nature portfolio

Peer Review File

Association of genetic scores related to insulin resistance with neurological outcomes in ancestrally diverse cohorts from the Trans-Omics for Precision Medicine (TOPMed) program

Corresponding Author: Dr Chloé Sarnowski

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)
Brief summary of the manuscript
Sarnowski et al completed an innovative study examining associations between multiple neurological traits and polygenic scores of metabolic phenotypes. These polygenic scores included fasting insulin and partitioned polygenic scores containing variants related to insulin resistant type 2 diabetes subtypes in a cross-population and population-specific design, leveraging a large sample size from the TOPMed program. They found numerous associations between cross population and population specific polygenic scores with brain volume and longitudinal cognition, indicating that genetic predisposition to these metabolic phenotypes is possibly involved in cognitive decline.

Overall impression of the work
The rationale for studying genetic predisposition for metabolic phenotypes in association with brain-related traits is scientifically sound and needed in the field. Using PRS-CSx was a methodologically current design for producing polygenic scores across diverse ancestries and the incorporation of partitioned polygenic scores yields greater biological specificity in an innovative manner. The results are intriguing and have potential implications for understanding how metabolic traits influence neurological phenotypes and cognitive trajectories. I have some minor comments listed below.

Specific comments, with recommendations for addressing each comment
1. It would be beneficial for the authors to expand upon the rationale for using fasting insulin as a proxy for insulin resistance (re: line 205). This is especially important considering the small and ethnically restricted sample included in the Laakso paper compared to the diverse populations in the present study.
2. The manuscript should clearly state that PRS-CSx does not require a p-value threshold to avoid confusion, especially for readers accustomed to clumping + thresholding methods (re: lines 205-210).
3. From the results tables it appears a Bonferonni correction was applied for multiple tests, please include this information in the main text (re: line 311).
4. In the Discussion, it would be helpful to elaborate on:
1. the specific Hispanic/Latino PS and reasons for the low variance explained,
2. strategies for PS generation in highly admixed populations,
3. alternate weighting strategies to account for unbalanced proportions between the training and validation sets.

Reviewer #2

(Remarks to the Author)
The current study aims to characterize the biological mechanisms underlying insulin resistance and dementia. To do so they analyze the associations of polygenic scores for fasting insulin and partitioned polygenic scores for T2D subtypes with neurological/cognitive outcomes in the TOPMED study. In this study they do not only focus on European ancestry, but also include other ancestries and used pooled genetic scores as well as population-specific genetic scores. In their results they

confirm genetic links between IR and dementia, brain volume and cognition.

The topic discussed in this study is of high relevance to the field and has the potential to be ground breaking. Strong points of this study are the focus on (genetic) links between insulin resistance and brain phenotypes and the much needed effort in the field to broaden the genetic links beyong European-only analyses.

Discussion points:
1. The background given is very minimal and misses the rationale why this study is investigating genetics (as opposed to only exploring the metabolic profiles in TOPMED)
2. Important references on genetic links between IR and AD/the brain/cognition are missing(at the current moment only mentioning 2 references from 2015 and 2020) and the statement that only "a few genetic loci" have been investigated in this form is not accurate, with many genetic correlation analyses including the full genome in their approach.
3. line 161 mentions the term "brain IR" but it is unclear what is meant with this, while it is an important concept for the subject
4. the rationale is missing for the authors to choose to only include the genetics of fasting insulin in their polygenic score approach, and not other relevant insulin resistance-related traits that also have large GWAS summary statistics available (like glycated hemoglobin or waist to hip circumference).
5. The main manuscript could benefit from highlighting which pPS's are tested and for which reason. Now the readers need to go to the supplement, and the rationale is then still not given.
6. To investigate the genetic link between two traits, like insulin resistance related measures and dementia/the brain/cognition, one could also perform genetic correlation analyses. Can the authors exlain why in this particular case they decided to perform polygenic score analyses in stead?
7. the "when considering suggestive signals" should not be in the first summary paragraph of the discussion, which should focus on the main and statistically sound results
8. where are the human brain enriched results discussed in the discussion coming from? They are not mentioned in the methods or the results.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed all my comments and I have no further concerns.

Reviewer #2

(Remarks to the Author)
Thank you for elaborating the introduction and the methods based on the provided feedback.

-The rationale for the use of polygenic scores is not completely sound, it will not provide causality and it is not essential to prove genetic links with other traits.
-The interpretation that findings are inconsistent if one study reports no global genetic correlation, while another study reports local genetic correlations is not correct, these methods are complementary and answering different questions.
-The choice of "protective" effects for polygenic score results is not reflective of the accepted interpretations in the field.

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
Thank you very much for the clarifications that now indeed reflect the nuance better and are complient with the field.

My only remaining point is that I don't agree with the solution to not show the local genetic correlations anymore, as the results there are more in line with the rationale of this study, and it would reflect the knowledge better to also show this as the global genetic correlation is clearly not providing the full picture.

We thank the reviewers for acknowledging the strengths of our paper and providing thoughtful and valuable comments to improve our manuscript. We have addressed all reviewer's comments and provided our answers hereafter in blue below each question. We have revised our manuscript accordingly and used tracked changes. Lines and pages numbers in our response refer to the revised manuscript with tracked changes.

We have corrected one affiliation for one coauthor that was incorrect in the original version of the manuscript (Dr. Psaty was affiliated with number 12 affiliation instead of 11). We have also revised **Figure 1** to update the year of publication for Dr. Sevilla-Gonzalez FI clustering analysis paper (preprint at the time of the submission, now published at *Nature Communications*).

Reviewer #1 (Remarks to the Author):

Brief summary of the manuscript
Sarnowski et al completed an innovative study examining associations between multiple neurological traits and polygenic scores of metabolic phenotypes. These polygenic scores included fasting insulin and partitioned polygenic scores containing variants related to insulin resistant type 2 diabetes subtypes in a cross-population and population-specific design, leveraging a large sample size from the TOPMed program. They found numerous associations between cross population and population specific polygenic scores with brain volume and longitudinal cognition, indicating that genetic predisposition to these metabolic phenotypes is possibly involved in cognitive decline.

Overall impression of the work
The rationale for studying genetic predisposition for metabolic phenotypes in association with brain-related traits is scientifically sound and needed in the field. Using PRS-CSx was a methodologically current design for producing polygenic scores across diverse ancestries and the incorporation of partitioned polygenic scores yields greater biological specificity in an innovative manner. The results are intriguing and have potential implications for understanding how metabolic traits influence neurological phenotypes and cognitive trajectories. I have some minor comments listed below.

Specific comments, with recommendations for addressing each comment
1. It would be beneficial for the authors to expand upon the rationale for using fasting insulin as a proxy for insulin resistance (re: line 205). This is especially important considering the small and ethnically restricted sample included in the Laakso paper compared to the diverse populations in the present study.

We thank the reviewer for this comment. We used FI as a proxy for IR in our genetic analyses based on very high genetic correlations reported between FI and HOMA-IR (Rg=[0.86-0.99]). [PMID: 17956454, PMID: 17646211] and GWASs which uncovered similar genetic regions for FI and IR quantified using HOMA-IR. [PMID: 22791750, PMID: 20081858]

We have expanded upon the rationale for using FI as a proxy for IR in the Methods section of the revised manuscript:

Methods

"The Homeostasis Model Assessment for Insulin Resistance [HOMA-IR] is the most extensively validated surrogate of IR. As few multi-ancestry GWASs have been performed on IR, we used FI as a proxy for IR based on high genetic correlation reported between FI and HOMA-IR". p10, lines 257-259

2. The manuscript should clearly state that PRS-CSx does not require a p-value threshold to avoid confusion, especially for readers accustomed to clumping + thresholding methods (re: lines 205-210).

We thank the reviewer for the suggestion. We now provide this clarification in the Method section of the manuscript:

"PRS-CSx, unlike other traditional methods based on clumping and thresholding, does not require a p-value threshold and thus uses all genetic variants." p11, lines 261-262

3. From the results tables it appears a Bonferonni correction was applied for multiple tests, please include this information in the main text (re: line 311).

We have clarified this in the Methods and Results section.

Methods

"For each analysis, we defined statistical significance using a multiple testing Bonferroni correction for the number of pPSs, traits and population groups tested." p14, lines 336-338

Results

"After multiple testing correction (i.e. Bonferroni), we detected significant associations of the cross-population and European FI PSs with total cranial volume (P=1.3×10-5; PEA=3.0×10-8 respectively), **Tables 2** and **Supplementary Table 10**." p15, lines 381-383

4. In the Discussion, it would be helpful to elaborate on:
1. the specific Hispanic/Latino PS and reasons for the low variance explained,

The Hispanic/Latino population is highly admixed and prone to heterogeneity. The low variance explained observed for the Hispanic/Latino PS might be the result of a combination of factors including an imperfectly matched LD reference panel [PMID: 35513724 and **Table 1R below**] as well as differences in the distribution and representation of Hispanic/Latino population groups in the studies included in the validation (HCHS/SOL) and the testing (MESA, SAFHS) sets (**Tables 1R and 2R below**). SAFHS includes Mexican Americans, MESA Hispanic/Latino group is largely

composed of Mexican Americans (>50%), while HCHS/SOL is represented in comparable proportions (>20%) by Puerto Ricans, Cubans, and Mexican Americans.

To illustrate this, we present below the population groups included in the LD reference panel used in PRS-CSx, as well as in the MAGIC FI meta-analysis, and in the TOPMed FI phenotype file.

Table 1R: Comparison of Hispanic/Latino populations included in the AMR reference panel, MAGIC FI GWAS meta-analysis, and TOPMed FI phenotype file

AMR Reference Panel	**MAGIC HISP studies in FI meta-analysis**	**TOPMed HISP studies**
Mexican ancestry (LA)	SIGMA (Mexican, Mexico)	HCHS/SOL (Hispanic, US)
Puerto Rican (Puerto Rico)	BetaGene (Mexican American)	MESA (Hispanic, US)
Colombian in Medellin (Colombia)	IRASFS (Hispanic, US)	SAFHS (Mexican American)
Peruvian in Lima (Peru)	IRAS (Hispanic, US)	
	TRIPOD (Mexican American)	
	HTN (Hispanic, US)	
	MACAD (Hispanic, US)	
	NIDDM (Hispanic, US)	

Table 2R: Comparison of Hispanic/Latino populations in TOPMed FI phenotype file for HCHS/SOL (included in the validation set) and MESA (included in the testing set)

HCHS/SOL

Population Group	**N**	**%**
NA	250	4.28%
Central American	413	7.07%
Cuban	1527	26.16%
Dominican	870	14.90%
Mexican	1326	22.71%
Puerto Rican	1449	24.82%
South American	253	4.33%
	5838	

MESA

Population Group	**N**	**%**
NA	10	1.16%
Central American	55	6.40%
Cuban	35	4.07%
Dominican	124	14.42%
Mexican	443	51.51%
Puerto Rican	117	13.60%
South American	76	8.84%
	860	

We now include a sentence in the limitations section of the Discussion of the revised manuscript and added **Table 2R** as a Supplementary Table (**Supplementary Table 15**):

"We observed a low variance explained for the Hispanic/Latino PS which could be due to an imperfectly matched LD reference panel as building such reference panel is challenging for admixed populations, as well as differences in the distribution and representation of Hispanic/Latino population groups in the studies included in the validation (HCHS/SOL) and the testing (MESA, and SAFHS) sets (**Supplementary Table 15**)." p20, lines 515-520

2. strategies for PS generation in highly admixed populations,

One important parameter when deriving PS using PRS-CSx is the ancestry-matched LD reference panel provided as input for each GWAS. Building such reference panel is challenging particularly for admixed populations. [PMID: 36873096] PRS-CSx has been shown to be robust to imperfectly matched LD reference panels [PMID: 35513724] but other methods leveraging local ancestry could be considered to better model association results from GWAS conducted in recently admixed populations. [PMID: 33462486, PMID: 23910464]

Additional methods could also be used for building PSs in admixed populations. Combining local ancestry deconvolution and partial PS computation can improve trait predictability in recently admixed individuals. [PMID: 32242022] Inclusive PGS (iPGS) for example can capture ancestry-shared genetic effects and does not require LD reference panels or need for local-ancestry inference. [PMID: 37890495] GAUDI is a penalized-regression-based method modeling ancestry-differential effects while borrowing information across segments with shared ancestry in admixed genomes. [PMID: 38310129]

We have added a paragraph in the Discussion about methods and strategies for PS derivation in admixed populations.

“Additional methods could be considered for building PSs in admixed populations including penalized-regression-based methods such as inclusive PGS (iPGS) that can capture ancestry-shared genetic effects and does not require LD reference panels or local-ancestry inference, and GAUDI, modeling ancestry-differential effects while borrowing information across segments with shared ancestry in admixed genomes.” p21, lines 520-524

3. alternate weighting strategies to account for unbalanced proportions between the training and validation sets.

We thank the reviewer for the opportunity to provide clarification and more information on the weighting strategy for the derivation of the cross-population PS.

In our analyses, we have run and compared two weighting strategies: 1) use weights learnt and derived from the PRS-CSx approach, and 2) re-weight each population specific PS contribution in the linear combination using the ratio of the proportion of populations in the testing and the validation sets. A comparison of the association results of the cross-population FI PSs with HOMA-IR with and without reweighting each population specific PS contribution is presented below in **Table 3R**.

Not accounting for the differences in the proportion of populations in the testing and the validation sets led to a drastic reduction in the proportion of variance explained of the cross-population PS (3.1% with original weights *versus* 11.6% with reweighting) due to unbalanced proportion of populations between the validation and the testing sets particularly Non-Hispanic White (33.2% in the validation set versus 66.5% in the testing set) and Hispanic/Latino (37.9% in the validation set versus 8.2% in the testing set).

Table 3R: Comparison of association results of the cross-population FI PSs with HOMA-IR under different weighting strategies

	B	SE	P	% Var explained
Weight estimated by PRS-CSx Cross-Population PS_{FI} = 0.06 × PSFI_EA + 0.12 × PSFI_AA + 0.18 × PSFI_HA				
Cross-population PS_{FI}	0.098341	0.003941	0.098341	0.031348
Re-weighted using the ratio of proportion of populations in the testing & validation sets Cross-Population PS_{FI} = 0.12 × PSFI_EA + 0.10 × PSFI_AA + 0.04 × PSFI_HA				
Cross-population PS_{FI}	0.188775	0.003941	2.23e-308	0.1155067

We have added **Table 3R** above as a Supplementary Table (**Supplementary Table 3**).

"To account for the unbalanced proportion of populations between the validation and the testing sets, we re-weighted each population specific PS contribution in the linear combination using the ratio of the proportion of populations in the testing and the validation sets (**Supplementary Table 3**). The exact formula used to derive the cross-population PS for each trait tested is provided in **Tables 1** and **2** and **Supplementary Table 3**." p12, lines 282-286

Reviewer #2 (Remarks to the Author):

The current study aims to characterize the biological mechanisms underlying insulin resistance and dementia. To do so they analyze the associations of polygenic scores for fasting insulin and partitioned polygenic scores for T2D subtypes with neurological/cognitive outcomes in the TOPMED study. In this study they do not only focus on European ancestry, but also include other ancestries and used pooled genetic scores as well as population-specific genetic scores. In their results they confirm genetic links between IR and dementia, brain volume and cognition.

The topic discussed in this study is of high relevance to the field and has the potential to be ground breaking. Strong points of this study are the focus on (genetic) links between insulin resistance and brain phenotypes and the much-needed effort in the field to broaden the genetic links beyond European-only analyses.

Discussion points:
1. The background given is very minimal and misses the rationale why this study is investigating genetics (as opposed to only exploring the metabolic profiles in TOPMED)

We agree with the reviewer and have extensively revised the Introduction section of the manuscript to expand upon our rationale to investigate genetics in this study.

"Metabolic traits and AD share common clinical or epidemiological risk factors which could be due to an overlap in causal genes and pathways. Some genetic variants have been reported associated with both AD and IR disorders in loci like *FTO, APOE, CLU,* and *TOMM40*. Several studies attempted to better understand the genetic link and etiology between glycemic/metabolic

traits including fasting insulin (FI) and AD/dementia using genetic correlation analyses, genetic and polygenic scores, and Mendelian Randomization (MR) as described hereafter.

Some studies evaluated the genetic overlap between insulin-related (including obesity, T2D, metabolic syndrome, fasting glucose, FI, and HOMA-IR) and neuropsychiatric disorders including AD. One study did not report significant global genetic correlation between AD and FI or HOMA-IR (Rg = 0.142, P=0.218). One study found that evidence of local correlations between IR-related conditions (T2D, obesity, and metabolic syndrome) and AD can be detected even in the absence of global genetic correlations. A cross-trait meta-analysis of AD and 10 metabolic traits reported a genetic correlation between FI and AD, with marginal statistical significance (Rg = −0.196, P= 0.087) and identified three loci common to AD and FI (CLU, CR1/CR2, and BCL3).

A few MR analyses have focused on characterizing the contribution of glycemic traits or IR to AD but did not find evidence of causality. These studies were conducted in participants of European ancestry and used a small number of genetic loci or variants associated with FI or IR (quantified using the Homeostatic Model Assessment for Insulin Resistance [HOMA-IR]), leading to a modest proportion of variance explained by these genetic instruments.

Using polygenic scores (PSs) as genetic instruments for IR can increase the power of the association analyses. Inconsistent findings have been reported regarding the association of genetic instruments for T2D (genetic scores or PSs) and AD or dementia risk, with some studies reporting significant associations with all-cause dementia and vascular dementia, while other studies did not identify significant association. One of this study, performed predominantly in White European participants, also evaluated association between glycemic traits PSs with dementia and reported a protective effect of PS of higher FI on AD and all-cause dementia (although not surviving Bonferroni correction). Leveraging newly developed methods for PS derivation in diverse populations and recently proposed partitioned PSs (pPSs) based on large clustering analyses of T2D and FI loci (25-28) could improve the genetic instruments for IR and help disentangle heterogeneity and pinpoint to specific biological mechanisms of IR in AD."

2. Important references on genetic links between IR and AD/the brain/cognition are missing (at the current moment only mentioning 2 references from 2015 and 2020) and the statement that only "a few genetic loci" have been investigated in this form is not accurate, with many genetic correlation analyses including the full genome in their approach.

We agree with the reviewer that the Introduction was missing important references and we have thus extensively revised the Introduction section of the manuscript to expand upon studies that attempted to better understand the genetic link and etiology between glycemic/metabolic traits and AD/dementia using genetic correlation analyses, genetic and polygenic scores, and Mendelian Randomization (MR).

"Some studies evaluated the genetic overlap between insulin-related (including obesity, T2D, metabolic syndrome, fasting glucose, FI, and HOMA-IR) and neuropsychiatric disorders including AD. One study did not report significant global genetic correlation between AD and FI or HOMA-IR (Rg = 0.142, P=0.218). One study found that evidence of local correlations between IR-related

conditions (T2D, obesity, and metabolic syndrome) and AD can be detected even in the absence of global genetic correlations. A cross-trait meta-analysis of AD and 10 metabolic traits reported a genetic correlation between FI and AD, with marginal statistical significance (Rg = −0.196, P= 0.087) and identified three loci common to AD and FI (CLU, CR1/CR2, and BCL3).

A few MR analyses have focused on characterizing the contribution of glycemic traits or IR to AD but did not find evidence of causality. (23,24) These studies were conducted in participants of European ancestry and used a small number of genetic loci or variants associated with FI or IR (quantified using the Homeostatic Model Assessment for Insulin Resistance [HOMA-IR]), leading to a modest proportion of variance explained by these genetic instruments.

Using polygenic scores (PSs) as genetic instruments for IR can increase the power of the association analyses. Inconsistent findings have been reported regarding the association of genetic instruments for T2D (genetic scores or PSs) and AD or dementia risk, with some studies reporting significant associations with all-cause dementia and vascular dementia, while other studies did not identify significant association. One of this study, performed predominantly in White European participants, also evaluated association between glycemic traits PSs with dementia and reported a protective effect of PS of higher FI on AD and all-cause dementia (although not surviving Bonferroni correction). Leveraging newly developed methods for PS derivation in diverse populations and recently proposed partitioned PSs (pPSs) based on large clustering analyses of T2D and FI loci (25-28) could improve the genetic instruments for IR and help disentangle heterogeneity and pinpoint to specific biological mechanisms of IR in AD."

3. line 161 mentions the term "brain IR" but it is unclear what is meant with this, while it is an important concept for the subject

We have now provided clarification on this sentence and defined the term brain IR in the Introduction:

"Decreases in the sensitivity of central nervous pathways to insulin, i.e., brain insulin resistance is an early, common, and major feature in patients with AD, whether they have T2D or not." p7 lines 165-168

4. the rationale is missing for the authors to choose to only include the genetics of fasting insulin in their polygenic score approach, and not other relevant insulin resistance-related traits that also have large GWAS summary statistics available (like glycated hemoglobin or waist to hip circumference).

Our rationale to use FI as a proxy for IR was based on strong genetic correlation reported between FI and HOMA-IR (Rg=[0.86-0.99]). [PMID: 17956454, PMID: 17646211] In genetic correlation analyses we conducted (not presented in this paper), the genetic correlation between FI [PMID: 34059833] and HOMA-IR [PMID: 20081858] was close to 1 (P=5.3E-33) while the genetic correlation between HbA1c [PMID: 34059833, PMID: 28898252] and HOMA-IR ranged from 0.20 to 0.24 (P=[0.0005-0.02]), and the genetic correlation between WHR [PMID: 30239722] and HOMA-IR was 0.68 (P=1.7E-24).

In addition, a cross-trait analysis of 10 metabolic traits and AD did not observe substantial genetic correlation between AD and WHR (Rg<0.05, *P*>0.3) but reported a genetic correlation between FI and AD, with marginal statistical significance (Rg = −0.196, P= 0.087). [PMID: 30805717] Another study investigating the genetic overlap between AD with metabolic syndrome, obesity, and T2D did not find evidence of significant genetic correlation between HbA1c and AD (Rg=-0.097, P=0.265). [PMID: 35165256] A study evaluating association of T2D and glycemic traits polygenic scores with dementia in 29,139 adults did not find significant association between HbA_{1c} PRS and dementia outcomes, but reported however some associations of FI PSs on AD and all-cause dementia. [PMID: 37091584]

We have revised the manuscript to provide clarification and included these references in the Discussion.

Methods

"The Homeostasis Model Assessment for Insulin Resistance [HOMA-IR] is the most extensively validated surrogate of IR. As few multi-ancestry GWASs have been performed on IR, we used FI as a proxy for IR based on high genetic correlation reported between FI and HOMA-IR". p10, lines 257-259

Discussion

"We opted to use FI as a proxy for IR to select GWAS to derive the PS based on high genetic correlation between FI and HOMA-IR. Derivation of PS using additional diverse GWAS conducted with other traits genetically correlated with HOMA-IR would be worth investigating in future studies." p20, lines 512-515

5. The main manuscript could benefit from highlighting which pPS's are tested and for which reason. Now the readers need to go to the supplement, and the rationale is then still not given.

We thank the reviewer for this suggestion. We have now clarified in the Method section which pPSs are tested and justified for which reason they were selected (positive association with FI and/or HOMA-IR).

"We included three clustering analyses of T2D (one European, set 1-Kim, and two multi-ancestry, set 2-Smith, Deutsch, and set 3-Suzuki) (25-27) and one clustering analysis of FI (European, set 4-Sevilla-Gonzalez).(28) We selected 19 clusters positively associated with FI and/or HOMA-IR to derive 19 pPSs: five clusters in set 1-Kim (Obesity, Lipodystrophy, Liver/Lipid, alkaline phosphatase (ALP) negative, and Hyper Insulin secretion), six clusters in set 2-Smith, Deutsch (Obesity, Lipodystrophy 1 and 2, Liver/Lipid, ALP negative, and Hyper Insulin secretion), four clusters in set 3-Suzuki (Obesity, Lipodystrophy, Liver/Lipid, and Metabolic syndrome), and four clusters in set 4-Sevilla-Gonzalez (IR mediated by visceral adiposity, Adiposity-driven hyperinsulinemia, IR-lipodystrophy, and Hepatic IR). A description of these clusters by study is provided in **Supplementary Tables 4** and **14**." p12, lines 292-301

6. To investigate the genetic link between two traits, like insulin resistance related measures and

dementia/the brain/cognition, one could also perform genetic correlation analyses. Can the authors explain why in this particular case they decided to perform polygenic score analyses instead?

Evaluating genetic correlation between two traits provide some information on a common underlying genetic etiology. Previous studies have evaluated the genetic overlap between insulin-related (including obesity, T2D, metabolic syndrome, fasting glucose, FI, and HOMA-IR) and AD, with some inconsistent findings. [PMID: 35165256, PMID: 40221434, PMID: 30805717] One study did not report significant global genetic correlation between AD and FI or HOMA-IR (Rg = 0.142, P=0.218). [PMID: 35165256] One study found evidence of local correlations between IR-related conditions (T2D, obesity, and metabolic syndrome) and AD even in the absence of global genetic correlations. [PMID: 40221434] A cross-trait meta-analysis of AD and metabolic traits reported a genetic correlation between FI and AD (Rg = −0.196, P= 0.087). [PMID: 30805717] In genetic correlation analyses we conducted (not presented in this paper) using more recent GWASs and selecting European populations, the genetic correlation between FI [PMID: 34059833] and AD [PMID: 35379992] was 0.08 (P=0.08). Importantly, population-specific summary statistics from large GWAS are not always available to perform these analyses in non-European populations.

In our analyses, we wanted to use polygenic scores as a method of causal inference to prioritize putative causal risk factors. [PMID: 32996171] By using polygenic scores, we aimed through our work to demonstrate that leveraging diverse populations improves genetic instruments for IR, to evaluate whether the genetic liability for IR is associated with AD and related traits, and to compare neurological trait associations of FI polygenic score and IR-related partitioned polygenic scores to provide insight into specific biological processes underlying IR and AD.

We revised the Introduction section of the manuscript to cite the genetic correlation analyses mentioned above and clarify the aims of our work.

“Some studies evaluated the genetic overlap between insulin-related (including obesity, T2D, metabolic syndrome, fasting glucose, FI, and HOMA-IR) and neuropsychiatric disorders including AD. One study did not report significant global genetic correlation between AD and FI or HOMA-IR (Rg = 0.142, P=0.218). One study found that evidence of local correlations between IR-related conditions (T2D, obesity, and metabolic syndrome) and AD can be detected even in the absence of global genetic correlations. A cross-trait meta-analysis of AD and 10 metabolic traits reported a genetic correlation between FI and AD (Rg = −0.196, P= 0.087).” p7, lines 179-186

“Here, we aimed to derive several genetic scores related to IR to demonstrate that leveraging diverse populations improves genetic instruments for IR, to evaluate whether the genetic liability for IR is associated with AD and related traits, and to compare neurological trait associations of IR-related PS and pPSs to provide insight into specific biological processes underlying IR and AD.” p9, lines 207-211

7. the "when considering suggestive signals" should not be in the first summary paragraph of the discussion, which should focus on the main and statistically sound results

We have moved the suggestive signals paragraph later in the Discussion in the revised manuscript. p18, lines 446-449

8. where are the human brain enriched results discussed in the discussion coming from? They are not mentioned in the methods or the results.

These findings are from the Suzuki et al paper (reference PMID: 38374256 cited in the text). To provide clarification, we have revised the related sentences as follow:

“The metabolic syndrome pPS has been reported by Suzuki et al to be, in the human brain, significantly enriched for regions of open chromatin in cell types including intratelencephalic (IT) projecting neurons and somatostatin-positive (SST+) GABAergic inhibitory neurons.” p18, lines 461-464

“The obesity pPS has been reported by Suzuki et al to be, in the human brain, significantly enriched for regions of open chromatin in cell types including IT projecting neurons, SST+ GABAergic inhibitory neurons and D1 medium spiny neurons.” p19, lines 473-476

Reviewers' comments:

Reviewer #2 (Remarks to the Author):

Thank you for elaborating the introduction and the methods based on the provided feedback.

-The rationale for the use of polygenic scores is not completely sound, it will not provide causality and it is not essential to prove genetic links with other traits.

Thank you for the opportunity to clarify the rationale for the use of polygenic scores.

Both polygenic scores and partitioned polygenic scores can be useful tools for disease risk prediction and risk stratification. Individuals with higher genetic liability to IR might be more susceptible to developing dementia or other related neurological outcomes and could thus benefit more from treatment preventing cognitive decline. Using genetic partitioned polygenic scores associated with T2D risk or contributing to elevated FI levels that have been shown to predispose differentially to cardiometabolic outcomes might help identify subgroups of individuals at higher risk of developing dementia or other related neurological outcomes. Using PS and pPS related to IR is also a complementary strategy to identify relevant pathways on a risk factor that can be intervened on.

We have clarified the rationale for using polygenic scores in the Introduction of the manuscript:

“Leveraging newly developed methods for PS derivation in diverse populations and recently proposed partitioned PSs (pPSs) related to IR based on large clustering analyses of T2D and FI loci (49-52) that predispose differentially to cardiometabolic disease risk could help disentangle heterogeneity and pinpoint to specific biological mechanisms of IR in AD. It represents thus a complementary strategy to identify relevant pathways on a risk factor that can be intervened on. Furthermore, such PS and pPSs related to IR could be used for risk stratification to help identify subgroup of individuals at higher risk of developing neurological outcomes and who could benefit more from preventive treatments.”

-The interpretation that findings are inconsistent if one study reports no global genetic correlation, while another study reports local genetic correlations is not correct, these methods are complementary and answering different questions.

We agree that the methods investigating global versus local genetic correlations are complementary and answering different questions. We have revised the Introduction to focus on studies that investigated global genetic correlations:

“Some studies evaluated the genetic overlap between insulin-related (including obesity, T2D, metabolic syndrome, fasting glucose, FI, and IR quantified using the Homeostasis Model Assessment for Insulin Resistance [HOMA-IR]) and neuropsychiatric disorders including AD. (39,41)

One study did not report significant global genetic correlation between AD and FI or HOMA-IR (Rg = 0.142, P=0.218). (41) A cross-trait meta-analysis of AD and 10 metabolic traits reported a genetic correlation between FI and AD (Rg = −0.196, P= 0.087) and identified three loci common to AD and FI (CLU, CR1/CR2, and BCL3). (39)“

-The choice of "protective" effects for polygenic score results is not reflective of the accepted interpretations in the field.

Thank you for this comment. Dybjer et al reported a significant negative association between a PRS (P-value threshold $P = 5 \times 10^{-4}$) for fasting insulin and mixed dementia (HR of 0.88, Bonferroni corrected *P*-value = 1.2e-02) and all-cause dementia (HR = 0.94; uncorrected *P*-value = 4.3e-02), and another PRS (P-value threshold $P = 5 \times 10^{-8}$) for fasting insulin with Alzheimer’s disease (HR = 0.86; uncorrected *P*-value = 1.6e-02).

We have rephrased the statement in the manuscript to accurately reflect those results:

Introduction

“One of this study, performed predominantly in White European participants, also evaluated association between glycemic traits PSs with dementia and reported negative associations of FI PSs on mixed dementia, and on all-cause dementia, and AD (although not surviving Bonferroni correction).(45)”

Discussion

“We did not observe significant association of the FI PSs with AD and dementia in our analysis which contrasts with the study from Dybjer et al who evaluated associations between PSs of T2D and glycemic traits with dementia diagnoses and reported negative associations of FI PSs on mixed dementia, and on all-cause dementia and AD (although not surviving Bonferroni correction).(45)”