Author’s response to reviews

Title: Association of Catechol-O-Methyltransferase Single Nucleotide Polymorphisms, Ethnicity, and Sex in a Large Cohort of Fibromyalgia Patients

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Dear Reviewers,

Please find attached a revised manuscript entitled “Association of Catechol-O-Methyltransferase Single Nucleotide Polymorphisms, Ethnicity, and Sex in a Large Cohort of Fibromyalgia Patients” for consideration in BMC Rheumatology. We have revised the manuscript, included here, along with a point-by-point response to the reviewers. We are confident we have addressed the major concerns and appreciate the feedback, as the manuscript is improved.

This manuscript presents the largest study, to date, that examines demographic and genetic associations of fibromyalgia in a diverse population. Some of the data in this manuscript were presented at the 2017 ASRA annual meeting and we received from great feedback from attendees and circulating editors. We believe this study will be of great interest to your readership.

Thank you for your consideration.
Fibromyalgia Paper

BMC Rheumatology operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.

Reviewer reports:

Jacob Ablin (Reviewer 1): Interesting and extensive study advancing existing knowledge regarding the associations of chronic pain and fibromyalgia with COMT polymorphisms. Well conducted and well written. Although the major results are negative (no association found between COMT haplotypes and fibromyalgia) the findings are worthy of publication.

Andrea G Nackley, PhD (Reviewer 2): Here, the authors conducted a retrospective analysis of demographic and genetic data collected from FM and non-FM controls to determine factors predictive of FM. Additional data from 1000 genomes was included for genetic analysis. Overall, the manuscript is well-written and data interesting. However, the methods lack rigor and the findings lack novelty. Specific concerns are indicated below.

Major Concerns:

1. Demographic risk factor analysis compares FM and non-FM groups, while genetic risk factor analysis compares FM and 1000 genomes groups. COMT SNP minor allele frequencies need to be reported for the non-FM controls as well as the FM and 1000 Genome subjects. Risk predictions should include genotypes of non-FM participants that were recruited in a similar fashion to the FM participants. The non-FM group also has age information that would be important to control for in genetic analysis.

We have added Supplementary Table 1 showing the minor allele frequencies of the COMT SNPs for all three groups.

The non-FM participants were recruited in a similar fashion as the FM participants. However, we acknowledge that because participants were recruited from doctor visits, mainly in offices that
deal with pain, our non-FM sample is skewed toward those who are sicker and/or also have chronic pain conditions, which informed our discussion point that COMT haplotypes may be informative of pain in general, not just fibromyalgia.

2. The chi-square analysis showing risk allele frequency for each COMT SNP is interesting from a descriptive standpoint, but not rigorous in terms of establishing an association of SNPs with FM. Logistic regression models should be used to determine the relationship between SNPs and case status, while including age/sex/ethnicity as covariates in order to show the independent contribution of each COMT SNP.

We agree that a logistic regression model would be ideal. However, logistic regression tends to be inaccurate when segments contain n<10. Because the 1000 Genomes population is small and did not include age, we went with simpler chi-square models but included gender and race. The results of which we show in Figure 1.

3. When looking at the relationship between ethnicity and FM risk, age group and sex were adjusted. Was the relationship between age and FM and sex and FM also evaluated while adjusting for other factors?

Yes, in Table 2 where we report demographics, the two noted significant associations with FM were from a model that adjusted for all demographics. It is also noted in the methods that the model is tested using all demographic features.

4. The manuscript is well-written overall, but lacks cohesion. All sections, including the methods, results and figures, and discussion should be presented in a logical order that parallels the abstract. Demographic data should be presented first, followed by demographic risk factors, then genetic factors. Suggested order should be:

a. Table 2
b. Table 3
c. Figure 2
d. Table 1
e. Figure 1 (COMT RAF panels- currently labeled as Figure 1)
f. Figure 3 (COMT diplotypes- currently labeled as Figure 1)
Thank you for this suggestion. We have updated the manuscript for cohesion, as well as reordered the tables and figures for clarity.

5. In the Introduction, the authors note that serotonin and catecholamines are key neurotransmitters in pain-inhibiting pathways. This is not entirely accurate and is counter to the premise of the current study. Catecholamines are known to either initiate or inhibit pain based on the cell type and context surrounding their expression. The premise for the current study is based on the idea that COMT SNPs associated with low activity (leading to increased levels catecholamines) are predictive of painful FM. The authors can refer to and cite the Smith et al., Pain, 2014 paper that examined these COMT SNPs and haplotypes for association with COMT activity levels.

Thank you for bringing this inconsistency to our attention. We have revised the paper to provide more accuracy and gratefully cite the Smith et al., Pain, 2014 paper.

6. The authors conclude that, based on their descriptive genetic findings, COMT activity and catecholamines are not likely driving the development of FM pain. There are many factors that influence COMT expression/activity and catecholamine bioavailability, so this is a very big assumption to make. Then it seems contradictory that COMT genetic variants associated with low activity are more prevalent in African-Americans who are at greater risk for FM.

We have revised the manuscript for clarity, as FM is a complex disease, and more studies are certainly needed.

Minor Concerns:

1. In the Abstract, authors state, "Females, younger individuals, and non-Caucasians were at higher risk…” As both the 'low' and 'middle' age groups were predictive of increased risk, it would be helpful to specify the age. The middle age group ranges 49-63 years, so the term "younger individuals" is misleading. Then in the Results section, Table 3 and Figure 2 suggest that the middle age groups are at greater risk. Please present findings and interpretation of findings in a consistent manner.

Thank you for pointing this out. We have revised the manuscript for more clarity.

2. In the Abstract, present the results in a consistently ordered way. Move the 2 African-American and Hispanic race results up a sentence, so as to follow the "Females had 1.72
increased odds…" sentence. Thank you for this suggestion. We have made the change in the abstract.

3. In the Abstract Conclusions, "This is the largest study to date that examines…" should be "This is the largest study, to date, that examines…" Thank you for catching that typo. We have added the missing commas.

4. The sentence in the Introduction, "Although criteria to diagnose FM exist, specifically a number of widespread painful tender points [9], solidifying the diagnosis is tricky since other presenting symptoms and comorbid diseases are likely to exist." does not clearly specify criteria for FM diagnosis. The criteria have changed over the past several years. Are you referring to the widely-used 1990 criteria (requiring presence of pain upon pressure applied to at least 11 of 18 specific points), or the updated 2010 criteria (that no longer use these tender points)?

The fact that the criteria for FM are subject to change, might even provide a compelling argument for an objective genetic biomarker.

Revised this paragraph to reflect more specifics around current diagnostic criteria and potential benefits of development of an objective biomarker.

5. Also, replace "tricky" with a synonym such as "challenging." Thank you for this suggestion. We have made the change.

6. In the Methods, authors note that only participants with genetic data for COMT SNPs were included in the analysis. Is this a subset of the 2,713 FM and 32,141 non-FM groups? Then, in the Statistical Analysis section the authors note the FM group was compared to individuals in the 1000 Genomes Project from US population (n=224). Does the 224 refer to those from the 1000 Genomes? Please clarify the exact number of FM, non-FM, and 1000 Genome subjects used for the analysis. This information is in Figure 2, but needs to be clear in the text.

We have reworded the methods to clarify that all participants had complete genetic data for each of the COMT SNPs. In the text, we do explicitly say that the 1000 Genomes group numbered 224 people: "To reduce bias due to patient recruitment and enrollment in the study, the FM group was compared to individuals in the 1000 Genomes Project [36] from US populations (n=224) to assess genetic associations with the COMT SNPs."
Wei Yang (Reviewer 3): This is a large-scale multi-racial association study of the mutations in the COMT gene with Fibromyalgia. I appreciate the great effort by the authors to carry out such an extensive study. However, I have great concerns about the study design and how racial differences have been handled when statistical analyses were performed.

More specially,

(1) How representative are the control samples?

(i) The control samples seemed recruited from clinical visits. It is possible that they have very different compositions with regard to genetic variations, gender, ethnicity compared to the general population, and may lead to many of the differences observed in the current manuscript.

(ii) The manuscript compared the allele frequencies between FM group and the 1000 genome data. I wonder why the comparisons have not been performed to compare the non-FM group to the 1000 genome data to see how the controls samples compares to the general population.

We agree that differences in race and gender composition can affect frequencies, which is why we broke down the comparisons to subgroups of different race and gender combinations in Table 1. Our non-FM group were not made of healthy individuals. Subjects were recruited from physician offices and were included in the study only if the physician deemed the genetic test was medically necessary to assess risk of FM. Thus these patients were seeing their doctors because of some pain, not necessarily FM. We did not include a comparison of the non-FM group with the 1000 genomes group because the non-FM group being so non-homogenous, any deviation from the 1000 genomes group would be difficult to interpret. We briefly noted our reasoning in the Methods section under Statistical Analysis: Genetic Associations with FM.

(iii) Page 8 line 35, I don't think diagnosis rate could be directly derived if the controls samples were not recruited from general populations. In the same paragraph, "Only those participants with genetics for the COMT single nucleotide polymorphisms… were included in the downstream analysis". What is the criteria that sample have been selected for genotyping? This could affect the distributions of sex, race, and genetical variations, also.

(iv) Page 11 line 24-25: "The FM group was compared of 71.6% females compared with 58.5% females in the non-FM group". Does the general population has female make up as high as 58.5%?

Subjects were recruited from physician offices and were included in the study only if the physician deemed the genetic test was medically necessary to assess risk of FM. Thus these patients were seeing their doctors because of some pain, not necessarily FM. Unfortunately since
this was an observational study, we could not control the gender and racial compositions of our groups. In addition to the COMT SNPs, we were also interested in how prevalent FM was in males vs females and different races. We agree that differences in race and gender composition can affect frequencies, which is why we broke down the comparisons to subgroups of different race and gender combinations in Table 1.

(2) In genetic association studies, the allele frequencies and effect of mutations generally show distinct differences across racial groups. Many statistical tests seem having been performed without consideration of the racial difference.

Figure 1 shows the allele frequencies in respect to FM vs 1000 Genomes (1) overall, (2) by gender, (3) by ethnicity group, and (4) by gender AND ethnicity group.

(2i) What is the frequencies of the haplotypes in each race group? Is there any difference in haplotype distribution across race groups? Is there any difference between FM and non-FM samples within each race group?

In table 2 we note that “% African-Americans is significantly increased as compared to % of Caucasians in the Non-FM group (p=3.11 x10^{-12}).” We also show in more detail in Figure 1 the difference in COMT haplotypes, by association with pain sensitivity, comparing African-Americans and Caucasians in the FM and non-FM groups.

(2ii) Table1 showed allele frequency difference across all race groups. This could be meaningless considering the generally huge difference between race groups, and the male:female compositions are different in FM and 1000 genomes data.

(2iii) Also in Table1, for SNPs that showed different MAFs between sexes, the difference might be driven by the difference in sex compositions.

Unfortunately since this was an observational study, we could not control the gender and racial compositions of our groups. In addition to the COMT SNPs, we were also interested in how prevalent FM was in males vs females and different races. We agree that differences in race and gender composition can affect frequencies, which is why we broke down the comparisons to subgroups of different race and gender combinations in Table 1. It is generally accepted that the 1000 Genomes project represents the allele frequencies of the larger population, which is why we used it as a control group for the FM group. We have added this criticism as a study limitation in the discussion section. We would like to note that when we found a difference in the prevalence of FM between sexes and among races, we were comparing the FM group to the non-
FM group. We reserved the comparison between the FM group and 1000 Genomes to COMT SNP associations only. Our interpretation of that comparison is how allele frequencies in the FM group deviated from the general population (represented by 1000 Genomes).

(3) Page 6 the last paragraph. Is the "sympathetic" nervous system or the "autonomic" nervous system the identified in the pathogenesis of FM? Page 7 Line 1 says that "The autonomic nervous system likely plays a role in regulation of fibromyalgia pain, as functional MRIs in FM subjects with higher levels of sympathetic nervous system activity demonstrated more temporal summation of pain". I am not able to see the logic in this sentence, how "higher levels of sympathetic nervous system" relates to the role of autonomic nervous system?

Clarified that it is abnormal catecholaminergic signaling and excess sympathetic NS activity that is thought to contribute to pathogenesis of FM.

(4) Page 12 line 22-29: "there was an association of COMT diplotypes when stratified by ethnicity: African American were 11.3 times more likely to have … than Caucasians". This is comparing the African American samples to the Caucasian samples, not "stratified" analysis by ethnicity. The latter means analyses performed within each ethnical group.

Thank you for bringing this to our attention. We have revised the manuscript for clarity.