nature portfolio

Peer Review File

Genetic risk factors underlying white matter hyperintensities and cortical atrophy

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

NCOMMS-24-19171-T

This paper examined the association between white matter hyperintensities (WMH) and cortical atrophy, and it explored the underlying neurobiological mechanisms of the WMH-cortical thickness associations through several genetic analyses among 51,064 dementia and stroke-free individuals from 10 independent cohorts with brain imaging data.

The authors have reported that WMH burden is inversely associated with cortical thickness, and this association is consistent across all 10 cohorts. By conducting a GWAS on the WMH-associated cortical atrophy, the authors have identified 20 genome-wide statistically significant loci. Subsequent genetic correlation analyses indicate relationships with multiple cardiometabolic, neurodegenerative, and neuropsychiatric traits. Cell-type and functional enrichment analyses additionally reveal insights into the biological processes of WMH-associated cortical atrophy and the cell types that mediate the genetic vulnerability of WMH-associated cortical thinning. Finally, a cell type enrichment analysis of the WMH-cortical thickness association was conducted to explore which cell types in the cerebral cortex mediate this association.

Critique:

1. When testing the association between WMH and cortical thickness, education should be adjusted as an additional covariate, some studies (PMID: 16887777, PMID: 31407822) have demonstrated that education is associated with both WMH and cortical thickness.

2. Findings from the current study reported some discrepancies compared to previous literature. For example, Tuladhar et al. (2015, citation 7) reported that higher WMH is related to higher cortical thickness in paracentral regions. However, current results indicate the association is negative. Although the discrepant results could be explained by differential sample sizes, it is worth replicating those previous findings with similar methods (e.g., same covariate adjustments and samples of similar age) to ensure that no other factors distort the observed WMH-cortical thickness association across regions in the current study.

3. The GWAS was conducted for the shared variance (PC1) between WMH and cortical thickness, and 20 loci were reported to reach genome-wide significance. How many of these genetic variants are unique compared to the variants identified when running a GWAS on WMH and cortical thickness separately? Do most of them overlap, or are there any unique genetic variants that are particularly relevant to WMH-associated cortical thinning?

4. Similarly, can a genetic correlation analysis of PC1 GWAS with other traits reveal a consistent trend when the PC1 GWAS is replaced with separate GWAS for WMH or cortical thickness? The summary statistics from different cardiometabolic, neurodegenerative, and neuropsychiatric traits should be cited in the 'Genetic Correlations of PC1 GWAS' section. If AD2 represents Alzheimer’s

disease, I am a bit surprised that there is almost no correlation between AD and the shared variance between WMH and cortical thickness. It is worth exploring if a similar correlation effect can be observed when replacing the PC1 GWAS with separate GWAS for WMH or cortical thickness.

5. A minor point is that WMH are not risk factors for AD. A risk factor is an antecedent factor that increases the risk of disease. That has not been shown in this study. The authors should remove that statement from the first sentence in the abstract and the manuscript.

Reviewer #2 (Remarks to the Author):

This manuscript leverages on 10 cohorts with available WMH, cortical thickness and GWAS data to study WMH-associated cortical thinning. The authors identified 20 loci, related to genes specific to endothelial cells, pericytes, astrocytes, and oligodendrocytes. The authors conclude that axonogenesis and features of cytoskeleton organization and intracellular transport determine genetic vulnerability to cortical atrophy in the presence of white matter lesions.

Despite the large sample size and innovative approach, I have a number of concerns.

The authors perform many analyses, and present many impressive results. Yet the rationale for this, or the research question is not entirely clear to me. A clear hypothesis should be stated.

I like the idea to disentangle the effect of WMH as a measure of small vessel disease on cortical thinning versus the effect of other processes (e.g. amyloid). When this would be the hypothesis, than I would not covary for vascular risk factors, as WMH would be expected to be a mediating factor between cardiovascular risk factors and cortical thinning. The fact that the associations don't change after adjustment for vascular risk factors rather suggests that the WMH may not represent vascular damage, but something else.

The suggestion is made that WMH cause cortical thinning, but being a cross-sectional study, this association does not need be causal.

Amyloid or other alzheimer-related factors are not mentioned at all. Although it is understandable that most epidemiological cohorts do not have PET or CSF measures of amyloid, most of them by

now have blood markers available, which would provide the possibility to take some measure of amyloid into account.

The methods is very concise, and many relevant details are missing. Such as – how were associations between WMH and cortical thickness determined? From the abstract and general wording of the text, conclusions seem to be made about cortical thickness in general (as a reader – I would think of global cortical thickness). When reading more closely, it becomes clear that the whole manuscript is based on insula thinning. Why would the association WMH~cortical thinning be very regional? The insula is chosen based on statistical significance, rather than biological considerations. I would at least like to see similar findings for global approach, or a number of different regions.

Reviewer #3 (Remarks to the Author):

• What are the noteworthy results?

- This study performs thorough analyses in a large sample to explore the relationship between White Matter Hyperintensities (WMH) and cortical atrophy and its underlying genetic risk factors and neurobiology.

o A meta-analyses was performed in over 51.000 persons from 10 cohorts showing that a higher White Matter Hyperintensity volume is associated with a lower thickness of the cerebral cortex.

o The meta-GWAS of the shared variance between WMH and insular cortical thickness identified 20 genome-wide significant loci.

o The cellular processes of WMH-associated cortical atrophy involve endothelial cells, pericytes, astrocytes (i.e. small vessel forming cells) and oligodendrocytes (providing support to axons) impacting excitatory neurons with long range axonal projections traversing through the white matter.

o 15 of the 20 genome-wide significant loci regulated expression of 54 genes in the cerebral cortex, that, together with their co-expressed genes, were enriched in biological processes of axongenesis, cytoskeleton organization, and intercellular transport. These processes determine an individual's genetic vulnerability to cortical atrophy in the presence of WMH.

• Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references.

- The work contributes to existing literature, by exploring the genetic risk factors and underlying neurobiology of the shared variance between WMH and cortical thickness, rather than investigating one or the other.

• Does the work support the conclusions and claims, or is additional evidence needed?

- The work support the conclusions drawn. Although important limitations remain regarding choice of certain region specific cerebral analyses.

• Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision?

- The most important design flaw of this study lies in studying the genetic underpinnings of the WMH-cortical thickness association in the insular region specifically, i.e. all analyses represented in Figure 2. The authors try to provide a rationale for the selection of this brain region in particular, by stating that the association between WMH and cortical thickness was strongest in the insula (see Figure 1c). However, looking at the forest plot in Fig 1c the effect sizes of the superiotemporal region does not seem to differ significantly from the effect sizes of the insula. The choice for performing GWAS analyses in the insula only is therefore not sufficiently supported by the data.

- The cross-sectional versus longitudinal nature of analyses is unclear. E.g. on page 7: “all individuals were stroke and dementia free” versus Figure 2c showing LD-score regression estimates between the GWAS of Principal Component 1 (i.e. the shared variance between WMH and cortical thickness) with various vascular (risk) factors and neurodegenerative traits, including stroke and Alzheimer’s Disease. Is this incident stroke and dementia in those cohorts that contributed to this meta-analysis with longitudinal data? Clarify which cohort has contributed with which longitudinal data.

- The authors mainly suggest that WMH leads to atrophy as a results of retrograde degeneration of neuronal bodies in the cerebral cortex. Could these processes however occur in parallel (but may not be necessarily causally related) due to a shared denominator such as other small vessel disease features and/or amyloid-beta pathologies.

- Amyloid- beta is not considered in analyses, while it often co-occurs with vascular cerebral damage, including WMH, and is a main driver of cortical atrophy. Although we understand that not all cohorts may have either PET, Cerebral Spinal Fluid and/or blood biomarkers available to determine amyloid-beta status, it should be mentioned in the discussion as a potential important confounding factor in the WMH & atrophy relationship.

- The multi-racial ARIC study is included potentially providing the opportunity to explore the impact of race to findings. Race is known to impact WMH load and dementia prevalence is also unequally distributed by race, underling the relevance considering this factor in analyses (although power may be too limited for all analyses).

• Is the methodology sound? Does the work meet the expected standards in your field?

- Included studies in this meta-analyses have used various methods to determine WMH and atrophy and moreover have varying field strengths, i.e. 1,5 T or 3 T. It should be stated how varying methods across cohorts may have impacted findings.

• Is there enough detail provided in the methods for the work to be reproduced?

- More detail should be given on the selection criteria that have been used for each cohort, i.e. a subset with MRI data at baseline? Several cohorts have longitudinal (MRI) data available but it is unclear if and how this data has been used in analyses.

Reviewer #1

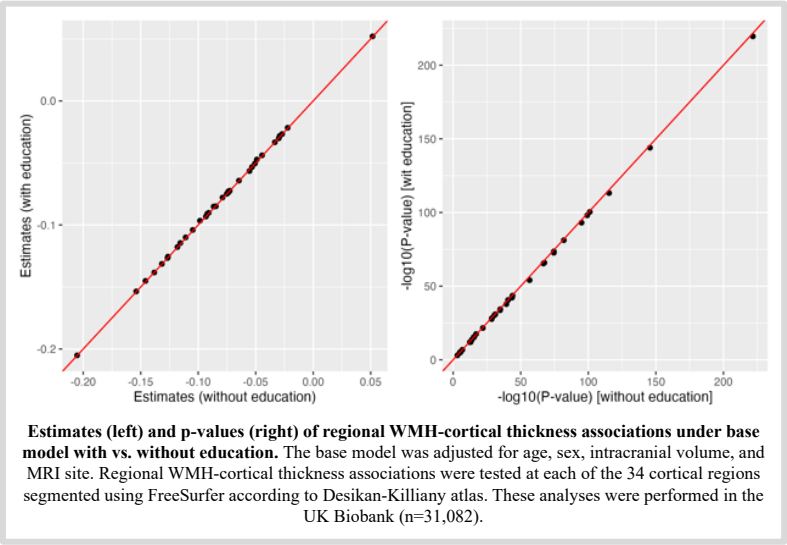
This paper examined the association between white matter hyperintensities (WMH) and cortical atrophy, and it explored the underlying neurobiological mechanisms of the WMH-cortical thickness associations through several genetic analyses among 51,064 dementia and stroke-free individuals from 10 independent cohorts with brain imaging data.

The authors have reported that WMH burden is inversely associated with cortical thickness, and this association is consistent across all 10 cohorts. By conducting a GWAS on the WMH-associated cortical atrophy, the authors have identified 20 genome-wide statistically significant loci. Subsequent genetic correlation analyses indicate relationships with multiple cardiometabolic, neurodegenerative, and neuropsychiatric traits. Cell-type and functional enrichment analyses additionally reveal insights into the biological processes of WMH-associated cortical atrophy and the cell types that mediate the genetic vulnerability of WMH-associated cortical thinning. Finally, a cell type enrichment analysis of the WMH-cortical thickness association was conducted to explore which cell types in the cerebral cortex mediate this association.

Critique:

1. When testing the association between WMH and cortical thickness (CT), education should be adjusted as an additional covariate, some studies (PMID: 16887777, PMID: 31407822) have demonstrated that education is associated with both WMH and cortical thickness.

Response: We have added education in our model, and the results remained similar, indicating that education is not confounding the associations between WMH and cortical thickness at any of the 34 cortical regions examined, as shown in the figure below.



Estimates (left) and p-values (right) of regional WMH-cortical thickness associations under base model with vs. without education. The base model was adjusted for age, sex, intracranial volume, and MRI site. Regional WMH-cortical thickness associations were tested at each of the 34 cortical regions segmented using FreeSurfer according to Desikan-Killiany atlas. These analyses were performed in the UK Biobank (n=31,082).

2. Findings from the current study reported some discrepancies compared to previous literature. For example, Tuladhar et al. (2015, citation 7) reported that higher WMH is related to higher cortical thickness in paracentral regions. However, current results indicate the association is negative. Although the discrepant results could be explained by differential sample sizes, it is worth replicating those previous findings with similar methods (e.g., same covariate adjustments and samples of similar age) to ensure that no other factors distort the observed WMH-cortical thickness association across regions in the current study.

Response: The results of the study of Tuladhar et al (2015) are not very different from the results of our study. Tuladhar et al (2015) reported that higher WMH are associated with lower CT in frontotemporal

regions but with higher CT in the paracentral region. Our study shows that higher WMH are associated with lower CT in 33 of the 34 tested regions, with the associations being most pronounced in frontotemporal regions and less pronounced in the paracentral region (**Fig. 1d**). To our knowledge, no other study than Tuladhar et al. 2015 has reported positive associations between WMH and CT[1–9].

Tuladhar et al study vs. our study was much smaller (426 participants from 1 cohort vs. 51,065 participants from 10 independent cohorts); it included participants of similar age and male/female proportion. Both studies excluded participants with dementia, but Tuladhar et al did not exclude participants with stroke (instead, it excluded participants with intracranial hemorrhage and intracranial space occupying lesion [often due to tumor]). Additional exclusion criteria in Tuladhar et al. study, but not in our study, were having visual/hearing impairments, language barrier, or psychiatric disease, and taking neuroleptics, acetylcholinesterase inhibitors, or L-dopa. Further, CT was estimated with different pipelines – CIVET in Tuladhar et al and FreeSurfer in all 10 cohorts from our study. The regional analyses of WMH-CT associations were different – vertex-wise in Tuladhar et al (thousands of vertices/comparisons may be more sensitive to false positives, especially in relatively small samples [e.g., [10],[11]) and region-wise (cortex parcellated into only 34 cortical regions) in our study. The statistical models were similar in that both studies adjusted for hypertension, diabetes, hypercholesterolemia, BMI, and smoking.

We have revised the relevant sentence in Introduction as follows: 'Previous research in smaller studies (n<2,000 participants) reported mostly inverse associations between WMH and cortical thickness[1–9,12] (and reviewed in[13]).'.

3. The GWAS was conducted for the shared variance (PC1) between WMH and cortical thickness, and 20 loci were reported to reach genome-wide significance. How many of these genetic variants are unique compared to the variants identified when running a GWAS on WMH and cortical thickness separately? Do most of them overlap, or are there any unique genetic variants that are particularly relevant to WMH-associated cortical thinning?

Table S4. Meta-GWAS of PC1 (WMH and insular CT) and their previous GWAS associations with WMH or CT

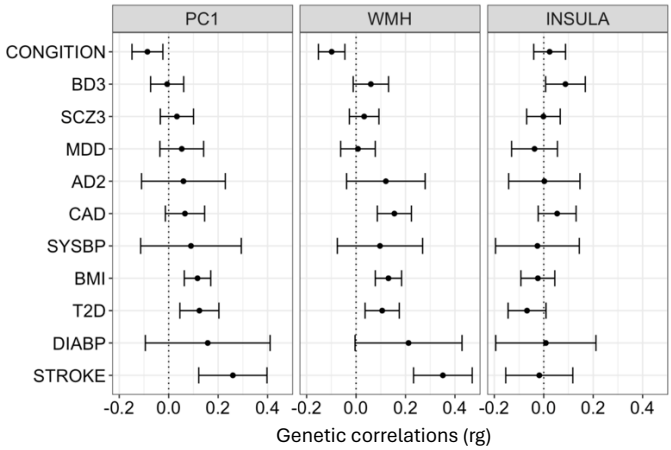
			Previous GWAS-significant loci*		References**	
PC1 locus #	rsID	chr:pos (hg19)	WMH	CT	WMH	CT
4	rs7454868, rs190945449	6:26799828, 6:26828359	no	no		
8	rs62477728	7:75132471	no	no		
9	rs11191163, rs11191268	10:103733624, 10:104115262	no	no		
14	rs11075976	16:51498626	no	no		
19	rs112783265	18:32358907	no	no		
7	rs798528	7:2772431	no	yes		9-11
12	rs3765066	15:75140854	no	yes		10,12
20	rs2072859	22:38322350	no	yes		9,10,12
5	rs13208741	6:45461253	no	yes		9-12
10	rs4630220	10:105459116	yes	no	2-5	
11	rs11838776	13:111040681	yes	no	2-4	
1	rs3762515	2:56150864	yes	no	2-5,7,8	
17	rs563065735, rs12950988	17:43129103, 17:43127708	yes	no	2-4	
18	rs3744027	17:73888743	yes	no	1-8	
6	rs4272224	6:151035800	yes	yes	2-4,6,8	9,10,12
2	rs147100405, rs72932753	2:203720774, 2:203670122	yes	yes	1-4	9,10
3	rs79934840, rs11711420	3:183403240, 3:183349010	yes	yes	2,4	9,10
13	rs17616633	16:51451683	yes	yes	2,4,6	9-13
15	rs9308343	16:87224857	yes	yes	2-6	9-14
16	rs1472932	17:19220666	yes	yes	2	10

*yes (no): Top SNP or it's LD-proxy with r^2>0.2 was (not) associated with WMH or CT at the genome-wide significance level of 5e-08
GWAS catalogue search was done on May 28, 2024.

Response: Thank you for this question. Of the 20 loci identified in the present study, 5 loci are 'unique' to WMH-associated cortical thinning (*i.e.*, not reported previously as being associated with either WMH or CT), 9 loci have been reported previously as being associated with either WMH or CT, and 6 loci have been reported previously as being associated with both WMH and CT. This information is now included in a new table – **Tab. S4** – and described in the text.

4. Similarly, (a) can a genetic correlation analysis of PC1 GWAS with other traits reveal a consistent trend when the PC1 GWAS is replaced with separate GWAS for WMH or cortical thickness?

Response: As shown in the figure below, genetic correlations of PC1 with vascular-risk, neurodegenerative, and psychiatric traits showed a similar trend to the genetic correlations of WMH (but not CT) with those traits. The relative lack of genetic correlations of CT with neurodegenerative and psychiatric traits is consistent with previous research[14].



LD-score regression estimates between our GWAS of PC1, white matter hyperintensities (WMH), or insular cortical thickness (INSULA) and the previous GWAS of various vascular risk factors, as well as neurodegenerative and neuropsychiatric traits. PC1 is the shared variance between WMH and INSULA. DIAB: Diastolic Blood Pressure, T2D: Type 2 Diabetes, BMI: Body Mass Index, SYSBP: Systolic Blood Pressure, CAD: Coronary Artery Disease, AD2: Alzheimer's Disease, MDD: Major Depressive Disorder, SCZ3: Schizophrenia PGC3, ALS: Amyotrophic Lateral Sclerosis, BD3 Bipolar disorder and Cognition.

(b) The summary statistics from different cardiometabolic, neurodegenerative, and psychiatric traits should be cited in the 'Genetic Correlations of PC1 GWAS' section.

Response: Apologies for this omission. The papers providing these summary statistics are now cited in the 'Genetic Correlations of PC1 GWAS' section, and reads as follows: 'We used linkage disequilibrium (LD)-score regression analysis[15] to test the genetic correlations between PC1 and vascular-risk (systolic blood pressure[16], diastolic blood pressure[16], stroke[17], BMI[18], diabetes[19], coronary artery disease[20]), psychiatric (schizophrenia[21], major depression[22], bipolar disorder[23]), neurodegenerative (Alzheimer's disease[24]) and general intelligence[25]. Plasma protein level GWASs were obtained from the Pharma Proteomics Project using individuals from the UK Biobank[26].'

(c) If AD2 represents Alzheimer's disease, I am a bit surprised that there is almost no correlation between AD and the shared variance between WMH and cortical thickness. It is worth exploring if a similar correlation effect can be observed when replacing the PC1 GWAS with separate GWAS for WMH or cortical thickness.

Response: AD2 indeed refers to AD. We have now removed the digits next to all disease abbreviations. As requested, we have carried out the suggested analyses. The results show that, like the PC1 phenotype, WMH and CT do not show significant genetic correlations with AD (as shown in the figure above). Importantly, this is consistent with our new results demonstrating that a polygenic risk score (PRS) generated from the GWAS summary-statistics of PC1 is associated with higher risk of vascular dementia, all-cause dementia but not AD (**Fig. 4**); this additional study was done in an independent cohort of ~500,000 participants of FinnGen. Note that the odds ratio is the highest for vascular dementia – individuals with the top (vs. bottom) decile of the PRS have a 52% higher risk of vascular dementia (whereas they had an 18%. higher risk of all-cause dementia, **Fig. 4**)

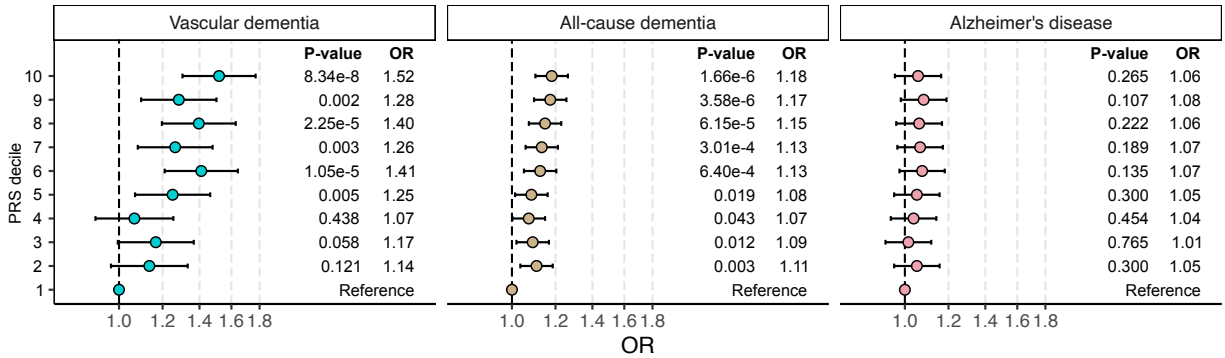


Figure 4. Association between polygenic risk score (PRS) of WMH and insular CT-derived PC1 and the risk of vascular dementia, all-cause dementia, and Alzheimer's disease. The odds ratios were calculated in FinnGen (n=500,348) by comparing each of the top nine PRS deciles to the lowest decile and adjusting for age, sex, the first 10 genetic principal components and genotyping arrays.

5. A minor point is that WMH are not risk factors for AD. A risk factor is an antecedent factor that increases the risk of disease. That has not been shown in this study. The authors should remove that statement from the first sentence in the abstract and the manuscript.

Response: In our original manuscript, we wrote: 'WMH is a major risk factor for dementia' based on previous research by others, reporting phenotypic WMH-dementia correlations in prospective studies[27], genetic WMH-dementia correlations in cross-sectional studies[28], and putative causal effects of WMH on dementia in Mendelian Randomization studies[29]. Note that all these analyses used 'all-cause' dementia rather than 'AD'. We deleted the sentence in Abstract, but, given this previous research and the new results of our present study (**Fig. 4**), we would prefer to keep the statement in Introduction in a slightly edited form: 'WMH is a risk factor for all-cause dementia'.

Reviewer #2

This manuscript leverages on 10 cohorts with available WMH, cortical thickness and GWAS data to study WMH-associated cortical thinning. The authors identified 20 loci, related to genes specific to endothelial cells, pericytes, astrocytes, and oligodendrocytes. The authors conclude that axonogenesis and features of cytoskeleton organization and intracellular transport determine genetic vulnerability to cortical atrophy in the presence of white matter lesions. Despite the large sample size and innovative approach, I have a number of concerns.

1. The authors perform many analyses and present many impressive results. Yet the rationale for this, or the research question is not entirely clear to me. A clear hypothesis should be stated.

Response: Our hypothesis was stated in Abstract (2nd sentence) as: "WMH may damage axons and in turn promote atrophy of the cerebral cortex…" and in Introduction (1st paragraph) as: "Injury of neuronal axons at the site of WMH may lead to retrograde degeneration of neuronal bodies and dendritic arbour within the cerebral cortex and thus promote cortical thinning[13]." To clarify the importance of axonal transport, we have added the following sentence (with 2 relevant references: "Retrograde axonal transport may contribute to this process by transporting various growth factors from the axon terminals to the cell body[30] and dendrites[31]".

Our aim was to provide insights into the underlying neurobiology, combining neuroimaging phenotypes with genetic and *in silico* transcriptomic.

2. I like the idea to disentangle the effect of WMH as a measure of small vessel disease on cortical thinning versus the effect of other processes (e.g. amyloid). When this would be the hypothesis, than I would not covary for vascular risk factors, as WMH would be expected to be a mediating factor between cardiovascular risk factors and cortical thinning. The fact that the associations don't change after

adjustment for vascular risk factors rather suggests that the WMH may not represent vascular damage, but something else.

Response: This is a difficult issue to address. Our results support strongly the relationship between the combined WMH-thickness phenotype (i.e., PC1) and processes related to the cerebral vasculatures: *(i)* the positive genetic correlations of PC1 with vascular risk factors (**Fig. 2c**), *(ii)* the cell type-specific enrichment of polygenic signals from the GWAS of PC1 in genes of endothelial cells, pericytes and astrocytes (among others, **Fig. 2d**), and, perhaps most importantly, the new finding showing *(iii)* the association of PC1 PRS with vascular dementia (**Fig. 4**). All these findings can be interpreted in two (mutually non-exclusive) ways: *(i)* vascular changes in white matter lead to its damage and, in turn, cortical atrophy via, for example, impaired axonal transport; and *(ii)* WMH are an index of impaired cerebral vasculature, including the vasculature in the cerebral cortex, which leads to cortical atrophy. We have added these two hypothetical scenarios in the Discussion section, pointing out that – of course – our results cannot speak to either the directionality of such possible relationships or the relative contributions of the two hypothetical pathways.

3. The suggestion is made that WMH cause cortical thinning, but being a cross-sectional study, this association does not need be causal.

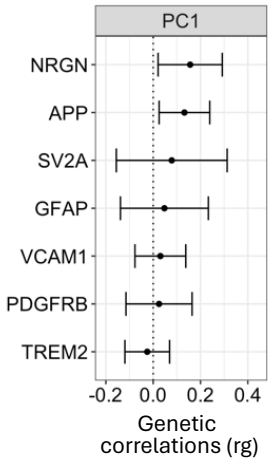
Response: We agree. Therefore, we performed 2-sample Mendelian randomizations to examine causality in the WMH-insula CT relationship. The results are inconclusive, being significant for inverse variance weighted MR only (**Tab. S5**). We have pointed this out in the Discussion section (see also our response Point 2 above).

Table S5. Two-sample Mendelian Randomization (MR) testing causal effects of WMH on insular or mean cortical thickness

		WMH (Sargurupremraj) -> CT (current meta-GWASs)				WMH (Sargurupremraj) -> CT (UKB)				WMH (UKB) -> CT (UKB)*			
	Method	**Number of SNPs**	**Beta**	**SE**	**P**	**Number of SNPs**	**Beta**	**SE**	**P**	**Number of SNPs**	**Beta**	**SE**	**P**
Insular cortical thickness	MR Egger	21	0.0711	0.2291	0.760	21	0.0844	0.2633	0.752	16	0.2374	0.3112	0.458
	Weighted median	21	-0.0044	0.0589	0.941	21	-0.1116	0.0669	0.095	16	-0.0592	0.0692	0.392
	Inverse variance weighted	21	-0.1214	0.0745	0.103	21	-0.2074	0.0873	0.017	16	-0.2036	0.0901	0.024
	Simple mode	21	-0.0388	0.1121	0.733	21	-0.1606	0.1120	0.167	16	-0.1673	0.1914	0.396
	Weighted mode	21	0.0206	0.0636	0.749	21	-0.0417	0.0743	0.581	16	-0.0441	0.0741	0.561
Mean cortical thickness	MR Egger	21	-0.0997	0.1594	0.539	21	-0.0986	0.1896	0.609	16	-0.2496	0.2149	0.265
	Weighted median	21	-0.0167	0.0440	0.704	21	-0.0507	0.0568	0.372	16	-0.0096	0.0581	0.869
	Inverse variance weighted	21	0.0382	0.0520	0.463	21	0.0393	0.0616	0.524	16	0.0654	0.0625	0.295
	Simple mode	21	-0.0119	0.0883	0.895	21	-0.0869	0.1015	0.402	16	0.0212	0.1191	0.861
	Weighted mode	21	-0.0524	0.0614	0.404	21	-0.0752	0.0767	0.339	16	-0.0331	0.0695	0.641

*Two-sample MR approaches were applied to single UK Biobank dataset included in the current work to account for potential horizontal pleiotropic effects of the genetic instrument variables [ref].

4. Amyloid or other Alzheimer disease-related factors are not mentioned at all. Although it is understandable that most epidemiological cohorts do not have PET or CSF measures of amyloid, most of them by now have blood markers available, which would provide the possibility to take some measure of amyloid into account.



Response: We thank you for this comment. We have now performed genetic correlation analyses between GWAS of PC1 and GWAS of plasma levels of proteins implicated in neurodegenerative processes, including amyloid-precursor protein (APP). These genes were *a priori* selected as related to biomarkers of neurodegeneration[32]. We find positive genetic correlations between our PC1 and plasma APP and plasma neurogranin (NRGN, a new **Fig. 2d**). Regarding APP, the correlations are interesting not only because APP fragments are the major constituent of AD-associated amyloid plaques and mutations or duplications of the gene coding for APP can cause familial AD, but also because APP and its fragments may function as long-distance sensors of cellular activity or damage, and the control axonal transport (among others); the latter function may be particularly important in large neurons[33], such as the excitatory neurons with long-range axonal projections implicated in WMH-associated cortical thinning

in the present study (**Fig. 3**). Regarding NRGN, previous research suggests that higher NRGN in cerebrospinal fluid is a marker of synaptic dysfunction[34].

5. The methods is very concise, and many relevant details are missing. Such as – how were associations between WMH and cortical thickness determined? From the abstract and general wording of the text, conclusions seem to be made about cortical thickness in general (as a reader – I would think of global cortical thickness). When reading more closely, it becomes clear that the whole manuscript is based on insula thinning. Why would the association WMH-cortical thinning be very regional? The insula is chosen based on statistical significance, rather than biological considerations. I would at least like to see similar findings for global approach, or a number of different regions.

Response: We apologise for the succinctness – most key methodological details were in Online Methods, and additional information (cohort description and characteristics, as well as information in MRI-data acquisition in each cohort) was in Supplement. We have now edited the manuscript to make clearer that we examined the associations between total WMH load and both mean (global) CT and regional CT at 34 regions. Our GWAS was performed on the shared variance between total WMH load and CT in a region showing the largest negative association, *i.e.*, insula. We chose insula based on statistical significance and consistency of the association across the 10 cohorts – the insular cortex was the most significantly associated region in 6 out of our 10 participating cohorts (in the remaining 4 cohorts, it was 2nd, 3rd, 4th, and 21st). Although we do not know why insula shows the strongest relationship with WMH, it is possible that this is related to the fact that 'Notably, the insula serves as a key node in multimodal integration networks[35] and a point of convergence for widespread cortical and subcortical input[36]' and, hence, it may be more vulnerable to axonal damage of multiple long-range neurons forming its afferent and efferent pathways.

Table S8. Genome-wide significant loci of PC1 with insular CT and their association with loci of PC1 with mean CT in the present set of cohorts

PC1 Locus #	rsID	chr:pos (hg19)	PC1 (WMH and insular CT)			PC1 (WMH and mean CT)				LD (r^2)**
			Estimate	SE	P-value	Estimate	SE	P-value	P-value*	
1	rs3762515	2:56150864	0.0841	1.23E-02	7.03E-12	0.0844	1.19E-02	6.52E-13	6.52E-13	1.00
2	rs147100405, rs72932753	2:203720774, 2:203670122	0.0744	1.11E-02	1.65E-11	0.0350	1.07E-02	1.02E-03	4.76E-05	1.00
3	rs79934840, rs11711420	3:183403240, 3:183349010	-0.0818	9.70E-03	4.05E-17	-0.0586	9.40E-03	4.36E-10	1.70E-11	0.99
4	rs7454868, rs190945449	6:26799828, 6:26828359	0.0846	1.28E-02	1.96E-11	0.0543	1.24E-02	5.11E-05	1.22E-08	0.40
5	rs13208741	6:45461253	0.0476	7.90E-03	2.72E-09	0.0204	7.70E-03	5.58E-03	6.87E-04	0.75
6	rs4272224	6:151035800	-0.0406	7.40E-03	3.19E-08	-0.0291	7.20E-03	4.85E-05	4.85E-05	1.00
7	rs798528	7:2772431	-0.0450	7.70E-03	8.70E-09	-0.0118	7.50E-03	1.02E-01	6.12E-03	0.25
8	rs62477728	7:75132471	0.0419	7.30E-03	1.10E-08	0.0218	7.00E-03	1.81E-03	3.83E-04	0.70
9	rs11191163, rs11191268*	10:103733624, 10:104115262	0.1183	2.16E-02	4.71E-08	0.1005	2.10E-02	2.10E-06	2.02E-07	0.55
10	rs4630220	10:105459116	-0.0606	8.00E-03	3.26E-14	-0.0346	7.80E-03	6.27E-06	4.15E-13	0.30
11	rs11838776	13:111040681	0.0496	8.00E-03	5.15E-10	0.0235	7.70E-03	1.86E-03	9.50E-05	0.90
12	rs3765066	15:75140854	0.0429	7.60E-03	1.24E-08	0.0218	7.30E-03	2.06E-03	7.38E-05	0.57
13	rs17616633	16:51451683	-0.0625	7.20E-03	1.78E-18	-0.0318	7.00E-03	3.81E-06	7.42E-07	0.95
14	rs11075976*	16:51498626	-0.0413	7.20E-03	8.63E-09	-0.0121	6.90E-03	8.37E-02	6.14E-03	0.26
15	rs9308343	16:87224857	0.0636	7.20E-03	8.37E-19	0.0321	7.00E-03	3.53E-06	1.88E-06	0.96
16	rs1472932	17:19220666	0.0599	9.10E-03	1.03E-10	0.0222	8.80E-03	9.71E-03	5.92E-05	1.00
17	rs563065735, rs12950988	17:43129103, 17:43127708	-0.0524	9.20E-03	1.17E-08	-0.0458	8.90E-03	2.38E-07	5.93E-10	0.67
18	rs3744027	17:73888743	0.0717	9.10E-03	2.19E-15	0.0759	8.80E-03	6.62E-18	1.33E-18	0.69
19	rs112783265	18:32358907	0.0482	8.10E-03	2.40E-09	0.0225	7.80E-03	4.19E-03	1.94E-03	0.97
20	rs2072859	22:38322350	0.0506	7.30E-03	4.42E-12	0.0269	7.10E-03	7.55E-05	2.08E-08	0.36

* Lowest P-value among those of the LD-proxies (r^2 >0.2) of the top SNP for PC1
**LD between the top PC1-SNP and its LD-proxy with the lowest p-value for PC1 with mean cortical thickness

Nevertheless, in response to this comment and Comment 1 of Reviewer 3, we now additionally show that: *(i)* all 20 GWAS-significant loci associated with PC1 derived from insular CT are also associated with PC1 derived from mean CT (at $p<6 \times 10^{-3}$; **Tab. S8** below). *(ii)* the pattern of genetic correlations with vascular, neurodegenerative, and neuropsychiatric traits is highly similar between PC1 with insular CT and PC1 with mean CT (**Fig. S2a** below); *(iii)* the pattern of genetic correlations with plasma proteins implicated in neurodegenerative processes is highly similar between PC1 with insular CT and PC1 with mean CT (**Fig. S2b** below); and *(iv)* the dementia risk associated with a polygenic risk score (PRS) generated based on GWAS summary-statistics of PC1 derived from insular CT is highly similar to that of PC1 derived from mean CT; for vascular dementia, for example, the risk is 52% higher in individuals with top (vs. bottom)

decile of the PRS with insular CT, and it is 33% higher in individuals with top (vs. bottom) decile of the PRS with mean CT bottom (as shown in **Fig. S3** and assessed in an independent cohort of ~500,000 participants of FinnGen).

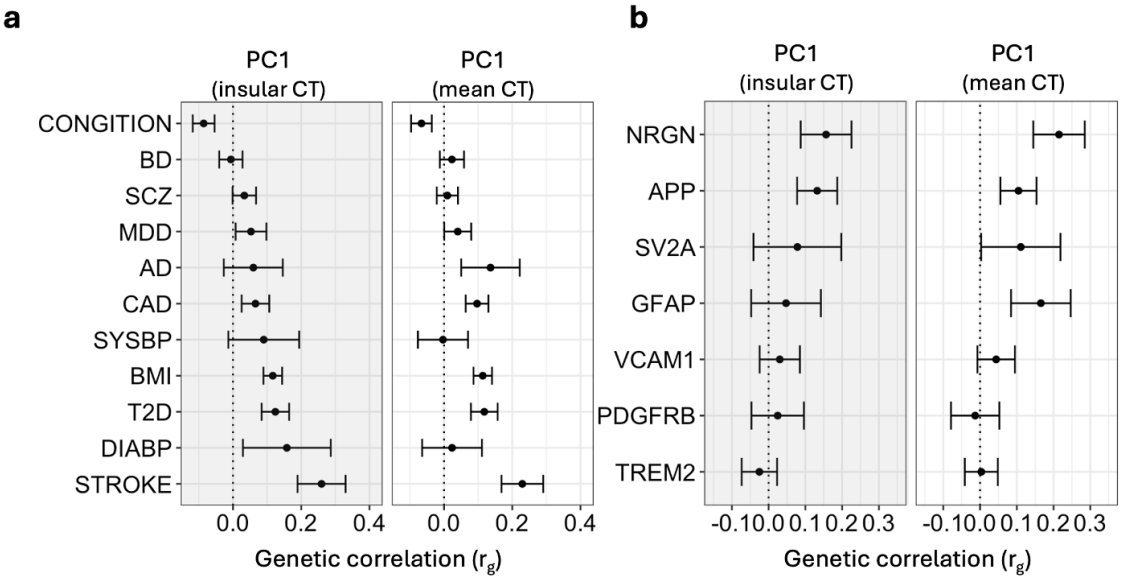


Figure S2. LD-score regression estimates between our GWAS of PC1 derived from WMH and insular or mean cortical thickness (CT) and the previous GWAS of (a) cognition, vascular risk, neurodegenerative and neuropsychiatric traits and of (b) plasma proteins related to neurodegenerative biological processes. DIAB: Diastolic Blood Pressure, T2D: Type 2 Diabetes, BMI: Body Mass Index, SYSBP: Systolic Blood Pressure, CAD: Coronary Artery Disease, AD: Alzheimer's Disease, MDD: Major Depressive Disorder, SCZ: Schizophrenia, BD Bipolar Disorder. NRGN: neurogranin, APP: amyloid precursor protein, SV2A: Synaptic vesicle protein 2, GFAP: Glial fibrillary acidic protein, VCAM1: Vascular cell adhesion protein 1, PDGFRB: platelet derived growth factor receptor beta, TREM2: Triggering receptor expressed on myeloid cells 2. These genes were *a priori* selected as related to biomarkers of neurodegeneration[29]. Error bars represent standard error.

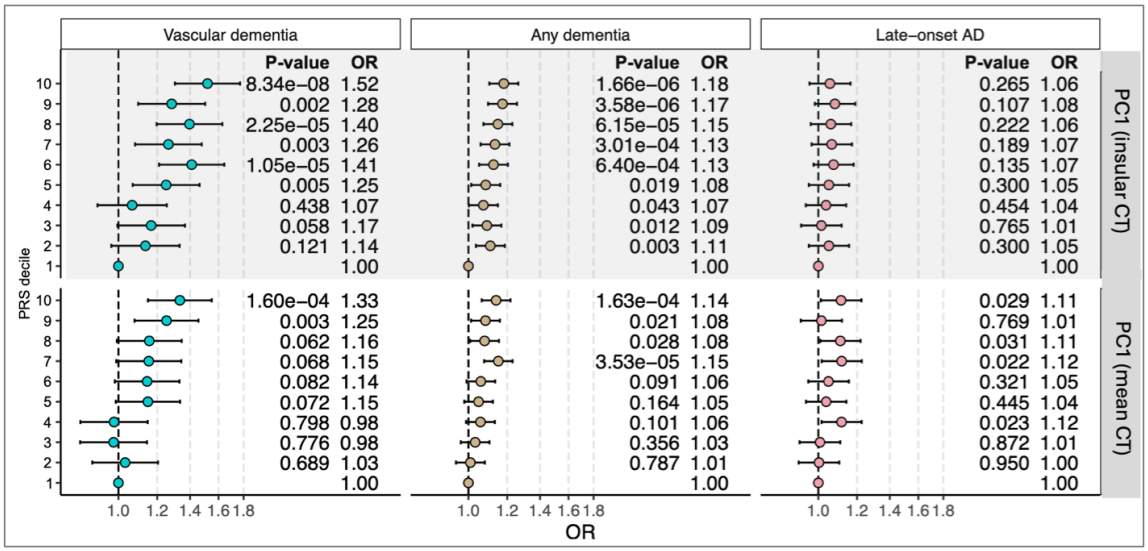


Figure S3. Association between polygenic risk score (PRS) of WMH and insular CT or mean CT-derived PC1 and the risk of vascular dementia, all-cause dementia, and Alzheimer's disease. The odds ratios were calculated in FinnGen (n=500,348) by comparing each of the top nine PRS deciles to the lowest decile and adjusting for age, sex, the first 10 genetic principal components and genotyping arrays.

Reviewer #3

What are the noteworthy results?

- This study performs thorough analyses in a large sample to explore the relationship between White Matter Hyperintensities (WMH) and cortical atrophy and its underlying genetic risk factors and neurobiology.
-A meta-analyses were performed in over 51,000 persons from 10 cohorts showing that a higher White Matter Hyperintensity volume is associated with a lower thickness of the cerebral cortex.
-The meta-GWAS of the shared variance between WMH and insular cortical thickness identified 20 genome-wide significant loci.
-The cellular processes of WMH-associated cortical atrophy involve endothelial cells, pericytes, astrocytes (i.e. small vessel forming cells) and oligodendrocytes (providing support to axons) impacting excitatory neurons with long range axonal projections traversing through the white matter.
-15 of the 20 genome-wide significant loci regulated expression of 54 genes in the cerebral cortex, that, together with their co-expressed genes, were enriched in biological processes of axon0genesis, cytoskeleton organization, and intercellular transport. These processes determine an individual's genetic vulnerability to cortical atrophy in the presence of WMH.

Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references.

-The work contributes to existing literature, by exploring the genetic risk factors and underlying neurobiology of the shared variance between WMH and cortical thickness, rather than investigating one or the other.

Does the work support the conclusions and claims, or is additional evidence needed?

1. The work supports the conclusions drawn. Although important limitations remain regarding choice of certain region-specific cerebral analyses.

Response: Please see our response to Reviewer 2/Comment 5.

Are there any flaws in the data analysis, interpretation, and conclusions? Do these prohibit publication or require revision?

2. The most important design flaw of this study lies in studying the genetic underpinnings of the WMH-cortical thickness association in the insular region specifically, i.e. all analyses represented in Figure 2. The authors try to provide a rationale for the selection of this brain region in particular, by stating that the association between WMH and cortical thickness was strongest in the insula (see Figure 1c). However, looking at the forest plot in Fig 1c the effect sizes of the superior temporal region does not seem to differ significantly from the effect sizes of the insula. The choice for performing GWAS analyses in the insula only is therefore not sufficiently supported by the data.

Response: Please see our response to Reviewer 2/Comment 5. Note that our analyses were not restricted to insula but included all cortical regions, both in a regional (34 regions) and global (mean thickness across the entire cerebral cortex) manner.

3. The cross-sectional versus longitudinal nature of analyses is unclear. E.g. on page 7: "all individuals were stroke and dementia free" versus Figure 2c showing LD-score regression estimates between the GWAS of Principal Component 1 (i.e. the shared variance between WMH and cortical thickness) with various vascular (risk) factors and neurodegenerative traits, including stroke and Alzheimer's Disease. Is this incident stroke and dementia in those cohorts that contributed to this meta-analysis with longitudinal data? Clarify which cohort has contributed with which longitudinal data.

Response: This study was cross-sectional only. We presented genetic correlations between the present GWAS of PC1 and the previously published GWAS of vascular, neurodegenerative, and psychiatric traits, including stroke and Alzheimer's disease. We now specify – for each cohort – the set of individuals that we analyzed in the present study in Supplement.

4. The authors mainly suggest that WMH leads to atrophy because of retrograde degeneration of neuronal bodies in the cerebral cortex. Could these processes however occur in parallel (but may not be necessarily causally related) due to a shared denominator such as other small vessel disease features and/or amyloid-beta pathologies.

Response: These are possible alternative explanations. Please see our response to Reviewer 2, Point 2, which includes additional text (added in the Discussion section) addressing this important issue.

5. Amyloid-beta is not considered in analyses, while it often co-occurs with vascular cerebral damage, including WMH, and is a main driver of cortical atrophy. Although we understand that not all cohorts may have either PET, Cerebral Spinal Fluid and/or blood biomarkers available to determine amyloid-beta status, it should be mentioned in the discussion as a potential important confounding factor in the WMH & atrophy relationship.

Response: Please see our response to Reviewer 2/Comment 4. New analyses included plasma levels of APP.

6. The multi-racial ARIC study is included potentially providing the opportunity to explore the impact of race to findings. Race is known to impact WMH load and dementia prevalence is also unequally distributed by race, underling the relevance considering this factor in analyses (although power may be too limited for all analyses).

Response: All participants in the present study were White Caucasians – this is due to limited availability of brain MRI in other ethnicities.

It is correct that the ARIC study includes 496 Black Americans with brain MRI – this may be a too small sample to draw meaningful conclusions. Of note, WMH, infarcts, and cortical volume were studied in the whole ARIC study previously, but no differences by race were reported[5].

Nevertheless, we fully agree that the lack of other ethnicities is a limitation of our study, as other multi-ethnic research of complex genetic traits indicates that simple trans-ethnic transferability of the results may be limited. For example, Pearson correlation of effect sizes of BP loci between European and African ancestries was only 0.37[37]. We now describe this limitation in text.

Is the methodology sound? Does the work meet the expected standards in your field?

7. Included studies in this meta-analysis have used various methods to determine WMH and atrophy and moreover have varying field strengths, i.e., 1,5 T or 3 T. It should be stated how varying methods across cohorts may have impacted findings.

Response: Stated as requested in Discussion as follows: 'Another potential limitation is the fact that WMH and cortical thickness were assessed with varying methods (e.g., 1.5T or 3T MRI scanners) across cohorts. Although the WMH-cortical thickness associations showed consistent direction of effect and spatial distribution across the cerebral cortex, we cannot exclude entirely the possibility that the varying methods impacted our findings.'

Is there enough detail provided in the methods for the work to be reproduced?

8. More detail should be given on the selection criteria that have been used for each cohort, i.e. a subset with MRI data at baseline? Several cohorts have longitudinal (MRI) data available, but it is unclear if and how this data has been used in analyses.

Response: We now clarify that only cross-sectional data were used in this study. For each cohort, we specify the sample that was used in the present study in Supplement.

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REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have addressed several of my concerns, but some points need clarification.

1. Several of the authors have been co-authors on similar publications. The authors should clarify any overlap.

2. I appreciate the use of the term "all-cause" dementia. However, the refer to "vascular dementia" and Alzheimer's disease. They provide no definitions for these designations.

3. I also appreciate the inclusion of biomarkers. However, those included are specific for Alzheimer's disease.

4. Might be best to leave it as "all-cause dementia".

Reviewer #2 (Remarks to the Author):

The authors have satisfactorily addressed my concerns.

Reviewer #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Thank you for revising your manuscript. You have adequately addressed my concerns and I have no further comments.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have addressed several of my concerns, but some points need clarification.

1. Several of the authors have been co-authors on similar publications. The authors should clarify any overlap.

Response: The neuroCHARGE group has published on WMH before[1], however, this is the first publication by the group to investigate the effect of WMH *on* cortical thickness using genetics.

2. I appreciate the use of the term "all-cause" dementia. However, the refer to "vascular dementia" and Alzheimer's disease. They provide no definitions for these designations.

Response: We have added the International Classification of Disease (ICD) code definitions for the phenotypes “vascular dementia”, “all-cause dementia”, and “late-onset Alzheimer’s disease” in the supplementary material under the project description of the FinnGen study. We have also pasted below, for your convivence.

Phenotype	FinnGen endpoint	Case definition	Control definition
Vascular dementia	F5_VASCDEM	ICD-10: F01 or ICD-9: 4378	Those not listed as cases and not having an entry of ICD-10: F00-F09; ICD-9: 290, 3310, 4378A; or ICD-8: 290.
All-cause dementia	KRA_PSY_DEMENTIA	ICD-10: F00-F09, F05.1, or G30; ICD-9: 290, 2912A, 2828C, 2941A, 3310A, 3311A, or 4378A; ICD-8: 290	Those not listed as cases.
Late-onset Alzheimer’s disease	AD_LO	ICD-10: F00.1*, F00.10*, F00.10*G30.1, G30.1, G30.1+F00.10	Those not listed as cases and not having an entry of ICD-10: G30 or ICD-9: 3310.

3. I also appreciate the inclusion of biomarkers. However, those included are specific for Alzheimer's disease.

Response: Although many of these biomarkers have been implicated in Alzheimer’s disease (AD), they are not specific to AD as one of the key reviews we used to derive them focused on biomarkers for neurodegenerative disease, *in general*[2]. For example, neurogranin (NRGN) is a marker for post-synaptic degeneration/dysfunction and is increased in cases of acute traumatic brain injury[3]. Similarly, GFAP is a marker for reactive astrocytes, and is implicated in many neurodegenerative disorders including frontotemporal dementia (FTD), progressive supranuclear palsy (PSP), and AD[44].

4. Might be best to leave it as "all-cause dementia".

Response: As per our response to Point 2, we have now provided the International Classification of Disease (ICD) code definitions for the phenotypes “vascular dementia”, “all-cause dementia”, and “late-onset Alzheimer’s disease”.

Reviewer #2 (Remarks to the Author):

The authors have satisfactorily addressed my concerns.

Response: Thank you.

Reviewer #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Thank you for revising your manuscript. You have adequately addressed my concerns and I have no further comments.

Response: Thank you.