nature research

Peer Review Information

Journal: Nature Genetics
Manuscript Title: Gene Discovery and Biological Insights into Anxiety Disorders from a Large-Scale Multi-Ancestry Genome-Wide Association Study
Corresponding author name(s): Dr Renato Polimanti

Reviewer Comments & Decisions:

Decision Letter, initial version:

21st Mar 2024

Dear Dr Polimanti,

Your Article, "Gene Discovery and Biological Insights into Anxiety Disorders from a Multi-Ancestry Genome-wide Association Study of >1.2 Million Participants" has now been seen by 2 referees. You will see from their comments below that while they find your work of interest, some important points are raised. We are interested in the possibility of publishing your study in Nature Genetics, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

To guide the scope of the revisions, the editors discuss the referee reports in detail within the team with a view to identifying key priorities that should be addressed in revision. In this case, we think both referees have provided constructive reviews aimed at strengthening the analyses and improving the presentation, and we particularly ask that you address their technical comments as thoroughly as possible with appropriate revisions. Please do not hesitate to get in touch if you would like to discuss these issues further.

We therefore invite you to revise your manuscript taking into account all reviewer and editor comments. Please highlight all changes in the manuscript text file. At this stage we will need you to upload a copy of the manuscript in MS Word .docx or similar editable format.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your manuscript:

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referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

*2) If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions, available
here.
Refer also to any guidelines provided in this letter.

*3) Include a revised version of any required Reporting Summary:
https://www.nature.com/documents/nr-reporting-summary.pdf
It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review.
A revised checklist is essential for re-review of the paper.

Please be aware of our guidelines on digital image standards.

Please use the link below to submit your revised manuscript and related files:

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Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We hope to receive your revised manuscript within 3 to 6 months. If you cannot send it within this time, please let us know.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

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We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,
Wei

Wei Li, PhD
Senior Editor
Nature Genetics
New York, NY 10004, USA

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Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
Anxiety is a latecomer to the field of psychiatric genetic association studies and the submission by Frikigkou is a major advance on current knowledge. It establishes, beyond doubt, that there is a molecular genetic basis to anxiety, that the architecture is probably shared (in part) across diverse ancestries, and provides some evidence of underlying mechanisms (although this work is at a relatively early stage).

1. The phenotypic correlation in anxiety (which is not defined early in the main text, a significant omission I think) is as low as 0.46 between 2 very large studies. This is really quite low, and dissimilar to similar estimates from studies of schizophrenia, bipolar disorder and major depression (with which it has much in common). What do the authors consider to be the main reason for this and have they addressed phenotypic heterogeneity, differences in QC/imputation/Neff per SNP, other technical differences and the arrays used in each study as possible explanations?
2. I am not familiar with the phenotypic dilution metric used and cannot make sense of the findings presented as they stand. Does the selection of FinnGen on the basis of SNP-h2 introduce potential bias, when other studies are larger and/or use more robust phenotypic measures? The method (or at least what it means) needs to be better explained in the main text.
3. The common factor leadings on anxiety measures from each study is reassuring, but the measures themselves need to be mentioned. The UKB measure used is no clearer, even after reading the methods section. The SNP-h2 of 5% is very low, especially with so few studies. SNP-h2 often diminishes as studies are added (see Wray paper on this topic from 2023), reflecting (possibly) increased heterogeneity and dilution across studies. Does this concern the authors, and do they think any further analyses may be needed to further understand why that may be?
4. The test of between ancestry PRS transferability was based on quintiles. I have never seen this approach used before for this purpose. Why not use the continuous PRS measure instead? Maybe the authors could explain the justification and/or clarify if the association with continuous measures is significant or not.
5. Do the authors think that the lack of transferability to SAS is somewhat concerning, as the genetic distance to this group is less than for most other comparisons?
6. The TWAS and PWAS analyses are very correlative, given that the authors do not first establish that altered RNA/protein is causally linked to anxiety liability. These studies are of some value, but do the authors think that going further and conducting co-localisation and SMR analyses might be worthwhile?
7. The high correlation with MDD is 0.9. This is exceptionally high. Is there any evidence that the correlations with other traits and inferred biology are in any way anxiety specific? Is there content overlap in the anxiety measures themselves that could be removed?
8. The phenome-wide LCV analysis is not standard are there are some concerns about this measure being less well-established that MR approaches and subject to biases that cannot be fully tested (again, unlike MR which has several variations that can be used to test for robustness to underlying

assumptions).

Reviewer #2:
Remarks to the Author:
Summary of the key results:

Friligkou and colleagues investigated the genetics of anxiety disorders using data from over 1.2 million participants across five ancestral groups. Through ancestry-specific and cross-ancestry GWAS, the authors identified 51 anxiety-associated loci (39 novel). Among the more notable results, the authors found PRS derived from individuals of European descent were associated with anxiety in African, Admixed-American, and East Asian groups. This suggests that genetic predispositions for anxiety may transcend ancestral boundaries. The heritability of anxiety was found to be enriched for genes expressed in several brain regions, highlighting the putative role neurobiological processes in anxiety disorders. Transcriptome- and proteome-wide analyses identified 115 genes associated with anxiety through brain-specific and cross-tissue regulation. The study found global and local genetic correlations with depression, schizophrenia, and bipolar disorder, suggesting shared genetic underpinnings among these psychiatric conditions. Additionally, putative causal relationships with several physical health conditions were identified.

Originality and significance:

This article substantially increased the number of loci associated with anxiety disorders. These additional loci enabled the identification of novel risk genes and discovery of pleiotropic loci with other disorders. However, an increase in genome-wide significant loci is expected by simply increasing sample size. The originality of this article primarily lies in the use of GWAS data from diverse ancestries. Although limited sample size only allowed the confirmation of a previously identified locus in individuals of African ancestry, a cross-ancestry meta-analysis found 10 novel anxiety risk loci.

Data & methodology:

The authors employed a range of commonly used and validated methods, including LDSC, transcriptome/proteome imputation, local analysis of covariance, and genomic structural equation modelling. Meta-analyses and secondary analyses were performed using standard parameters, thresholds, etc. I cannot see any issues with the validity/appropriateness of the methods employed by the authors. All methods are frequently applied in the analysis of complex traits.

Suggested improvements: experiments, data for possible revision:

I would like to see some additional secondary analyses to prioritise gene candidates. This would allow a more detailed examination of targets for follow-up functional studies/drug-target analyses.

• Can the authors perform fine-mapping of loci using established tools like PAINTOR or polyfun? Many of these approaches may only be used in European cohorts, however cross-population methods are available (e.g., XMAP).
• Can the authors employ co-localisation and/or SMR HEIDI approaches to prioritise risk genes from TWAS.

• There are larger brain eQTL datasets available for TWAS (e.g., PsychENCODE, MetaBrain, eQTLGen [blood]). Why did the authors opt for GTEx over these resources?
• There is also a joint-tissue imputation approach for PrediXcan which may improve power (PMID: 33020666).
• Is it possible to perform drug-target analyses? Establishing whether the novel genes are known targets for psychotropic drugs may increase the translational potential of results.

Appropriate credit to previous work:

Authors cite and discuss previous anxiety disorders GWASs, although I would like to see a discussion/comparison with results from Thorp et al. 2021 (PMID:33859377) (large GWAS of anxiety symptoms).

Clarity and context:

No issues with clarity and context.

Overall impressions:

The outstanding feature of this paper is the curation and analysis of genome-wide data on anxiety disorders from multiple sources and diverse ancestries. This provided a large (four-fold) increase in the number of genome-wide significant loci anxiety disorders which informed the discovery of novel risk loci and pleiotropic mechanisms with other traits/disorders. I would like to see a more in-depth secondary analysis of risk loci, including statistical fine-mapping, colocalization, and drug-gene target analyses.

Author Rebuttal to Initial comments

RESPONSE TO REVIEWERS

Reviewer #1:

Anxiety is a latecomer to the field of psychiatric genetic association studies and the submission by Friligkou is a major advance on current knowledge. It establishes, beyond doubt, that there is a molecular genetic basis to anxiety, that the architecture is probably shared (in part) across diverse ancestries, and provides some evidence of underlying mechanisms (although this work is at a relatively early stage).

1. The phenotypic correlation in anxiety (which is not defined early in the main text, a significant omission I think) is as low as 0.46 between 2 very large studies. This is really quite low, and dissimilar to similar estimates from studies of schizophrenia, bipolar disorder and major depression (with which it has much in common). What do the authors consider to be the main reason for this and have they addressed phenotypic heterogeneity, differences in QC/imputation/Neff per SNP, other technical differences and the arrays used in each study as

possible explanations?

RESPONSE: The genetic correlation of anxiety phenotypes reported among the cohorts investigated is in line with that reported by previous anxiety GWAS (PMID: 31906708; 31116379; 31748690; 37945807). To a lesser extent, cross-cohort variability was also reported by a recent GWAS of depression (medrxiv 2024.04.29.24306535). Because the LDSC analysis is limited to HapMap variants (as recommended to avoid differences in imputation quality and Neff per SNP), this heterogeneity cannot be explained by technical differences. In the revised manuscript, we included information regarding the heritability, intercept, and lambda values for all anxiety cohorts (revised Supplemental Table 1). These statistics show no evidence of systematic bias due to technical artifacts or other confounders in these datasets. We clarified this point in the revised manuscript (page 10).

2. I am not familiar with the phenotypic dilution metric used and cannot make sense of the findings presented as they stand. Does the selection of FinnGen on the basis of SNP-h2 introduce potential bias, when other studies are larger and/or use more robust phenotypic measures? The method (or at least what it means) needs to be better explained in the main text.

RESPONSE: PheMED estimates phenotypic dilution φ using one of the GWASs as a reference. Phenotypic dilution is defined by the authors as the phenotype misclassification that would occur if we assigned a case status to a control or vice-versa. The authors of PheMED define a measure of such phenotypic misclassification as $\varphi=\frac{PPV_2+NPV_2-1}{PPV_1+NPV_1-1}$, where PPV denotes the positive predictive value and NPV the negative predictive value of the reference phenotype 1 and the tested phenotype 2. If the phenotypic misclassification (dilution) of the reference GWAS is greater than the one of the tested GWAS, then φ>1. If the phenotypic dilution of the reference GWAS is smaller, then φ<1.

Regarding the reviewer's comment on the choice of a reference phenotype, PheMED developers (PMID: 36711948) recommend using the GWAS that achieves the smallest dilution value as a reference to improve the interpretability of the results. Changing the reference dataset would change the relative phenotypic dilution estimates φ but not the ranking of phenotypes from least to most diluted. Comparing with the least diluted phenotype also aids in the interpretation of the significance threshold. Let us suppose we use a phenotype far from the extremes of the phenotypic dilution. In this case, datasets with similar dilution values would not appear as significantly diluted, while datasets on both extremes of high and low dilution would appear as more or less diluted (with φ>1 and φ<1, respectively). As the reviewer correctly pointed out, the selection was conducted based on the dilution value and FinnGen was used as reference because it exhibited the smallest dilution value. Following Reviewer #1's recommendation, we updated the manuscript to explain this method and our analyses more accurately (pages 3, 16, and 17).

3. The common factor leadings on anxiety measures from each study is reassuring, but the measures themselves need to be mentioned. The UKB measure used is no clearer, even after reading the methods section. The SNP-h2 of 5% is very low, especially with so few studies. SNP-h2 often diminishes as studies are added (see Wray paper on this topic from 2023), reflecting (possibly) increased heterogeneity and dilution across studies. Does this concern the authors, and do they think any further analyses may be needed to further understand why that may be?

RESPONSE: We updated the Methods section (page 16) and Table 1 to more information regarding anxiety definitions. The SNP-h2 heritability of the ANX factor (0.05±0.002) is in line with what was reported for GWAS conducted in large single cohorts (i.e., MVP SNP-h2=0.056±0.004; AoU SNP-h2=0.06±0.009). Accordingly, the fact that it is similar to that observed in single-cohort anxiety GWAS supports that ANX SNP-h2 heritability may be driven more by within-cohort heterogeneity rather than cross-cohort heterogeneity. In this context, iPSYCH cohort showed high SNP-h2 for both anxiety and depression, suggesting that this dataset may be more homogenous in terms of severity and age of onset. We clarified this point in the revised manuscript (page 4).

4. The test of between-ancestry PRS transferability was based on quintiles. I have never seen this approach used before for this purpose. Why not use the continuous PRS measure instead? Maybe the authors could explain the justification and/or clarify if the association with continuous measures is significant or not.

RESPONSE: PRS quintile comparisons allowed us to report odds ratios, which can be easily interpretable. Similar analyses were reported in other GWAS of psychiatric disorders (e.g., PMID 38637617; medrxiv 2024.04.29.24306535). To facilitate the interpretation of our findings to all readers, we added the statistics related to anxiety association considering PRS continuous spectrum in the revised manuscript (page 5) and the revised Supplemental Table 4.

5. Do the authors think that the lack of transferability to SAS is somewhat concerning, as the genetic distance to this group is less than for most other comparisons?

RESPONSE: Unfortunately, SAS population in AoU (Neff=499) has a much smaller effective sample size than other AoU population groups: AFR=17,799; AMR=14,741; EAS=1,259; EUR=61,153. Accordingly, the lack of transferability is due to the low statistical power of the SAS PRS analysis. We clarified this point in the revised manuscript (pages 5 and 14).

6. The TWAS and PWAS analyses are very correlative, given that the authors do not first establish that altered RNA/protein is causally linked to anxiety liability. These studies are of some value, but do the authors think that going further and conducting co-localisation and SMR analyses might be worthwhile?

RESPONSE: Following Reviewer #1's recommendation, we included a colocalization analysis to investigate further TWAS and PWAS significant genes (pages 8, 11, 12, 13, 17, 19, and 20).

7. The high correlation with MDD is 0.9. This is exceptionally high. Is there any evidence that the correlations with other traits and inferred biology are in any way anxiety specific? Is there content overlap in the anxiety measures themselves that could be removed?

RESPONSE: We agree with Reviewer #1 that there is a high genetic correlation between ANX and MDD. This is in line with known comorbidity between them and the presence of shared symptoms. However, our study uncovered genetic mechanisms specific to anxiety. Our MixeR analysis showed that although there is a high genetic overlap between them, there could be 10% anxiety-specific SNPs (Figure 5; Supplemental Table 15). This is in line with the local genetic correlation analysis where among the loci identified, 13% was not shared with MDD (Supplementary Table 16). We clarified this important point in the revised Discussion (page 12).

8. The phenome-wide LCV analysis is not standard are there are some concerns about this measure being less well-established that MR approaches and subject to biases that cannot be fully tested (again, unlike MR which has several variations that can be used to test for robustness to underlying assumptions).

RESPONSE: While LCV approach has been used by many studies (e.g., PMID: 36525587, 36192376, 36151087; 35754104; 35394011; 34907416; 34897459), we agree that using additional methods can permit us to characterize better the relationship between anxiety and other health outcomes. Unfortunately, most MR methods can be biased by sample overlap. Accordingly, we decided to use two MR methods that estimate effects using genetic information while controlling for sample overlap: MRlap (PMID: 37036286) and CAUSE (PMID: 32451458). We revised the manuscript based on the results obtained from these methods (pages 10, 13, 20, and 21).

Reviewer #2:

Friligkou and colleagues investigated the genetics of anxiety disorders using data from over 1.2 million participants across five ancestral groups. Through ancestry-specific and cross-ancestry GWAS, the authors identified 51 anxiety-associated loci (39 novel). Among the more notable

results, the authors found PRS derived from individuals of European descent were associated with anxiety in African, Admixed-American, and East Asian groups. This suggests that genetic predispositions for anxiety may transcend ancestral boundaries. The heritability of anxiety was found to be enriched for genes expressed in several brain regions, highlighting the putative role neurobiological processes in anxiety disorders. Transcriptome- and proteome-wide analyses identified 115 genes associated with anxiety through brain-specific and cross-tissue regulation. The study found global and local genetic correlations with depression, schizophrenia, and bipolar disorder, suggesting shared genetic underpinnings among these psychiatric conditions. Additionally, putative causal relationships with several physical health conditions were identified.

Originality and significance:
This article substantially increased the number of loci associated with anxiety disorders. These additional loci enabled the identification of novel risk genes and discovery of pleiotropic loci with other disorders. However, an increase in genome-wide significant loci is expected by simply increasing sample size. The originality of this article primarily lies in the use of GWAS data from diverse ancestries. Although limited sample size only allowed the confirmation of a previously identified locus in individuals of African ancestry, a cross-ancestry meta-analysis found 10 novel anxiety risk loci.

RESPONSE: We thank Reviewer #2 for appreciating the significance of our study.

Data & methodology:
The authors employed a range of commonly used and validated methods, including LDSC, transcriptome/proteome imputation, local analysis of covariance, and genomic structural equation modelling. Meta-analyses and secondary analyses were performed using standard parameters, thresholds, etc. I cannot see any issues with the validity/appropriateness of the methods employed by the authors. All methods are frequently applied in the analysis of complex traits.
RESPONSE: We are glad that Reviewer #2 did not find any issue with the methodology used.

Suggested improvements: experiments, data for possible revision:
I would like to see some additional secondary analyses to prioritise gene candidates. This would allow a more detailed examination of targets for follow-up functional studies/drug-target analyses.
RESPONSE: We thank Reviewer #2 for their suggestions and revised the manuscript, including novel findings generated from the analyses recommended.

• Can the authors perform fine-mapping of loci using established tools like PAINTOR or polyfun? Many of these approaches may only be used in European cohorts, however cross-population methods are available (e.g., XMAP).

RESPONSE: We performed fine-mapping analyses of the loci identified in the EUR, cross-ancestry, and AFR GWAS meta-analyses. These novel findings are now included in the revised manuscript (page 6, 11, and 18).

• Can the authors employ co-localisation and/or SMR HEIDI approaches to prioritise risk genes from TWAS.

RESPONSE: Following Reviewer #2's suggestion, we conducted a colocalization analysis to follow up on the loci identified from TWAS and PWAS (pages 8, 11, 12, 13, 17, 19, and 20).

• There are larger brain eQTL datasets available for TWAS (e.g., PsychENCODE, MetaBrain, eQTLGen [blood]). Why did the authors opt for GTEx over these resources?

RESPONSE: The eQTL datasets mentioned by Reviewer #2 are derived from a large number of individuals, but a limited number of tissues. We decided to use GTEx v8, because it provides a large number of brain and non-brain tissues (N= 13 and 35, respectively). This permitted us to explore a broader spectrum of tissue-specific regulatory mechanisms. We clarified this point in the revised manuscript (page 19).

• There is also a joint-tissue imputation approach for PrediXcan which may improve power (PMID: 33020666).

RESPONSE: We agree with Reviewer #2 that joint-tissue imputation can further boost tissue-specific transcriptomic associations. Since our brain TWAS identified more than 150 associations, we decided to use a conservative approach to more reliably discuss the possible tissue associations of the associations observed.

• Is it possible to perform drug-target analyses? Establishing whether the novel genes are known targets for psychotropic drugs may increase the translational potential of results.

RESPONSE: In the revised manuscript, we investigated the loci identified with respect to their translation potential considering information available from the Drug Gene Interaction Database (page 7, 12, and 19).

Appropriate credit to previous work:
Authors cite and discuss previous anxiety disorders GWASs, although I would like to see a discussion/comparison with results from Thorp et al. 2021 (PMID:33859377) (large GWAS of anxiety symptoms).

RESPONSE: We thank Reviewer #2 for highlighting this previous study. In the revised manuscript, we compared and discussed our results with respect to these previous findings (page 12, Supplemental Table 22).

Clarity and context:
No issues with clarity and context.
RESPONSE: We are glad that the text was clear.

Overall impressions:
The outstanding feature of this paper is the curation and analysis of genome-wide data on anxiety disorders from multiple sources and diverse ancestries. This provided a large (four-fold) increase in the number of genome-wide significant loci anxiety disorders which informed the discovery of novel risk loci and pleiotropic mechanisms with other traits/disorders. I would like to see a more in-depth secondary analysis of risk loci, including statistical fine-mapping, colocalization, and drug-gene target analyses.

RESPONSE: We thank Reviewer #2 for appreciating our study. Following their suggestions, we performed additional analyses and included these novel results in the revised manuscript.

Decision Letter, first revision:

17th Jul 2024

Dear Dr. Polimanti,

Thank you for submitting your revised manuscript "Gene Discovery and Biological Insights into Anxiety Disorders from a Multi-Ancestry Genome-wide Association Study of >1.2 Million Participants" (NG-A64712R). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Genetics, pending minor revisions to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements soon. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Genetics Please do not hesitate to contact me if you have any questions.

Sincerely,
Wei

Wei Li, PhD
Senior Editor
Nature Genetics
www.nature.com/ng

Reviewer #1 (Remarks to the Author):

I am satisfied with the responses to reviewers' comments

Reviewer #2 (Remarks to the Author):

The authors addressed my comments and concerns. No further comments.

Final Decision Letter:

13th Aug 2024

Dear Dr. Polimanti,

I am delighted to say that your manuscript "Gene discovery and biological insights into anxiety disorders from a large-scale multi-ancestry genome-wide association study" has been accepted for publication in an upcoming issue of Nature Genetics.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Genetics style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

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Sincerely,
Wei

Wei Li, PhD
Senior Editor
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