Peer Review File

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Reply to the reviewer A:

We feel great thanks for your professional suggestions.

Comment 1: This is an interesting analysis of BHD patients with, likely, a genetically not too different background. The observation of a higher prevalence of pulmonary cysts and pneumothorax compared to cases found in other areas is intriguing. The question not answered in this analysis is: which mutation is found? Is there a more frequent one and if so differs this from findings outside Asia?

Reply 1: Thanks for your nice suggestion. We have added the details of genetic mutations of 10 BHD patients diagnosed in our hospital in table 2 and we have also collected information of genetic mutations of all patients from the articles we enrolled according to your suggestion. All genetic mutations of patients we enrolled were shown in a new table (Table 7). Our new data and analysis have shown that the most frequent genetic mutations in East Asian patients were c.1285delC on exon 11 (18.4%), c.1285dupC on exon 11 (18.4%), and c.1347_1353dupCCACCCT on exon 12 (8.2%). Exon 11 was the most common site of mutation (37.4%), followed by exon 14 (10.2%), exon 12 (10.2%), and exon 6 (9.5%). From studies conducted outside of Asian patients, the most frequent mutations were c.1285dupC and c.1285delC too (Laura S. Schmidt et al, Am. J. Hum. Genet 2005. 76:1023–1033; J R Toro et al, J Med Genet 2008;45:321–331.). It seems that there was no obvious difference in major genetic mutations between East Asian patients and patients from other areas. But whether the clinical difference was determined by other less frequent genetic mutations is still unknown, we believe a large comparative study is necessary and needed in the future to be conducted in East Asia and Europe/USA concurrently to explore the underlying reasons including the gene discrepancy. In addition to genetic mutation, there may be some other underlying reasons which cause the clinical differences inside and outside East Asia, such as different background of healthy systems or medical habits of patients,

and we've added these discussion in the new manuscript. We hope these changes would make the manuscript better and be acceptable to you.

Changes in the text: We have added data of mutations in Table 2 and Table 7. We also have added some data in the part of results, including "The mutations of BHD in enrolled patients are listed in Table 7. The details of genetic mutations were not clear in 19 patients. Among 147 BHD patients with definite information about mutation site, c.1285delC on exon 11 (18.4%), c.1285dupC on exon 11 (18.4%), and c.1347_1353dupCCACCCT on exon 12 (8.2%) were the most frequent BHD mutations. The identified mutation sites included introns 4, 5, 7, 9, 10, 11, and exons 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14. Exon 11 was the most common site of mutation (37.4%), followed by exon 14 (10.2%), exon 12 (10.2%), and exon 6 (9.5%)" (please see line 182-188). Furthermore, we discussed the difference of mutations of BHD patients in or outside East Asia in the part of discussion, including "No obvious differences in major genetic mutations between East Asian patients and patients from other areas were found, as the most frequent mutations in our study were c.1285delC on exon 11, c.1285dupC on exon 11, and c.1347_1353dupCCACCCT on exon 12, which were similar to those in Europe and the USA (4, 10). Whether the clinical difference was determined by other, less frequent genetic mutations is still unknown; a comparative study is needed in the future to explore the underlying gene discrepancy" (please see line 235-241), and "There were several limitations to our study. Although it revealed that fewer typical skin lesions and renal tumors were present in East Asian patients with BHD and that pulmonary cysts with pneumothorax were the most common manifestations, we could not find the fundamental causes of these different clinical characteristics. The main genetic mutations of East Asian patients were similar to those in other areas, and it is still unknown whether other, less frequent genetic mutations determined the clinical differences. Medical habits or diagnostic processes may be different in these regions. Patients presenting with only skin lesions may potentially be overlooked, and those who had pulmonary cysts with pneumothorax may be misdiagnosed. All of these reasons can lead to clinical discrepancies between East Asia and Europe/USA. A large comparative study is necessary and needed in the future, concurrently in East Asia and

Europe/USA, to explore these discrepancies" (please see line 282-293). We hope these changes would be acceptable to you.

Comment 2: Furthermore, the speculation that BHD is very rare in China is strange (line 144 manuscript), as in one excellent paper by Ren et al (Clin Genet 2008;74:178–183) describing a prospective study in spontaneous pneumothorax (PSP) cases testing on FLCN mutations, these authors describe a rather high frequency of BHD in PSP patients. A suggestion is to include these case in the overview as well. A confirmation of this rather high frequency comes from a different paper (Ebana H et al. Respirology (2018) 23, 414–418). Not sure whether information from this study might be of value for this manuscript as well. The conclusion of Liu (ref 7) might be true if PSP is rare in China, which I doubt.

Reply 2: We are really sorry for our unprecise statement that BHD is very rare in China. As the articles about BHD syndrome in China are limited, and there is no epidemic data about this disease in China to now, we indeed couldn't make a statement that BHD is rare. As you suggested, we have carefully read the article you recommended (Ren et al, Clin Genet 2008;74:178–183), and this article is really excellent where we could find more information about BHD in China, and their data have shown the BHD may not rare in China. So we deleted the related statement that indicating the BHD is rare in China. Moreover, we enrolled the data of Ren et al (Clin Genet 2008;74:178–183) and reanalyzed the data of East Asian patients with BHD as you suggested, and we hope the new data would be more comprehensive. Also, we have carefully read another article you suggested (Ebana H et al. Respirology (2018) 23, 414–418), from their study in Japan, the BHD syndrome may not so rare in East Asia. But as this study only introduced the pneumothorax of BHD patients without information about skin or renal lesions and genetic mutations, we didn't enroll their study in our data. We really thank you for your nice advice and recommended articles. We have corrected all statement that the BHD is rare in China or East Asia. We hope this would be acceptable to you.

Changes in the text: We have added data of patients from Ren et al(Clin Genet 2008;74:178–183) in Table 3. Thus, we further changed data in Table 6. We hope these changes will meet your approval. We appreciate your warm work earnestly and thank you again.

Reply to the reviewer B:

We sincerely appreciate your professional work and valuable comments.

Comment 1: The study is trying to give a proof for a different type of appearance of the Birt-Hogg-Dubé syndrome in east-Asia, especially China, Japan and Korea compared to Europe and the United States. This aim was not fully reached as I will try to explain in the following lines. First of all we find a very thorough list of literature carefully worked up from the east asian area of Korea, Japan and China which is very positive. Nevertheless I miss such a thorough work up of the European and US literature to really have the possibility to compare the patients in each region and to give proof for their hypothesis. One of the studies the authors picked out to try to proof the difference between BHD in East Asia and Europe/US is the paper of Toro et al. (No. 4). In this paper the different types of mutations of the FLCN Gene are very well described on the basis of 50 families and a report of the recent literature. Toro did not make any differences from the ethnic origin of the patients and I would consider there are a lot of the families with origin from Asia as well as patients with Caucasian and afro-american origin. To take this study population to compare with East Asian population, where I would not believe to find any diversity like in the US can be very misleading. Toro as well tried to find out which type of mutation could be more responsible with which symptom picking out two of them (c.17733insC and c.1733delC). None of them showed any statistical significance. It would be as a matter of fact very interesting to find out if there are any differences in the appearance of the FLCN gene in Asia compared to Europe or America. This was unfortunately not done by the authors. There is no analysis of the mutations in the paper. This could by the only way to find out if there are any differences existing like supposed.

Reply 1: Thank you so much for your nice suggestions. We really learned a lot from your comments. As you suggested, the study from Toro et al (No. 4) didn't introduce the ethnic background of their BHD patients, it seems that we couldn't make a conclusion that East Asian patients show fewer skin or kidney lesions compared with patients from USA or Europe. Though in their study, there were 34% patients with renal tumors and 90% of BHD families had typical FFs, while from our data, only 22.9% of BHD patients had kidney lesions including 7.2% renal cancers, and 36.7% of BHD patients had skin lesions including 13.3% confirmed FFs. We believe this difference

may be indicative. To find out more evidence to support our speculation, we reviewed many studies from Europe/USA and East Asia not only including the study from Toro et al. For example, in another study from USA (Laura et al, No.2), they showed 45% BHD patients with renal tumors and 84% proven FFs. Moreover, a study from Netherlands showed 13.4% BHD patients with renal cancers (Paul C. Johannesma et al, PLoS One. 2019; 14(3): e0212952; we've added this article as No.5) and a French study showed 82% BHD patients with FFs (Kluger N et al, Br J Dermatol. 2010;162(3):527-537; we've added this article as No.6). In contrast, studies from Japan, Korea, China (No.5, No.6, and No7 have been changed into No.7, No 8, No 9 in new manuscript), they showed relatively fewer patients with skin lesions and kidney lesions. We think this phenomena is interesting and worthy to explore the underlying causes, and that's the reason we conducted this study and enrolled BHD patients from East Asia to summarize their clinical manifestations and make a more reliable and comprehensive analysis. We think there may be some reasons to explain this clinical difference between Europe/USA and East Asia, including possible different gene mutations or different background of healthy systems or medical habits of patients. We think the information of our study could be helpful to the clinicians especially in East Asia, as there are not enough studies about BHD syndrome in these areas. We believe our study may help researchers and doctors in East Asia to know more about the clinical characteristics of BHD. And as you suggested, we've added details of gene mutations of all BHD patients in our study. We hope these new information would make the study better.

Changes in the text: We have added data of mutations in Table 2 and Table 7. We also have added some data in the part of results, including "The mutations of BHD in enrolled patients are listed in Table 7. The details of genetic mutations were not clear in 19 patients. Among 147 BHD patients with definite information about mutation site, c.1285delC on exon 11 (18.4%), c.1285dupC on exon 11 (18.4%), and c.1347_1353dupCCACCCT on exon 12 (8.2%) were the most frequent BHD mutations. The identified mutation sites included introns 4, 5, 7, 9, 10, 11, and exons 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14. Exon 11 was the most common site of mutation (37.4%), followed by exon 14 (10.2%), exon 12 (10.2%), and exon 6 (9.5%)" (please see line 182-188). Furthermore, we discussed the difference of mutations of BHD patients in or outside East Asia in the part of discussion, including "No obvious differences in major

genetic mutations between East Asian patients and patients from other areas were found, as the most frequent mutations in our study were c.1285delC on exon 11, c.1285dupC on exon 11, and c.1347_1353dupCCACCCT on exon 12, which were similar to those in Europe and the USA (4, 10). Whether the clinical difference was determined by other, less frequent genetic mutations is still unknown; a comparative study is needed in the future to explore the underlying gene discrepancy" (please see line 235-241), and "There were several limitations to our study. Although it revealed that fewer typical skin lesions and renal tumors were present in East Asian patients with BHD and that pulmonary cysts with pneumothorax were the most common manifestations, we could not find the fundamental causes of these different clinical characteristics. The main genetic mutations of East Asian patients were similar to those in other areas, and it is still unknown whether other, less frequent genetic mutations determined the clinical differences. Medical habits or diagnostic processes may be different in these regions. Patients presenting with only skin lesions may potentially be overlooked, and those who had pulmonary cysts with pneumothorax may be misdiagnosed. All of these reasons can lead to clinical discrepancies between East Asia and Europe/USA. A large comparative study is necessary and needed in the future, concurrently in East Asia and Europe/USA, to explore these discrepancies" (please see line 282-293). We hope these changes would be acceptable to you.

Comment 2: But there is a relevant difference in the health care system of Europe, Japan, Korea and the US compared to China like the author is obviously aware of: lines 62-64:"…frequently mislabeled as having COPD, emphysema…"This leads to the fact that in Europe or the US, as well as in Japan and Korea the patients have other possibility of visiting a doctor. And it is very logical that if a patient has a more difficult access to the health care system because of the lack of financial abilities or greater distance to a hospital. The symptoms have to be more severe to call of visit a doctor. That is probably the main reason why skin lesions are rare and pneumothorax is more often diagnosed. (lines 150-153). If health care is basic who is interested in genetic testing of a pneumothorax or a skin lesion? Therefore it is simple to explain why - line 144 - „…BHD is rare in China…"For me there is a huge bias in the study that the authors cannot rule out. It is obvious that the differences seen by the authors are mainly

based on the fact that there is BHD underdiagnosed in certain regions of the world due to the health care system and not explained by a different character of the disease in another region of our world. Unfortunately the data presented by the authors cannot rule out this bias and a more thorough analysis of this interesting questions has to be done as stated above.

Reply 2: Thanks again for your professional work and valuable advice. As you suggested, there are some limitations in our study. Though our study showed fewer patients with skin lesions including typical FFs and kidney lesions including renal tumors in East Asian patients, to now, we couldn't achieve a final conclusion of the underlying causes of these clinical characteristics. Firstly, the main gene mutations of East Asian patients seem similar to those reported in other areas, and we didn't find obvious different mutations in East Asia. Secondly, as you suggested, the different healthy system may cause this discrepancy. Moreover, medical habits of patients or the diagnosis of process may be different in different regions. For example, some patients only with skin lesions may not go to hospital for a diagnosis. Or some doctors who find the skin lesions may not further check the chest x-ray or kidney ultrasound because their limited knowledge of BHD syndrome. All of these reasons may lead to clinical discrepancy in East Asia and Europe/USA. We've added a paragraph in the discussion to talk about these limitations and underlying causes in our study. But without doubts, our study and other relatively small studies conducted in Japan or China indicated these clinical differences in BHD patients from different regions. We believe what we found would be helpful for clinicians in this area to deepen the understandings of BHD syndrome and make an early diagnosis and treatment for these patients. And we think a large comparative study is necessary and needed in the future to be conducted in east-Asia and Europe/USA together to confirm this discrepancy. We think our study could be indicative to doctors and researchers in East Asia and other regions.

Changes in the text: We have added in our text as "There were several limitations to our study. Although it revealed that fewer typical skin lesions and renal tumors were present in East Asian patients with BHD and that pulmonary cysts with pneumothorax were the most common manifestations, we could not find the fundamental causes of

these different clinical characteristics. The main genetic mutations of East Asian patients were similar to those in other areas, and it is still unknown whether other, less frequent genetic mutations determined the clinical differences. Medical habits or diagnostic processes may be different in these regions. Patients presenting with only skin lesions may potentially be overlooked, and those who had pulmonary cysts with pneumothorax may be misdiagnosed. All of these reasons can lead to clinical discrepancies between East Asia and Europe/USA. A large comparative study is necessary and needed in the future, concurrently in East Asia and Europe/USA, to explore these discrepancies" (please see line 282-293). We hope these changes would be acceptable to you.

Reply to the reviewer C:

Comment: The authors studied the clinical characteristics of BHDS in East-Asian patients based on their own personal experience and review of the literature. The study is well executed with useful clinical conclusions.

Reply: We feel great thanks for your comments on our article.

Reply to the reviewer D:

Thank you so much for your comments and nice suggestions. We carefully studied these comments and revised our manuscript according to your suggestions.

Comment 1: I enjoyed reading this manuscript. The theme is an important one and I do feel it will add to this area of rare diseases. I do have some comments that are largely related to syntax, grammar and punctuation. See below. It is frequently mentioned throughout the text that pulmonary cysts are a 'symptom' (e.g. line 23, 139, 151, 170, 201). Pulmonary cysts are a radiological finding and are nearly universally asymptomatic unless a pneumothorax develops.

Reply 1: We are really sorry for this mistake. We've corrected them according to your suggestion. We've changed 'symptom' into 'radiological finding' or 'manifestation'. We hops these would be better.

Changes in the text: We have modified 'symptom' into 'radiological finding' or 'manifestation' in our text as advised (please see line 67, 203, 217, 232, 276)

Comment 2: The term ‘systemic literature review’ is used frequently throughout the text. I believe this should be systematic (e.g. line 11, 60, 90).

Reply 2: Thanks for your nice advice. We’ve changed ‘systemic’ into ‘systematic’ in the new manuscript.

Changes in the text: We have modified ‘systemic’ into ‘systematic’ in our text as advised (please see line 52, 111, 144).

Comment 3: There should be a space between a word and the start of parentheses (i.e. 135 patients (86.5% NOT 135 patients(86.5%). This error occurs throughout the article (e.g. line 17, 18, 19, 20, 21, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 146, 184,)

Reply 3: Thank you so much for your work. We have added a space in all these parts you mentioned.

Changes in the text: We have modified our text as advised (please see line 58, 59, 60, 61, 62, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 211, 254).

Comment 4: Birt-Hogg-Dube is an autosomal dominant condition. Therefore it affects all ages (and sexes) equally and it is incorrect to say that it most commonly affects subjects aged 30-40 years. This needs to be clarified. The age at presentation or age at diagnosis may be in this age range. Is that what the authors meant? There is no reference given for this information.

Reply 4: We are sorry for our negligence. As an autosomal dominant disorder, BHD syndrome affects all ages equally as you said. We actually wanted to express that many patients were diagnosed between 30-40 years (Park et al, AJR Am J Roentgenol 2019 212(4):766-772.). While considering this information came from a small study and different studies showed different mean age of diagnose, we decided to delete it. We hope this new change would be more precise.

Changes in the text: We have modified our text as advised (please see line 90-92).

Comment 5: RCC is used frequently throughout the text. I cannot see anywhere that this acronym has been explained. My understanding is that RCC is a renal cell carcinoma, which is a histological subtype of renal cancer. The authors must make clear whether they are talking about benign renal tumours, malignant renal tumours or a specific subtype of renal malignant tumour.

RCC is not a symptom (see line 138).

Also see line 147: ‘no patient developed RCC’, did they develop a different renal cancer like an onchocytoma?

Reply 5: We are really sorry for our unclear use of RCC. Firstly, we’ve added the full name of RCC in the text according to your suggestion, which is **‘**renal cell carcinoma (RCC)’. Next, we’ve checked the whole manuscript to confirm whether the use of RCC is right or not. We’ve changed many ‘RCC’ into ‘renal tumors’ in the text as this term may be more accurate, except for some patients mentioned were histologically verified as RCC. Especially, in line 204 **‘**FFs and RCC are also present in more than 80% and 27% of these BHD patients, respectively’, from the two articles referenced, we found that they used ‘renal tumors’ in their articles, so we’ve changed ‘RCC’ into ‘renal tumors’. In line 252 ‘as Pavlovich[52] et al indicated that 27% BHD individuals had RCC and Zbar[53] et al found a seven-times increase in the risk of RCC for BHD-affected patients.’, as the two studies included patients with oncocytoma, we’ve changed ‘RCC’ into ‘renal tumors’. We hope these changes would be better and more accurate.

As ‘RCC is not a symptom (see line 138, in new manuscript, it has been changed into line 203) ’, we’ve changed the ‘symptom’ into ‘manifestation’. In line 147 ‘no patient developed RCC’ (in new manuscript, it has been changed into line 212), which was referenced from Liu et al (Orphanet Journal of Rare Diseases (2017) 12:104), there were 27 BHD patients in their study, where 4 patients had renal cysts and 2 patients had hamartoma, so they didn’t develop a renal cancer like onchocytoma at the time of diagnosis. We hope this could answer your question.

Changes in the text: We have modified our text as suggested, the ‘renal cell carcinoma (RCC)’ has been added (please see line 176). In line 204, we have changed it into ‘Renal tumors and FFs are also present in more than 27% and 80% of these BHD patients, respectively (10, 52)’. In line 252, we have changed it into ‘Pavlovich et al. (55) indicated that 27% of BHD individuals had renal tumors, and Zbaret al. (56) found a 7 fold increase in the risk of renal tumors for BHD-affected patients’. We’ve changed the ‘symptom’ into ‘manifestation’ (please see line 203). We hope these changes would be better.

Comment 6: Line 162: Pulmonary cysts are ‘main signs’. This is an ambiguous

statement and needs to be clarified.

Reply 6: Sorry for our unclear statement. We wanted to express that the pulmonary cysts are main manifestations in Japanese BHD patients, and 'signs' might be misused. So we've changed the 'signs' into 'manifestations'. We hope this would be better.

Changes in the text: We have modified the 'signs' into 'manifestations' (please see line 228).

Comment 7: Line 164: It seems quite odd to me to mention someone by name (Dr. Furuya) in this way. If this information is required then perhaps best to say "Furuya et al."

Reply 7: Thanks for your nice suggestion. We've changed Dr. Furuya into Furuya et al.

Changes in the text: We have modified Dr. Furuya into Furuya et al as advised (please see line 230)

Comment 8: Line 202: 'which may be mixed with COPD'. This needs to be clarified or expanded on. I don't understand what the authors are trying to convey here.

Reply 8: We are really sorry for our unclear statement. We wanted to express that BHD may be misdiagnosed as other disease like COPD, because skin and renal lesions are less common, and pulmonary cysts as well as pneumothorax are more frequent manifestations in East Asian populations. So we've changed 'In east-Asian patients, skin lesions and RCC are not common, according to our literature review and PC as well as pneumothorax are main symptoms, which may be mixed with COPD. Especially in China, COPD mainly occurs in males with smoking history. So we speculate some male BHD patients haven't been properly diagnosed for reasons above' into 'In East Asian patients, according to our literature review, skin lesions and renal tumors are not common, and PCs, as well as pneumothorax, are the main manifestations, which may be misdiagnosed as other diseases. In China, particularly, COPD mainly occurs in males with a history of smoking (59). So we speculate that some males with BHD have not been properly diagnosed, and have been misdiagnosed with COPD'. We hope this change would be better and easier to understand.

Changes in the text: We have modified our text as advised (please see line 274-279).

Comment 9: Line 194 – 205: The authors point here is important and well received but

needs some clarification and more clarity in use of language for the reader. As mentioned above BHD is autosomal dominant. There is no sex preference. This is not made clear enough in this section. The language used is somewhat ambiguous (i.e. line 204/205). It should be stressed that the question is whether men express the clinical phenotype of BHD to a lesser extent than women or is there some bias in terms of presentation/symptoms/investigations.

Reply 9: We are sorry for our poor language. We've polished the language with the help of the AME editing service, and we hope the new manuscript would be better. In this paragraph, we wanted to express that different studies showed gender difference in BHD patients, and we have added more references to make this opinion much clear. Many studies showed no sex preference in BHD patients. However, we found some studies from East Asia showed a higher frequency of female patients. We think this phenomena may be a bias in terms of symptoms. As skin lesions and kidney lesions are less frequent in East Asia, and COPD are frequent here, we guess some male patients may be misdiagnosed as COPD. More data and further large studies are needed to verify our speculation.

Changes in the text: We have modified our text as "Although the majority of patients in our study were female, BHD syndrome is usually regarded as an autosomal dominant disease without gender discrimination, and some studies have supported this aspect. For example, a large Canadian family involving 36 members with *FLCN* mutations did not show a discrepancy in genders (58), and the study from Zbar et al. (56) also showed similar rates in males and females. Meanwhile, some other articles show a higher frequency of these mutations in females. Toro et al. (4) studied the clinical information of 89 individuals with *FLCN* mutations, and among them, 52 (58%) were women. In their studies from East Asia, Lee et al. (8) and Liu et al. (9) showed that there was a higher frequency in females. This phenomenon may be associated with the bias of symptoms. In East Asian patients, according to our literature review, skin lesions and renal tumors are not common, and PCs, as well as pneumothorax, are the main manifestations, which may be misdiagnosed as other diseases. In China, particularly, COPD mainly occurs in males with a history of smoking (59). So we speculate that

some males with BHD have not been properly diagnosed, and have been misdiagnosed with COPD. More epidemiological data are needed to confirm whether there is a gender dominance for BHD syndrome in East Asia."(please see line 265-281).

Comment 10: Line 20: 'trichodiscom' should be trichodiscoma.

Reply 10: We are sorry for this mistake. We've corrected it according to your suggestion.

Changes in the text: We have changed 'trichodiscom' into 'trichodiscoma' (please see line 61)