Peer Review File
Article information: https://dx.doi.org/10.21037/jtd-21-1064

Reviewer A

Overall comment: The authors present a comprehensive narrative review on the respiratory manifestations of Marfan syndrome…This review provides valuable information on the respiratory complications of Marfan syndrome, that can further aid clinicians to improve the management of this patient population.
Reply : thank you for the positive comments and helpful suggestions below.

Minor comments:
Comment 1: I would suggest to include 'Marfan syndrome' among the keywords.
Reply 1 : Done

Comment 2: In line 84, LAP is incorrectly stated as latency activating peptide. The correct form is latency associated peptide.
Reply 2 : Corrected.

Comment 3: The sentence in line 87-90 would be more precise, if beside abnormal fibrillin-1, reduced amount of fibrillin-1 was also mentioned (mutations either cause abnormal protein structure or a decreased amount of protein), which also leads to an increased bioavailability of TGFbeta.
Reply 3: Thank you for this information. This text has been clarified in Section 3 (Fibrillin-1 Structure and Function), 3rd paragraph.

Comment 4: … I would recommend to explain TGFbeta sequestration with more details including the various complexes (e.g.: small latent complex) and also mentioning the important non-canonical signaling pathway beside the canonical one… the following review can provide a good basis: Benke K et al. The role of transforming growth factor-beta in Marfan syndrome. Cardiol J. 2013;20(3):227-34.
Reply 4: Thank you for this suggestion. We have now greatly expanded on these points in Section 3. 3rd paragraph. This addition also prefaces the losartan discussion later when we discuss the role of the ERK1/2 signaling pathways of the non-canonical TGFβ signaling pathway.

Comment 5: Comment to line 242-244: according to the current knowledge, Loeys-Dietz syndrome is associated with 6 genes (SMAD2, SMAD3, TGFB2, TGFB3, TGFBR1 and TGFBR2) and the mutations of these genes are loss of function mutations.
Reply 5: Thank you for the information. This has been corrected in Section 4.5, 2nd paragraph.

Comment 6: In line 245, I would recommend to add aortic dissection as a high-risk event in MFS and LDS populations.
Reply 6: Done. Please see Section 4.5, 2nd paragraph.

Comment 7: In line 335 the gene FBN1 should be written in italics.
Reply 7: Agreed. To avoid confusion, fibrillin-1 gene will be written as "*FBN1*" as suggested whereas fibrillin-1 protein will be written as "fibrillin-1."

Comment 8: When referring to mouse genes, the following form should be used: Fbn1 (e.g.: line 352)
Reply 8: Corrected.

Comment 9: I would suggest to add and comment on the following paper, as it also provides relevant information on respiratory manifestations in MFS patients:
Kolonics-Farkas AM, Agg B, Benke K, Odler B, Bohacs A, Kovats Z, Szabolcs Z, Müller V. Lung Function Changes are More Common in Marfan Patients Who Need Major Thoracic Surgery. Lung. 2019 Aug;197(4):465-472.
Reply 9: We had already included this important manuscript in Section 4.3, 2nd paragraph.

Reviewer B
Overall comment: A well written English review of pulmonary manifestations reported in Marfan syndrome (MFS). The subject of respiratory manifestations in MFS is highly appreciated as research in the area is sparse…citations throughout the manuscript is erroneous…uncritical listing of manifestation and figures. Statements and figures are reproduced in the review without a background understanding of the subject. The main objective of enlightening the reader in the field of respiratory manifestations of MFS does not seem to be meet. Instead the authors present a flimsy hypotheses on Losartan effect on the pulmonary system.
Reply : We thank Reviewer B for advising us to cite more appropriate references and to discuss them more completely, and have incorporated the numerous suggestions noted below.

Major comments:
Citations:
Comment 1: Many references are irrelevant, obsolete or in some cases incorrect. Several statements are without references. Ex. a review from 2006 is obsolete when a new set of diagnostic guidelines were implemented in 2010.
Reply 1: We agree with the Reviewer that we should have included the 2010 Revised Ghent Criteria for MFS (Loeys BL et al. J Med Genet 2010). The new criteria emphasized two cardinal manifestations, or in absence of those, *FBN1* mutation and/or combination of clinical features with a scoring system. By refining the criteria, the incidence and prevalence of MFS may fall, and older manuscripts may have overestimated the prevalence/incidence. The new criteria make a literature review for a disease initially described 125 years ago somewhat more difficult as it is not possible to determine if all patients enrolled would meet today's criteria and many trials simply state "Patients diagnosed with Marfan syndrome." While a large number of MFS literature were published prior to this 2010 revised Ghent Criteria, they helped refine the guidelines. We have also added a new reference (Groth KA et al. Am J Cardiol 2018) which estimated the prevalence of MFS after publication of the revised Ghent Criteria in the Introduction, 1st paragraph.

We believe the "2006 review" Reviewer B is referring to is a 2005 paper by Judge and Dietz. We have now included a new Table 2 on the revised Ghent criteria for MFS and a brief discussion of it in the last sentence of Section 3 of the manuscript. Please also see response to Comment 2.

Comment 2: I haven't checked all references, but I'm concerned on the ones I have checked.
Reply 2: We agree and thank the Reviewer for bringing this to our attention. We agree that many of the references are older and predate the most recent nosology; however, many of

them were kept in our review because of a paucity of data in several topics relevant to the review. Additionally, prior to the first submission, we had removed about 50 references from the list that were not felt to be adequate or representative of current knowledge. With this round of edits, we have added 15 more contemporary and relevant ones, many of which were recommended by the Reviewers.

Comment 3: I do believe the manuscript leak several relevant citations. Ex. the diagnostic criteria is not cited in the manuscript. This piles to the impression that the authors have a sparse knowledge on MFS literature.
Reply 3: Thank you. Please see response to Comments 1 and 2 above.

Comment 4: Parts of the review seems to be of a better standard (astma section). I believe the authors have a specific knowledge on respiratory diseases but I'm not sure the knowledge also concerns MFS. There still seems to be large areas of the review that is outside authors knowledge area.
Reply 4: We apologize for not being clearer in bringing the different topics into a more cohesive document. We believe we have assembled a writing team with good breadth to discuss the topics included in the manuscript well. Drs. Borg and Chan are pulmonologists, Dr. Godfrey is a MFS scientist who has been awarded the Antoine Marfan Award from the National Marfan Foundation for his work, and Dr. Hadley-Miller is an orthopedic surgeon with clinical and research interests in the skeletal abnormalities of MFS. Dr. Tun is an epidemiologist. We believe the background of the authors contribute significantly to the topics discussed in the manuscript. Based on the suggestions of both Reviewers, we have revised Section 3 (Fibrillin-1 Structure and Function) to be more complete and accurate on the pathophysiology of MFS.

Comment 5: I'm not sure why this section (Section 3 FIBRILLIN-1 STRUCTURE AND FUNCTION) is included in this manuscript. It is highly complex and I do not believe it is of benefit of understanding the review. I would not expect a full review of the biochemical and histological aspects of MFS in a review of respiratory manifestations in MFS. I acknowledge that some parts of the manuscript refer to some biochemical issues bus these could be explained together with each point.
Reply 5: We understand the Reviewer B's concern of including this section but we respectfully wish to keep this relatively brief discussion on Fibrillin-1 Structure and Function as we have observed that most clinicians do not understand the pathophysiology of MFS. While our goal is not to provide the comprehensive review on the pathophysiology of MFS, we believe a basic understanding of it would be welcome by clinicians. As the Reviewer noted, it helped put subsequent discussion on the respiratory manifestations in better pathophysiological context, particularly those regarding emphysema and pneumothorax development and the potential role for angiotensin receptor blockade. Also based on Reviewer A's suggestion, we have clarified and discussed more completely some of the components of the TGFβ-related complexes. Please see modified Section 3.

Comment 6 (Critical review of the references): In the references are used uncritical. Ex. "In one study of all patients referred to a MFS center-of-excellence, 44% of MFS subjects had an obstructive spirometry pattern". No study critics of using percentages and no control cohort. The study is rather small of only 64 patients. This is only an example as most stated figures and symptoms are not evaluated with a research critical mindset. Ex. when screening the

manuscript I only found two mentioning of “significance” related to references, not a single mentioning of “confidence interval” or “P-value”.

Reply 6: You are absolutely right that these random percentages seem without context. Unfortunately, the field of data (particularly with regard to respiratory disease) in MFS is overwhelmingly case series and case reports as opposed to more ideal case-control type reviews which would allow these types of statistical significance comparisons between patients with MFS and the general population. We are left in many cases to contrast them to known prevalence of certain diseases in the general population. Even without directly citing CDC or other health agency prevalence statistics on certain respiratory conditions, we believe some of the prevalence numbers of abnormalities are intuitively very high. For example, under section 4.1, we discuss a recent review of pectus abnormalities, stating that they may be present in up to 70% of those with MFS. These defects (carinatum in particular) are given diagnostic points in the revised Ghent criteria for the reason that it has been well recognized to be of higher proportion in those with MFS than without.

Putting into context the aforementioned paper citing that 44% of MFS have an obstructive spirometry, recent estimates of asthma and COPD prevalence in the American population are on the order of ~10% and 6-7%, respectively, indicating indirectly that obstructive physiology is more frequent in MFS subjects than the general population. Unfortunately, we cannot find a case-control of COPD/asthma/obstructive lung disease in the available databases, especially none within the last 11 years since the revised criteria were released. In addition, due to their abnormal appendicular to axial skeleton height, one would expect most patients with Marfan to be falsely diagnosed with restrictive lung disease (due to reliance of spirometry standard ranges like GLI and NHANES III) on height measured traditionally. We believe this point, in particular, makes a number like this 44% stand out as evidence of pulmonary pathology. Where able, we have gone through our manuscript and added control or contemporaneous background prevalence for reader’s comparison. For example, in Section 4.3, 2nd paragraph, we added: *“In comparison, the contemporaneous prevalence of obstructive lung disease in Italy was around 11% for COPD and around 6% for asthma.”*

Comment 7: I would like the authors to elaborate on issues like very old references ex. incidences of pneumothorax to 4-14% references from 1984. Since these references, three (the first set was in 1986) new set of diagnostic criteria and the discovery of the FBN1 gene have passed! The fact that MFS was not even defined in 1984, could have an impact on how relevant the 1984 cohort is defined?

Reply 7: We thank the Reviewer for this insightful comment. As in our reply to comment 6, there is a paucity of case-control studies in MFS. To elaborate on your example here: The Marfan Syndrome has had various definitions and diagnostic criteria over the years since its initial description in the 19th century. Prior to both of the 1984 studies on pneumothorax we cited, there was a publication in the NEJM in 1979 attempting to standardize diagnosis of the disease. The Hall and Pyeritz retrospective study was a case series in a medical genetics clinic at a prominent American research hospital. There were 249 cases of MFS who met “strict criteria for diagnosis” in that clinic, defined as the presence of abnormalities compatible with MFS in at least two body systems (skeletal, cardiovascular, ocular) or in one body system together with a family history of MFS. Within this cohort, there were 11 cases of pneumothorax for a prevalence of 4.4%. Similarly, the Wood paper from 1984 with 11% rate of pneumothorax lists similar criteria for inclusion: “Marfan abnormalities in at least two separate body systems (Skeletal, Cardiovascular, ocular) or in one body system where there was a family history of a classically affected first degree relative”. While as not refined as the 2010 nosology, these inclusion criteria could potentially meet revised Ghent criteria #1,3,5, 6,

and 7, only leaving out criteria #2 and 4 where *FBN1* positivity is required. We have added incidence rates for the general population for comparison. In addition, a newer study from 2011 utilizing CT scanning in MFS to identify risk and also quantifying rate of pneumothorax in their cohort (4.8%) is now cited.

In addition, we have added a qualifying statement in Section 4.4, 1st paragraph: *"Based on these limited available data, it is unclear whether spontaneous pneumothorax is more common in MFS than the general population."*

Comment 8: I would also like the authors the elaborate on studies without a control cohort. A pneumothorax incidence rate of 4-14% in MFS is that high or low compared to other groups. Are the study population representative of MFS or are the study biased?
Conclusion:
Reply 8: Please see our responses to comment 6 and 7 above. We apologize we did not mention that there was no control group to be compared to the MFS group regarding the incidence of pneumothorax. This is due to the paucity of case-control studies in the literature. We have added information on the incidence of spontaneous pneumothorax in the general population for the reader's comparison.

Comment 9: The conclusion is partly based on misinterpretation of a minor study claiming a respiratory morbidity of 10%. Se the comments to the study line 49-50. I believe it is a major issue that the authors do not know when research is of high or of low quality and how to interpretate research data. This should be the entire foundation of a review.
Reply 9: Thank you for this point, as this statistic does require strengthening to be a valid argument. Recent manuscripts from 2018 (Groth KA et al. Am J Cardiol 2018) and 2019 (Pyeritz RE. Genet Med 2019) cite a range of respiratory morbidity/symptoms of 10-11%, and the Groth case control study found an increased odds ratio of death from respiratory causes in MFS. These papers are now cited. The prior reference is removed from that section. Please see these additions to Section 1 (Introduction), 1st paragraph.

Comment 10: The second part of the conclusion is focused on Losartan potential effect on paraseptal emphysema and pneumothorax complications. These conclusions are partly based on research of Losartan effect on aorta. These effects are debatable and I do believe the authors have an incorrect understanding of the current research status in this area.
Reply 10: We agree and thank the Reviewer for bringing this to our attention. We agree there is no direct evidence that losartan, which antagonizes TGFβ signaling, has any beneficial effect on MFS-associated lung disease. However, based on existing evidence that TGFβ may be involved in the pathogenesis of emphysema due to smoking or *FBN1* mutation, we speculated that there is the potential for losartan to help with this. But we have modified the language to reflect this speculation (**Section 5, 3rd paragraph**): *"The translation of the aforementioned pathogenic signaling mechanisms in the context of vascular disease, TGFβ, and losartan to what occurs in human lungs in MFS remains to be determined. However, based on the pathophysiologic mechanisms of MFS-associated emphysema, losartan may prevent the progression of this lung disease, albeit this is highly speculative…. Since overexpression of TGFβ may also play a role in smoking-related emphysema in humans, of mice with Fbn1 gene mutation, and in mouse lungs following exposure to cigarette smoke, losartan may potentially mitigate the development of emphysema in humans although this remains speculative."*

Comment 11: The pulmonary angle on Losartan is based on research in rodents mainly exposed to smoking hereafter extrapolating these theses to an MFS mice model. I do believe this part of the conclusion to be very speculative and somehow outside the scope of a review.
Reply 11: Please see response to Comment 10.

Comment 12: Why use all the space in this manuscript on chest wall deformity, spinal deformity, asthma and reactive airways, bronchiectasis and sleep apnea when not mentioning these areas in the conclusion?
Reply 12: We apologize for omitting in the conclusion of not mentioning these respiratory disorders. We have now mentioned them.

Minor comments:
Comment 13: Line 12 and 44 it is stated prevalence is 1 in 3,000 to 10,000. This is a very large span. Only one reference is a relevant study (Chiu et al 2014) on MFS prevalence stating a prevalence of 10.2 in 100,000. A reference of a review from 2006 (Jugde et al), before the new Ghent criteria is irrelevant. There are several relevant publications on prevalence of MFS that could be cited.
Reply 13: In addition to the Chiu reference (= prevalence of 1 in 10,000), an excellent 2018 review from Denmark (Groth KA et al. Am J Cardiol 2018) suggests a prevalence using the newer criteria of closer to 1 in 15,000, more consistent with the expected drop in prevalence seen after diagnostic refinement from the revised 2010 Ghent criteria. This new text and reference have been added. The majority of websites including that of the National Marfan Foundation and the CDC, still list prevalence at around 1 in 5,000, which seems largely based on the numbers cited in the Judge review from 2005. We did not find a recent (later than 2010) review that revises prevalence for the United States or the UK in our literature search. We have revised Section 1 (Introduction), 1st paragraph to reflect this: *"After revision of the diagnostic criteria in 2010, the estimated prevalence has fallen from 1 in 5,000 to a more current estimate of 1 in 10,000 to 15,000 individuals."*

Comment 14: Line 47 the citation of Jugde et al from 2006 is still obsolete. In 2010 the new diagnostic criteria were published. These criteria would be a better citation than a 15 year old citation of a review based on old diagnostic criteria.
Reply 14: We agree with the Reviewer of adding the Revised Ghent Criteria (please see last sentence of Section 3 and new Table 2). Please see our Response to Comment 1 of Reviewer 2 regarding the Judge et al (2005) citation.

Comment 15: Line 49-50 state "Up to 10 percent of MFS individuals will have respiratory symptoms or complications or may have a respiratory disease-related cause of death". The citation state 7 deaths are caused by "Pulmonary disease" representing 10% of deaths. The 7 deaths are of a total of 69 deaths. The total cohort was 2329 and the 7 deaths of pulmonary disease represents only 0.3 % of the cohort. The data is not comparable as the study do not have a control cohort. The misinterpretation of these data is very vital as this figure is (mis-)used in the conclusion section. The study of 69 MFS deaths with a percentage statement is not a relevant scientific way of evaluating either morbidity nor mortality. It is possible that MFS actually cause an increased mortality by respiratory causes compared to non-MFS. The study of 2329 MFS with 69 deaths cannot evaluate this question without a control cohort. A similar study with a control cohort by Groth et al found a respiratory cause of death in MFS of HR of 3.0 (CI 1.4 – 6.3), so there might be a point but not by the used reference.

Reply 15: Thank you for this important comment and suggestion. We have addressed this in Comment 9 above. We will instead use the odds ratio of 3 from the Groth paper (Groth KA et al. Am J Cardiol 2018) with its given CI and P values. This new text has been added to Section 1 (Introduction), 1st paragraph.

Comment 16: Line 53-54: State "The goal of this review is to delineate the molecular consequences of a defective FBN1 protein and the skeletal defects, lung abnormalities, and sleep disordered breathing in MFS". The study is not a phenotype-genotype study! How can this review "delineate the molecular consequences".
Reply 16: We have clarified this sentence so as to not mislead our readers in Section 1 (Introduction), 1st paragraph: "The goal of this review is to elaborate how a defective fibrillin-1 protein contributes to the skeletal defects, lung abnormalities, and sleep disordered breathing in MFS that may contribute to respiratory compromise."

Comment 17: Line 55-56: States "We will also discuss how losartan, presently used to prevent the vascular abnormalities associated with MFS". Losartan has been a buzz but I believe the idea of Losartan preventing aorta dilatation is definitely proven not to be true. At least there is no documentation of the effect of Losartan in MFS.
Reply 17: We thank the Reviewer for pointing out thus controversial area. While some major trials have not shown a benefit of losartan to beta blockade for aortopathy, a long-term clinical outcome study (COMPARE trial) published in 2020 showed that combined losartan and b-blocker therapy significantly reduced the incidence of aortic dissection and death (Van Andel MM et al. Eur Heart J 2020). Thus, there remains a theoretical pathway in which this pharmaceutical class may be of use for some of the pathophysiologic mechanism in MFS abnormalities. We have modified aforementioned sentence in the Introduction to reflect that that benefits of losartan in the vascular abnormalities (aortopathy) is controversial: *"We will also discuss how losartan, presently used by some to prevent the vascular abnormalities (aortopathy) associated with MFS, may or may not benefit the respiratory complications of MFS despite conflicting evidence for its efficacy in aortopathy."* We hope to stimulate interest in this pathway for studies from a pulmonary/respiratory perspective given the promising data in mice.

Comment 18: Line 101-104 state: "Monitoring of lung function in patients with MFS is conventionally done by using an extrapolated sitting height, as opposed to standing height, to standardize expected spirometry results to comparison databases due to the appendicular skeletal elongation (particularly in leg length) seen in MFS". The use of extrapolated sitting height as a conventional monitoring method is new to me. I mark the reference is from 1987. Do the authors have a newer reference of this "conventional" method.
Reply 18: We apologize for this oversight as it was inaccurate to say that extrapolated sitting height is "conventionally done." We have revised Section 4.1 to detail how that one study attempted to correct for a propensity for false-positive restrictive lung disease on spirometry. Spirometric standard guidelines such as GLI or NHANES III being dependent on either standing height or extrapolated ulnar length, both of which would lead to overestimation of the thoracic cage size and "underperformance" or restrictive type defects falsely appearing. Perhaps a diagnosis of MFS should lead to an alteration in extrapolated height to address this propensity, but we are not in a position to alter the standards at this time. We have removed the text of "conventionally done."

Comment 19: Line 201: State "The incidence of spontaneous pneumothorax (SP) in individuals with MFS is estimated to be 4-14%". In what time period is the incidence? Lifetime, per week or per year? Incidences are not mentioned in percentages! Is this a high or low incidence? Do the authors have a comparable cohort to compare this incidence?
Reply 19: This is a good question. These rates are from retrospective reviews in adults with MFS who have suffered a pneumothorax at any time, so more closely reated to "lifetime" or rather lifetime up to the time of the analysis. To the best of our knowledge, there is not a prospective collection of MFS and pneumothorax incidence, but the presence of spontaneous pneumothorax is given points in the scoring system for the 2010 diagnostic criteria for MFS. We have added information about incidence of pneumothorax in the general population and also qualified the statement of concern as "lifetime" in Section 4.4.

Comment 20: Line 216-217 state: "definitive surgical treatment with pleurodesis, wedge resection, and/or bullectomy performed by video assisted thoracoscopic surgery is recommended". In the reference article MFS is not mentioned once. How can the treatment be "recommended" without any comment for MFS without testing the treatment on MFS patients? It might be a reasonable postulation, but the authors must at least comment on why this treatment is recommended to MFS.
Reply 20: Thank you for this comment as it indicates further clarification is needed in our manuscript. Pleurodesis is standard of care for secondary spontaneous pneumothorax (pneumothorax in the presence of underlying structural lung abnormality) for all since there is a high risk for recurrence (>50% in some trials), particularly if they experience prolonged air leak and lack of re-expansion. We have now clarified this in Section 4.4, 2nd paragraph and also referenced the guideline from the British Thoracic Society.

Comment 21: Line 302-303 state: "The prevalence of sleep apnea in patients with MFS is much higher than in age- and sex-matched controls, with a range of 31 to 64% of patients being affected". What is the comparison? What is prevalence in the background population or control cohort? One reference did not have a control cohort and the second study had 25 MFS patients and 12 control patients. The second study again is very old and the diagnostic criteria of MFS have changed twice since etc. etc.
Reply 21: We have revised the section to include more details from one reference already cited, but more importantly also added details of the background rates of OSA in the general population for comparison. The following text was added to Section 4.8, 1st paragraph: "A case-control polysomnographic study of 61 patients who fulfilled the revised Ghent criteria for MFS and 26 matched controls revealed that both mild and moderate obstructive sleep apnea were more common in patients with MFS than controls, with 32.8% vs 11.5% having mild disease and 18% vs 0% having moderate disease, respectively. In contrast, the prevalence of symptomatic sleep apnea in in American adults is 5 to 15%, depending on gender and age."

Comment 22: Line 321-322 state: "Losartan, an angiotensin II type-1 receptor blocker, has been shown to prevent the vascular complications of MFS in murine models and humans." This is a highly controversial statement. Other researchers have the opposite conclusion ex Hofmann et al 2019 in JAMA. I believe "shown" should be changed to "suggested" or even better "misproven".
Reply 22: Thank you. We have modified the language as suggested by Reviewer 2. Although there is mixed data for the benefit of losartan in aortopathy, Hofmann et al (JAMA Cardiology 2019) suggests that although the available evidence does not suggest that progression of aortic dilatation is less with losartan and beta-blocker compared to beta-blocker alone, there

are inadequate studies on whether losartan may still play a role in reducing aortic dissections, need for surgery, or sudden cardiac death. A longer-term follow-up study of COMPARE released in Nov 2020 indicated those who continued to use both losartan + beta-blocker have significant decreases in aortic dissection and death (van Andel MM et al. Eur Heart J 2020). Studies on reducing morbidity and mortality from MFS-associated respiratory diseases are needed; we hope to stimulate such interest with our review article. We have now cited both articles.

Comment 23: Line 334-336 state: "The efficacy of losartan in MFS patients may also be related to the type of FBN1 mutation. Positive drug effects are seen in patients with mutations that cause haploinsufficiency rather than those that are dominant-negative." Reference? I do believe the reference is the COMPARE study. The COMPARE study also has some design/bias issues.
Reply 23: Thank you. We have expanded on describing the murine models of fibrillin-1 mutations and signaling pathways in which the potential effects of AT1R modification with losartan may have theoretical efficacy for respiratory diseases felt related to underlying MFS and have added an relevant reference (Takeda N et al. Int Heart J 2016).

Comment 24: Line 337-338 state: "Moreover, skeletal manifestations do not improve on therapy," Reference? I have no knowledge of studies examining medical therapy of skeletal manifestations. If stated that medical therapy does "not improve" on skeletal manifestations a reference is needed. I believe the issue have never been examined and therefore you cannot make the statement.
Reply 24: Reviewer is correct. Based on our search, we are also not aware of any long-term effects of losartan on the skeletal manifestations of MFS. We have modified the statement in Section 5, 3rd paragraph to the following: *"However, it remains unclear if the skeletal manifestations improve on therapy, or whether some of the defects may develop as severely or rapidly. Therefore, some of the mechanical problems related to scoliosis and pectus excavatum and the concomitant respiratory issues may still be problematic despite losartan use. Nevertheless, undertaking a prospective study – perhaps analyzed in parallel in current studies examining the effects of treatment on vascular pathology – using both subjective and objective measures on the long-term effects of losartan on the skeletal manifestations of MFS may be of great value."*

Comment 25: Line 347-348 state: " TGFb is induced and excessive in the lungs of mice with cigarette smoke exposure and in the lungs of humans with emphysema." Reference?
Reply 25: Thank you for pointing this out. We have added the correct reference (Podowski M et al. J Clin Invest 2012) in Section 5, 4th paragraph.

Comment 26: Line 358-359 state: "induce a variety of effects on the respiratory system, inducing substantial morbidity in up to 10 percent of individuals with MFS and also being associated with increased mortality." As mentioned earlier, the study is 10% of the diseased cohort and therefore you cannot state morbidity it would be mortality! The statement of 10% in combination with an increased mortality based on a study without a control cohort is simply not correct. The authors need to get these simple and basic, but very important figures correct.
Reply 26: Thank you. We address this in comment 9, and have clarified the text. We have clarified this statement in Section 6 (Conclusion): *"…induce a variety of effects on the respiratory system – including thoracic cage abnormalities that mechanically compromise respiratory function, airways disease (emphysema, asthma, and bronchiectasis), and sleep*

apnea – inducing substantial morbidity in individuals with MFS and being associated with increased mortality." The Groth article you have referenced addresses increased mortality in a more recent and case-controlled manner and we greatly appreciate this suggestion. Please see text regarding risk of death from respiratory causes in the Section 1 (Introduction).

Comment 27: Table 1: From where are these features? This is not the recent Ghent criteria nor the older criteria. Why are these clinical features chosen?
Reply 27: This Table 1 was a compilation of two review papers by noted authorities on MFS and a more recent one. While the two older review papers were published before the revised Ghent criteria, we considered it important to include because they were by individuals from an institution with great expertise in MFS. Table 1 is not a list of diagnostic criteria but rather a list of described clinical manifestations, which we believe would be useful for clinicians. The revised Ghent criteria for diagnosis of MFS is now listed in Table 2.