variable as illustrated in our study, suggesting modifier effects of additional genetic or environmental factors. Indeed, ADPKD patients are prone to develop hypertension via multiple mechanisms, and hypertension in turn could cause tear in already vulnerable aortic walls. In accordance, hypertension has been reported to be more prevalent in ADPKD-associated aortic dissection patients. Long-term hypertension may therefore serve as superimposed risk factor that could modify the propensity toward dissection formation in ADPKD”

3. In the description of the case, it's affirmed "He thereafter received blood pressure control therapy with multiple antihypertensive drugs"; can you better precise the therapy instaured? Are there in literature any drug associated with a slower decline of renal function and a low mortality rate in ADPKD patients?

As for the referee’s concern, we have provided the antihypertensive drug information in the revised manuscript. “He thereafter received strict blood pressure control therapy with intravenous sodium nitroprusside, which is converted to oral antihypertensive drugs captopril and betaloc”. Based on the current understanding of the pathogenesis of hypertension and results of clinical trials in ADPKD, we suggest that the optimal treatment of this disease is RAAS
inhibitors with ACEI or ARBs. These agents remain the most recommended drugs to treat hypertension in patients with ADPKD although clinical studies have not convincingly demonstrated evident benefit. Patients who develop a significant decline in renal function may be more safely treated with another agent such as a beta-blocker or calcium channel blocker; we prefer to use a beta blocker as a second agent given the potentially detrimental effects of calcium blockers on cyst formation. You can refer to the review article-Update on Pathogenesis, Management, and Treatment of Hypertension in Autosomal Dominant Polycystic Kidney Disease. Saudi J Kidney Dis Transpl 2017;28(2):253-260.

4. The gene-mutation positive members of the family, even if asymptomatic, could benefit of an accurate screening program for cardiovascular complication; please underline in the discussion the importance to perform close clinical and echocardiographic screenings already at a young age in these subjects, suggesting the importance to introduce a molecular testing of ADPKD in similar situation. Moreover, what kind of instrumental examinations do you suggest for the follow-up of the patient of this case?

We have revised the manuscript as you suggest. “Silverio et al. summarized clinical features of ADPKD-associated aortic dissection patients and found that they were markedly younger than those in IRAD (International Registry of acute Aortic Dissection), despite a small sample number of patients.” “Our case emphasized the importance of aorta imaging in young ADPKD patients to exclude aortic diseases.”

Regarding instrumental examinations, “Transesophageal echocardiography is a useful screening method, but enhanced computed tomography is recommended when aortic dissection is strongly suspected.”

Minor Comments
1. All references must be numbered consecutively, in square brackets, in the order in which they are cited in the text and not listed in it with first author's name; please correct them.

We have revised the reference format as you suggest.
2. The legend of figure 1 and 2 have been inverted; please change it in the correct order.

Sorry for the mistake. We have corrected it.

3. In the manuscript there are some grammar errors, particularly some sentences are too prolix. Please briefly review the language of entire article.

We have carefully reviewed the manuscript and made some corresponding changes, hoping that it meets your expectation.

Referee 3
This is a report of a stop gain pkd2 mutation in a family with 3 PKD-affected individuals. The proband has aortic dissection, but it is not clear whether the pkd2 carriers in the family have been phenotyped for aortic dissection.

All family members were subjected to enhanced computed tomography and no aortic lesions were found except the proband, suggesting low penetrance of this specific vascular phenotype in ADPKD patients.

Whether pkd2 has anything to do with aortic dissection in this family is unclear. Currently, as stated, only 1 out of 3 individuals in this family with the pkd2 mutation have aortic dissection.

Polycystins play a critical role in maintaining vascular integrity. Consequently, ADPKD patients are at an increased risk of developing intracranial aneurysms and aortic dissection. ADPKD belongs to congenital syndromes associated with aortic and/or arterial aneurysm and dissection (Bradley, T.J., et al., The Expanding Clinical Spectrum of Extracardiovascular and Cardiovascular Manifestations of Heritable Thoracic Aortic Aneurysm and Dissection. Can J Cardiol, 2016. 32(1): p. 86-99.)

What about other genes for aortic dissection? It appears as though no other variants were identified from exome sequencing of the proband.
Whole exome sequencing (WES) could simultaneously unbiasedly sequence all coding genes. We almost excluded all possible pathogenic variants located in causal or related genes of aortic dissection by employing WES.

What was father's creatinine?

His father’s creatinine is normal, 83umol/L

How was rare and pathogenic defined. Did they functionally assess all variants?

The variants were then filtered using standard methods, focusing on rare (minimal allele frequency < 0.01) and pathogenic (predicted deleterious by SIFT or PolyPhen2) variants.

What about aortic dissection in other individuals in the family, including the probands mother? Has she had Thoracic CT?

All family members were subjected to enhanced computed tomography and no aortic lesions were found except the proband, suggesting low penetrance of this specific vascular phenotype in ADPKD patients.