An analysis of genetically regulated gene expression across multiple tissues implicates novel gene candidates in Alzheimer’s disease

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Abstract

Introduction: Genome-wide association studies (GWAS) have successfully identified multiple independent genetic loci that harbour variants associated with Alzheimer’s disease, but the exact causal genes and biological pathways are largely unknown.

Methods: To prioritise likely causal genes associated with Alzheimer’s disease, we used S-PrediXcan to integrate expression quantitative trait loci (eQTL) from the Genotype-Tissue Expression (GTEx) study and CommonMind Consortium (CMC) with Alzheimer’s disease GWAS summary statistics. We meta-analysed the GTEx results using S-MultiXcan, prioritised disease-implicated loci using a computational fine-mapping approach, and performed a biological pathway analysis on the gene-based results.

Results: We identified 126 tissue-specific gene-based associations across 48 GTEx tissues, targeting 50 unique genes. Meta-analysis of the tissue-specific associations identified 73 genes whose expression was associated with Alzheimer’s disease. Additional analyses in the dorsolateral prefrontal cortex from the CMC identified 12 significant associations, 8 of which also had a significant association in GTEx tissues. Fine-mapping of causal gene sets prioritised gene candidates in 10 Alzheimer’s disease loci with strong evidence for causality. Biological pathway analyses of the meta-analysed GTEx data and CMC data identified a significant enrichment of Alzheimer’s disease association signals in plasma lipoprotein clearance, in addition to multiple immune-related pathways.

Conclusions: Gene expression data from brain and peripheral tissues can improve power to detect regulatory variation underlying Alzheimer’s disease. However, the associations in peripheral tissues may reflect tissue-shared regulatory variation for a gene. Therefore, future functional studies should be performed to validate the biological meaning of these associations and whether they represent new pathogenic tissues.

Keywords: Alzheimer’s disease, Gene expression, Genome-wide association study, Genetics, Genetic epidemiology, Computational biology
Background

An estimated 5.5 million Americans were living with Alzheimer’s disease in 2017, with a prevalence of 10% for people over the age of 65 years [1]. In the absence of a significant medical breakthrough, the number of people living with Alzheimer’s disease is estimated to reach 13.8 million in the USA alone by 2050 [1]. Alzheimer’s disease is the sixth leading cause of death in the USA, but this is likely to be an underestimation as complications of the disease, such as pneumonia, are often recorded as the primary cause of death. Alzheimer’s disease is characterised by neuronal death and key neuro-pathological changes, including the deposition of β-amyloid and hyperphosphorylated tau tangles. Genome-wide association studies (GWAS) have identified genetic risk factors for Alzheimer’s disease and provided novel insights into disease aetiology. A GWAS meta-analysis of 74,046 individuals (25,580 cases and 48,466 controls) identified 19 genetic risk loci [2], which has since increased to some 24 loci with larger sample sizes [3]. Biological pathway analyses of these data implicate the immune system and lipid metabolism as well as tau binding and amyloid precursor protein metabolism [2], although a disease mechanism of action has yet to be established.

In GWAS, significant associations are reported for a single nucleotide polymorphism (SNP) with the lowest $P$ value, but the signal could be explained by one (or more) variant within the linkage disequilibrium block where that SNP resides. Furthermore, GWAS loci may contain multiple genes or regions that affect the expression of other genes. Additional analyses are required to elucidate the biological mechanisms that underlie statistical associations between genetic variants and disease risk. One method is to identify loci where SNP variation is associated with differences in gene expression, called expression quantitative trait loci (eQTL). Genome-wide gene expression data has been successfully integrated with SNP genotype data to prioritise risk genes and reveal possible mechanisms underlying susceptibility to a range of psychiatric disorders [4–7]. This approach may be performed in cases and controls for whom both gene expression and SNP genotype data are available. However, these data sets are likely to have limited sample size and suffer from confounding from reverse causality as variation in gene expression may be influenced by disease status or drug treatment.

An alternative method is to integrate GWAS findings with independent gene expression data provided by large international consortia, such as the multi-tissue Genotype-Tissue Expression (GTEx) project [8] and the Common-Mind Consortium (CMC). GTEx (version 7) contains SNP genotype data linked to gene expression across 53 tissues from 714 donors, including 13 brain regions, and the CMC contains gene expression data from the dorsolateral prefrontal cortex of 646 donors. These data represent a valuable resource with which to quantify the association between genetically regulated expression in multiple tissues and the phenotype of interest. Association testing can be carried out using a gene-based approach implemented by transcriptomic imputation approaches [5, 9, 10] which reduce the high level of multiple testing from single-variant tests and increase power to identify trait-associated loci both from a strong functional SNP signal and from a combination of modest signals. The application of transcriptomic imputation using GWAS summary statistics without the need for individual-level data allows these methods to be applied to large-scale GWAS meta-analysis results. Here, we apply a transcriptomic imputation approach called S-PrediXcan to Alzheimer’s disease GWAS summary statistics in order to explore the genetic component of gene expression associated with the disorder. We then use these data in a fine-mapping approach to prioritise candidate causal genes with disease-implicated loci, and identify peripheral tissues that might provide biologically meaningful information on Alzheimer’s disease pathways and processes.

Materials and methods

Alzheimer’s disease GWAS summary statistics

Detailed methods, including a description of population cohorts, quality control of raw SNP genotype data, and association analyses for the Alzheimer’s disease GWAS, are described in detail elsewhere [2]. The Alzheimer’s disease GWAS, performed by members of the International Genomics of Alzheimer’s Project (IGAP), included an initial meta-analysis of 4 samples of European ancestry (17,008 cases and 37,154 controls) followed by an analysis of moderately associated SNPs ($P < 1 \times 10^{-3}$) in an independent sample of 8572 cases and 11,312 controls of European ancestry. All cases received clinical confirmation of late-onset Alzheimer’s disease. SNPs were imputed using the European population reference from the 1000 Genomes Project 2010 interim release based on the sequence data freeze from 4 August 2010 and phased haplotypes from December 2010 [11]. Logistic regression association tests were conducted for imputed marker dosages with age and sex as covariates, as well as principal components to control for possible population stratification. Summary statistics for 7,055, 881 autosomal SNPs were made available by IGAP and were utilised in our study.

Identification of genes with differential expression levels between Alzheimer’s disease cases and controls

We used S-PrediXcan to integrate eQTL information with GWAS summary statistics to identify genes of which genetically predicted expression levels are associated with
Alzheimer’s disease status. S-PrediXcan estimates gene expression weights by training a linear prediction model in a reference sample with both gene expression and SNP genotype data. The weights are used to predict gene expression from GWAS summary statistics, while incorporating the variance and co-variance of SNPs from a linkage disequilibrium (LD) reference panel. We used expression weights for 48 tissues with S-PrediXcan expression weights from the GTEx Project (version 7), the dorsolateral prefrontal cortex from the CommonMind Consortium (CMC), and LD information from the 1000 Genomes Project Phase 3 [11]. These data were processed with beta values and standard errors from the Alzheimer’s disease GWAS to estimate the expression-GWAS association statistic.

To increase power to identify genes whose expression is similarly differentially regulated across tissues, we meta-analysed GTeX S-PrediXcan results using the S-MultiXcan algorithm [12]. We used Bonferroni correction to adjust for the number of tests performed within each tissue as well as across all tissues and genes (Table S1).

Results

A cross-tissue transcriptome-wide association study identifies peripheral tissues enriched with Alzheimer’s disease association signals

Using S-PrediXcan, we identified 126 significant associations (Supplementary Table S2) targeting 50 unique genes (Supplementary Table S3) after multiple testing correction for all genes and tissues ($P < 2.68 \times 10^{-5}$). Among significant associations, there was a slight bias towards positive $Z$ scores ($N = 75$ [60%]). The number of significant associations per tissue was largely a function of sample size, with the skin (sun-exposed lower leg) (number of RNA-seq samples $N = 473$) harbouring the largest number of associations ($n = 9$), followed by the lung ($n = 8$) (Table 2) (Supplementary Figure S1). For significant genes identified in multiple GTeXx tissues, effect directions were largely consistent across tissues (Fig. 1). The most significant gene association in GTeX data was for APOE; genetic variants associated with increased Alzheimer’s disease risk are predicted to down-regulate expression levels of APOE in three peripheral tissues, including the sun-exposed skin ($Z = 19.50, P = 1.03 \times 10^{-64}$) and the non-sun-exposed skin ($-16.56, P = 1.27 \times 10^{-61}$) (Table 1; Supplementary Table S3) after multiple testing correction (Bonferroni correction for 186,230 tests $[0.05/186,230] \ P < 2.68 \times 10^{-5}$). Of note, although APOE is expressed more widely in the brain compared to most other tissues (Supplementary Figure S2), the eQTL associations with APOE are only found in non-brain tissues. While these associations are likely to be due, at least in part, to the increased sample size (and therefore statistical power) of peripheral tissues, they highlight the importance of interrogating multiple (accessible) tissues in eQTL analyses of complex (brain-related) traits.

We removed genes flanking the APOE region ($\pm 500$ kb) due to its strong association with Alzheimer’s disease and identified 29 significant associations (Supplementary Table S3). The most significant gene outside the APOE region was the vasodilator-stimulated phosphoprotein VASP ($Z = -11.30, P = 1.24 \times 10^{-24}$) in the testis (Table 1). The most significant association outside chromosome 19 was observed for the clusterin CLU in the skin (sun-exposed lower leg) (Table 1; Supplementary Table S3). Taken together with findings for APOE, these data suggest the skin (together with other peripheral tissues) may be used as an accessible surrogate tissue for peripheral biomarker discovery and molecular studies of causal disease processes (Table 2).

To improve power relative to the single-tissue analyses, we combined results from different single-tissue models into a single aggregate statistic using S-MultiXcan. We identified 73 gene-level S-MultiXcan associations after correction for multiple testing.

Pathway analysis of gene-based analyses

We performed a biological pathway analysis using generalised linear model regression, with the Z score from the GTeX S-MultiXcan or CMC S-PrediXcan association data as the dependent variable and membership in Reactome pathways as a linear predictor. Pathways containing fewer than 10 cis-heritable genes (i.e. genes whose average expression across tissues is influenced by proximal $<1$ Mb from the gene start or end) SNPs were removed, resulting in 1318 biological pathways for pathway enrichment analysis. A Bonferroni-corrected $P$ value of $P = 3.79 \times 10^{-5}$ (adjusting for 1318 tested pathways) was used to correct for multiple testing.
Table 1: Top 5 S-PrediXcan associations by APOE region

| Gene   | Chr | Most significant tissue | SNPs | Tissues | Z score   | P value       |
|--------|-----|-------------------------|------|---------|-----------|--------------|
| Inside APOE |     |                         |      |         |           |              |
| APOE   | 19  | Skin, sun-exposed leg   | 10   | 3       | −19.50    | 1.03 × 10^{-84} |
| NECTIN2| 19  | Oesophagus muscularis   | 3    | 8       | −19.28    | 8.32 × 10^{-83} |
| APOC1  | 19  | Adrenal gland           | 3    | 3       | −19.13    | 1.48 × 10^{-81} |
| BLOC1S3| 19  | Oesophagus muscularis   | 9    | 1       | −15.63    | 4.29 × 10^{-55} |
| RELB   | 19  | Lung                    | 24   | 1       | 11.55     | 7.14 × 10^{-31} |
| Outside APOE |    |                         |      |         |           |              |
| VASP   | 19  | Testis                  | 53   | 1       | −11.30    | 1.24 × 10^{-29} |
| SIX5   | 19  | Skin, sun-exposed leg   | 40   | 2       | 10.28     | 8.60 × 10^{-25} |
| CD3EAP | 19  | Substantia nigra        | 6    | 1       | 10.06     | 8.65 × 10^{-24} |
| ZNF155 | 19  | Minor salivary gland    | 62   | 2       | −8.45     | 3.02 × 10^{-17} |
| CLU    | 2   | Skin, sun-exposed leg   | 5    | 2       | 8.22      | 2.04 × 10^{-16} |

Chr chromosome; N SNPs are the number of eQTLs included in the MetaXcan prediction model; N tissues are the N tissues with P value < 7.63 × 10^{-7}; Z score represents the strength of association between gene expression and disease risk. Positive values indicate that an increased level of gene expression is associated with increased disease risk, while negative values indicate that a reduced level of gene expression increases disease risk.
Table 2 Number of significant S-PrediXcan associations per tissue

| Tissue                                      | Tissue sample size (N) | Gene associations (N) | Genes                                                                 |
|---------------------------------------------|------------------------|-----------------------|----------------------------------------------------------------------|
| Skin, sun-exposed lower leg                 | 414                    | 10                    | APOE, APOC1, NECTIN2, SIX5, CLU, CLPTM1, ZNF229, ZYX, PPP1R13L, KLC3 |
| Lung                                        | 383                    | 8                     | RELB, APOE, CEACAM19, APOC2, APOC1, APOC4, MS4A2, DMPK              |
| Oesophagus mucosa                           | 358                    | 8                     | PPP1R13L, KLC3, EPHA1, ZNF234, MS4A2, RP11-385F7.1, TOMM40, PVR    |
| Oesophagus muscularis                       | 335                    | 6                     | NECTIN2, BLOC1S3, CR1, CEACAM19, BIN1, PVR                          |
| Skin, not sun-exposed suprapubic            | 335                    | 6                     | APOE, APOC2, ZNF229, CLPTM1, MS4A2, PVR                             |
| Adrenal gland                               | 175                    | 4                     | APOC1, APOC4, QPCTL, CEACAM19                                      |
| Brain hippocampus                           | 111                    | 4                     | CEACAM19, CR1, NECTIN2, HLA-DQA2                                    |
| Pancreas                                    | 220                    | 4                     | CEACAM19, CBLC, FO8, BCAM                                          |
| Spleen                                      | 146                    | 4                     | PVR, FZD4, CEACAM19, SIX5                                          |
| Stomach                                     | 237                    | 4                     | MS4A2, ZNF45, CBLC, CEACAM19                                       |

Table 3 Top 5 S-MultiXcan associations by APOE region

| Gene       | Top tissue               | N tissues | P             | Z score | Min  | Max  | Mean | SD  |
|------------|--------------------------|-----------|---------------|---------|------|------|------|-----|
| Inside APOE|                          |           |               |         |      |      |      |     |
| PVRL2      | Oesophagus muscularis    | 17        | 2.64 × 10^{-11} | −19.28  | 5.78 | 4.94 |      |     |
| APOE       | Skin, sun-exposed leg    | 7         | 4.25 × 10^{-10} | −19.50  | 7.51 | 3.58 | 10.50|     |
| APOC1      | Adrenal gland            | 4         | 4.05 × 10^{-9}  | −19.13  | 5.98 | 6.24 | 13.43|     |
| BLOC1S3    | Oesophagus muscularis    | 6         | 9.00 × 10^{-7}  | −15.63  | 3.48 | 1.78 | 7.07 |     |
| APOC4      | Adrenal gland            | 4         | 1.40 × 10^{-5}  | −9.53   | 5.91 | 0.92 | 7.96 |     |
| Outside APOE|                         |           |               |         |      |      |      |     |
| SIX5       | Skin, sun-exposed leg    | 4         | 1.24 × 10^{-3}  | −6.18   | 10.3 | 0.41 | 7.40 |     |
| VASP       | Testis                  | 3         | 5.58 × 10^{-28} | −11.3   | 2.61 | 2.29 | 7.82 |     |
| BIN1       | Oesophagus muscularis    | 23        | 3.58 × 10^{-16} | −6.32   | 4.09 | 2.21 | 3.26 |     |
| CLU        | Skin, sun-exposed leg    | 8         | 7.51 × 10^{-14} | −3.07   | 8.22 | 1.28 | 4.12 |     |
| CR1        | Oesophagus muscularis    | 7         | 1.69 × 10^{-11} | −0.38   | 7.33 | 4.29 | 3.45 |     |

N tissues, number of tissues with significant gene-based association; Z score, minimum, maximum, mean, and standard deviation of the Alzheimer’s disease association coefficient from S-MultiXcan.

A comparison of multi-tissue GTEx results with brain-specific eQTL database from the CommonMind consortium

We performed an S-PrediXcan analysis using expression weights for a single brain region (dorsolateral prefrontal cortex) collected by the CMC and identified 12 significant (P < 5.08 × 10^{-6}) gene-based associations (Supplementary Table S5). We compared these data with the meta-analysed results from 48 tissues in GTEx (Table 4). Of 12 significant gene-based associations in CMC, 8 also showed a significant association in GTEx tissues (P < 1.93 × 10^{-6}). The Z scores between CMC and GTEx were concordant where the mean absolute GTEx Z score was ≥ 1, highlighting the consistency of the datasets. The CMC results were influenced by APOE, with the top association (TOMM40; P = 1.37 × 10^{-101}) located within the APOE gene cluster on chromosome 19q13.
GTEx tissue with the strongest association for TOMM40 was the oesophagus mucosa (Z score = 5.57, \( P = 2.61 \times 10^{-8} \)) (Supplementary Table S2), a tissue that contains over twice the number of samples as the largest brain tissue (\( N = 407 \) versus \( N = 173 \) in the cerebellum). One TOMM40 association was observed in GTEx brain tissue (putamen basal ganglia); the association was insignificant (\( P = 6.73 \times 10^{-2} \)), but the Z score direction of effect was consistent with CMC data (GTEx: Z = -1.83; CMC = -21.40).

Fine-mapping further prioritises genes within GWAS risk loci

We applied the fine-mapping of causal gene sets (FOCUS) algorithm to prioritise genes within GWAS risk loci. Genes with a higher posterior inclusion probability tended to have a higher S-PrediXcan Z score (Spearman correlation = 0.8269, \( P = 1.64 \times 10^{-87} \)) (Fig. 2). Candidate casual genes not nearest the GWAS index SNP included GRIK4 (SROL1 locus; S-PrediXcan Z score = 5.16, PIP 0.985) and UNC79 (SLC24A4 locus: S-PrediXcan Z score 4.77; PIP 0.793) (Supplementary Table S6). Both GRIK1 and UNC79 are involved in ion transmembrane transport and were not prioritised as likely causal genes in a recent GWAS of Alzheimer’s disease [3], highlighting the potential utility of FOCUS in gene prioritisation.

A biological pathway analysis identifies altered expression of lipoprotein clearance pathways in Alzheimer’s disease

We tested for the enrichment of Alzheimer’s disease associations in Reactome biological pathways by regressing gene pathway membership against the (signed) Z score from the S-MultiXcan analyses. This approach allowed us to assess the enrichment of Alzheimer’s disease associations within biological pathways, as well as the mean effect size and effect direction of gene expression within the enriched pathways. In the (multi-tissue) GTEx S-MultiXcan analysis, one pathway—plasma lipoprotein clearance—was significantly downregulated in Alzheimer’s disease cases after correction for multiple testing (beta coefficient = -0.7861, \( P = 6.64 \times 10^{-6} \)) (Table 5, Supplementary Table S7). Plasma lipoprotein clearance was also significantly downregulated in cases using the CMC data (beta = -0.5646, \( P = 8.31 \times 10^{-26} \)). Furthermore, we identified the upregulation of multiple immune-related pathways, especially related to toll-like receptor (TLR) cascades (e.g. toll-like receptor TLR1: TLR2 cascade; beta = 0.3684, 1.32 \times 10^{-9}) (Table 5, Supplementary Table S8), using the CMC data.

**Discussion**

We performed multi-tissue analysis of gene expression underlying Alzheimer’s disease to identify and prioritise candidate causal genes and pathogenic tissues. Using the transcriptome-wide association study method S-PrediXcan and tissue-specific eQTL information from GTEx, we identified 50 unique candidate risk genes for Alzheimer’s disease. A meta-analysis of these tissue-specific data found 73 genes associated with Alzheimer’s disease. Because GTEx-derived brain tissues may lack sufficient power to identify robust association signals underlying complex diseases, we ran S-PrediXcan using expression weights derived from 646 dorsolateral prefrontal cortex samples from the CommonMind Consortium. We identified 12 gene-based associations, 8 of which were also significant in the meta-analysed GTEx analysis. Fine-mapping of causal gene sets further prioritised novel gene candidates within 10 independent risk

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**Table 4** Significant associations in the CMC dorsolateral prefrontal cortex and corresponding GTEx association statistics

| CMC (DLFPC) | | | | GTEx (48 tissues) |
|---|---|---|---|---|
| Gene | Z | P | Tissue | Z | Z SD | P |
| TOMM40 | -21.40 | 1.37 \times 10^{-10} | Oesophagus mucosa | 0.57 | 3.44 | 1.10 \times 10^{-7} |
| ZNF222 | 13.93 | 4.11 \times 10^{-44} | – | – | – | – |
| IRF2BP1 | 12.28 | 1.21 \times 10^{-34} | Heart atrial appendage | -0.94 | 0.73 | 4.34 \times 10^{-1} |
| EML2 | 7.98 | 1.50 \times 10^{-15} | Cerebellar hemisphere | -0.22 | 2.72 | 4.01 \times 10^{-10} |
| CR1 | 7.91 | 2.63 \times 10^{-15} | Oesophagus muscularis | 4.30 | 3.45 | 1.69 \times 10^{-11} |
| CLPTM1 | -6.30 | 2.94 \times 10^{-10} | Skin, sun-exposed leg | -2.98 | 4.89 | 3.35 \times 10^{-23} |
| TRAPC6A | -6.29 | 3.22 \times 10^{-10} | Thyroid | -1.02 | 2.84 | 2.26 \times 10^{-13} |
| ZNF45 | -6.12 | 9.18 \times 10^{-10} | Stomach | 0.19 | 2.72 | 2.47 \times 10^{-23} |
| DMWD | 5.91 | 3.48 \times 10^{-9} | Adipose visceral omentum | 3.45 | 1.45 | 3.08 \times 10^{-8} |
| ZNF223 | 5.90 | 3.69 \times 10^{-9} | Brain cerebellum | 1.03 | 1.17 | 5.88 \times 10^{-4} |
| PVR | -4.82 | 1.41 \times 10^{-6} | Spleen | -4.64 | 2.05 | 9.68 \times 10^{-27} |
| AP2A2 | -4.65 | 3.30 \times 10^{-6} | Heart atrial appendage | -0.30 | 1.93 | 2.48 \times 10^{-2} |

**DLFPC** dorsolateral prefrontal cortex
loci. Biological pathway analysis of the meta-analysed GTEx data and CMC data found a downregulation of genes involved in plasma lipoprotein clearance. Furthermore, the CMC data strongly implicated the upregulation of genes involved in immune-related pathways and processes, particularly toll-like receptor activity. These results highlight the utility of investigating multiple tissues underlying complex disorders, including peripheral tissues unrelated to the pathogenic tissue of interest (such as skin tissue for brain-related processes in Alzheimer’s disease) [7]. Our results demonstrate a multi-tissue approach to gene discovery in Alzheimer’s disease may identify not only candidate causal genes and pathways, but peripheral (i.e. accessible) surrogate tissues for diagnostic biomarkers and the discovery of causal mechanisms.

Table 5: Biological pathways associated with Alzheimer’s disease association signals in the dorsolateral prefrontal cortex from the CMC

| Pathway ID       | Pathway name                                      | Coef  | SE     | P       |
|------------------|---------------------------------------------------|-------|--------|---------|
| GTEx S-MultiXcan |                                                    |       |        |         |
| R-HSA-8964043    | Plasma lipoprotein clearance                      | −0.7861| 0.1745 | 6.64 × 10^{-6} |
| CMC DLPFC        |                                                    |       |        |         |
| R-HSA-168179     | Toll-like receptor TLR1:TLR2 cascade               | 0.3684| 0.0263 | 1.32 × 10^{-44} |
| R-HSA-167044     | Signalling to RAS                                 | 0.6713| 0.0493 | 2.78 × 10^{-42} |
| R-HSA-187687     | Signalling to ERKs                                | 0.5786| 0.0438 | 6.52 × 10^{-40} |
| R-HSA-447115     | Interleukin-12 family signalling                  | 0.6109| 0.0468 | 7.27 × 10^{-39} |
| R-HSA-354192     | Integrin alphaIIb beta3 signalling                | 0.6337| 0.0486 | 8.14 × 10^{-39} |

Coef beta coefficient from a logistic regression model testing the enrichment of genes associated with Alzheimer’s disease in Reactome pathways.
Two recent studies performed transcriptome-wide association analyses of brain samples in Alzheimer’s disease. Raj et al. [14] used TWAS FUSION [15] with eQTL data derived from 450 frontal cortex samples and genotype data from the Religious Order Study or the Memory and Aging Project (ROS/MAP), while Marioni et al. [16] applied Summary-data-based Mendelian Randomization (SMR) [17] to GWAS summary data from a meta-analysis of proxy Alzheimer’s disease cases from the UK Biobank and IGAP meta-analysis summary data, and eQTL data from over 600 frontal cortex samples from the Common Mind Consortium. These analyses identified a total of 9 candidate genes whose expression in brain tissue was associated with Alzheimer’s disease. We found a significant association with 4 of these candidate genes (CRI, TOMM40, PVR, CLPTM1) in at least one peripheral tissue. The effect direction of the beta coefficients in our study had the same effect directions for the candidate genes CRI, PVR, and CLPTM1, and the strongest associations were found in peripheral tissues, including the skin.

We observed largely concordant effect directions in the S-PrediXcan association statistics (Z scores) across the brain and peripheral tissues. While the brain is the critical pathogenic tissue in Alzheimer’s disease, genetic (i.e. regulatory) effects on gene expression underlying the disorder are enriched in less biologically obvious tissues, such as vascular tissues and the skin [7]. More generally, there is a high level of tissue-shared eQTL regulation at GWAS loci for complex diseases [7], particularly between (embryonically related) brain and skin tissue [18]. These results suggest the study of accessible peripheral tissues such as the skin may capture regulatory effects on gene expression and/or new pathogenic tissues underlying Alzheimer’s disease. Such an approach is supported by surrogate tissue analyses of Parkinson’s disease, which identified alpha-synuclein deposits—a hallmark of dementia—in the nerve fibres of the skin [19, 20]. Future studies of peripheral tissues may therefore increase power to identify gene-based associations in Alzheimer’s disease; however, functional studies will be required to assess the biological relevance on the associations in relation to disease onset and progression.

Transcriptome imputation methods such as S-PrediXcan are prone to false-positive associations due to linkage disequilibrium between SNPs used to build the expression weights, which induce spurious gene-trait associations within chromosomal regions [13]. We used fine-mapping of causal gene sets to further prioritise genes within risk loci. We found the probability for each gene in a region to be causal was largely a function of its S-PrediXcan Z score, where genes with larger Z scores had larger posterior inclusion probabilities as the causal gene. Nonetheless, we identified 6 genes that were not reported as the closest gene within ± 100 kb of the top SNP of known GWAS-defined associated genes at the time of publication of Lambert et al. [2], which represent novel, functionally relevant candidate causal genes in Alzheimer’s disease. Among these novel candidates are GRIK4 at the SORL1 locus and LINC79 at the SLCA4A4-RIN3 locus. Both GRIK4 (glutamate ionotropic receptor kainate type subunit 4) and LINC79 (unc-79 homologue, NALCN channel complex subunit) have biased expression in the brain and encode ion channel subunits, and it is conceivable that their dysfunction may contribute to altered synaptic plasticity, learning, and development in Alzheimer’s disease [21].

Biological pathway analysis of genes in both our meta-analysed GTEx and CMC results found a downregulation of ‘plasma lipoprotein clearance’ in Alzheimer’s disease. These results are consistent with a recent meta-analysis of cross-tissue expression imputation of 44 GTEx tissues [22], which found the enrichment genes whose expression was associated with Alzheimer’s disease in gene ontology terms related to lipoprotein clearance. Lipoprotein clearance may play an important role in Alzheimer’s disease pathogenesis through the association of APOE and several other genes that function in lipid or lipoprotein metabolism, including Clusterin (CLU) and ATP binding cassette (ABC) transporter A7 (ABCA7, [23]). Specifically, it has been hypothesised that dysfunctional lipoprotein clearance in the central nervous system may facilitate the formation of two critical neuroanatomical features in Alzheimer’s disease: amyloid plaques and neurofibrillary tangles. These neuroanatomical features may be indicated by global changes in gene (mRNA) and protein expression of lipid and lipoprotein-related genes in both brain tissue and peripheral blood [24]. The association of lipoprotein-related genes with Alzheimer’s disease in the skin and other non-brain tissues, together with concordant effect directions across tissues (including brain tissue), suggests peripheral tissues may provide a biologically valid substrate for the study of genetic factors and their impact on higher order molecular processes in Alzheimer’s disease.

Pathway analysis of the CMC gene-based data found the upregulation of genes involved in immune-related processes, most notably toll-like receptor cascades. Toll-like receptors are involved in many physiological and pathological responses, and their activity is thought to play a role in several neurological disorders, including Alzheimer’s disease [25, 26]. The receptors are widely expressed on microglial cells—the chief immune cells of the central nervous system—and their activation is associated with Aβ plaque deposition [27] and enhanced neurodegeneration [28]. Although we cannot draw mechanistic conclusions, our results suggest a potential relationship between altered immune signalling, impaired plasma lipoprotein clearance, and Aβ plaque deposition in Alzheimer’s disease.
Our multi-tissue transcriptome imputation approach has a number of advantages over traditional expression quantitative locus studies of complex diseases. First, transcriptome imputation methods allow the study of genetically regulated gene expression without directly measuring expression data from an appropriate cell type in diseased cases and health controls. Second, by estimating the genetically regulated component of gene expression, transcriptome imputation methods remove the impact of unmeasured (i.e. uncontrolled) environmental factors on gene expression, thereby improving the interpretability of expression association signals. Third, transcriptome imputation aggregates SNP-level associations to individual genes, reducing the multiple testing burden and increasing statistical power. A multi-tissue meta-analysis, such as S-MultiXcan, further reduces the multiple testing burden by combining association statistics across all interrogated tissues. Fourth, TWAS methods utilise eQTL information from large eQTL databases with uniform sample collection and strict quality control protocols which improves the reliability of results and enables replication across disorders/traits.

A disadvantage of GTEx is the use of bulk tissue samples to measure gene expression. As a result, GTEx cannot accurately account for cellular heterogeneity and may under represent certain cell populations in a given tissue. Many of the Alzheimer’s disease risk loci identified through GWAS are not highly expressed in bulk brain tissues and therefore previous attempts to identify brain eQTLs have likely been affected by cellular heterogeneity [29, 30]. A large proportion of Alzheimer’s disease risk loci have been linked to immune function, and our results in (dorsolateral prefrontal cortex) brain tissue corroborate these findings. However, the study of immune function in the brain is complicated by the heterogeneous cell populations, which may dilute disease-specific expression signatures from immune-related populations such as microglia. Analyses of primary cell-type-specific expression from the Immune Variation project have shown that Alzheimer’s disease risk alleles are enriched among monocyte-specific eQTLs [31]. Future studies may therefore use more easily accessible monocytes as a proxy to examine the (immune) cell-specific effects of susceptibility variants in Alzheimer’s disease.

Conclusions
In summary, we performed a multi-tissue transcriptome-wide association study of Alzheimer’s disease. We confirmed an association between DNA sequence variation and gene expression for known Alzheimer’s disease candidate genes and identified multiple genes whose expression has not previously been associated with the disease. Many disease associations were observed in both brain and peripheral tissues, most notably skin tissue, and the effect directions for the association statistics were largely consistent across tissues. A meta-analysis of 48 GTEx tissues, including 13 brain tissues, confirmed the association of candidate genes identified in the single-tissue analyses, in addition to several novel genes, most of which were also identified in an analysis of gene expression in the dorsolateral prefrontal cortex from the CMC. These results suggest the joint analysis of brain and peripheral tissues may capture regulatory effects underlying Alzheimer’s disease; however, functional studies will be required to assess their biological relevance in disease activity. The use of skin tissue—which had the largest number of associations with Alzheimer’s disease in our study—represents a promising avenue for the study of regulatory variation and risk genes in Alzheimer’s disease.

Supplementary information
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Authors’ contributions
Conceptualization: ZFG; formal analysis: ZFG and DE; methodology: ZFG, ERG, and EMD; supervision: ERG, EMD, and MLK; writing—original draft: ZFG; writing, review, and editing: ZFG, EMD, DE, and MLK. The author(s) read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article and its supplementary information files.

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Not applicable.

Consent for publication
Not applicable.

Competing interests
ERG receives an honorarium from the journal Circulation Research of the American Heart Association, as a member of the Editorial Board. He also performs consulting on pharmacogenetic analysis with the City of Hope Research Institute.

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