nature portfolio

Peer Review File

Cross-Ancestry Comparison of Aptamer and Antibody Protein Measures

Corresponding Author: Dr Laura Raffield

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript by Nicholas et al. presents a systematic comparison of the relative abundances of ~2,000 proteins measured by Olink and SomaScan technologies in plasma samples from ~1,900 participants of the Multi-Ethnic Study of Atherosclerosis (MESA). This work examines cross-platform similarities and differences in total protein abundance, as well as in pQTL effects. The authors find that protein-altering variants (PAVs), i.e. coding variants that may affect protein structure and/or post-translational modifications (PTMs), are responsible for 25-30% of pQTL discrepancies observed across proteomic platforms and may partially explain the variability in platform agreement across ancestral populations. The authors suggest that PAVs should be considered in future analyses of proteomic data.

Overall, the manuscript describes an important and timely study that supports and extends prior observations on the variability of protein measurements and pQTL effects across proteomic platforms. A key strength of this study is the use of two proteomic technologies (Olink and SomaScan) on the same plasma samples, enabling a direct comparison between the platforms.

Below, I provide a series of comments intended to increase the clarity and impact of the presented findings.

1. The authors should provide clear and concise definitions for key concepts and data sources that are used extensively throughout the manuscript. While most of these are defined in the Methods section, they should be introduced more prominently and formally upon first usage. Specifically, please introduce:
- Protein-altering variant (PAV)
- Credible set
- Sentinel variant
- Data source for the mass-spectrometry-based pQTL data

2. The following sentence in the abstract requires clarification: “one-third of the pQTL signals were driven by protein-altering variants (PAVs)”. It is unclear which figure or table reports the evidence for this sentence. If the authors refer to Fig. 2B, the sentence should be revised to state that “25-30% of platform-specific pQTL signals were driven by protein-altering variants”.

3. Extended Data Figure 1 (the distribution of correlations between Olink and SomaScan protein measurements) should be elevated to a main figure as it is central to the study’s main motivation. I would suggest replacing the current Fig. 1 with this figure. In addition, 2-3 specific examples of proteins representing the extremes of the distribution (highly positive, highly negative and near-zero correlations) should be included.

4. Similarly, Supplementary Figure 5 should be elevated to a main figure and the 19 proteins with platform-discordant pQTLs should be clearly labeled on the scatter plot.

5. I would discourage the authors from using 3D pie charts (Fig. 2B, D). A table would convey the relative proportion of platform concordant and discordant pQTLs much more effectively.

6. The observation that 80 proteins “exhibited significant differences in assay agreement across ancestries” (i.e., their Olink- and SomaScan-derived measurements were correlated in some ancestries but not others) is very interesting. The authors

should provide a list of these 80 proteins, ideally in a table or figure within the main text.

7. To provide further insight into the nature of this ancestry-related phenomenon, the authors should more explicitly report the following:
- How many of these 80 proteins have cis- or trans-pQTLs with different minor allele frequency (MAF) across ancestries?
- In how many cases are differences in platform agreement explained by differences in pQTL MAF versus differences in pQTL effect size/direction?

8. Are there examples of proteins with multiple independent cis-pQTLs, such that some are platform-concordant, while others are platform-discordant or platform-specific?

9. Are proteins with platform-specific/concordant/discordant cis-pQTLs more/less likely to exhibit platform-specific/concordant/discordant trans-pQTLs?

10. There appears to be a discrepancy between Figures 2A and 2C that requires clarification. Fig. 2A suggests that the number of proteins with platform-specific pQTLs is relatively similar across platforms (1,036 proteins for Olink and 840 proteins for SomaScan). However, in Fig. 2C, the cumulative area representing Olink-specific proteins (blue bars) is noticeably larger than the area of SomaScan-specific proteins (red bars). Please clarify if these figures are intended to show different information.

11. The discussion regarding trans-pQTL pleiotropy (page 11) requires further clarification. “We identified 10 pleiotropic trans-pQTL regions that were associated with at least 5 proteins on both platforms”. Does this imply that a given trans-pQTL is associated with the same set of 5 proteins on both platforms? Or could it be one set of 5 on Olink and another set of 5 on SomaScan? Depending on the definition, the interpretation of pleiotropy would be different.

12. The Excel file containing the main and supplementary tables is excessively large and difficult to manage. Please consider reducing the number of data columns to the most essential ones. In addition, the number of significant digits per value is unrealistically large and can be reduced dramatically.

13. Page 13: the reference to Extended Data Figure 7 should be changed to Extended Data Figure 6.

14. This work, while rich in statistical observations, would greatly benefit from more substantial biological interpretation. I would strongly encourage the authors to propose specific hypotheses that could explain the observed cross-platform discrepancies in protein measurements and pQTL effects. Specifically:
- What factors may contribute to the wide range of correlations among protein measurements (Extended Fig. 1)? In particular, while near-zero correlations can be explained by various sources of technical/experimental noise, what mechanisms could explain strong negative correlations across platforms?
- Similarly, what mechanisms could explain pQTLs with significant, yet directionally opposite, effects on protein measurements? While assay interference may explain the presence or absence of a pQTL signal, explaining strong, oppositely signed pQTL signals is more challenging. Would the same hypothesis apply for cis- and trans-pQTLs?
- Can the authors hypothesize why the majority of platform-specific and platform-discordant pQTLs are not PAVs (Fig. 2B)?
- Given that 16% of the 80 proteins with significant cross-platform correlation differences between ancestries contain ancestry-differentiated PAVs, what hypotheses can explain the remaining 84%?

15. Finally, it is important to recognize that platform dependency is not necessarily just a technical artifact. Platform-discordant pQTLs could reflect important biological phenomena, such as PTMs, differences in protein structure and/or association with its functional neighbors (e.g., chaperons, transporters, binding partners). As the authors likely agree, the platform dependency of pQTL effects could provide incredibly valuable insights not only into the reliability of proteomic technologies but also into the state and connectivity of the proteome itself. I would encourage the authors to emphasize this point in their manuscript.

Reviewer #2

(Remarks to the Author)
Reviewer comments

The study by Nicholas et al. compared two state-of-the-art plasma proteomic platforms with >2k proteins commonly measured in about >1.8k ancestral diverse participants of the MESA study. The study is well presented but mainly confirms previous observations for factors, including host genetic ones, affecting platform concordance. The observation that PAVs account for ancestral difference in cross-platform concordance is interesting but also somewhat incremental, since we already know that PAVs can affect assay concordance and that allele frequencies for some PAVs differ between ancestries. The authors do also not provide a comparison of how their correlation estimates compare to previous publications to judge to validity of the presented results or provide enough detail on how pQTL discovery was done.

One notable aim of the study it the attempt to provide tangible solutions to overcome technical shortcomings when using such proteomic technologies, ie., by adjusting for potentially epitope artefacts induced by PAVs. However, the results presented might be misleading, since 1) for a given proteomic platform, it is not immediately clear whether a PAV is causing altered abundance or infers with the affinity reagent (or both for that matter), and 2) the biological interpretation massively

changes, ie., the value actually lies in understanding differences not trying to smooth those (see comments below).

1) The relevance for PAVs has been well acknowledged, but it is somewhat unclear whether they really represent an issue and at which scale, since one would naively assume that PAVs are one of the most common mechanisms by which the secretion/stability of proteins, in particular those readily detectable in plasma, might be altered.
2) The adjustment for PAVs may improve correlation across platforms, but may also fall short of biological interpretation, in a sense that both platforms target different isoforms of the same gene product. For example, it had been already outlined that for PILRA the missing concordance of both platforms aligns well with the differential role of the isoforms/cleavage products in the pathology of Alzheimer's disease (Pietzner et al. 2021 Nat Comms). This raises the question whether adjusting for PAVs is a desirable outcome. The improvement in the PILRA example presented here is also mainly by bringing the average value within each genotype to a similar level rather than truly improving correspondence within genotypes. In other words, the PAV-adjusted value is even harder to understand than the original diverging examples.
3) The rationale to why assay performance should be dependent on genetic ancestry is somewhat artificial, and it is upfront clear, that missense variants prevalent in one compared to another ancestry may exert such effects. It is interesting to demonstrate that for some protein targets but the overemphasize on such difference is more harm- then helpful for the field.
4) What can the authors say about the overall performance of both techniques to identify credible (cis-)pQTLs? For example, more than twice as much cis-pQTLs are identified for Olink compared to SomaScan, which may raise concerns in the performance of the updated SomaScan platform. This more important message is somewhat lost in the disproportional focus on ancestral differences.
5) Also, PAVs are only one among other potential genetically induced reasons for platform discordance, including splicing QTLs or effects in trans that mediate PTMs.
6) I am not sure whether I follow the logic presented line 226 onwards. PAVs do not necessarily, maybe even mostly, have an effect on transcript abundance that is why we look at protein and not gene expression.
7) Line 284 onwards: The presented analysis does not provide any evidence that 'artificial' pQTLs confound epidemiological associations but rather may mask them. Also, the interpretation of the resulting models becomes very different, which is not acknowledged by the authors. Look for example at insights of platform discordance for UMOD (https://pubmed.ncbi.nlm.nih.gov/35446786/) and APOL1 (https://www.biorxiv.org/content/10.1101/2025.01.30.635763v2). Such insights are much more valuable than trimming cross-platform association for generic characteristics to align.
8) The section on how pQTL mapping was done lacks a lot of detail. Multiple ancestries are referred to, but not mentioned how those have been treated during pQTL discovery and fine-mapping.

Minor:

Line 109 onwards: I do not understand, why trans-pQTLs should be affected by epitope effects. Also, epitope effects are not confounders in an epidemiological sense, worse case they mask effects, ie., lead to false-negative signals. The authors do also not provide evidence that cis-MR studies are systematically affected by PAVs.

Line 198 onwards: Those statements are somewhat circular. That is, why would one expect differential cis-pQTLs if both platforms actually measure the same thing. It would be more interesting to establish thresholds to what extent cross-platform correlations might still be considered 'good enough' to enable integration of platforms to optimize pQTL discovery.

Line 215 onwards: What is the difference between an eQTL and genetic variants affecting transcript abundance?

Line 238 onwards: I am struggeling to understand the message here. Are MS-PAVs those pQTL that change the protein signature but not the abundance of peptides? If so, how do the authors ensure that MS-based measurements are valid, ie., MS-based methods usually have high coefficients of variations and mapping peptides to proteins is a challenge.

Line 636 onwards: I might be wrong but isn't adjusting for the lead cis-pQTL the very same as identifying independent credible sets, ie., the correspondence of identifying secondary signals with, e.g., GCTA-cojo compared to SuSiE?

Figure 2: Please do not use 3D-pie charts, the latter are itself already misleading in presenting proportions but putting them into this shape makes it even worse.

Supplementary Table 3: Many credible sets have exactly identical association statistics for 5 or more distinct variants. Can the authors explain why this is?

Reviewer #3

(Remarks to the Author)
Nicholas et al. present a cross-platform evaluation of plasma protein measurements obtained using two proteomics technologies: SomaScan 7k and Olink Explore 3072. Utilizing a diverse population from the MESA cohort and whole-genome sequencing data, the authors systematically dissect platform concordance, identify genetic determinants. The findings are timely and relevant, with practical implications for biomarker discovery, causal inference using pQTLs, and clinical proteomics.

Major Comments
1. The manuscript emphasizes cross-ancestry evaluation, which is commendable. Given the growing importance of multi-ancestry studies in proteogenomics and drug development, this reviewer recommend the authors to further highlight the necessity and translational relevance of cross-population analyses in the result section.

2. While the authors acknowledge the challenge in distinguishing affinity binding artifacts from true abundance differences, further classification of discordant signals (e.g., those with consistent eQTL direction but opposite pQTL effect) could strengthen the biological interpretation. Consider discussing whether specific protein classes (e.g., immune vs metabolic) are enriched for platform-discordant PAVs.

3. The manuscript briefly mentions the risk of biased causal inference. However, the implications are profound. Consider adding a short paragraph or summary table discussing how the use of affected pQTLs as instruments may impact current Mendelian randomization studies. It would also be valuable to explore how such biases might be amplified in ancestry-diverse datasets.

4. Beyond protein-altering variants (PAVs), it would be helpful to report whether the enriched variants (e.g., lead cis-pQTLs) disproportionately localize to exonic versus intronic regions. This would provide greater clarity regarding the genomic architecture of the detected signals.

Minor Comments
1. Could protein expression patterns, particularly the tissue or organ of origin, affect cross-platform measurement similarity? A brief discussion or exploratory analysis could be informative.

2. The authors adjusted for 10 protein PCs in the pQTL analysis. It would be helpful to briefly clarify the rationale for this choice (e.g., was this empirically determined or standard practice?), and whether results are robust to vary this number.

3. Please consider providing more technical details about the pQTL analysis, such as the software version used, statistical models (linear vs. mixed), and how related individuals were handled or filtered in the association testing.

4. The analysis of pleiotropic trans-pQTLs is rigorous, but the interpretation remains cautious. If possible, comment on whether some platform-specific pleiotropic loci correspond to known biological hubs (e.g., APOE, ABO).

5. Data/code availability: The authors mention supplemental tables but do not provide links to raw pQTL summary statistics. Public release would be a substantial asset to the field.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors' response and revised manuscript adequately address my concerns. The authors made a significant effort to provide thoughtful and detailed answers to my questions. I have no further comments.

Reviewer #2

(Remarks to the Author)
Reviewer comments

I agree with the authors, that differentially frequent PAVs can contribute to poor transferability of proteomic findings across ancestries, and this is well executed in the manuscript. My general concern remains the overall relevance and novelty of the findings. Unlike a genetic association, this is an entirely analytical question as faced daily in clinical chemistry, if new assays or lots of chemicals emerge/are supplied. Both proteomic platforms have staggering overlap, and it is well documented for the comparison at hand, but also other platforms, that they do not align well. This can be demonstrated in as few as fifty samples (see here https://www.nature.com/articles/s42004-025-01665-1), since one would expect not less than identical values for each and every sample, as the same protein is supposedly measured. Increasing sample size, as the authors do, may have the added benefit to explore potential factors accounting for poor correspondence. The authors identify such for ~1% of the targeted proteins, even less with clear PAV evidence, which makes me wonder about the overall relevance compared to other issues those technologies face. I like to note, that while adjusting for PAVs seems to be an appealing idea, the achieved improvements are still way off properly correlating assays. One might suggest, that simply omitting the 80 something proteins from cross-ancestry analysis would be the most robust solution until companies develop better assays.

Also, the additional findings appear rather incremental, and the work would have benefitted from demonstrating a wider relevance of the findings, such as for powerful proteomics prediction models. That is models that show considerable improvement over and above clinical practice.

- The claim of novel biology following PAV-adjustment is posthoc, and only possible because multiple platforms were available. In settings in which only one platform is available, it would not be clear whether adjusting for PAVs is a good or bad thing to do. Consider the example of PILRA, for which inference based on both technologies differs considerably, and all the adjustment does, is raising the average level, but not improving the correlation within each genotype group. A more appropriate analysis would be to stratify the analysis by genotype or demonstrate an interaction effect to clearly demonstrate that the PAV is a measurement artefact.
- I appreciate the thoughtful response regarding PAV adjustment concerns, though I have reservations about some conclusions. MR, for example, aims to make inference on the relevant biological entity suspected to be causal. Beyond other known challenges with plasma protein MR, inability to instrument the truly causal disease mechanism limits the utility of the approach in particular since evidence at the locus may already imply altered isoform expression or similar. Additionally, the value of the 'unmasked' associations warrants clarification—conditional analysis already accounts for the leading cis-pQTL and subsequently selected variants, and it remains unclear whether these associations arise from residual LD or other forms of collider bias.
- I would appreciate clarification on the difference between adjusting for PAVs first (which should represent the lead signal at the locus) and running SuSiE afterwards. SuSiE uses linear regression models to compile credible sets that are conceptually similar to classic conditional analysis (e.g., GCTA-cojo). It would strengthen the manuscript to demonstrate that accounting for PAVs in this manner yields the expected primary and secondary signals observed with the unaffected assay, as this would provide evidence that the new signals are biologically meaningful.
- The newly added MR analysis strengthens the study's impact, though additional analyses would enhance the robustness of the findings. Specifically, correction for multiple testing and heterogeneity estimates for proteins with >1 cis-pQTL would help distinguish statistical noise from genuine signal. Rather than focusing on a single trait where the discordant pQTL tags the mechanism of action for AD, a demonstration across multiple traits would more comprehensively establish the utility of accounting for PAVs. Methods designed to address this scenario have been proposed (https://www.cell.com/cell-genomics/fulltext/S2666-979X(24)00329-X).
-
Minor

Line 255 onwards: There seems to be something missing.

Reviewer #3

(Remarks to the Author)
The authors have well addressed all my concerns/comments.

Throughout this document, responses to reviewers are written in blue.
Text that was added to the manuscript was also pasted into this document in red.

Reviewer #1 (Remarks to the Author):

The manuscript by Nicholas et al. presents a systematic comparison of the relative abundances of ~2,000 proteins measured by Olink and SomaScan technologies in plasma samples from ~1,900 participants of the Multi-Ethnic Study of Atherosclerosis (MESA). This work examines cross-platform similarities and differences in total protein abundance, as well as in pQTL effects. The authors find that protein-altering variants (PAVs), i.e. coding variants that may affect protein structure and/or post-translational modifications (PTMs), are responsible for 25-30% of pQTL discrepancies observed across proteomic platforms and may partially explain the variability in platform agreement across ancestral populations. The authors suggest that PAVs should be considered in future analyses of proteomic data.

Overall, the manuscript describes an important and timely study that supports and extends prior observations on the variability of protein measurements and pQTL effects across proteomic platforms. A key strength of this study is the use of two proteomic technologies (Olink and SomaScan) on the same plasma samples, enabling a direct comparison between the platforms.

Below, I provide a series of comments intended to increase the clarity and impact of the presented findings.

1. The authors should provide clear and concise definitions for key concepts and data sources that are used extensively throughout the manuscript. While most of these are defined in the Methods section, they should be introduced more prominently and formally upon first usage. Specifically, please introduce:
- Protein-altering variant (PAV)
- Credible set
- Sentinel variant
- Data source for the mass-spectrometry-based pQTL data

We thank the reviewer for this suggestion. We replaced “sentinel variant” with “lead variant” throughout to increase clarity. We now introduce each term formally in the introduction and/or results.

We now introduce protein-altering variants in the introduction as (red font indicates new text):

Genetic variants may alter circulating protein abundances through many biological mechanisms, including protein altering variants (PAVs) such as missense variants, or alterations to transcriptional or translational efficiencies and post-translational modifications (PTMs) that impact downstream protein stabilities.

We introduce the mass-spectrometry based pQTL studies in the introduction as:
Recent MS-based pQTL studies (Suhre et al. 2024) (Suhre et al. 2024) have further suggested that some pQTL may reflect isoform differences (although these studies are limited in number and encompass fewer proteins/participants).

And again, with more detail, in the results:
While mass-spectrometry (MS)-based pQTL studies are relatively limited in size and number (with some of the broadest studies, used for pQTL comparison here, currently including 1,260 Americans and 1,980 proteins (Suhre et al. 2024), or 325 participants and ~2,800 proteins (Suhre et al. 2024)), comparing affinity-based pQTL to pQTL identified in MS studies may facilitate signal interpretation.

We now introduce the concept of credible sets in the results:
SuSiE (Wang et al. 2020) fine-mapping of pQTL was used to define *cis*-pQTL credible sets – which are sets of variants predicted to contain the causal variant and are used here to define genetic signals. Here, we observed significant credible sets (containing at least one variant $p<5x10^{-8}$) for 840 proteins on SomaScan (**Table ST4**) and 1,036 proteins on Olink (see Methods, **Table 4, ST5**, **Figure 3A, Methods**).

2. The following sentence in the abstract requires clarification: “one-third of the pQTL signals were driven by protein-altering variants (PAVs)”. It is unclear which figure or table reports the evidence for this sentence. If the authors refer to Fig. 2B, the sentence should be revised to state that “25-30% of platform-specific pQTL signals were driven by protein-altering variants”.

We apologize for the prior lack of clarity.

The abstract is revised to state:
“Protein measures from each platform were associated with genetic variants (pQTLs), and approximately 25-30% of the pQTL signals on each platform were likely driven by protein-altering variants (PAVs).”

3. Extended Data Figure 1 (the distribution of correlations between Olink and SomaScan protein measurements) should be elevated to a main figure as it is central to the study’s main motivation. I would suggest replacing the current Fig. 1 with this figure. In addition, 2-3 specific examples of proteins representing the extremes of the distribution (highly positive, highly negative and near-zero correlations) should be included.

We elevated Extended Data Figure 1 to main text figure 2, and now provide examples of proteins representing extremes of the distribution.

Proteins with highly discordant inter-platform correlations included Growth arrest and DNA damage-inducible proteins-interacting protein 1 (G45IP, Pearson’s r=-0.38) and Caspase-8 (Pearson’s r=-0.30), while proteins with highly concordant inter-platform correlation included Chitosidrase-1 (Pearson’s r=0.88) and insulin-like growth factor-binding protein 1 (Pearson’s

r=0.90). Several proteins showed near-zero correlations, including interleukin-15 (Pearson's $r=7.2x10^{-6}$) and angiotensin converting enzyme 2 (Pearson's $r=-5.9x10^{-4}$). Correlation coefficients for each protein generally agreed with those observed in prior, single population studies (FIGURE S3).

4. Similarly, Supplementary Figure 5 should be elevated to a main figure and the 19 proteins with platform-discordant pQTLs should be clearly labeled on the scatter plot.

While we kept this plot in the supplement (now Supplementary Figure 9 A and B) due to spacing issues (we could not clearly label all platform-discordant PAV proteins cleanly in the plot), we added a main text table 3 to explicitly list these proteins and information about them for the reader.

5. I would discourage the authors from using 3D pie charts (Fig. 2B, D). A table would convey the relative proportion of platform concordant and discordant pQTLs much more effectively.

We apologize for the lack of clarity and revised Figure 2 (now Figure 3) to replace the 3D pie charts with tables (see Figure 3B and D).

6. The observation that 80 proteins "exhibited significant differences in assay agreement across ancestries" (i.e., their Olink- and SomaScan-derived measurements were correlated in some ancestries but not others) is very interesting. The authors should provide a list of these 80 proteins, ideally in a table or figure within the main text.

We thank the reviewer for recognizing the importance of this finding and apologize for not highlighting these 80 proteins more prominently before. Given the size of this table, we highlight the 20 proteins with the highest degree of inter-platform correlation heterogeneity between the four ancestries (Bonferroni-significant Cochran's Q p-value ($p<0.05/2,708$), largest range in inter-platform correlation estimates across ancestries) in the main text table 2. We display all 80 proteins with highly significant cross-ancestry heterogeneity in Supplemental Table 3.

7. To provide further insight into the nature of this ancestry-related phenomenon, the authors should more explicitly report the following:
- How many of these 80 proteins have cis- or trans-pQTLs with different minor allele frequency (MAF) across ancestries?

While few proteins (180 proteins) have any ancestry-differentiated *trans*-pQTL association at this participant sample size, we observe a higher proportion of proteins with ancestry-heterogenity in platform agreement associate with an ancestry-differentiated *trans*-pQTL (20% of proteins with ancestry-heterogeneous correlations compared to 8.8% of all proteins). A much larger proportion of ancestry heterogeneous proteins (56%, versus 26% overall) are associated with an ancestry-differentiated *cis*-pQTL, consistent with the notion that *cis*-pQTL often have larger effects than *trans*-pQTL on overall protein abundance.

Revisions Table 1. Percentage of all proteins vs. ancestry-heterogeneous proteins with ancestry-differentiated *cis*- and/or *trans*-pQTL

	Total n proteins	With any *cis*-pQTL	With ancestry differentiated *cis*-pQTL	With any *trans*-pQTL	With ancestry differentiated *trans*-pQTL	With any *cis*- + *trans*-pQTL	With ancestry differentiated *cis*- + *trans*-pQTL
All proteins	2,157 (100%)	1239 (57%)	563 (26%)	605 (28%)	180 (8.8%)	348 (16%)	58 (2.7%)
Proteins with ancestry-heterogenous inter-platform correlation estimates *(Cochran's Q for inter-platform correlation heterogeneity p<0.05/2,708 probe pairs)*	80 (100%)	69 (86%)	45 (56%)	19 (36%)	16 (20%)	24 (30%)	10 (13%)

We expanded main text table 1 to show these numbers. We also added text to the results (lines 302-307) to report these numbers:
While proteins with ancestry-differentiated correlation may have multiple contributors to inter-platform variation, including ancestry-differentiated *cis*-pQTL (which may be non-coding) or *trans*-pQTL (which 56% and 20% of proteins with ancestry-heterogeneous correlation were associated with, respectively (Table 1), confounding of ancestry with environmental and lifestyle factors, etc, we focus on the potential effects of ancestry-differentiated PAVs on protein measures here.

- In how many cases are differences in platform agreement explained by differences in pQTL MAF versus differences in pQTL effect size/direction?

While we share the reviewer's interest in understanding the extent to which heterogeneity in platform-agreement by ancestry is impacted by allele frequency versus variant effect size, within and across genetic ancestries, it is not feasible to evaluate this question without considering local ancestry. We cannot naively interpret effect size heterogeneity across ancestries, as LD patterns, and thus causal variant tagging, may vary by local ancestry.

Future studies may choose to evaluate this question by evaluating pQTL variant effect size heterogeneity in participants with the same local ancestry background, determining gene by gene the local ancestry in the region and evaluating whether there is the same effect size for participants from differing global ancestry or race or ethnicity population groups with the same local ancestry assignment, as has been done with gene expression (PMID: 35716666), perhaps suggesting a gene environment interaction, or if within a population cluster differing effect sizes exist exist by local ancestry, as has been observed for APOE4/Alzheimer's Disease (PMID: 36071110, PMID: 31606368), suggesting a gene-gene interaction may exist. However, without

such analysis, we may see spurious effect size heterogeneity due to differences in power (for example,due to allele frequency or imperfect tagging of the causal variant PMID: 36941441), and this can be a lot of work to disentangle. We now note in the discussion that consideration of differences in pQTL effect sizes by local ancestry background may be important to consider in future work.

8. Are there examples of proteins with multiple independent cis-pQTLs, such that some are platform-concordant, while others are platform-discordant or platform-specific?

In main analyses (considering only one probe per UniProt ID), we do observe several proteins associated with multiple signals of varying platform concordance on each platform. The table below displays counts. As shown, it is more common for a protein to associate with a platform-concordant and platform-specific *cis*-pQTL than to associate with a platform-concordant and platform-discordant *cis*-pQTL. We speculate that an association with both a platform-concordant and platform-specific *cis*-pQTL may reflect common ability of assays to detect genetic loci with large effects on protein abundance or shape, and different sensitivities of assays to genetic loci which have smaller effects on protein abundance or may interact with other environmental factors (and that differences in assay variability or noise may also contribute to some platform specific findings). This is supported by the fact that, for the majority of proteins (87% on SomaScan, and 80% on Olink, respectively) associated with both a platform-concordant and platform-specific *cis*-pQTL, the platform-specific *cis*-pQTL signal is not the primary signal.

Revisions Table 2. Number of Proteins Associated with Multiple *cis*-pQTL Signals, Per Platform/Signal Annotation

	N Proteins SomaScan*	**N Proteins Olink***
Multiple Platform-Concordant *cis*-pQTL (>1 signal per protein, all associated signals platform-concordant)	125	163
Platform-Concordant + Platform-Specific *cis*-pQTL	210	167
Platform-Concordant + Platform-Discordant *cis*-pQTL	6	7
Platform-Discordant + Platform-Specific *cis*-pQTL	3	6
Platform-Concordant + Platform-Discordant + Platform-Specific *cis*-pQTL	2	3

*Counts correspond to analyses including one probe per-platform per UniProt ID

9. Are proteins with platform-specific/concordant/discordant cis-pQTLs more/less likely to exhibit platform-specific/concordant/discordant trans-pQTLs?

Overall, we note similar proportions of proteins with platform-concordant/discordant /specific *cis*-pQTL, or no *cis*-pQTL at all have *trans*-pQTL (as shown in column 2 of the table below). However, there are very few (1) proteins with platform-discordant *trans*-pQTL, and relatively few proteins (96) with platform-concordant *trans*-pQTL.
The majority of *trans*-pQTL are platform-specific, and are for proteins with no *cis*-pQTL on either platform, or for proteins with a platform-specific *cis*-pQTL.

Revisions Table 3. Proteins according to platform-specificity of *cis*- and *trans*-pQTL

	N Proteins	**N Proteins with *trans*-pQTL in Group (%)**	**N Proteins with platform-concordant *trans*-pQTL (%)**	**N Proteins with platform-discordant *trans*-pQTL (%)**	**N Proteins with platform-specific *trans*-pQTL (%)**
All proteins	2,157	524 (24%)	96 (4.5%)	1 (0%)	427 (20%)
Proteins with platform-concordant *cis*-pQTL	514	106 (21%)	54 (11%)	0 (0%)	52 (10.1%)
Proteins with platform-discordant *cis*-pQTL	11	2 (18%)	1 (9%)	0 (0%)	1 (9%)
Proteins with platform-specific *cis*-pQTL	587	152 (26%)	10 (2%)	0 (0%)	142 (24%)
Proteins with no *cis*-pQTL	1045	264 (25%)	31 (3%)	1 (0.1%)	232 (22%)

10. There appears to be a discrepancy between Figures 2A and 2C that requires clarification. Fig. 2A suggests that the number of proteins with platform-specific pQTLs is relatively similar across platforms (1,036 proteins for Olink and 840 proteins for SomaScan). However, in Fig. 2C, the cumulative area representing Olink-specific proteins (blue bars) is noticeably larger than the area of SomaScan-specific proteins (red bars). Please clarify if these figures are intended to show different information.

Figure 2A (now 3A) displays a cumulative UpSet plot which illustrates the total number of proteins in each category (a protein may be counted in more than one bar if it falls in more than one category). Figure 2C (now 3C) displays the histogram of SomaScan vs. Olink platform correlations, with bars now colored according to whether the proteins contributing have a *cis*-pQTL on SomaScan-only, Olink-only, or both platforms. In total, 1,036 proteins associate with a *cis*-pQTL on Olink and 840 associate with a *cis*-pQTL on SomaScan, as shown by the left-most 2 bars. The 3rd bar from the left shows that 644 of these proteins are associated with a *cis*-pQTL on both platforms (no bar in this plot shows the counts of proteins with a platform-specific *cis*-pQTL). In total, there were more *cis*-pQTL unique to the Olink platform, and these were more common for proteins with lower inter-platform correlation (as shown in Figure 3C). Hence, Figure 2A (now 3A) supports the conclusion from Figure 2C (now 3C) - more

proteins have a significant *cis*-pQTL on Olink overall, and a greater number of platform specific *cis*-pQTL are observed for Olink measured proteins.

11. The discussion regarding trans-pQTL pleiotropy (page 11) requires further clarification. “We identified 10 pleiotropic trans-pQTL regions that were associated with at least 5 proteins on both platforms”. Does this imply that a given trans-pQTL is associated with the same set of 5 proteins on both platforms? Or could it be one set of 5 on Olink and another set of 5 on SomaScan? Depending on the definition, the interpretation of pleiotropy would be different.

We thank the reviewer for this question and apologize for the lack of clarity in this statement. In the current definition of pleiotropy, we do not require the signal to associate with the same set of 5 proteins on both platforms; these signals could be one set of 5 on Olink and another set of 5 on SomaScan.

We added text to clarify this point at line 414-416 in the results:
We identified 10 pleiotropic *trans*-pQTL regions that were associated with at least 5 proteins on each platform (although not necessarily the same proteins on both platforms),

12. The Excel file containing the main and supplementary tables is excessively large and difficult to manage. Please consider reducing the number of data columns to the most essential ones. In addition, the number of significant digits per value is unrealistically large and can be reduced dramatically.

We apologize for the difficulty navigating the prior file of tables. We greatly reduced the number of data columns in tables (particularly those displaying *cis*- and *trans*-pQTL credible sets), and reduced redundancy between tables. For several tables, we also added legends and/or descriptions of the information in each column. We also added hyperlinks to each table on a sheet at the beginning of the tables document, to make it easier to navigate between tables and to quickly see an overview of what information is present in each table.

13. Page 13: the reference to Extended Data Figure 7 should be changed to Extended Data Figure 6.

We thank the reviewer for finding this error and apologize for the confusion. The figure reference is now updated.

14. This work, while rich in statistical observations, would greatly benefit from more substantial biological interpretation. I would strongly encourage the authors to propose specific hypotheses that could explain the observed cross-platform discrepancies in protein measurements and pQTL effects. Specifically:
- What factors may contribute to the wide range of correlations among protein measurements (Extended Fig. 1)? In particular, while near-zero correlations can be explained by various sources of technical/experimental noise, what mechanisms could explain strong negative correlations across platforms?

An existing 2021 SomaScan and Olink platform-comparison study from Pietzner et al performed a feature selection approach (PMID: 34819519) to explore biological and technical factors contributing to variation in protein measure correlation across platforms. They report a variety of factors associating with protein-measure correlation, with percentage of outliers, percentage of measures below the limit of detection (SomaScan) or percentage of missing measures (Olink), probe binding affinity for a given target, and presence of a transmembrane domain in the protein as the top five variables explaining variation in protein correlation.

While we observe 581 out of the total 2,708 probe pairs with negative inter-platform correlations. While most of these negative correlations are weak, the strongest negative correlation we observe in this study is Pearson's r=-0.42 for GRB-2 related adapter protein 2. Strong negative correlations observed are interesting as they suggest that platforms are consistently measuring differing, yet biologically related values for the same proteins. We suggest that in many cases, these values may reflect probes consistently measuring different isoforms of the same protein.

Recent papers have evaluated factors influencing protein measures on each platform, with Carrasco-Zanini et al exploring SomaScan 5k protein measures in 8,350 Fenland study participants (PMID: 39327534, 2024), and Pietzner et al exploring Olink Explore 3072 measures among 43,240 UK Biobank participants (*medRixv* 2025, doi: doi.org/10.1101/2025.01.09.25320257). To briefly explore potential drivers of negative correlation coefficients, we looked at proteins with negative inter-platform correlations reported in Eldjarn et al., 2023 and low correlations in MESA (Pearson's r<0.2) for which at least 15% of protein variance could be explained on each platform. For the four proteins meeting this criteria (neurexophilin-3 (MESA Pearson's r =0.01), EF-hand domain-containing protein D1 (MESA Pearson's r = 0.04), cysteine-rich motor neuron 1 protein (MESA Pearson's r = 0.11), and microfibrillar-associated protein 5 (MESA Pearson's r = -0.01),) a *cis*- or *trans*- genetic pQTL score was the top contributor to protein variance on at least one platform, while triglycerides, cystatin C, or smoking status were the top contributor on the other platform.

We added text at lines 608-614 in the discussion:
We observe a wide range of inter-platform correlations for given protein measures, as in prior studies. One prior study demonstrated that biological (such as presence of a trans-membrane domain, protein length) and technical factors (percentage of outlier or below limit of detection protein measures, etc), can explain this wide range of correlation coefficients (Pietzner et al. 2021). While relatively uncommon, negatively correlated protein measures are interesting, as these suggest that both platforms measure consistently different, but biologically related values for the same proteins, such as different isoforms.

- Similarly, what mechanisms could explain pQTLs with significant, yet directionally opposite, effects on protein measurements? While assay interference may explain the presence or absence of a pQTL signal, explaining strong, oppositely signed pQTL signals is more challenging. Would the same hypothesis apply for cis- and trans-pQTLs?

In cases where a platform-discordant *cis*-pQTL (i.e. a pQTL with significant, yet directionally opposite effect on protein measurements from different platforms) is detected, we hypothesize that one of the *cis*-pQTL effect sizes likely tags overall protein abundance, while the other effect size likely tags a different isoform (or, alternatively, both assays tag different isoforms, and neither measures overall abundance). For these proteins, effect direction support from expression QTL (eQTL) and mass-spectrometry based pQTL (MS-pQTL) data may help disentangle which effect tags abundance versus specific isoforms. However, the challenge is that eQTL and MS-pQTL studies may not always detect an effect at a true causal locus for both biological and technical reasons (transcript degradation, lack of power in the tissue of protein origin, etc), as we found in our study. Hence, we propose that while affinity pQTL effect direction support from eQTL and MS-pQTL is helpful, the absence of an eQTL or MS-pQTL at an affinity-pQTL locus does not mean that the locus is not a true QTL. Further MS-pQTL and eQTL studies with larger sample sizes will be useful for interpretation of affinity-based pQTL, especially in cases of platform-discordant signals.

We added text at lines 234-238 to clarify these points in the results:
While the majority of *cis*-pQTL variants were non-coding on both platforms (Figure S7), we observe several *cis*-pQTL signals driven by protein altering variants (PAVs – most commonly missense variants) on each platform, raising concerns as to whether the signal captures abundances or differences in isoform, as has been shown in other studies (Pietzner et al. 2025; Wang et al. 2025; Suhre et al. 2024). As protein altering variants (PAVs) can impact affinity-probe binding, *cis*-pQTL associations driven by PAVs may reflect assay interference due to different affinities for encoded isoforms, particularly when the association is detected on only one platform or detected on both with different effect-directions between platforms.

We added text at lines 636-644 to expand these points in the discussion.
cis-pQTL driven by coding genetic variation, such as protein altering variants (PAVs) may, in some cases, reflect impacts to the target protein structure that affect protein measurements, particularly for *cis*-pQTL with platform-discordant directions of effect or platform-specific significance. While PAVs may certainly alter a protein's overall abundance through impacts on protein stability and other mechanisms, studies incorporating comparisons to mass-spectrometry (MS) protein measures, measures of gene transcript abundance, and/or functional validation have established cases where platform-discordant PAV *cis*-pQTL reflect differences in probe binding to genetically encoded isoforms, including for uromodulin (Li et al. 2022) and apolipoprotein-1 (Wang et al. 2025).

While the same may be true for a *trans*-pQTL, where one effect size tags abundance, and the other effect captures the impact of a change to protein structure, the underlying causal variant likely acts through a less direct mechanism than an encoded change to the target protein structure. Instead, the variant may alter post-translational modifications, where, for example, the efficiency of perhaps a glycosylase is altered, resulting in less glycosyl groups on downstream protein targets, which may then change the affinity of probes for the downstream protein target. This type of mechanism could also influence platform-specific *trans*-pQTLs, for example the

FUT6/FUT3 locus identified as pleiotropic on Olink only. While the impact of post-translational modifications on platform measurements may differ by platform, at this sample size, we see very few platform-discordant *trans*-pQTL; this is likely a function of power. Additional studies with expanded sample size will be needed to further explore this effect.

- Can the authors hypothesize why the majority of platform-specific and platform-discordant pQTLs are not PAVs (Fig. 2B)?

While only ~30% of platform-specific pQTLs are PAVs, the majority of platform-discordant pQTLs *are* in fact PAVs. We apologize that the figure did not clearly indicate this before, and have revised the figure to a table (now in Figure 3) to in attempt to better convey this information. We hypothesize that the platform-discordant *cis*-pQTL containing PAVs reflect differential binding of each platform's probes to the encoded isoforms. This is likely true for some of the platform-specific *cis*-pQTL containing PAVs as well. However, there are other reasons we may see platform-specific *cis*-pQTL, other than the different affinity reagent binding due to PAV. For example, perhaps one platform is underpowered to detect a true *cis*-pQTL due to a noisier measure or lower probe affinity for the target protein, as discussed in the response to question 8 above. For *trans*-pQTL, platform-specificity of genetic signals may have a variety of interpretations, including off-target binding or post-translational modifications.

- Given that 16% of the 80 proteins with significant cross-platform correlation differences between ancestries contain ancestry-differentiated PAVs, what hypotheses can explain the remaining 84%?

While only 16% of these 80 proteins associate with an ancestry differentiated PAV *cis*-pQTL, we note that in total, 56% of these proteins associate with an ancestry-differentiated *cis*-pQTL (mostly non-coding). Hence, some ancestry differences may be driven by platform differences due to different contributions of non-coding *cis*-pQTL to protein variance for the isoforms with highest affinity for aptamers/antibodies; similarly, as in question 7 above, ancestry differentiated *trans*-pQTL may contribute. Furthermore, ancestry-heterogeneity in inter-platform correlations may be driven by residual correlation of ancestry with various other environmental factors (for example, region, diet, etc), that researchers may not want to adjust away in association studies with phenotypes.

We added text in the discussion (lines 617-625) to highlight these points:
Although this study focuses on the contribution of coding genetic variation to cross-ancestry differences, additional factors such as ancestry-differentiated non-coding *cis*- genetic variation or *trans*-pQTL, differential recruitment of participants of varied ancestry by site, or other environmental or clinical factors, may also contribute to ancestry-heterogeneity in correlation estimates.

And at lines 741-749:
However, the improvement in inter-platform correlation across ancestry groups was not linear, and several proteins with ancestry-heterogeneity were also associated with

ancestry-differentiated non-coding *cis*-pQTL or *trans*-pQTL, further suggesting the interplay of environmental or other genetic effects apart from PAVs. Researchers may not always want to adjust for non-PAV factors that explain ancestry differences in these settings. Nonetheless, careful consideration of the potential for assay-interference differentially impacting protein measurements across ancestry groups will be imperative to maximize utility of proteomics data in future research and clinical settings.

15. Finally, it is important to recognize that platform dependency is not necessarily just a technical artifact. Platform-discordant pQTLs could reflect important biological phenomena, such as PTMs, differences in protein structure and/or association with its functional neighbors (e.g., chaperons, transporters, binding partners). As the authors likely agree, the platform dependency of pQTL effects could provide incredibly valuable insights not only into the reliability of proteomic technologies but also into the state and connectivity of the proteome itself. I would encourage the authors to emphasize this point in their manuscript.

We thank the reviewer for this comment, and agree with the statement. We added text at lines 636-644 and lines 666-670 to expand these points in the discussion.
cis-pQTL driven by coding genetic variation, such as protein altering variants (PAVs) may, in some cases, reflect impacts to the target protein structure that affect protein measurements, particularly for *cis*-pQTL with platform-discordant directions of effect or platform-specific significance. While PAVs may certainly alter a protein's overall abundance, studies incorporating comparisons to mass-spectrometry (MS) protein measures and/or functional validation have established cases where platform-discordant PAV *cis*-pQTL reflect differences in probe binding to genetically encoded isoforms, including for uromodulin (Li et al. 2022) and apolipoprotein-1 (Wang et al. 2025).

…
When *cis*-pQTL do reflect differential binding to encoded isoforms, we note that studying the impact of individual isoforms can be biologically informative in studies of disease, as established with GDF8/11 for cardiovascular events (Walker et al. 2025). However, it may not be the case that all protein isoforms to have biologically distinct impacts, and careful interpretation and analysis on a protein-by-protein fashion is needed.

Reviewer #2 (Remarks to the Author):

Reviewer comments

The study by Nicholas et al. compared two state-of-the-art plasma proteomic platforms with >2k proteins commonly measured in about >1.8k ancestral diverse participants of the MESA study. The study is well presented but mainly confirms previous observations for factors, including host genetic ones, affecting platform concordance. The observation that PAVs account for ancestral

difference in cross-platform concordance is interesting but also somewhat incremental, since we already know that PAVs can affect assay concordance and that allele frequencies for some PAVs differ between ancestries. The authors do also not provide a comparison of how their correlation estimates compare to previous publications to judge to validity of the presented results or provide enough detail on how pQTL discovery was done.

We thank the reviewer for the positive comments regarding the study presentation. While we acknowledge that we are, by no means, the first group to publish on the potential for genetic variants to interfere with affinity-based proteomics measures, we provide new insight into the agreement of SomaScan 7k and Olink Explore 3072, which are relatively recent versions of these platforms, in a larger number of participants with measures on both platforms than prior studies, which allowed us to find new insights. Additionally, we emphasize that the ability to explore ancestry-differentiated PAVs and their contribution to protein measurement noise between ancestries was noted as a major (and timely) novelty and strength of this study by the other reviewers. While the concept may be relatively intuitive, there is no existing study (to our knowledge) that has investigated the issue at a large scale across high throughput proteomics platforms. An understanding of differences in platform-concordance across ancestries, and potentially ancestry-differentiated drivers in assay-discordance, is important to maximize the utility of data from both of these platforms.

Prior inter-platform correlation estimates for each protein are present in Supplemental Table 2, 29, are compared to inter-platform correlation estimates calculated in this study in Supplemental Figure 3. We agree with the reviewer such information is important to present to the reader.

We apologize for the prior lack of detail in pQTL mapping methods and expanded this section (see lines 935-968).

One notable aim of the study it the attempt to provide tangible solutions to overcome technical shortcomings when using such proteomic technologies, ie., by adjusting for potentially epitope artefacts induced by PAVs. However, the results presented might be misleading, since 1) for a given proteomic platform, it is not immediately clear whether a PAV is causing altered abundance or infers with the affinity reagent (or both for that matter), and 2) the biological interpretation massively changes, ie., the value actually lies in understanding differences not trying to smooth those (see comments below).

1) The relevance for PAVs has been well acknowledged, but it is somewhat unclear whether they really represent an issue and at which scale, since one would naively assume that PAVs are one of the most common mechanisms by which the secretion/stability of proteins, in particular those readily detectable in plasma, might be altered.

We agree with the reviewer that PAVs are a highly relevant consideration both because they may alter protein abundance in circulation, and because prior studies have established PAVs

may alter protein shape (and thus, in some cases, probe binding affinity for a protein target). We do not propose that all *cis*-pQTLs driven by PAVs interfere with assay performance; rather, there is likely a subset of PAV-associated proteins for which this is true. In the absence of extensive comparisons to mass-spectrometry and lack of *in vitro* follow-up, it is challenging to determine the extent to which PAV-mediated assay interference is an issue. However, as studies continue to use data from these proteomics platforms, and platforms continue to evolve, it may be important to characterize and consider accounting for PAVs in analyses, and determine whether doing so unveils any new biology, as we show in the manuscript, for a subset of PAV-impacted protein measures. Additionally, we further emphasize that multiple potentially-impacted proteins are potential drug targets or biomarkers.

While we briefly mentioned these points in the discussion, we now clarify and highlight these points further with additional text in the introduction (lines 101-104):

Genetic variants may alter circulating protein abundances through many biological mechanisms, including protein altering variants (PAVs) such as missense variants which may influence protein stability, or alterations to transcriptional or translational efficiencies and post-translational modifications (PTMs) that could also impact downstream protein stabilities.

And discussion (lines 634-642):
However, *cis*-pQTL driven by coding genetic variation, such as protein altering variants (PAVs) may, in some cases, reflect impacts to the target protein structure that affect protein measurements, particularly for *cis*-pQTL with platform-discordant directions of effect or platform-specific significance. While PAVs may certainly alter a protein's overall abundance, studies incorporating comparisons to mass-spectrometry (MS) protein measures and/or functional validation have established cases where platform-discordant PAV *cis*-pQTL reflect differences in probe binding to genetically encoded isoforms, including for uromodulin (Li et al. 2022) and apolipoprotein-1 (Wang et al. 2025).

And lines 644-647:
It remains challenging to establish the pervasiveness of assay-interference effects; however, we note that several putatively impacted proteins are potential biomarkers or drug targets (for example, suPAR (Reisinger et al. 2021; Jehn et al. 2021) and PILRA (Zhan et al. 2025)).

2) The adjustment for PAVs may improve correlation across platforms, but may also fall short of biological interpretation, in a sense that both platforms target different isoforms of the same gene product. For example, it had been already outlined that for PILRA the missing concordance of both platforms aligns well with the differential role of the isoforms/cleavage products in the pathology of Alzheimer's disease (Pietzner et al. 2021 Nat Comms). This raises the question whether adjusting for PAVs is a desirable outcome. The improvement in the PILRA example presented here is also mainly by bringing the average value within each genotype to a similar level rather than truly improving correspondence within genotypes. In other words, the PAV-adjusted value is even harder to understand than the original diverging examples.

We thank the reviewer for this comment, and acknowledge that whether a researcher decides to adjust for a PAV should depend on the context and goals of an analysis. The reviewer raises a valid point, that probes claiming to target the same protein across platforms may, in fact, measure different isoforms, and in some cases, it may be helpful to keep protein measures separate and not meta-analyze values across platforms or adjust for PAVs, to allow better resolution of these isoform differences.

However, the challenge is that platforms often do not report which isoform of a protein a given probe will measure. In the 2021 Pietzner et al. study, developing a hypothesis to explain platform discrepancies for the PILRA probes required characterizing genetic signals for each platform's measure and comparing these to previously reported GWAS signals for several outcomes. Other studies that demonstrate the value of platform-discordant PAV to reveal isoform-specific biology have since emerged (PMID: 35446786; Wang et al., *bioRxiv,* 2025, www.doi.org/10.1101/2025.01.30.635763), we added these as citations in our study. However, these processes are not feasible in all studies, especially as less may be known about protein isoforms and protein-disease relationships for other, less characterized protein targets. For many proteins, platforms do not report a specific isoform that is measured. Even in cases where platforms do report a specific isoform is measured, these often lack extensive validation and off-target binding is possible. Hence, due to the lack of clarity about what each platform is measuring right now, fully exploring platform differences may not be feasible or informative for many proteins, without additional work to clarify which isoform is being measured. Future studies incorporating mass-spectrometry comparisons will help further establish variants that drive assay interference versus abundance effects (Sissala et al., *Research Square* 2025, www.doi.org/10.21203/rs.3.rs-6501601/v1; Kirsher et al., *bioRxiv* 2025, www.doi.org/10.1101/2025.02.14.638375).

Beyond this, the overall protein abundance may in fact be the value of interest in many studies. For example, Mendelian Randomization (MR) studies are often interested in relating overall protein abundances to an outcome. When interested in protein abundances and improving detection of factors associated with overall abundance, we may be better powered to find these associations when we do adjust for PAVs in protein measures where affinity-reagent based measurement is impacted by them. This is corroborated by the fact that when we do adjust protein measures for platform-discordant PAVs in analyses, we often find new associations. For example, when adjusting PILRA measures for platform-discordant PAVs, we unmask a plausible non-coding *cis*-pQTL signal that was not detected in models that did not account for the PAV, suggesting such increased power. Similar unmasking was observed for non-coding cis pQTLs for other proteins like CPPED1 and ACP1 on SomaScan.

In summary, we agree with the reviewer that careful consideration is needed on whether information may be lost regarding differential isoform abundance by adjusting for discordant PAV, counterbalanced by the potential to have better power to detect correlates of overall protein abundance with such adjustment. We have added to the discussion text on lines 667-681 and cited some newer papers on interpretations of isoform-specific biology from proteomics study to the discussion.

When *cis*-pQTL do reflect differential binding to encoded isoforms, we note that studying the impact of individual isoforms can be biologically informative in studies of disease; as established with GDF8/11 for cardiovascular events (Walker et al. 2025). However, it may not be the case that all protein isoforms to have biologically distinct impacts. Also, platforms often do not report which isoform a given probe targets (or do not provide evidence of specific targeting) and disentangling which isoform each platform measures requires additional analyses incorporating genetic or MS evidence, which may not be feasible in all studies. If the measure of interest is overall protein abundance, rather than the abundance of specific protein isoforms, PAV-adjustment may provide an avenue of accounting for potential isoform differences in affinity-reagent binding. Careful consideration is needed on whether information may be lost regarding differential isoform abundance by adjusting for discordant PAV, counterbalanced by the potential to have better power to detect correlates of overall protein abundance with such adjustment.

3) The rationale to why assay performance should be dependent on genetic ancestry is somewhat artificial, and it is upfront clear, that missense variants prevalent in one compared to another ancestry may exert such effects. It is interesting to demonstrate that for some protein targets but the overemphasize on such difference is more harm- then helpful for the field.

We thank the reviewer for raising this concern and assure that we have no intention of reducing interest in proteomics. Researchers would certainly be less interested in proteomics data if platforms were shown to have no or very limited ancestry-transferability, which is not the case. In our analyses, we show that there do not seem to be widespread systematic differences in platform agreement by ancestry. *Most* proteins do not show dramatic differences in platform correlation by ancestry, or discordant ancestry-differentiated PAVs. However, for a subset of proteins, there may be genetic interference that differs in frequency by ancestry, and thereby interferes with protein measures from different differentially across ancestries. Where these PAVs *do* exist, knowing about them and accounting for them early in research will improve the utility of measures and prevent problems with bias in protein measurement and differential/inaccurate findings by ancestry downstream. This concept has already been seen in the field of genetics with polygenic risk scores (PRS), which are clearly impacted by ancestry-differentiated variants and may differ in transferability by ancestry if GWAS effect-estimates or the variants included in scores are biased in their representations of ancestries. While the impact may not be as extreme, genetic interference by ancestry for proteins may reduce ancestry-transferability of measures and genetic/epidemiological associations for a subset of potentially influential proteins, and may bias protein genetic risk scores. Hence, we emphasize that evaluating genetic interference with protein measures across ancestry is a highly relevant task, and will only improve the utility of proteomics data, both across platforms and diverse ancestries.

We added text at lines 191-193 to clarify for readers that the majority of proteins do not show differences in platform agreement by ancestry.

Results:
While the majority of individual proteins showed similar cross-platform agreement across ancestries, 80 protein targets exhibited significant differences in assay agreement across ancestries (Cochran's Q $P<1.85*10^{-5}$, Bonferroni adjustment for the total number of probe pairs (2,708)).

Discussion (lines 613-614):
Importantly, we show that, while most proteins did not show systematic differences in platform agreement by ancestry, a subset of 80 proteins had significantly heterogeneous correlation across ancestries, suggesting that one or both platforms may vary in performance across ancestries.

4) What can the authors say about the overall performance of both techniques to identify credible (cis-)pQTLs? For example, more than twice as much cis-pQTLs are identified for Olink compared to SomaScan, which may raise concerns in the performance of the updated SomaScan platform. This more important message is somewhat lost in the disproportional focus on ancestral differences.

We agree this could be better discussed, and added additional text to highlight this point in the

Results (lines 214-229):
More proteins showed a *cis*-pQTL on Olink compared to SomaScan (48% vs. 39%, respectively); additionally, we observed nearly twice as many credible sets on Olink compared to SomaScan (although we note this is only among the 2,157 proteins measured on both platforms).

And discussion (lines 626-630):
We observed similar proportions of protein measures associated with any *cis*-pQTL on each platform (39% SomaScan, 48% Olink), with more distinct *cis*-pQTL identified for Olink overall (for the set of 2,157 proteins measured on both platforms), consistent with single-ancestry genetic comparisons of earlier versions of SomaScan and Olink platforms (Eldjarn et al., 2023; Katz et al., 2022; Pietzner, Wheeler, Carrasco-Zanini, Kerrison, et al., 2021).

5) Also, PAVs are only one among other potential genetically induced reasons for platform discordance, including splicing QTLs or effects in trans that mediate PTMs.

We agree with the reviewer that splicing QTLs or *trans*-pQTL based mediation of PTMs may impact the concordance of protein measures across platforms. While we performed a preliminary investigation of *trans*-pQTL concordance and pleiotropy across platforms to look at PTMs, effects like splicing are even harder to fully assay with current affinity-based methods. This necessitates additional studies utilizing mass-spectrometry based proteomics to help incorporate these lesser-understood effects into our understanding. Additionally, splice QTL will provide a valuable target for future work.

We expand on these points in the discussion (lines 660-663):

Integration of larger MS, expression, and splicing datasets will further facilitate identification of assay-interference pQTL.and help differentiate, in situations of discordant pQTL effects, what a given platform's measure captures, as affinity reagents may have differential binding to different protein isoforms (Pietzner et al. 2025)(Wang et al. 2025; Suhre et al. 2024).

And limitations (lines 849-852):
Platform comparisons incorporating MS protein measures are already proving informative to further depict isoform differences and impacts of splicing on detected protein structures (Pietzner et al. 2025)(Kirsher et al. 2025; Sissala et al. 2025), and will be especially important to expand across ancestrally-diverse sets of participants.

6) I am not sure whether I follow the logic presented line 226 onwards. PAVs do not necessarily, maybe even mostly, have an effect on transcript abundance that is why we look at protein and not gene expression.

We agree that PAVs may not necessarily affect transcript abundance. In addition, many true pQTL effects may not necessarily show up in steady-state eQTL studies, and many eQTL studies are also underpowered, especially in tissues other than blood. Hence, when you *do* see pQTL/eQTL effects in the same direction, it can be a helpful indication to suggest that a pQTL captures overall protein abundance due to the variant's similar genetic impacts on transcript abundance. However, when you do *not* see a shared pQTL/eQTL signal, it is challenging to make conclusions, as there may be an undetected eQTL at the same locus (or, the variant may influence protein stability (and therefore circulating abundance) but not transcript abundance, as the reviewer suggests).

We added text to the results to clarify this point for the reader:
While we consider the presence of a directionally concordant eQTL within a pQTL signal as helpful indication to suggest that a pQTL captures differences in protein abundance, we note that the *absence* of a shared eQTL is challenging to interpret, as there may be an undetected eQTL due to power, context specificity of eQTL effects, etc, or the variant may alter protein abundance through a mechanism other than expression.

7) Line 284 onwards: The presented analysis does not provide any evidence that 'artificial' pQTLs confound epidemiological associations but rather may mask them. Also, the interpretation of the resulting models becomes very different, which is not acknowledged by the authors. Look for example at insights of platform discordance for UMOD (https://pubmed.ncbi.nlm.nih.gov/35446786/) and APOL1 (https://www.biorxiv.org/content/10.1101/2025.01.30.635763v2). Such insights are much more valuable than trimming cross-platform association for generic characteristics to align.

We thank the reviewer for sharing these citations and now cite them in the discussion of this paper to show that delving into the impacts of platform-discordant PAVs can suggest meaningful

biology and isoform differences. However, as discussed above, when the value of interest is the overall protein abundance, and the relationship between a protein's overall abundance and an outcome, accounting for PAVs in models may indeed provide the best power to detect these relationships.

However, we agree that "mask," rather than "confound", is the correct term to describe how platform-discordant PAVs may impact genetic and epidemiological associations. We have removed the word "confound" from the paper in this context, and replaced it with correct descriptors throughout. We also have tried to edit our paper to better emphasize that PAV adjustment should be considered carefully, given trade-offs between potentially improving understanding of different isoforms vs improved power for understanding relationships (genetic or epidemiological) with overall protein abundance, as discussed above in response to Reviewer 2 major comment #2.

Discussion:
While PAVs may certainly alter a protein's overall abundance through impacts on protein stability and other mechanisms, studies incorporating comparisons to mass-spectrometry (MS) protein measures, measures of gene transcript abundance, and/or functional validation have established cases where platform-discordant PAV *cis*-pQTL reflect differences in probe binding to genetically encoded isoforms, including for uromodulin (Li et al. 2022) and apolipoprotein-1 (Wang et al. 2025).

…

When *cis*-pQTL do reflect differential binding to encoded isoforms, we note that studying the impact of individual isoforms can be biologically informative in studies of disease; as established with GDF8/11 for cardiovascular events (Walker et al. 2025). However, it may not be the case that all protein isoforms to have biologically distinct impacts. Also, platforms often do not report which isoform a given probe targets (or do not provide evidence of specific targeting) and disentangling which isoform each platform measures requires additional analyses incorporating genetic or MS evidence, which may not be feasible in all studies. If the measure of interest is overall protein abundance, rather than the abundance of specific protein isoforms, PAV-adjustment may provide an avenue of accounting for potential isoform differences in studies with data from one platform only.

8) The section on how pQTL mapping was done lacks a lot of detail. Multiple ancestries are referred to, but not mentioned how those have been treated during pQTL discovery and fine-mapping.

We apologize for the lack of detail in our pQTL mapping methods previously, and have added several additional details to the pQTL methods section. We clarify that our pQTL mapping and fine-mapping was performed ancestry pooled, as has been done in TOPMed metabolite QTL and expression QTL studies. (PMID: 39211135, PMID: 40034763; PMID: 40162269). We perform pQTL mapping ancestry pooled as assigning an individual to, for example, an African or

Admixed American ancestry group can be artificial and reduce power, while making fine-mapping much more complex, given the continuum of genetic ancestry observed in diverse cohorts such as PAGE, All of Us, and TOPMed; hence, stratifying by ancestry may reduce overall study power, given that many variants *are* shared across ancestry groups, as recently demonstrated for other quantitative traits (PMID: 40162269).

Minor:

Line 109 onwards: I do not understand, why trans-pQTLs should be affected by epitope effects. Also, epitope effects are not confounders in an epidemiological sense, worse case they mask effects, ie., lead to false-negative signals. The authors do also not provide evidence that cis-MR studies are systematically affected by PAVs.

As above, we acknowledge that "confound" was the wrong word for us to use in this sense - rather "mask effects" is a much better way to describe the potential impact of both platform-discordant PAVs and pleiotropic *trans*-pQTLs on probe binding to protein targets, and we thank the reviewer for this suggestion.

To clarify how a *trans*-pQTL detection may be due to assay interference, here we hypothesize that, if a genetic variant impacts deposition of post-translational modifications that impact affinity probe binding for one or several proteins, a *trans*-pQTL signal may arise at the locus of this variant. However, this signal may not be tagging different protein abundances, but rather differences in affinity probe binding to forms of protein with different post-translational modifications (for example glycosylation). This has also been discussed in the 2023 Eldjarn et al platform comparison in deCODE/UK Biobank participants for loci such as *FUT6* and *ABO*. Additional studies incorporating mass-spectrometry and functional follow-up for individual proteins and their PTMs *in*-vitro will provide additional clarity on the extent to which this is true for individual proteins.

We added text to the discussion at lines 755-776 to further emphasize these points:
The same trend has been observed in the UK BioBank / deCODE SomaScan and Olink platform-comparison, where significant platform-specific pleiotropic *trans*-pQTL were seen at some of the same regions, such as complement factor gene *CFH* and glycoprotein-encoding *VTN for* SomaScan protein measures, and proteins associated with these loci often showed lower inter-platform correlation compared to other proteins (Eldjarn et al. 2023). Eldjarn et al propose such loci may capture an interaction between SomaScan technical processes and the protein pathways influenced by the *trans*-pQTL. In a similar vein, we observe platform-shared and/or platform-specific pleiotropic *trans*-pQTL regions at loci encoding proteins involved in coagulation processes (*F12*, *F5, CPB2*), blood type (*ABO*), fat-transport proteins *(APOE*), other glycoproteins (*HPX, DAG1*), and proteins with roles in glycosylation (*FUT6, FUT2, HRG, GNPTAB*), rather than for transcription factors (as might be expected if a locus captures a true "master-regulator" effect -- for example, *trans*-eQTL hotspots are often located in transcription factors (Yao et al. 2017)(Orchard et al. 2025)). Such loci may result in widespread changes to, for instance, protein glycosylation, which could induce epitope-effects for several proteins based on differential binding to proteins with different PTMs. Similarly, the pleiotropic associations with

coagulation factors may impact protein isoforms or indicate susceptibility of one or both platforms to sample preparation variability. Further work, particularly with MS, will be needed to better understand the biological and/or technical effects these pleiotropic *trans*-pQTL capture.

We have now added an analysis to describe the impact of platform-discordant PAVs on MR study results, and demonstrate that inclusion of platform-discordant PAVs as MR instruments can result in platform-discordant or platform-specific significant MR results. We expand more on the results of these findings in the results and discussion section of this text (see response to Reviewer 3's main comment #3).

Line 198 onwards: Those statements are somewhat circular. That is, why would one expect differential cis-pQTLs if both platforms actually measure the same thing. It would be more interesting to establish thresholds to what extent cross-platform correlations might still be considered 'good enough' to enable integration of platforms to optimize pQTL discovery.

We share the reviewer's interest in understanding what proteins may have high potential for cross-platform meta-analysis. To this end, we added an analysis binning proteins measured on both platform by correlation coefficient and evaluating how many pQTL for proteins in each category were platform-shared (overlapping genetic signal, concordant direction of effect), or platform-specific (only detected on one platform or the other). We show that proteins with inter-platform Pearson's r>0.6 are most likely to have platform-shared *cis*-pQTL signals (includinga substantial proportion of PAV signals). We suggest that these proteins may have highest potential for cross-platform meta-analysis and added text to the results at lines 288-294 and Supplemental Figure 10 to help better emphasize our point in the discussion.

Results:
Although we note that some platform-specific *cis*-pQTL were observed even for proteins with highly-correlated measures (Figure 3C, Figure S10) (which may be due to factors such as differences in intra-assay variability, or differing sensitivities for assays to environmental factors interacting with genetic variation to regulate protein abundance), overall, we propose that *cis*-pQTL presence/absence may be a strong predictor of inter-platform concordance, and that proteins with high inter-platform correlation, for example Pearson's r>0.6, may hold high potential for meta-analysis of pQTL across SomaScan and Olink platforms.

Our hope is that this text places the following, pre-existing text in the discussion, into better context now:
"Proteins associated with concordant *cis*- genetic signals exhibited the highest average inter-platform correlation, increasing confidence that the affinity probes for these proteins captured the same entities and may have high potential for cross-platform meta-analysis."

Line 215 onwards: What is the difference between an eQTL and genetic variants affecting transcript abundance?

We apologize for the confusion; these terms are essentially synonymous, with an eQTL specifically referring to a statistically significant association between a genetic variant and a transcript abundance detected by a prior study.

We added the following text in the results (lines 314-317) to clarify this definition and our rationale:
Incorporating orthogonal lines of genetic evidence, such as expression quantitative trait loci (eQTL), which are statistically significant genetic variant associations with transcript abundance, may increase confidence that pQTL associations capture true alterations in protein abundance. When a pQTL variant is also associated with a gene's transcript abundance, that may support the variant has a true impact on protein abundance.

As well as the methods (lines 999-1001):
To assess whether pQTL may exhibit shared effects with GTEx expression quantitative trait loci (eQTL), which are statistically significant associations between a genetic variant and a transcript abundance…

Line 238 onwards: I am struggling to understand the message here. Are MS-PAVs those pQTL that change the protein signature but not the abundance of peptides? If so, how do the authors ensure that MS-based measurements are valid, ie., MS-based methods usually have high coefficients of variations and mapping peptides to proteins is a challenge.

Here, "MS-PAVs" are variants reported by Suhre et al in "Nanoparticle enrichment mass-spectrometry proteomics identifies protein-altering variants for precise pQTL mapping" (*Nature Communications*, 2024) to associate with one of a protein's constituent peptide levels, but not necessarily the protein's overall abundance, as measured by mass-spectrometry. We acknowledge that this terminology could be confusing. Variants identified by Suhre at al as associating with constituent peptides but not overall protein abundance could induce artifactual pQTL. Here, we compare affinity-based *cis*-pQTL signals identified in MESA to these likely non-abundance related PAVs reported in Suhre et al to see which may have additional lines of evidence to suggest they do not capture overall differences in protein abundances. Notably, for 2 out of 19 proteins (APOL1 and PGLYRP2) with a platform-discordant *cis*-pQTL, the affinity-based signal is also an MS-PAV signal reported in Suhre et al.; unfortunately most of our platform-discordant *cis*-pQTL were not investigated in this paper.

We agree that MS-based protein measurements come with their own set of challenges in interpretation and well-powered pQTL mapping, such as coefficients of variation and peptide-to-protein mappings. For this reason, we do not suggest here or in our manuscript that MS-based protein measurements necessarily reflect ground truth. However, MS measurements do provide more granularity regarding peptide sequence and structure, as well as post-translational modifications, than affinity-based proteomics measurements do. Hence, when MS-pQTL and affinity-based pQTL align, this can be a good indication that an affinity-based pQTL is, in fact, capturing impacts on protein abundance. Similarly, when a PAV within a

significant affinity-based pQTL shows evidence for being an MS-PAV, this may suggest the PAV does not impact protein abundance but rather induces isoform differences.

We removed the term “MS-PAV” throughout our text, as this term is to our knowledge specific to the Suhre et al. study at this time and may cause confusion. Instead, we introduce and describe the term in the results (lines 349-357) as:

MS-pQTL studies may also help differentiate PAV-containing pQTL that do not reflect differences in overall protein abundance, but may, for example, reflect differences in specific peptide abundances, altered affinity reagent binding, or technical errors. Platform-discordant signals for 2 proteins, APOL1 and PGLYRP2, had evidence of impacting specific peptides but not overall protein abundance in an MS study (Suhre et al. 2024) (**Table S8, ST4, ST5**); unfortunately, many of the proteins and pQTLs with platform-discordant signals were not studied in the relatively limited current MS-pQTL literature but will hopefully be interrogated in future studies. Hence, MS pQTL findings may be used to better disentangle true biological abundance signals from potentially artifactual signals.

Line 636 onwards: I might be wrong but isn’t adjusting for the lead cis-pQTL the very same as identifying independent credible sets, ie., the correspondence of identifying secondary signals with, e.g., GCTA-cojo compared to SuSiE?

Here, we adjust genetic models, for a subset of proteins, for platform-discordant PAV *cis*-pQTL (which may or may not be the primary genetic signal for a given protein). While this is similar in principle to conditional analyses, we note that here we use SuSiE systematically across all proteins with a QTL to approximate conditional analyses across individual level data and to identify credible sets. By contrast, in our select number of direct conditional analyses, we adjust for platform-discordant PAVs suggesting that these are signals that are very likely to increase noise in the protein abundance measure. Hence, adjustment for these signals may result in detection of genetic signals that were previously masked, and thus undetectable (or showed very low significance) with SuSiE or other methods for approximating conditional analysis on original summary statistics. Conversely, if a PAV did *not* impact the measure of a protein, we would expect no change in genetic signals detected.

Figure 2: Please do not use 3D-pie charts, the latter are itself already misleading in presenting proportions but putting them into this shape makes it even worse.

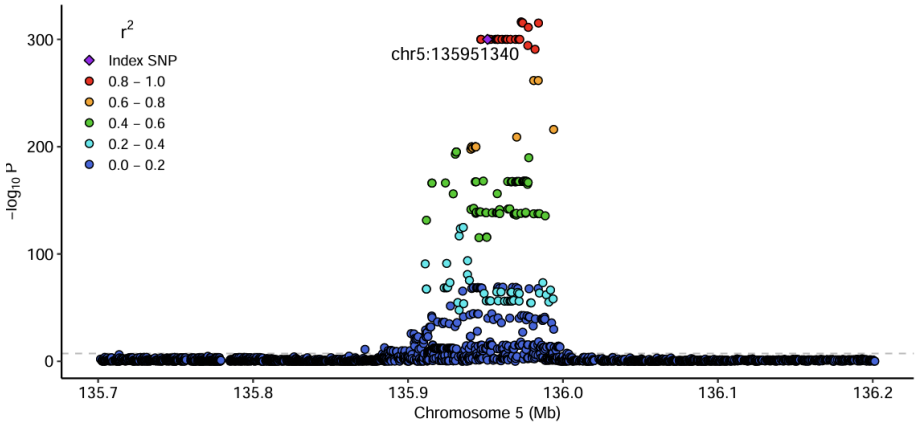
We apologize for the lack of clarity in Figure 2, and replaced the pie charts with tables to emphasize our point (that a large portion of platform-discordant *cis*-pQTL, and 25-30% of platform-specific *cis*-pQTL are driven by PAVs) more clearly.

We now additionally list the proteins associated with a platform-discordant PAV *cis*-pQTL in main text Table 3.

Supplementary Table 3: Many credible sets have exactly identical association statistics for 5 or more distinct variants. Can the authors explain why this is?

These are often variants in high or nearly perfect linkage-disequilibrium (LD) in participants included in our study, which are inherited with one another and thus highly correlated.

For example, see a Locus Zoom plot of the primary Olink *LECT2 cis*-pQTL signal (Olink credible set ID OID30320_1) below. This pQTL credible set contains six highly significant variants that are in very high LD with one another (r^2~1). These SNPs have essentially identical pQTL association statistics because they are nearly perfectly correlated, and as SuSiE cannot distinguish between the variants, they are all included in the credible set.



Revisions Figure 1. Locus Zoom plot of primary Olink *LECT2 cis*-pQTL signal (credible set OID30320_1), demonstrating identical p-values for variants that are in high LD (B= -1.02, SE=0.02, P=1x10^{-300} for the six variants in the credible set).

Reviewer #3 (Remarks to the Author):

Nicholas et al. present a cross-platform evaluation of plasma protein measurements obtained using two proteomics technologies: SomaScan 7k and Olink Explore 3072. Utilizing a diverse population from the MESA cohort and whole-genome sequencing data, the authors systematically dissect platform concordance, identify genetic determinants. The findings are timely and relevant, with practical implications for biomarker discovery, causal inference using pQTLs, and clinical proteomics.

Major Comments
1. The manuscript emphasizes cross-ancestry evaluation, which is commendable. Given the growing importance of multi-ancestry studies in proteogenomics and drug development, this reviewer recommend the authors to further highlight the necessity and translational relevance of cross-population analyses in the result section.

We thank the reviewer for recognizing the importance of considering proteomics platform agreement across diverse genetic ancestries. We now emphasize the importance of these

analyses in the results section (lines 183-185, lines 195-197), and added text to further highlight this point in the introduction (lines 96-100) and discussion (lines 727-730).

Introduction:

Furthermore, existing comparisons of SomaScan and Olink protein measures are limited to populations of predominantly one ancestry; it remains unknown if assay performance varies across diverse ancestries. Recognizing which protein measures commonly agree – or disagree – across platforms and ancestries will be critical for equitable application of proteomics data in research and clinics.

Results:

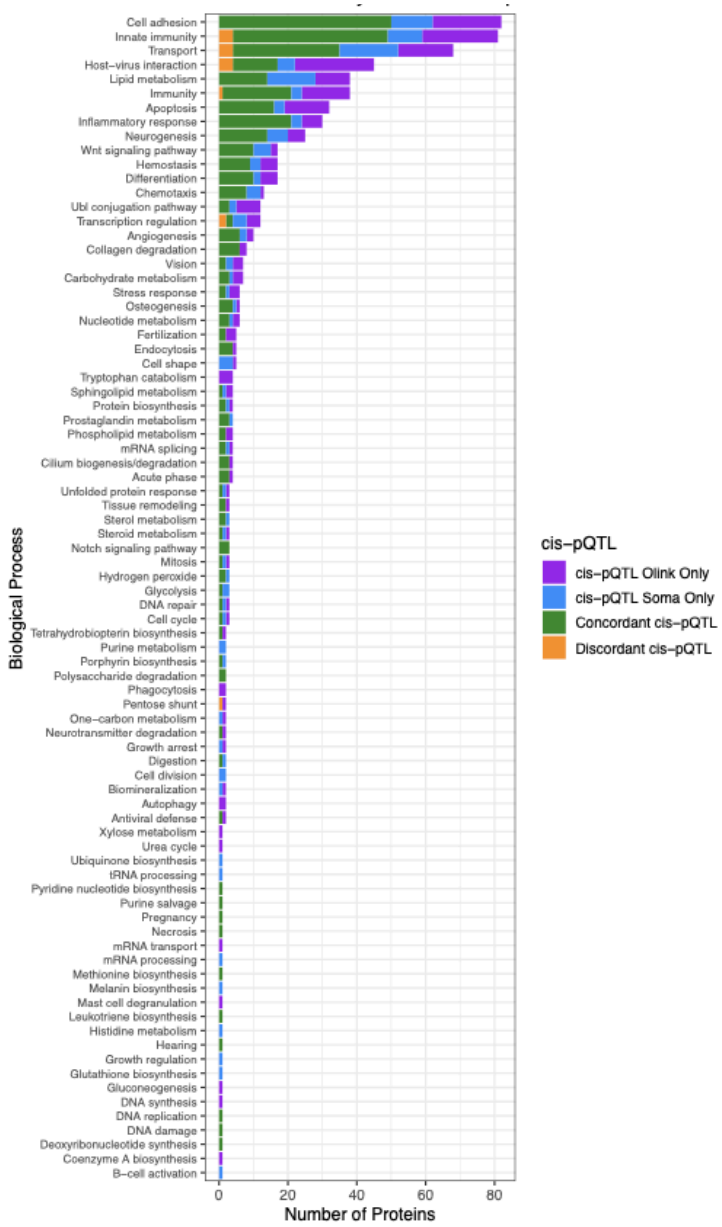
Given the multi-ancestry composition of MESA, we had the unprecedented opportunity to explore protein measure agreement within ancestry groups and establish whether there were any proteins with significant differences in platform-agreement across ancestry groups. … These differences may be of high relevance to note as research using affinity-based proteomics platforms across diverse populations continues to expand.

Discussion:

Careful consideration of the potential for assay-interference differentially impacting protein measurements across ancestry groups will be imperative to increase utility of proteomics data in future research and clinical settings.

2. While the authors acknowledge the challenge in distinguishing affinity binding artifacts from true abundance differences, further classification of discordant signals (e.g., those with consistent eQTL direction but opposite pQTL effect) could strengthen the biological interpretation. Consider discussing whether specific protein classes (e.g., immune vs metabolic) are enriched for platform-discordant PAVs.

We thank the reviewer for the suggestion. In practice, it is challenging to map proteins to only one class; while several resources offer suggested classes (for example, Human Protein Atlas, UniProt, GeneOntology), there is not one consistent class mapping across resources, and proteins often map to multiple classes within a given resource. However, to address this comment, we performed an additional analysis, where we mapped proteins to classes based on the first biological process listed in the Human Protein Atlas, then explored the platform-concordance of *cis*-pQTL for proteins with *cis*-pQTL in each category. We see proteins with platform-discordant *cis*-pQTL map to Innate immunity, transport, host-virus interaction, and transcription regulation functions, although given the very relatively small number of proteins associated with a platform-discordant pQTL at this sample size, there is no marked enrichment in any one class. Full breakdown of pQTL signal concordance by protein class can be seen in the figure attached below.



Revisions Figure 2. Protein counts within protein class (as derived from the Human Protein Atlas), with bars colored according to platform specificity of the *cis*-pQTL.

3. The manuscript briefly mentions the risk of biased causal inference. However, the implications are profound. Consider adding a short paragraph or summary table discussing how the use of affected pQTLs as instruments may impact current Mendelian randomization studies. It would also be valuable to explore how such biases might be amplified in ancestry-diverse datasets.

We thank the reviewer for this suggestion and agree that the implications of platform-discordant *cis*-pQTL in Mendelian Randomization (MR) results could be profound. To this end, we added MR analyses using SomaScan, then Olink, *cis*-pQTL mapped in the present study as MR instruments, and compared results between the two models.

Given that a number of studies reporting genetic liability for PILRA (which is one of the proteins with lowest inter-platform correlation, and a platform-discordant PAV *cis*-pQTL that has been reported in multiple studies - see PMID 40050982, PMID 390111113, PMID 38820305) in Alzheimer's Disease (AD), and the different directions of effect reported between studies using pQTL instruments obtained using SomaScan vs. Olink (PMID 40050982, PMID 390111113, PMID 38820305), we chose to perform an exploratory, two-sample MR analysis for AD using Bellinguez et al. (2024) GWAS summary statistics for outcome data. We analyzed only the probes corresponding to the 2,157 proteins measured on both platforms.

Overall we find that similar proportions of tested proteins in MR analyses using *cis*-pQTL instruments from each platform had nominally significant MR results (Wald/Inverse Variance Weighted $p<0.05$). Of the 51 proteins with a nominally significant MR result on both platforms, 5 have discordant directions of effect between platforms - all of these proteins are associated with either a platform-discordant PAV *cis*-pQTL (3) or a platform-specific PAV *cis*-pQTL (2).

We next wanted to determine whether excluding platform-discordant and platform-specific PAV *cis*-pQTL resulted in more concordant MR results. For many proteins with a platform-discordant *cis*-pQTL instrument, the platform-discordant *cis*-pQTL was the only one contributing SNP instrument; hence, many of these proteins could not be tested in analyses excluding these SNPs as instruments.

Interestingly, 10 proteins were significant only in the model excluding platform-specific PAV *cis*-pQTL instruments (5 SomaScan, 5 Olink), and include Growth Differentiation Factor-15 (GDF-15), which is highly discussed in current literature around cardiometabolic diseases (PMID: 34381196), as well as Osteoclast-Associated Receptor (OSCAR), Lymphotoxin Alpha (LTA), Hepatocyte Growth Factor Activator (HGF), and others. We hypothesize that, for these proteins, the associated platform-specific PAV *cis*-pQTL may perhaps tag non-abundance effects, and including them in MR models thereby biases results towards the null, whereas excluding these instruments may increase power to detect true effects.

We added a results section, main text Figure 6, and as well as text from the discussion (below, lines 732-744) to emphasize the importance of these results:
We show that platform-specific and platform-discordant PAVs may bias Mendelian Randomization (MR) results if used as genetic instruments for circulating protein abundances (Lemmelä et al., 2022), in some cases resulting in platform-specific and/or platform-discordant MR estimates. Furthermore, we establish that excluding platform-specific PAV instruments from MR models may increase power to detect significant associations for a subset of proteins. This is particularly surprising, as, in theory, these instruments explain less of total trait variance, supporting that the platform-specific PAV may in some cases be masking other signals (for example, from non-coding or regulatory variants) that do reflect protein abundance. Similarly, if proteomics platforms are employed clinically, failure to account for genetic variation that does not capture overall protein abundance measures may result in systematically different measures

among carriers of such genetic variants, thereby creating challenges for downstream applications in disease diagnosis/prognosis.

4. Beyond protein-altering variants (PAVs), it would be helpful to report whether the enriched variants (e.g., lead cis-pQTLs) disproportionately localize to exonic versus intronic regions. This would provide greater clarity regarding the genomic architecture of the detected signals.

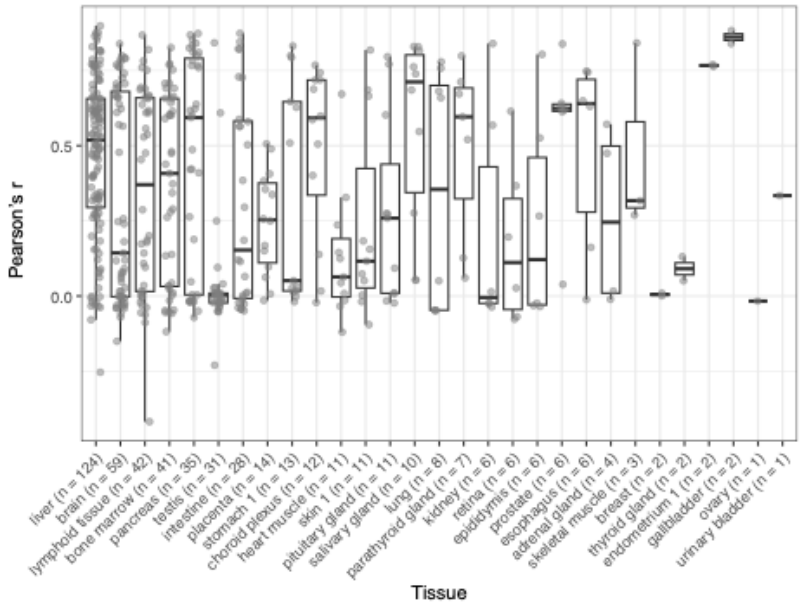
In an additional analysis, we found that the most common lead *cis*-pQTL functional classifications are (in order from most to least common), intronic for the protein-encoding gene, regulatory (i.e. in regions immediately upstream or downstream of the protein-encoding gene), and exonic for the protein encoding gene. In the present study, we focus on the exonic protein-altering *cis*-pQTL variants, given their potential to directly impact protein measurement through structural changes to the protein. However, the non-coding and other classes of *cis*-pQTLs, which are even more abundant, are interesting and valuable to dissect further in future studies.

Full results for this analysis are now added in Supplemental Figure 7.

Minor Comments

1. Could protein expression patterns, particularly the tissue or organ of origin, affect cross-platform measurement similarity? A brief discussion or exploratory analysis could be informative.

We thank the reviewer for this question. Notably, it is challenging to map proteins to a single tissue or organ of origin, as this is not fully resolved for many proteins. Protein-encoding gene expression may take place in >1 tissue, and further relevant modifications may be made to proteins in other tissues as well. In this analysis, however, we took a gene-expression based approach to map proteins to a tissue of origin, and only assessed proteins classified as tissue-enriched in the Human Protein Atlas based on RNA expression patterns. We then took only the top tissue per protein (based on the highest number or transcripts per million detected per tissue) and considered this the tissue of origin. Unsurprisingly, we see that the majority of these proteins map to liver as the tissue of origin, while only a few proteins mapped to many other tissues of origin (such as ovary or urinary bladder). We did not see that that protein tissue / organ of origin has a large effect on cross-platform measurement similarity. Full results are shown in the figure attached below.



Revisions Figure 3. SomaScan 7k and Olink Explore 3072 protein measure correlations (Pearson's r) according to tissue (as determined from the Human Protein Atlas RNA expression data).

2. The authors adjusted for 10 protein PCs in the pQTL analysis. It would be helpful to briefly clarify the rationale for this choice (e.g., was this empirically determined or standard practice?), and whether results are robust to vary this number.

There is not an established standard practice within the field, however, several ongoing proteomics studies within the TOPMed program choose to adjust for 10 protein PCs. We performed a full analysis, keeping all other covariates the same and adjusting for 0 to 100 protein PCs, to evaluate how sensitive lead *cis*-pQTL detection is to the number of protein PCs included in the QTL mapping model. We found that adjusting for 100 protein PCs maximized the number *cis*-pQTL detected. However, we chose to adjust for 10 protein PCs on each platform in this study due to concerns of adjusting away subtle *trans*-pQTL effects that we were interested in evaluating in this study with adjustment for later PCs, as was found for *trans*-eQTL analyses in TOPMed (for example, PMID: 40034763). Adjustment for 10 protein PCs increased the number of *cis*-pQTL discovered relative to the analysis without any protein PCs in the model on both platforms, but without risking large impacts on *trans*-pQTL analysis. For this paper, we felt it would be easiest to interpret to use the same modelling strategy for *cis*- versus *trans*-effects, but we acknowledge in methods that this may not maximize *cis*- pQTL discovery.

We added Supplementary Figures 4 and 5 to show the results of this sensitivity analysis and the results of GWAS for protein PCs on each platform, and expanded our text in the methods to justify our choice of adjustment of 10 protein PCs for the readers (lines 919-930):

Protein PCs were calculated across all inverse-normal transformed protein measures for each platform. Early protein PCs likely capture the impacts of latent variables such as technical processes or platform sample handling, impacts of kidney function or disease status among participants. Here, we show that adjustment for protein PCs improves power for *cis*-pQTL discovery on both platforms (Figure S4). While adjustment for 10 protein PCs does not maximize *cis*-pQTL detection, we performed a GWAS of the first 100 protein PCs on each platform to show that several later PCs likely capture impacts of *trans*-pQTL (Figure S5), which we did not want to adjust away for the purposes of this study. Hence, we chose 10 PCs for consistency between platforms and *cis*- and *trans*-pQTL analyses, although future research endeavors may want to consider adjusting for different numbers of protein PCs between *cis*- and *trans*-pQTL analyses to maximize discovery.

3. Please consider providing more technical details about the pQTL analysis, such as the software version used, statistical models (linear vs. mixed), and how related individuals were handled or filtered in the association testing.

We apologize for the lack of description in pQTL methods and have added several details in the methods (lines 917-950) and results (lines 206-212) sections. Linear models were implemented in tensorQTL version 1.0.8, with direct relatives excluded from the analysis entirely.

4. The analysis of pleiotropic trans-pQTLs is rigorous, but the interpretation remains cautious. If possible, comment on whether some platform-specific pleiotropic loci correspond to known biological hubs (e.g., APOE, ABO).

We remain cautious in our interpretation of pleiotropic *trans*-pQTL as we cannot make conclusive statements without additional empirical evidence; it is possible that some of these loci are true *trans*-pQTL for at least some protein targets, and may interfere with protein measures for some other targets. We find both platform-shared and platform-specific pleiotropic loci at known biological hubs (i.e. *ABO* as a platform-shared pleiotropic locus, and *APOE* as a platform-specific pleiotropic locus for SomaScan). However, some of these loci may impact the functions of proteins that deposit post-translational modifications (for example, *ABO* - which encodes a glycosyltransferase that adds glycosyl groups on the H-antigen - and *GNPTA* - which is another platform-shared pleiotropic locus and encodes GlcNAc-1-phosphotransferase, which adds mannose-6-phosphate groups to proteins), and could, in theory, result in alterations to affinity probe binding to downstream targets. This may explain the much lower inter-platform correlation of protein measures for proteins associated with a platform-specific pleiotropic *trans*-pQTL on SomaScan (mean Pearson's r=0.09, SD=0.21), compared to all proteins included in analyses, and proteins with no pQTL at all. Future studies applying mass-spectrometry, which can have resolution for post-translational modifications, in larger sample sizes, will prove invaluable for further dissecting these loci and their impact on protein abundance.

We added text to the discussion to expand on these known pleiotropic loci (lines 755-765), as well as others:

The same trend has been observed in the UK BioBank / deCODE SomaScan and Olink platform-comparison, where significant platform-specific pleiotropic trans-pQTL were seen at some of the same regions, such as complement factor gene *CFH* and glycoprotein-encoding *VTN* for SomaScan protein measures, and proteins associated with these loci often showed lower inter-platform correlation compared to other proteins (Eldjarn et al. 2023). They propose such loci may capture an interaction between SomaScan technical processes and the encoded biological changes. In a similar vein, we observe platform-shared and/or platform-specific pleiotropic trans-pQTL regions at loci encoding proteins involved in coagulation processes (*F12*, *F5*, *CPB2*), blood type (*ABO*), fat-transport proteins (*APOE*), other glycoproteins (*HPX*, *DAG1*), and proteins with roles in glycosylation (*FUT6*, *FUT2*, *HRG*, *GNPTAB*).

5. Data/code availability: The authors mention supplemental tables but do not provide links to raw pQTL summary statistics. Public release would be a substantial asset to the field.

We agree with the reviewer's point, and emphasize that our goal is to create an asset to the field that can increase the utility of proteomics measures and both SomaScan and Olink pQTL for all researchers. To this end, we plan to release raw pQTL summary statistics via the GWAS Catalog in parallel to final publication of this paper, and are currently in the process of uploading.

Reviewer #2 (Remarks to the Author):

Reviewer comments

I agree with the authors, that differentially frequent PAVs can contribute to poor transferability of proteomic findings across ancestries, and this is well executed in the manuscript. My general concern remains the overall relevance and novelty of the findings. Unlike a genetic association, this is an entirely analytical question as faced daily in clinical chemistry, if new assays or lots of chemicals emerge/are supplied.

We thank the reviewer for complimenting our execution of this study. Our primary goal with this paper, based on our results, was to raise awareness of the potential for genetic interference to bias protein measures obtained from proteomics assays, particularly across ancestries, and to demonstrate the power of adjustment for protein-altering variants (PAVs) to improve discovery when they are likely driving assay discordance. Certainly, others have discussed the potential of PAVs to impact affinity-based protein measures. But to our knowledge, we are the first study to look at genetic drivers of assay discordance across diverse ancestries at scale in the context of high-throughput proteomics, and among the first to illustrate the value of adjustment for PAVs likely driving assay interference.

We believe proteomics data - from both SomaScan and Olink technologies - are an exciting addition to the field. However, as with any data, its value rests in our ability to properly interpret the results we obtain from it. While examples are currently limited in number, when genetic interference does impede discovery or bias genetic and/or phenotypic associations found with proteomics data, it is important to account for, in order to obtain the most biological insight from the data.

Both proteomic platforms have staggering overlap, and it is well documented for the comparison at hand, but also other platforms, that they do not align well. This can be demonstrated in as few as fifty samples (see here https://www.nature.com/articles/s42004-025-01665-1), since one would expect not less than identical values for each and every sample, as the same protein is supposedly measured. Increasing sample size, as the authors do, may have the added benefit to explore potential factors accounting for poor correspondence.
We agree that proteomics platform comparisons in much smaller sample sizes also report that the majority of proteins measured on both of these platforms are poorly correlated. In the manuscript text, we state that the bimodal distribution of inter-platform correlation coefficients is consistent with what has been observed in other studies, with multiple references (including the Kirsher et al study linked above - a pre-print at the time of resubmission, reference updated in the manuscript text now).

The scope of our study differs from these existing comparisons in that we had the opportunity to examine protein measure correlation across diverse ancestry groups, and specifically examine whether any proteins show evidence of genetic variants with ancestry-differentiated allele frequencies interfering with protein measures.

The authors identify such for ~1% of the targeted proteins, even less with clear PAV evidence, which makes me wonder about the overall relevance compared to other issues those technologies face. I like to note, that while adjusting for PAVs seems to be an appealing idea, the achieved improvements are still way off properly correlating assays. One might suggest, that simply omitting the 80 something proteins from cross-ancestry analysis would be the most robust solution until companies develop better assays. Also, the additional findings appear rather incremental, and the work would have benefitted from demonstrating a wider relevance of the findings, such as for powerful proteomics prediction models. That is models that show considerable improvement over and above clinical practice.

We agree with the reviewer on multiple points. First, it is correct that when considering all probe pairs, only 18 proteins showed what we considered to be the highest tier of evidence for a protein-altering genetic variant (PAV) interfering with at least one platform's protein measures (i.e. PAV-driven pQTL signal proximal to the protein-encoding gene was significantly associated with both platform's protein measures, with discordant directions of effect). However, we were stringent in the examples we chose here. We believe there are likely additional cases of assay interference due to PAVs. For example, several PAVs were significantly associated with only one platform's measure of a protein (48 for SomaScan, 119 for Olink). Many of these PAVs do not have support from eQTLs, mass spectrometry or other protein measurement methods, etc, and we (and others in prior reports) suspect some but not all are affinity-binding artifacts. While we did not have the data to delve into the meaning of these platform-specific associations, future large studies incorporating mass-spectrometry will be critical to assess which of these PAVs with platform-specific associations drive assay interference.

Second, we also noted that adjustment for PAVs does not result in perfectly correlated protein measures. There are likely several factors beyond the PAVs discussed here which may play into assay discordance - including additional coding genetic drivers of assay discordance that were not significantly associated with protein measures in this study, technical factors including study site effects or assay protocols that disproportionately influence one platform, or environmental effects. Certainly, we cannot account for all drivers of assay discordance. However, the fact that using PAV-adjusted protein measures still, in multiple cases, improved our ability to detect genetic and/or phenotypic associations for affected proteins, and generally improved the concordance of these associations across platforms (despite the fact that protein measures were still not perfectly correlated) exemplifies the power in and utility of accounting for genetic drivers of assay discordance when we can.

Finally, we emphasize that excluding 80 protein measures with evidence for cross-ancestry heterogeneity in inter-platform correlation is not a solution to the issue of platform-discordance. Many studies have already reported significant associations between these protein measures and various phenotypes; some, including suPAR (which we discuss at length in the manuscript) could be valuable clinical biomarkers - despite cross-ancestry differences. We argue that where cross-ancestry differences in protein measurements exist, they are valuable to know about and account for in studies to ensure proper interpretation of results and transferability of results across participants of different ancestries.

Here, our goal was to advance the conversation surrounding genetic drivers of assay interference. But we agree that certainly, future studies with additional data and assessing the role of genetic assay interference in clinical prediction models will be needed.

- The claim of novel biology following PAV-adjustment is posthoc, and only possible because multiple platforms were available. In settings in which only one platform is available, it would not be clear whether adjusting for PAVs is a good or bad thing to do. Consider the example of PILRA, for which inference based on both technologies differs considerably, and all the adjustment does, is raising the average level, but not improving the correlation within each genotype group. A more appropriate analysis would be to stratify the analysis by genotype or demonstrate an interaction effect to clearly demonstrate that the PAV is a measurement artefact.

It is indeed challenging (and not always possible with existing data) to detect assay-interference due to PAVs, especially in the absence of “gold-standard” or “ground-truth” measures for most proteins. In order to develop hypotheses of suspected assay interference, we first had to integrate and compare data from both platforms.

We believe that the evidence we present (particularly in figures 4 and 5) is compelling to suggest that some PAVs likely do not impact protein abundances, but rather drive differences in protein measures not related to overall protein abundances (such as isoform differences). A genotype-stratified analysis would be limited by different numbers of participants with different genotypes; power differences would make interpreting results difficult, and this would not be a scalable solution to accounting for differences in protein measurements in other studies. Interaction models between cis and trans-pQTLs for a given protein could be an interesting future direction but is outside our current scope.

We agree that for most proteins, with data currently available, we do not know which, if either platform’s measure is impacted by assay-interference due to PAVs. This necessitates future studies incorporating orthogonal strategies of protein measurement (such as mass-spectrometry) in the same sets of participants.

- I appreciate the thoughtful response regarding PAV adjustment concerns, though I have reservations about some conclusions. MR, for example, aims to make inference on the relevant biological entity suspected to be causal. Beyond other known challenges with plasma protein MR, inability to instrument the truly causal disease mechanism limits the utility of the approach in particular since evidence at the locus may already imply altered isoform expression or similar.

We agree that MR aims to make inferences on the biological entity suspected to be causal. As proteomics platforms claim to measure protein abundances, the common assumption in MR analyses using pQTL instruments is that the entity modeled with these instruments is overall protein abundance. We agree (and argue throughout this paper) that pQTL instruments do not always capture relationships between genetic variants and overall protein abundances. However, we do not know if the instrument is capturing isoform differences, post-translational

modifications, etc, and often cannot make nuanced assumptions as to what the entity we are modeling when a subset, but not all, pQTLs used in MR are associated with a feature other than overall protein abundance.
Thus, we generally assume when interpreting MR models we are analyzing the causal relationship between overall protein abundance and another trait, even if this is not what our instrument is modeling. This is likely why excluding PAV instruments from MR analyses, in some cases, improved our power to detect significant protein-Alzheimer's disease relationships in MR.

Additionally, the value of the 'unmasked' associations warrants clarification—conditional analysis already accounts for the leading cis-pQTL and subsequently selected variants, and it remains unclear whether these associations arise from residual LD or other forms of collider bias.

Adjustment for PAVs in genetic analyses is essentially an informed conditional analysis. We chose to adjust for PAV signals that we suspected may be driving assay interference. We then evaluated which non-coding signals remained robust, or showed stronger associations with the protein measure, when we adjusted for the PAV signals that we believed were likely driving assay-interference on at least one platform.

In the present study, we adjusted for copies of the PAV allele as a covariate in pQTL mapping, then took resulting pQTL summary statistics obtained and performed fine-mapping in SuSiE to define credible sets of variants comprising each genetic signal. To ensure putative novel associations did not arise from residual LD with the PAV signal, we determined LD between variants in credible sets obtained from PAV-adjusted pQTL mapping, and variants in credible sets obtained from the pQTL mapping model without PAV adjustment, and discarded any credible sets obtained from PAV-adjusted models that contained a variant in LD (R^2>0.1) with a variant in a credible set obtained without PAV adjustment. Hence, the credible sets we report from PAV-adjusted pQTL mapping as putatively newly associated are independent of the credible sets we found in pQTL mapping without adjustment for the PAV.

This is described in our methods section:

"To account for a subset of signals that appeared to increase in significance post-PAV adjustment due to residual LD with the adjusted-PAV, we filtered PAV-adjusted credible sets that contained variants in LD with non-PAV adjusted credible sets. Here, "newly-associated" *cis*-pQTL are defined as *cis*-pQTL credible sets obtained in PAV-adjusted models that do not overlap, and are not in LD (R^2>0.1) with variants in credible sets from pre-PAV adjusted models."

- I would appreciate clarification on the difference between adjusting for PAVs first (which should represent the lead signal at the locus) and running SuSiE afterwards. SuSiE uses linear regression models to compile credible sets that are conceptually similar to classic conditional

analysis (e.g., GCTA-cojo). It would strengthen the manuscript to demonstrate that accounting for PAVs in this manner yields the expected primary and secondary signals observed with the unaffected assay, as this would provide evidence that the new signals are biologically meaningful.

In our pQTL mapping pipeline, we first ran tensorQTL to generate pQTL summary statistics with adjustment for copies of the PAV in each participant's sequencing data as a covariate (i.e. we directly conditioned on the PAV). This step was most similar to the approximate conditional analysis performed in GCTA-cojo in many QTL studies. We then input these summary statistics to SuSiE in order to generate credible sets of variants likely defining each genetic signal.

In this paper, we focused on PAVs significantly associated with both platforms' protein measures with discordant effect-directions of association. We do not know whether one, or both platform's measures are impacted by assay interference in these cases, or to what degree they are impacted. Furthermore, a given platform's assay might be affected by both genetic interference and technical/environmental noise, which could reduce power for genetic discovery. Hence, it is challenging, if not impossible to confidently define an "unaffected" assay for reference.

However, we note that for suPAR, when we adjusted for each individual's copies of the *PLAUR* PAV in pQTL mapping, a second, non-coding signal in the region became more significantly associated with each platform's measure.

- The newly added MR analysis strengthens the study's impact, though additional analyses would enhance the robustness of the findings. Specifically, correction for multiple testing and heterogeneity estimates for proteins with >1 cis-pQTL would help distinguish statistical noise from genuine signal. Rather than focusing on a single trait where the discordant pQTL tags the mechanism of action for AD, a demonstration across multiple traits would more comprehensively establish the utility of accounting for PAVs. Methods designed to address this scenario have been proposed (https://www.cell.com/cell-genomics/fulltext/S2666-979X(24)00329-X).

We thank the reviewer for sharing this reference. Here, our aim was to start a conversation regarding the impact of using PAV instruments that do not capture protein abundances in MR. For this reason, we took a very lenient approach to MR, using only a nominal significance threshold ($p<0.05$). We also did not systematically assess horizontal pleiotropy. However, future work will certainly expand MR analyses to additional traits.

Minor

Line 255 onwards: There seems to be something missing.

We apologize for any errors in file loading. We cannot see the issue from our end but would be happy to revise if the reviewer or editor provides additional information on the missing information.