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Prevailing antagonistic risks in pleiotropic associations with Alzheimer's disease and diabetes

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Abstract

Background: The lack of efficient preventive interventions against Alzheimer's disease (AD) calls for identifying efficient modifiable risk factors for AD. As diabetes shares many pathological processes with AD, including accumulation of amyloid plaques and neurofibrillary tangles, insulin resistance, and impaired glucose metabolism, diabetes is thought to be a potentially modifiable risk factor for AD. Mounting evidence suggests that links between AD and diabetes may be more complex than previously believed.

Objective: To examine the pleiotropic architecture of AD and diabetes mellitus (DM).

Methods: Univariate and pleiotropic analyses were performed following the discovery-replication strategy using individual-level data from 10 large-scale studies.

Results: We report a potentially novel pleiotropic *NOTCH2* gene, with a minor allele of rs5025718 associated with increased risks of both AD and DM. We confirm previously identified antagonistic associations of the same variants with the risks of AD and DM in the *HLA* and *APOE* gene clusters. We show multiple antagonistic associations of the same variants with AD and DM in the *HLA* cluster, which were not explained by the lead SNP in this cluster. Although the ε2 and ε4 alleles played a major role in the antagonistic associations with AD and DM in the *APOE* cluster, we identified non-overlapping SNPs in this cluster, which were adversely and beneficially associated with AD and DM independently of the ε2 and ε4 alleles.

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Conclusions: This study emphasizes differences and similarities in the heterogeneous genetic architectures of AD and DM, which may differentiate the pathogenic mechanisms of these diseases.

Keywords

Alzheimer's disease; diabetes; pleiotropy; apolipoprotein E gene; major histocompatibility complex

INTRODUCTION

Alzheimer's disease (AD) is an age-related neurodegenerative disorder with limited interventions available to partly ameliorate its symptoms. Alzheimer's Association (AA) alarms about sharp increases in AD with age and over time that increase AD-related emotional, physical, and economic burdens on people, their families, societies, and health systems [1]. Prior studies argue that AD is closely related to diabetes [2, 3] implicating AD even as a type 3 diabetes [4, 5].

Diabetes mellitus (DM)—an endocrine/metabolic disorder in aging—occurs when the body becomes resistant to insulin or does not make enough insulin and it can be considered a modifiable risk factor for AD. Insulin plays a key role in the brain including food intake control and regulation of cognitive function. Insulin/insulin-like-growth-factor (IGF) signaling is involved in synaptic formation; neuronal plasticity; learning; memory; neuronal stem cell activation; neurite growth and repair [6]. Insulin resistance induces hyperinsulinemia that leads to inhibition of insulin-degrading enzyme—a regulator of amyloid beta (Aβ) concentrations in neuronal and microglial cells—and decreasing Aβ clearance [7]. Dysfunction of the insulin/PI3K/Akt signaling pathway leads to hyperphosphorylation of the microtubule-associated protein tau in the brain of AD patients through the GSK-3β kinase and the formation of neurofibrillary tangles [8]. Several cardiovascular risk factors can increase the severity of DM and AD including obesity, high blood pressure, high cholesterol, and triglycerides [1]. These and other evidence support insulin deficiency, insulin resistance, and, consequently, impaired glucose metabolism as potential mechanisms linking DM and AD [9–16].

The analyses of summary statistics from genome-wide association studies of AD and DM have been launched to gain insights into potential commonalities and differences in the genetic architectures of these traits. They provided mixed evidence suggesting that some genetic variants could be involved in the regulation of both AD and DM in a concordat manner, while the other variants can confer their risks in an antagonistic manner when the same variant increases the risk of one trait but decreases it for the other trait [17–22]. A growing literature suggests that the links between AD and DM might be more complex than previously believed [22–25].

In this study, we leveraged individual-level data from 10 large-scale datasets, rather than summary statistics from published studies, to identify loci harboring pleiotropic associations of single nucleotide polymorphisms (SNPs) with AD and DM. Having individual-level data,

we examined whether the same variants in each locus conferred risks of AD and DM in a consistent or antagonistic manner.

METHODS

Study cohorts

We used data on populations of European ancestry from three Alzheimer's Disease Centers (ADCs), which are a part of the National Institute on Aging (NIA) Alzheimer's Disease Genetics Consortium (ADGC) initiative [26], the NIA collaborative study from the Alzheimer's Disease Sequencing Project (ADSP) [27], the Atherosclerosis Risk in Communities (ARIC) study [28], the Cardiovascular Health Study (CHS) [29], the Framingham Heart Study (FHS) parental and offspring cohorts [30], the Multi-Site Collaborative Study for Genotype-Phenotype Associations in Alzheimer's disease (GenADA) [31, 32], the NIA Late-Onset Alzheimer Disease Family-Based Study (LOADFBS) [33], the Multi-Ethnic Study of Atherosclerosis (MESA) [34], the Religious Orders Study and Memory and Aging Project (ROSMAP) data [35], and the UK Biobank (UKB) [36]. The basic characteristics of the available samples are given in Table 1.

AD phenotype

We used AD affection status defined by investigators from ADGC, ADSP, FHS, GenADA, LOADFBS, and ROSMAP primarily based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria [37, 38]. In CHS, AD affection status was determined using the International Classification of Disease codes, ninth revision (ICD-9, 331.0). In UKB, AD definition was done using ICD-10 (G30, F00) codes.

DM phenotype

Diabetes mellitus (DM) phenotype was defined based on having fasting blood glucose levels of 126 mg/dl or larger or using glucose-lowering medications in MESA (2003 American Diabetes Association fasting criteria algorithm) [39]. FHS and ARIC defined DM as having fasting blood glucose ≥126 mg/dl, non-fasting blood glucose ≥200 mg/dl, or taking glucose-lowering medications. In CHS and UKB, DM was defined based on ICD-9 (250) and ICD-10 (E11) codes. In LOADFBS and GenADA, DM was self/proxy reported. We did not separate type 1 and type 2 diabetes because the vast majority of DM cases were of type 2 and the largest sample of DM in UKB (Table 1) was defined based on ICD-10 E11 (type 2 diabetes mellitus) code.

Genotypes

To facilitate cross-platform comparison, we imputed SNPs for all studies except UKB using the HRC reference panel at the Michigan Imputation Server (MIS) [40]. The following genotyping arrays were used for imputation: Affymetrix 6.0 (~1M SNPs) chip in ARIC and MESA; Illumina HumanCNV370v1 (~370K SNPs) in the CHS; Affymetrix 500K (~500K SNPs) in FHS and GenADA; Illumina Human 610Quadv1B (~610K SNPs) in the LOADFBS; Illumina Human 660WQuadv1A (~600K SNPs) and Illumina HumanOmniExpress-12v1C (~700K SNPs) in the ADGC; the

quality-controlled whole-genome sequencing (WGS) GATK pipeline in ADSP; WGS, Illumina HumanOmniExpress, and Affymetrix GeneChip 6.0 (from Synapse, https://www.synapse.org/#!Synapse:syn17008936) in the ROSMAP cohort. SNPs, which were submitted to the MIS, were selected by using Rayner's quality control tools with the HRC reference panel (https://www.well.ox.ac.uk/~wrayner/tools/).

For the UKB cohort, the imputation was performed by the UK Biobank team using HRC and UK10K panel (https://biobank.ndph.ox.ac.uk/ukb/label.cgi?id=100319).

The imputation process resulted in about 6742K overlapping SNPs in most cohorts, which were used in the analyses.

Statistical Analyses

We followed the discovery-replication strategy and performed univariate (i.e., phenotype-specific) and pleiotropic analyses as detailed below. The discovery analysis used studies, which included both AD and DM phenotypes, i.e., CHS, FHS, GenADA, LOADFBS, and UKB (Table 1 and Figure 1). For replication analysis, we used independent datasets assessing AD in each of three ADC samples (ADC1, ADC2, and ADC3), ADSP, ROSMAP, and DM from ARIC and MESA.

Our univariate analyses focused on AD and DM separately. We conducted a genome-wide scan at the discovery stage and examined associations for the selected SNPs (selection is detailed in the Results section) at the replication stage in each study (except FHS and LOADFBS) separately using *Plink* [41]. The logistic regression model was adjusted by basic covariates, i.e., sex, age, and first five principal components (PCs) (all studies); year of birth (ARIC, CHS); field center (ARIC, CHS, MESA); an indicator of ROS or MAP sample in ROSMAP. Because FHS and LOADFBS included participants from families, we used models from the GCTA package to adjust for familial correlation [42]. Other adjustments in FHS and LOADFBS included sex, age, first five PCs, and the cohort indicator in FHS. Mediation effects of the lead SNPs in the selected loci were evaluated using the same models with additional adjustment by the lead SNP in each locus separately.

The results of the univariate analyses were aggregated using fixed-effects meta-regression with inverse-variance weighting implemented in METAL software [43]. This test provided summary statistics (effect sizes, standard errors, and *p*-values) for AD and DM separately and for the discovery and replication samples separately.

Pleiotropic meta-analysis was implemented using Fisher's method and an omnibus test to aggregate the summary statistics for AD and DM. Fisher's method [44] combines *p*-values from the meta-analysis of AD and DM assuming that *p*-values are from independent tests. The omnibus test [45–47] adjusts the analyses for correlation between phenotypes and takes into account the direction of the effect. In the case of two phenotypes, it takes the following form:

$$\chi^2_K \sim \left[\left(\hat{z}_1^2\Sigma_{22} - \hat{z}_1\hat{z}_2\Sigma_{21}\right) + \left(\hat{z}_2^2\Sigma_{11} - \hat{z}_1\hat{z}_2\Sigma_{12}\right)\right]/\det\left(\boldsymbol{\Sigma}\right).$$

Here χ^2_K is a chi-squared distribution with K=2 degrees of freedom equal to the number of phenotypes, from which the p-value is calculated. Parameter $\hat{z}_i = \hat{\beta}_i/\hat{\sigma}_i$ is a z-score of the associations of SNPs with an i^{th} phenotype (i=1,2), $\hat{\beta}_i$ is an estimated effect size and $\hat{\sigma}_i$ is its standard error. The $\mathbf{\Sigma}$ is the 2×2 correlation matrix of the z-scores. We evaluated the correlation between AD and DM in each study, in which both phenotypes were available, and constructed one correlation coefficient as an average weighted by the study sample size, r=0.02. The same correlation coefficient was used at the discovery and replications stages. Fisher's method and omnibus test adjust for tests with multiple phenotypes by increasing the degree of freedom.

In the case if the signs of the correlation between AD and DM ($r = \Sigma_{21} = \Sigma_{12}$) and the product of z-scores are the same, the p-value can be larger in the omnibus test than in Fisher's method. This situation indicates mediation pleiotropy. The opposite signs indicate the antagonistic genetic heterogeneity when the p-value can be smaller in the omnibus test than in Fisher's method [48–50].

Pleiotropic associations

Pleiotropic effects can be naturally defined using the inherent property of Fisher's method and the omnibus test, which defines the probability of events. That is, assuming no correlation between phenotypes, a smaller p-value in Fisher's method compared to those in the meta-analyses of AD and DM implies a larger probability of associations with both these phenotypes than the probabilities of the associations with each of these phenotypes separately. The same logic holds for the omnibus test, except that it corrects for correlation between phenotypes.

RESULTS

Univariate and pleiotropic analyses of AD and DM identified 250 SNPs in three loci

Univariate analysis at the discovery stage identified 5186 SNPs with high imputation quality (r^2≥0.75), which attained genome-wide (GW) significance (p<5E-8) in at least one individual study or a univariate meta-analysis across five studies for AD or DM. In addition, we included 54 SNPs for which the significance of the associations was below the GW threshold after removing the estimates from studies with lower imputation quality. These 5240 SNPs were mapped to 79 loci on all chromosomes, except chromosome 21 (Figure 1, Supplementary Table 1).

Pleiotropic meta-analysis at the discovery stage identified that 4333 SNPs in 63 loci attained the GW significance either in Fisher's method or the omnibus test. We further excluded SNPs by requiring that either Fisher's or omnibus p-value was less than p-values in the meta-analysis of AD and DM (see "Pleiotropic associations" section in Methods) and SNPs, which were not available or excluded in the replication studies. This selection resulted in 740 SNPs with pleiotropic associations in 19 loci.

Replication analysis of independent datasets identified a subset of 379 of 740 SNPs in four loci with pleiotropic associations having the same effect directions in the meta-analyses of

each disease separately (AD or DM) at the discovery and replication stages and attaining $p<0.05$ either in Fisher's method or the omnibus test.

Univariate and pleiotropic meta-analyses of the results from the discovery and replication stages confirmed pleiotropic associations for 250 of 379 SNPs by showing smaller p-values in these analyses compared to those at the discovery stage. This set included seven SNPs, —the *APOE* ε4 encoding rs429358 SNP and six more SNPs in the *APOE* cluster,—for which Fisher's, omnibus, and AD p-values were nearly zero, $p<10^{-302}$. We considered them as pleiotropic because they attained either GW (four SNPs) or suggestive-effect (three SNPs with p~1E-7) significance for DM. These 250 SNPs with pleiotropic effects were on chromosomes 1p12 (rs5025718 mapped to *NOTCH2*), 6p21.32, and 6p21.33 (174 SNPs mapped to an *HLA* gene cluster), and 19q13.32 (75 SNPs mapped to an *APOE* gene cluster) (Tables 2 and 3, and Supplementary Table 1).

Pleiotropic association in the NOTCH2 1p12 region

The minor allele of the rs5025718 *NOTCH2* SNP was adversely associated with DM at the GW level (Table 2) showing consistent effect directions in all studies, except LOADFBS (Supplementary Table 1). The same allele was also adversely associated with AD in all studies except GenADA and ADC3.

Antagonistic pleiotropic associations in the APOE and HLA gene clusters

All 75 pleiotropic SNPs in the *APOE* locus showed antagonistic associations with AD and DM, i.e., if a minor allele conferred a risk of AD then the same allele was also associated with decreased risk of DM (Table 2 and Supplementary Table 1). As expected, the *APOE* ε4 allele showed the strongest adverse association with AD, $\beta=1.305$, $p<$1E-302. This allele also showed the most significant favorable association with DM, $\beta=-0.080$, $p=$1.91E-10. Likewise, all 174 SNPs mapped to an *HLA* gene cluster showed antagonistic associations with AD and DM (Table 2 and Supplementary Table 1). Although the most significant associations with AD (rs9275476) and DM (rs9275599) were observed for different SNPs in this cluster, these SNPs were in nearly perfect linkage disequilibrium (LD) with $r^2=87\%$.

The role of sex in the associations of the lead SNPs in the NOTCH2, APOE, and HLA gene clusters with AD and DM.

Consistent with previous studies [51, 52], our analysis fitting the models for each sex separately showed a somewhat smaller (but significantly, $p=$3.32E-3, chi-square test [53]) risk of AD for rs429358 in men than women (Table 2). No other significant differences in the risks of AD or DM between men and women for the same genetic variants were identified.

Mediation of pleiotropic associations by the lead SNPs in the APOE and HLA gene clusters

Next, we examined whether pleiotropic associations of the identified SNPs with AD and DM in the *APOE* and *HLA* gene clusters could be mediated by the lead SNPs. As lead SNPs, we selected ε4-encoding rs429358 in the *APOE* cluster and rs9275476 in the *HLA* cluster.

All 75 pleiotropic SNPs in the *APOE* cluster were associated with AD at the GW level (Supplementary Table 1). After adjustment of the model by rs429358, the significance of all associations decreased, i.e., *p*-values increased (Supplementary Table 2). Still, 36 SNPs were associated with AD at $p<0.05$, including three AD-associated SNPs at the GW level (Table 3 and Supplementary Table 2). For 19 of these 36 SNPs, the effect directions in the rs429358 adjusted model were the same as in the unadjusted model. For the remaining 17 SNPs, including the three AD-associated SNPs at the GW level, the effects changed directions from positive (adverse) association with AD in the unadjusted model to negative (beneficial) association with AD in the rs429358 adjusted model.

These 75 pleiotropic SNPs were associated with DM at $1.91E\text{-}10\leq p\leq 0.0466$ in the unadjusted model (Table 3 and Supplementary Table 1). Only four of them were associated with DM at $p<0.05$ after adjustment by rs429358. The effect directions for them were the same in the adjusted and unadjusted models. Minor alleles of two SNPs—rs440277 and rs73052307—were associated with both AD and DM oppositely at $p<0.05$ in the rs429358 adjusted models.

Of 174 pleiotropic SNPs associated with AD in the *HLA* cluster, 91 SNPs remained significant at $p<0.05$ after adjustment by rs9275476 (Table 3 and Supplementary Table 3). For DM, 105 SNPs were significant at $p<0.05$ after adjustment by rs9275476, including 26 DM-associated SNPs at the GW level. None of the associations with AD or DM changed the directions in the rs9275476 adjusted models. All 91 AD-associated SNPs were also oppositely associated with DM.

Mediation of the associations with AD and DM by the ε4 and ε2 alleles in the APOE cluster

Change of the effect direction for the same allele in the model adjusted for the ε4-encoding rs429358 SNP compared to the unadjusted model suggests a potential role of the ε2 allele (encoded by a minor allele of rs7412). We selected 36 SNPs associated with AD at $p<0.05$ (Table 3 and Supplementary Table 2) and identified 11 clumps (*plink* 1.9) with LD between SNPs from different clumps less than $r^2=0.7$ (Supplementary Table 4). Then, we evaluated associations of 11 SNPs representing these clumps with AD using a model adjusted for rs429358 and rs7412. Favorable GW significant associations with AD—represented by *APOC1* rs438811 SNP ($\beta=-0.322$, $p=1.81E\text{-}11$)—were explained by the protective association of the ε2 allele with AD, i.e., highly significant strong protective effect for rs438811 became weak and non-significant (Table 4). For the other six SNPs favorably associated with AD in the rs429358 adjusted models, adjustment by rs7412 showed no significant associations ($p>0.05$) for three SNPs (rs111371860, rs11668327, rs10119) and significant protective associations for the remaining three SNPs (rs440277, rs118170342, rs1081105). For two (rs10414043 and rs79701229) of the four SNPs adversely associated with AD in the rs429358 adjusted models, adjustment by rs7412 had little effect and resulted in non-significant associations for the remaining two SNPs, rs12972156 and rs2927481 (Table 4).

Of four SNPs associated with DM in the rs429358 adjusted models (Supplementary Table 2, rs57537848, rs11666329, rs440277, rs73052307), we selected three SNPs, as rs57537848 and rs11666329 were in perfect LD ($r^2=100\%$). Adjustment of the rs429358-adjusted

models for DM by rs7412 had a trivial effect on the estimates for these three SNPs (Table 5 and Supplementary Table 2). SNPs associated with AD and DM at $p<0.05$ did not overlap (Tables 4 and 5).

DISCUSSION

Our univariate and pleiotropic analyses following the discovery-replication strategy identified three clusters on chromosomes 1p12 (rs5025718 SNP mapped to *NOTCH2* gene), 6p21.32–33 (174 SNPs mapped to the *HLA* gene cluster), and 19q13.32 (75 SNPs mapped to the *APOE* gene cluster). The results for the *HLA* and *APOE* gene clusters support previous findings, including antagonistic relationships when the same allele conferred a risk of one trait but was beneficially associated with the other trait [17, 22]. Our sex-stratified analysis showed consistent associations of the lead SNPs in these clusters with AD and DM in men and women with the only significant difference indicating a smaller AD risk for rs429358 in men than women [51, 52].

The *NOTCH2* gene appears to be a plausible candidate for pleiotropic predisposition to AD and DM, which was not reported in recent pleiotropic analyses powered by summary statistics from large-scale GWAS of AD and diabetes [17, 54]. Unlike the *HLA* and *APOE* gene clusters, *NOTCH2* was characterized by concordant directions of the associations with AD and DM. The Notch signaling pathway was extensively studied for its links with neurodegeneration, AD, and cardiovascular conditions including diabetes [55–59]. In addition, rs10923931 mapped to the *NOTCH2* gene was reported as a diabetes-associated variant [60]. However, unlike potentially pleiotropic rs5025718 *NOTCH2* SNP (Table 2), rs10923931 was associated with DM in our study (β=0.084, p=1.44E-9), but not with AD (β=0.060, p=0.124).

Our analysis showed that the majority of SNPs in the *HLA* cluster were still associated with AD (91 of 174 SNPs) and DM (105 of 174 SNPs) after adjustment for the potential mediation effect of the lead SNP. We also observed that all 91 AD-associated SNPs were associated with DM in the same antagonistic manner as the lead SNP (Table 2 and Supplementary Table 3). The presence of many disease-associated SNPs in the lead-SNP adjusted analysis and their antagonistic associations with AD and DM is in line with the complex structure of the *HLA* cluster, which includes 224 genes. The critical role of this cluster in the immune system and the dense coverage of the short chromosome region by many genes suggest a complex interplay of different genes in the immune defense that is likely the result of evolutionary adaptation to various conditions in the past [61]. The evolutionary-driven complexity of this cluster makes it difficult to identify a specific pathogenic mechanism of age-related diseases such as AD and DM [62]. This complexity also suggests that disease risks can be attributed not only to individual variants but also to their combinations [61].

In the *APOE* gene cluster, the major contribution to the AD and DM risks was from the ε4 allele, as evidenced by the fact that adjustment of the model by the ε4-encoding rs429358 SNP resulted in weaker associations for the other SNPs, many of which became non-significant. However, our analysis also showed GW's significant favorable associations

with AD (Table 3) even though the same variants were adversely associated with AD in the ε4-unadjusted model at the GW level. These GW significant favorable associations were explained by the contribution of the ε2 allele as they became non-significant after adjustment by rs7412 (Table 4, rs438811). Nevertheless, our analysis conditional on rs429358 and rs7412 SNPs identified the ε4- and ε2- independent adverse and beneficial associations with AD and DM. Unlike the *HLA* gene cluster, SNPs associated with AD and DM in the *APOE* gene cluster did not overlap (Tables 4 and 5). Antagonistic associations of the ε4 allele with AD and DM in the *APOE* cluster, complemented by the non-overlapping AD- and DM-associated SNPs, support different genetic mechanisms for AD and DM in this cluster.

Prior epidemiological studies suggested links between DM and AD (see Introduction). Mounting evidence indicates, however, that DM may be tighter linked to non-AD neuropathology, such as cerebrovascular pathology and vascular dementia, rather than AD pathology [23–25]. These insights from neuropathological studies are complemented by the results of correlation analyses, which suggest a weak genetic and phenotypic correlation between AD and DM, e.g., r=0.09 [63], and phenotypic, e.g., r=0.02 (in this study and in [48]). These results are further complemented by the increasing body of studies, including the current work, reporting antagonistic associations of the same genetic variants with AD and DM [17, 22]. Mechanisms driving divergence between the AD and DM pathologies are currently unclear; this divergence may impact, however, potential interventional strategies aimed to manage DM-related conditions to affect the incidence of AD [22].

Deeper analyses of the genetic architectures of AD and DM may help in identifying the overlap and divergence of pathogenic mechanisms of these highly heterogeneous diseases. Possible explanations of the divergence and overlap could be related to more pronounced roles of combinations of genetic variants in the form of haplotypes or combinations of genotypes, than previously believed, in susceptibility to AD and DM. Indeed, prior studies showed the substantial impact of such combinations on the AD risk [64–68], including strong modulation of the association of the ε4 allele with AD by other variants in the *APOE* gene cluster [69]. The effects of combinations of genetic variants can be driven by evolutionary adaptation and survival selection during the life course. These genetic mechanisms can be affected by environmental factors contributing to AD and DM heterogeneity. Discovering such genetic mechanisms will contribute to identifying more homogeneous pathogenic mechanisms of AD and DM.

Concluding, our study leveraging individual-level data reports a potentially novel pleiotropic *NOTCH2* gene, on chromosome 1p12, with a minor allele of rs5025718 concordantly conferring risks of both AD and DM. Our study also supports antagonistic associations of the same variants with the risks of AD and DM in the *HLA* and *APOE* gene clusters previously reported by studies utilizing summary statistics from large-scale meta-analyses. Access to the individual-level data allowed us to identify the effects of SNPs, which were independent of the lead SNPs in the *HLA* and *APOE* gene clusters. We report antagonistic associations of the same variants with the risks of both AD and DM in the *HLA* gene clusters, independently of the lead SNP. We also found non-overlapping SNPs in the *APOE* gene cluster, which effects were not explained by the ε2 and ε4 alleles. This study supports

the view that pathogenic mechanisms of highly heterogeneous conditions such as AD and DM may have different origins. Dissecting overlaps and differences of these mechanisms may be facilitated by the analyses of the roles of combinations of genetic variants in the form of haplotypes or combinations of genotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

This article was prepared using data obtained through dbGaP (accession numbers phs000372.v1 [ADGC], phs000572, v.8 [ADSP], phs000280, v.5 [ARIC], phs000007.v32 [FHS], phs000287.v7 [CHS], phs000219, v.1 [GenADA], phs000168.v2 [LOADFBS], phs000209, v.13 [MESA]), Synapse (https://www.synapse.org/#!Synapse:syn3219045) (ROSMAP), and UK biobank repository (UKB). See the extended acknowledgment in the Supplemental Acknowledgement file.

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Data Availability Statement:

The data were obtained through designated repositories with controlled access. Data users cannot share this data.

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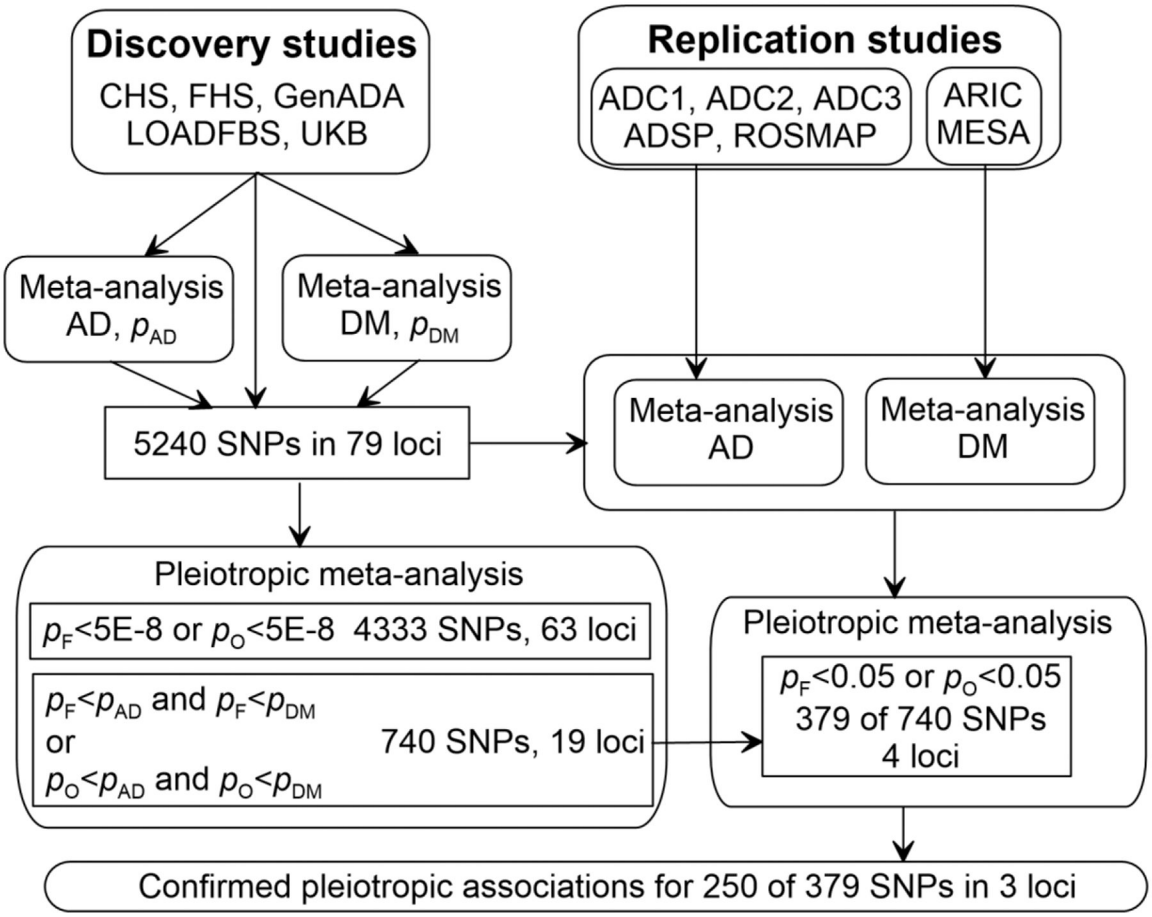

Figure 1. A chart representing the flow of the analyses.

Symbols p_{AD}, p_{DM}, p_F, and p_O denote values from the univariate meta-analyses of Alzheimer's disease (AD) and diabetes mellitus (DM) and pleiotropic meta-analysis using Fisher's method and the omnibus test, respectively. Study abbreviations are given in Table 1.

Table 1.

Basic characteristics of genotyped participants of European ancestry in the selected studies.

Sample	All			AD cases			DM cases		
	N	Men (%)	Age, mean (SD), years	N	Men (%)	Age, mean (SD), years	N	Men (%)	Age, mean (SD), years
ADGC	5349	44.59	78.53 (8.5)	3585	46.97	79.72 (7.71)			
ADSP	1568	60.52	76.86 (10.92)	998	61.72	74.20 (12.02)			
ARIC	9631	47.00	75.34 (6.85)				2044	53.91	75.23 (7.03)
CHS	3262	39.79	83.63 (5.16)	201	34.83	85.35 (4.86)	440	50.68	83.08 (5.11)
FHS	3761	43.84	79.23 (10.13)	361	31.02	89.41 (6.94)	660	56.52	79.48 (9.06)
GenADA	1588	38.98	75.76 (8.59)	806	42.18	78.05 (8.60)	143	44.76	77.23 (7.37)
LOADFBS	3299	38.83	75.62 (11.61)	1507	35.70	85.50 (8.45)	309	52.10	77.99 (10.01)
MESA	2526	47.74	72.69 (9.92)				382	53.14	73.10 (9.77)
ROSMAP	871	32.95	86.28 (4.65)	486	30.25	87.31 (3.91)			
UKB, 60+	333208	45.99	69.32 (5.10)	913	51.04	72.67 (4.63)	24723	61.22	68.78 (6.85)

AD denotes Alzheimer's disease; DM denotes diabetes mellitus; SD denotes standard deviation.

ADGC is the National Institute on Aging (NIA) AD Genetics Consortium (ADGC) dataset, which includes data from three AD Centers (ADC1, ADC2, and ADC3); ADSP is the NIA AD Sequencing Project; ARIC is the Atherosclerosis Risk in Communities study; CHS is the Cardiovascular Health Study; FHS is the Framingham Heart Study parental and offspring cohorts; GenADA is the Multi-Site Collaborative Study for Genotype-Phenotype Associations in AD; LOADFBS is the NIA Late-Onset Alzheimer Disease Family-Based Study; MESA is the Multi-Ethnic Study of Atherosclerosis; ROSMAP is the Religious Orders Study and Memory and Aging Project; and UKB is the UK Biobank dataset.

Age was defined as the age of death or censoring for ADGC, ARIC, CHS, FHS, MESA, ROSMAP, and UKB. For LOADFBS and GenADA, age was provided at the launch of the study. ADSP defined age as the age at the onset of AD or the age at the last follow-up.

The UKB sample was limited to subjects who were 60 years and older.

Blank cells indicate not available information.

Table 2.

The results of the univariate and pleiotropic meta-analyses of the associations of the lead SNPs in three clusters with Alzheimer's disease (AD) and diabetes mellitus (DM) from the discovery and replication samples.

SNP ID	Position	Cytoband	Cluster	Alleles	Sex	Pleiotropy		Univariate AD			Univariate DM		
						P_{Fisher}	$P_{omnibus}$	β_{AD}	SE_{AD}	P_{AD}	β_{DM}	SE_{DM}	P_{DM}
rs5025718	119935662	1p12	*NOTCH2*	C/T	M&W	3.88E-10	6.37E-10	0.111	0.038	3.48E-03	0.076	0.013	4.30E-09
					W	1.21E-04	1.02E-04	0.058	0.060	3.36E-01	0.071	0.017	2.88E-05
					M	1.93E-06	3.10E-06	0.150	0.052	4.06E-03	0.084	0.020	2.80E-05
rs9275476	32704847	6p21.32	*HLA*	T/C	M&W	3.38E-25	1.62E-25	−0.241	0.042	1.16E-08	0.125	0.014	4.75E-19
					W	4.43E-13	3.22E-13	−0.216	0.064	7.42E-04	0.122	0.018	1.81E-11
					M	1.13E-12	9.82E-13	−0.261	0.059	1.03E-05	0.129	0.022	3.44E-09
rs9275599	32714652	6p21.32	*HLA*	C/T	M&W	6.73E-24	3.40E-24	−0.205	0.042	1.20E-06	0.130	0.014	9.63E-20
					W	4.28E-14	2.66E-14	−0.205	0.065	1.46E-03	0.133	0.019	8.31E-13
					M	4.13E-10	3.31E-10	−0.192	0.059	1.08E-03	0.126	0.022	1.48E-08
rs429358	44908684	19q13.32	*APOE*	T/C	M&W	<1E-302	<1E-302	1.305	0.031	<1E-302	−0.080	0.013	1.91E-10
					W	1.42E-136	4.84E-138	1.179	0.048	5.57E-133	−0.080	0.016	7.98E-07
					M	1.89E-210	2.58E-212	1.371	0.044	6.58E-209	−0.079	0.020	5.86E-05

Column **Position** is a chromosome position in base pairs given in Genome Reference Consortium Human Build 38 (GRCh38).

Column **Alleles** shows major/minor alleles. A minor allele was used as an effect allele.

Column **Sex** indicates samples of men (M) and women (W) combined (M&W) and separately.

Column **Pleiotropy** shows *p*-values from the pleiotropic meta-analysis using Fisher's method (P_{Fisher}) and the omnibus test ($P_{omnibus}$).

Columns **Univariate AD** and **Univariate DM** show effect sizes (β), standard errors (SE), and *p*-values (P) from the univariate meta-analysis of AD and DM, respectively.

Table 3.

Summary information on 249 SNPs showing pleiotropic associations with Alzheimer's disease (AD) and diabetes mellitus (DM) in the *APOE* and *HLA* gene clusters.

Disease	Chr	Cluster	Lead-SNP-unadjusted models		Lead-SNP-adjusted models	
			N_{SNPs}	Significance	N_{SNPs}	Significance
AD	6	*HLA*	174	1.16E-8≤*p*≤3.29E-2	91	3.68E-3≤*p*≤4.69E-2
DM	6	*HLA*	174	9.63E-20≤*p*≤3.79E-7	105	7.47E-12≤*p*≤3.90E-2
AD	19	*APOE*	75	*p*≤1.70E-14	36	1.81E-11≤*p*≤4.74E-2
DM	19	*APOE*	75	1.91E-10≤*p*≤4.66E-2	4	1.76E-2≤*p*≤4.27E-2

Chr denotes chromosome

Columns **Lead-SNP-unadjusted models** and **Lead-SNP-adjusted models** show the number of AD- or DM-associated SNPs (N_{SNPs}) and the range of *p*-values in the univariate meta-analyses of the discovery and replication samples in *HLA* and *APOE* gene clusters. Detailed information on the univariate and pleiotropic associations is given in Supplementary Table 1 for the lead-SNP-unadjusted models and Supplementary Tables 2 and 3 for the lead-SNP-adjusted models in the *APOE* and *HLA* gene clusters, respectively.

Table 4.

The results of the meta-analysis of the associations of SNPs representing 11 clumps in the *APOE* gene cluster on chromosome 19 with Alzheimer's disease after adjustment for the ε4- and ε2-encoding SNPs.

SNP ID	Position	Alleles	Gene	Gene distance	*β*	SE	*P*-value	Direction	I^2	*P*-het
rs2927481	44833992	C/A	NECTIN2	12305	0.039	0.029	1.72E-01	++-?+++-+-	0.3	4.31E-01
rs111371860	44842530	A/T	NECTIN2	3767	−0.109	0.066	9.75E-02	+---+-?-+-	65.9	2.81E-03
rs440277	44857967	G/A	NECTIN2	0	−0.058	0.029	4.03E-02	--+----+--	0	5.72E-01
rs79701229	44881674	G/A	NECTIN2	0	0.209	0.091	2.15E-02	+++---+-++	0	5.45E-01
rs12972156	44884202	C/G	NECTIN2	0	0.081	0.046	7.64E-02	+++-++?-++	49.8	4.33E-02
rs11668327	44895376	G/C	TOMM40	0	−0.036	0.041	3.86E-01	--+--+?++-	3.3	4.07E-01
rs118170342	44898611	T/C	TOMM40	0	−0.123	0.062	4.76E-02	------?++-	0	6.38E-01
rs10119	44903416	G/A	TOMM40	0	−0.041	0.041	3.18E-01	+-+--+?-+-	46.6	5.97E-02
rs1081105	44909698	A/C	APOE	305	−0.174	0.067	9.90E-03	------?+--	41.1	9.34E-02
rs10414043	44912456	G/A	APOC1	1869	0.136	0.056	1.55E-02	+++-++?-++	39.9	1.01E-01
rs438811	44913484	C/T	APOC1	841	−0.057	0.070	4.21E-01	+?+---?-++	81.5	3.24E-06

Column **Position** is a chromosome position in base pairs given in Genome Reference Consortium Human Build 38 (GRCh38).

Column **Alleles** shows major/minor alleles. A minor allele was used as an effect allele.

SE denotes a standard error.

Column **Direction** shows signs of the effects in individual cohorts, ordered as ADC1, ADC2, ADC3, ADSP, CHS, FHS, GENADA, LOADFS, ROSMAP, and UKB (see Table 1 footnotes for abbreviations). Positive (+) and negative (−) signs indicate positive and negative values of beta. Question mark (?) refers to not available estimates.

I^2 is a heterogeneity coefficient across cohorts; P-het is the heterogeneity *p*-value.

Table 5.

The results of the meta-analysis of the associations of SNPs with diabetes mellitus in the *APOE* gene cluster on chromosome 19 adjusted for the ε4- and ε2-encoding SNPs.

SNP ID	Position	Alleles	*β*	SE	*P*-value	Direction	I^2	*P*-het
rs11666329	44851039	G/A	−0.022	0.009	1.55E-02	--+----	26.6	2.26E-01
rs440277	44857967	G/A	0.019	0.010	4.29E-02	-++++++	0	6.55E-01
rs73052307	44881148	T/C	0.030	0.013	2.66E-02	--++++-	12.8	3.32E-01

Column **Position** is a chromosome position in base pairs given in Genome Reference Consortium Human Build 38 (GRCh38). All SNPs are mapped to the *NECTIN2* gene.

Column **Alleles** shows major/minor alleles. A minor allele was used as an effect allele.

SE denotes a standard error.

Column **Direction** shows signs of the effects in individual cohorts, ordered as ARIC, CHS, FHS, GENADA, LOADFS, MESA, and UKB (see Table 1 footnotes for abbreviations). Positive (+) and negative (−) signs indicate positive and negative values of beta. Question mark (?) refers to not available estimates.

I^2 is a heterogeneity coefficient across cohorts; P-het is the heterogeneity *p*-value.