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Identification of blood metabolites associated with risk of Alzheimer's disease by integrating genomics and metabolomics data

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Abstract

Backgrounds—Specific metabolites have been reported to be potentially associated with Alzheimer's disease (AD) risk. However, the comprehensive understanding of roles of metabolite biomarkers in AD etiology remains elusive.

Methods—We performed a large AD metabolome-wide association study (MWAS) by developing blood metabolite genetic prediction models. We evaluated associations between genetically predicted levels of metabolites and AD risk in 39,106 clinically diagnosed AD cases, 46,828 proxy AD and related dementia (proxy-ADD) cases, and 401,577 controls. We further conducted analyses to determine microbiome features associated with the detected metabolites and characterize associations between predicted microbiome feature levels and AD risk.

Results—We identified fourteen metabolites showing an association with AD risk. Five microbiome features were further identified to be potentially related to associations of five of the metabolites.

Conclusions—Our study provides new insights into the etiology of AD that involves blood metabolites and gut microbiome, which warrants further investigation.

Keywords

Alzheimer's disease; metabolomics; genetic instruments; risk; associations

Introduction

Alzheimer's disease (AD) is the most common form of dementia. The late-onset AD (LOAD) occurring after age 65 contributes to the majority of AD cases. The number of people with dementia increased to 57.4 million in 2019 globally, and this number was estimated to reach 152.8 million in 2050 [1], resulting in a great challenge to healthcare systems worldwide. Reduced glucose [2–4] and increased glucose-6-phosphate (G6P) [5]

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levels were reported in AD patients compared with cognitively normal individuals in several studies. Several metabolites (kynurenine, 3-Hydroxy-L-kynurenine, 3-hydroxyanthranilic acid, quinolinic acid, kynurenic acid, Xanthurenic acid, picolinic acid, and cinnabarinic acid) involved in Kynurenine Pathway (KP), a metabolic pathway involved in the production of nicotinamide adenine dinucleotide (NAD^+), were also reported to be associated with AD [6]. The glucose, G6P, and KP were related to energy metabolism in organisms. Furthermore, the gamma-glutamylglutamine [7], alpha-glutamyltyrosin [8], carnosine, and kynurenic acid (KYNA) [9] were also reported to be associated with AD risk. The carnosine was reported to have multiple biological functions, including pH buffering, metal-ion chelation, and antioxidant activities, which are beneficial for human health [10]. The KYNA could antagonize the function of α7 nicotinic and N-methyl-d-aspartate receptors, which are critical for normal brain development and cognitive processes. Thus, decreased cerebral KYNA levels may have beneficial effects [11].

Metabolite abundance can be influenced by normal physiological processes, development of diseases, environmental factors, and genetic variations [12, 13]. The conventional metabolome-wide association study (MWAS) that focuses on measured overall metabolite levels is a powerful method to identify biomarkers of risk of disease [14], which could provide new insights into the roles of specific metabolism pathways in disease etiology. To date, only a few studies assessing metabolites levels have been performed for AD [15–18]. A recent study [19] evaluating 249 blood metabolites in 118,021 individuals reported two metabolites (β-hydroxybutyrate and acetone) to be associated with AD risk. Another study [20] reported four metabolites (S-HDL-free cholesterol, M-HDL-phospholipids, 22:6, docosahexaenoic acid, and glutamine) to be associated with AD. The results from these studies are not always consistent. For example, one glycerophospholipid (PC ae C34:0) was identified to be associated (false discovery rate (FDR) < 0.1) with global cognition, episode memory, perceptual speed, and semantic memory in Huo's study. While in Varma's study, authors did not observe an association between PC ae C34:0 and memory ($p = 0.94$). Several potential reasons may explain such inconsistencies, including general population attributes and analytical and preprocessing approach differences across studies. To better characterize metabolite markers related to AD risk, it is critical to conduct studies with reduced limitations of existing research. In the current study, we employed genetic instruments to assess the associations between genetically predicted metabolite levels and AD risk. This approach should be less susceptible to selection bias, reverse causation, and confounding effects because the alleles are transferred from parents to offspring randomly when the gamete is formed. GWAS has reported multiple genetic variants associated with levels of many metabolites in blood [21–23]. Many of these genetic variants collectively can provide strong instrumental variables for evaluating the associations of genetically predicted metabolite levels with AD risk in a sufficiently powered study. In the current study, comprehensive metabolite genetic prediction models were established to serve as genetic instruments. Such instruments synthesizing predictive effects of multiple variants have been demonstrated to capture higher proportion of exposure levels compared with instruments of individual quantitative trait loci (QTL) single nucleotide polymorphisms (SNPs) (24). In our earlier work focusing on DNA methylation, association analyses using prediction

models could almost fully capture all associations that can be identified using the QTL as instruments, while additionally identifying multiple novel associations [24].

Gut microbiota, another layer of omics markers that is getting rapid attention in the research community, plays a key role in the metabolic metabolism of the human host. The abnormal abundance of microbiota gut has been reported to potentially lead to multiple human diseases, including AD [25]. To better characterize the relationship between the identified metabolites and AD risk, we further assessed whether specific microbiome features may be also associated with these metabolites and AD risk. To test this, we leveraged gut microbiota GWAS summary statistics from the FINRISK study to evaluate the bi-directional metabolite-microbiome associations, as well as the associations of predicted microbiome levels with AD risk.

Materials and Methods

Genetic Instruments for Metabolite Levels in Blood

The included study subjects were twins enrolled in the TwinsUK registry [26], the largest cohort of community-dwelling adult twins in the UK. A total of 1,696 subjects, containing data of both genotypes and metabolites at three clinic visits, were involved in the current study. The abundance of metabolites in blood was performed at Metabolon (Durham, North Carolina, USA), resulting in 901 quantified metabolites. Among them, 644 consistently heritable (coefficient of variation (the ratio of the standard deviation and mean) of the heritability values estimated from all three visits was less than 0.5) metabolites were used for further study, as described in the original study publication [23]. Then, the identity-by-descent (IBD) was used to identify cryptic relatedness with independent genotyped SNPs. Individuals with an IBD proportion greater than 0.95 were identified as twins, and individuals who had an IBD proportion ranging from 0.2 to 0.95 were identified to be related. All individuals falling into these categories were subsequently removed from the downstream analysis. A total of 881 unrelated European ancestry subjects were determined for further analyses. The mean of the quantile normalized metabolite values from the three visits were rank-based inverse normal transformed using the R package *RNOmni* [27] before downstream analyses. Principal components were generated using EIGENSOFT [28] with 72,646 variants, which were composed of disequilibrium (LD)-pruned ($R^2 < 0.2$) common (MAF ≥ 5%) genotyped variants with 200 SNPs window. The abundance of each metabolite was adjusted by sex, mean age across the three visits, mean body mass index (BMI) across the three visits, and the first ten principal components. Then, the metabolite residuals were rank-based inverse normal transformed again for downstream analysis.

Genotyping of the TwinsUK dataset was done with a combination of Illumina arrays (HumanHap300, HumanHap610Q, 1M-Duo, and 1.2MDuo 1M). Genotype imputation was further performed using the Michigan Imputation Server [29], and the HRC (Version r1.1 2016) was used as a reference panel. We removed single-nucleotide polymorphism (SNPs) with imputation quality (R^2) < 0.8, minor allele frequency (MAF) < 0.05, or with > 5% missing data in the 881 subjects. A total of 5,288,025 SNPs and normalized metabolite residuals were set as trait values to perform linear regression with an additive genetic model using PLINK (v2.00a2LM). For SNPs showing an association at a p-value below 5×10^{-7},

their cis-SNPs (within 100 kb) were extracted as potential predictors for each metabolite of interest for model building. To include potential predictors from multiple chromosomes, we converted all the chromosome numbers to Z and combined them as a single pseudo chromosome. Here, we selected 5×10^{-7} as the threshold to maximize potentially informative SNPs while avoiding too much noise. The strand-ambiguous SNPs (A-T/T-A and C-G/G-C) were excluded. The TWAS/FUSION framework [30] was used to establish genetic prediction models with four statistics methods, including best linear unbiased predictor (BLUP), least absolute shrinkage and selection operator (LASSO), Elastic Net, and top SNPs. The model showing the most significant cross-validation *P*-value was selected for each metabolite of interest and only prediction models with a cross-validation performance R^2 (percentage of the variances of metabolite levels explained by the model) ≥ 0.01 were retained for the downstream association analysis, which is a common threshold used in such studies [31–36].

Association Analysis Between Genetically Predicted Metabolite Abundances and AD risk

For the association analyses between predicted metabolite levels and AD risk, we analyzed publicly available GWAS summary statistics of 39,106 clinically diagnosed AD cases, 46,828 proxy-ADD cases, and 401,577 controls from European ancestry, as described elsewhere [37]. In brief, the subjects of AD, proxy-ADD cases, and controls were collected from The European Alzheimer & Dementia Biobank dataset (EADB), GR@ACE/DEGESCO study, The Rotterdam Study (RS1 and RS2), European Alzheimer's Disease Initiative (EADI) Consortium, Genetic and Environmental Risk in AD (GERAD) Consortium/Defining Genetic, Polygenic, and Environmental Risk for Alzheimer's Disease (PERADES) Consortium, The Norwegian DemGene Network, The Neocodex–Murcia study (NxC), The Copenhagen City Heart Study (CCHS), Bonn studies, and UK Biobank. The Illumina or Affymetrix microarray was used to genotype the polymorphisms in each study. For the subjects from UK Biobank, a combined panel (HRC, UK10K, and 1000G) was used to perform the genotype imputation and the SAIGE software was used to assess associations between SNPs and AD risk with forty PCs and batch as covariates. All subjects from The Rotterdam Study (RS1 and RS2) were imputed with the HRC reference panel and the PLINK software was used to perform the genome-wide association with three PCs as covariates. The genotyping data of all remaining subjects was imputed with TOPMed or HRC reference panel and SNPTEST was used to identify the associations between SNPs and AD risk with or without PC as covariates. We used TWAS/FUSION [30] to investigate the main associations. The modified script FUSION.compute_weights.modified.R (https://github.com/Arthur1021/Metabolites-prediction-models) was used to establish genetic prediction models for metabolites, omitting the filtering step based on estimated heritability. The FUSION.assoc_test.R script was further used for testing the associations of predicted metabolite levels with AD risk. For this, we used the LD reference panel derived from the TwinsUK data. We used the false discovery rate (FDR) corrected *p*-value threshold of ≤ 0.05 to determine significant associations between genetically predicted metabolite abundances and AD risk. To further investigate potential regulatory mechanisms of SNPs associated with the identified metabolites, we annotated the SNPs used in genetic prediction models, as well as those in proximity ($r^2 > 0.60$) to the SNPs using ANNOVAR software. The SNPs

were annotated into one of nine functional categories, including exonic, intronic, intergenic, upstream, 3′-UTR, 5′-UTR, ncRNA intronic, ncRNA exonic, and downstream.

Sensitivity analyses

To further examine whether the identified associated metabolites from the main analyses may be robust to our analysis strategies, two alternative strategies were used to test their associations under different scenorios. First, in the main analyses we used rank-based inverse normalization to fit the normal distribution of the metabolite levels for adjusting for covariates. Before fitting the metabolite prediction models, we applied rank-based inverse normalization to the residuals again. To test if the second step influence downstream associations, in a sensitivity analysis we used the residuals without normalization for the downstream analysis. Second, four available covariates (sex, mean age across the three visits, mean BMI across the three visits, and the first ten principal components) were used in the main analyses to reduce potential confounding effects. In another sensitivity analysis we did not adjust for mean BMI across the three visits, then performed the same steps to test if adjustment of BMI may affect the downstream metabolite associations. Furthermore, we assessed the robustness of the significant association results by examining different *p*-value cutoffs (*p*-value < 1×10^{-7}, *p*-value < 5×10^{-8}, and *p*-value < 1×10^{-8}) for selecting genetic variants for model building.

Identify causal effects of metabolites on AD

To further explore the likely causal effects of metabolites associated with AD risk, we used Summary-data-based Mendelian Randomization (SMR) [38] and Polygenic risk scores-continuous shrinkage (PRS-CS) [39] methods to replicate the main results. The SMR v.1.30 software was used to explore the potential mechanisms linking metabolites and AD risk, we implemented the SMR by leveraging quantitative trait loci (mQTL) of the identified metabolites and summary-level data from the AD GWAS. For PRS-CS, PRS-CS software was used to infer mQTL weights using mQTL summary data and an external LD reference panel (1000 Genome Project Phase 3) of the European populations. Then the SNP weights were applied to conduct association analysis with AD GWAS summary statistics.

Two-sample MR Analysis involving microbiome features

Two-sample Mendelian randomization (MR) analysis is a statistical method aiming to identify potential causal effect of an exposure on an outcome using summary statistics from genome-wide association studies (GWAS) in two independent samples [32, 34, 40–43]. Employing a two-sample MR approach can effectively mitigate or reduce biases arising from unmeasured confounding or reverse causality. Additionally, this method offers enhanced statistical power and increased flexibility when selecting exposure and outcome variables [44]. In the current study, potential causal relationships between identified blood metabolites, gut microbiota, and AD risk were further investigated using two-sample MR [45]. GWAS summary results for the 14 identified metabolites were collected in the TwinsUK study and GWAS summary results for 468 gut microbiota features were collected from the FINRISK study which focuses on European ancestry participants [46]. For the microbiome study, briefly, 5,959 subjects with matched gut microbial metagenomes and genome information were involved. The Illumina genome-wide

SNP arrays (the HumanCoreExome BeadChip, the Human610-Quad BeadChip, and the HumanOmniExpress) were used to detect variations in the population and two Finnish-population-specific reference panels (2,690 high-coverage whole-genome sequencing and 5,092 whole-exome sequencing samples) were used to perform imputation using IMPUTE2 v.2.3.0 software. The gut microbiome from stool samples was characterized by shallow shotgun metagenomics sequencing with Illumina HiSeq 4000 Systems and the taxonomic profiling was performed using Centrifuge v.1.0.4. Then, the GWAS was performed using a linear mixed model (LMM) using BOLT-LMM v.2.3.2 with the top ten PCs, age, sex, and genotyping batch as covariates. The bidirectional associations were further performed to infer the associations between metabolite abundance (as exposure or outcome) and microbial abundance (as outcome or exposure), and between microbial abundance (exposure) and AD risk (outcome). We used SNPs associated with each of the metabolites of interest at $p < 5 \times 10^{-8}$ as genetic instruments. Of the 14 identified metabolites, eleven have at least one variant included as instruments. For the analyses treating the gut microbiota as exposure, consistent with suggestions from authors of the original study, the 147 microbial features associated with at least one variant at $p < 1 \times 10^{-8}$ were included. Aligned with suggestions from the previous study [46], all SNPs showing an association at $p < 1 \times 10^{-5}$ for each of the 147 microbial features were selected as potential SNP instruments. Subsequently, LD-clumping with a strict threshold ($r^2 < 0.001$ in the 1000 Genomes European data within 10 kb windows) was conducted to select independent instruments for metabolite and microbial abundance. Data harmonization was conducted using TwoSampleMR software, by selecting default parameter option 2 to ensure the effect of SNPs on the exposure and the outcome correspond to the same allele [45]. Then, the Wald ratio (WR) (when only one instrument SNP was available) or inverse variance weighted (IVW) method (when there was more than one instrument SNP) was used to perform the two-sample MR analysis. The threshold of $p < 0.05$ was used to determine significant associations for further consideration. For the implicated microbial features, we further evaluated their associations with AD risk, using the same instruments.

Results

Metabolites genetic prediction models

The overall design of this study was displayed in Fig. 1. After testing 644 consistently heritable metabolites with 5,288,025 SNPs, at the p-value threshold of 5×10^{-7}, a total of 447 metabolites were associated with at least one SNP. We tried to establish prediction models for these 447 metabolites. Satisfactory models with prediction performance of at least 0.01 (the proportion of variation that can be explained by the predicting SNPs, or 10% correlation between predicted and measured metabolite levels) were established for 422 metabolites. The proportion of the metabolite level variance that can be explained by the predicting SNPs range from 1% to 54.80%, with a median value of 3.95% (Fig. S1). The distribution of the genetic prediction models was similar to a previous study [47]. The detailed information for the performance of the prediction models established for the 422 metabolites was included in Table S1.

Associations of Predicted Metabolite levels in Blood with AD Risk

Using the GWAS summary statistics of AD, we identified 14 metabolites whose genetically predicted abundances in blood were associated with AD risk at a false discovery rate (FDR) of < 0.05 (Fig. 2 and Table 1). Among them, nine metabolites exhibited a significant association with AD risk after Bonferroni correction (p-value = 1.18×10^{-4}). The association results for all 422 tested metabolites each with four different prediction modeling methods were included in Table S1. The detailed information for the SNPs included in the prediction models of 14 associated metabolites was shown in Table S2. Twelve of them were known metabolites and involved in amino acid, peptide, lipid, energy, and carbohydrate super pathways, and X - 17178 and X - 21735 were unknown compounds. All the metabolites were observed to be inversely associated with AD risk except for 3-(3-hydroxyphenyl)propionate sulfate (Z-score = 8.52, p-value = 1.62×10^{-17}) (Table 1). Five metabolites (phenylalanylserine, leucylalanine, alpha-glutamyltyrosine, alpha-glutamylglycine, and gamma-glutamylglutamine) were involved in peptide pathway and four (androsterone sulfate, 5alpha-androstan-3beta,17beta-diol disulfate, epiandrosterone sulfate, and 5alpha-androstan-3alpha,17beta-diol monosulfate (1)) metabolites were involved in lipid pathway. This indicates potential importance of peptides and lipids in the etiology of AD. The succinylcarnitine was involved in the tricarboxylic acid cycle (TCA) cycle and the arabonate/xylonate was involved in the pentose phosphate pathway. Both of them play key roles in carbohydrate metabolism and could interact with each other through glycolysis, suggesting that energy metabolism could be important for AD development. Furthermore, functional annotation using ANNOVAR suggested that of the predicting SNPs of associated metabolites, there were nonsynonymous SNPs in six genes, namely *TIMELESS*, *GLS2*, *CYP3A43*, *KCNH6*, *MS4A6A*, and *HEATR4* (Table S3). Interestingly, all the six genes, *TIMELESS* [48], *GLS2* [49], *CYP3A43* [49], *KCNH6* [50], *MS4A6A* [51], and *HEATR4* [52], had been previously reported related to AD. For example, the gene *GLS2* had significantly lower expression (FDR < 0.05) in the hippocampus/entorhinal cortex of AD patients compared with controls [53]. The *KCNH6* [50] was reported as a candidate target for AD by combining the Genotype-Tissue Expression (GTEx) and Drug Gene Interaction (DGI) databases. The transcripts level of *MS4A6A* was found to be significantly higher ($p < 0.003$) in the blood of individuals with AD compared with the control group [51].

Sensitivity analyses

We further performed sensitivity analyses as described above. Regardless of whether we conduct rank-based inverse normalization for the residuals, the associations of the 14 identified metabolites remained highly significant with the same directions of effect (Table S4). When we remove BMI as a covariate in outcome adjustment, 13 out of the 14 (92.86%) metabolites (except for M02730 (gamma-glutamylglutamine)) demonstrated significant associations with the same directions. In addition, we set different p-value thresholds (1×10^{-7}, 5×10^{-8}, and 1×10^{-8}) for selecting potential SNP predictors. Among the 14 metabolites that displayed significance associations in the main analyses, 11 exhibited potential SNP predictors at the different thresholds, whereas three metabolites (M45415, M48255, and M02730) did not have any SNPs associated at the corresponding thresholds. Notably, ten out of the 11 metabolites were successfully replicated using different p-value

thresholds, except for M41530 (alpha-glutamylglycine) (Table S4). These support the robustness of our findings.

Robustness of the identified metabolites-AD risk associations using SMR and PRS-CS methods

To further evaluate whether the identified blood metabolites-AD risk associations may be robust for different methods, we additionally applied SMR and PRS-CS analyses. Results from the SMR suggest that 12 out of the 14 metabolites showed suggestive causal effects on AD risk at nominal significance level (p-value < 0.05) with consistent effect directions, except for alpha-glutamylglycine and X - 21735 (Table S5). Subsequently, we employed the Heterogeneity in Dependent Instrument (HEIDI) approach to examine the null hypothesis that the associations identified by the SMR test are attributed to pleiotropy, and no metabolites were rejected with PHEIDI < 0.01 (Table S5). Furthermore, the PRS-CS analysis demonstrated that all the 14 identified associated metabolites showed an association with AD risk at a nominal significance level (p-value < 0.05) with consistent effect directions (Table S6). The corresponding weights obtained from the PRS-CS analysis were included in Table S7.

Analyses involving microbiome features

We further applied two-sample MR analysis to better understand relationships among gut microbiota, blood metabolites, and AD risk. There were five microbiota-metabolite-AD and two metabolite-microbiota-AD with consistent directions of association. The genetically predicted UBA1066 sp900317515 abundance was positively associated with levels of 5alpha-androstan-3beta,17beta-diol disulfate and X – 21735. Genetically predicted levels of 5alpha-androstan-3beta,17beta-diol disulfate and X – 21735 were negatively associated with AD risk. There was also a negative association between genetically predicted UBA1066 sp900317515 abundance and AD risk. These results suggested the gut microbiota UBA1066 sp900317515 abundance could potentially be associated with AD risk by regulating the levels of 5alpha-androstan-3beta,17beta-diol disulfate or X – 21735 in blood (Fig. S2 and Table S8). The genetically predicted CAG-884 sp000433875, Bifidobacterium adolescentis, and actinobacteria abundances were negatively associated with leucylalanine, araboname/xylonate, and gamma-glutamylglutamine levels, respectively. For these three metabolites, their predicted levels were negatively associated with AD risk. Interestingly, there were positive associations of predicted abundances of CAG-884 sp000433875, Bifidobacterium adolescentis, and actinobacteria with AD risk. These results suggested that the three gut microbial features (CAG-884 sp000433875, Bifidobacterium adolescentis, and actinobacteria) could be positively associated with AD risk by regulating the levels of metabolites (leucylalanine, araboname/xylonate, and gamma-glutamylglutamine, respectively) in blood (Fig. S2 Table S8). Furthermore, we identified that the predicted levels of X – 21735 were negatively associated with CAG-552 abundance, and predicted CAG-552 abundance was positively associated with AD risk. There was also a negative association between predicted levels of X – 21735 and AD risk. These results indicated that X – 21735 levels in blood could be negatively associated with AD risk by influencing the CAG-552 abundance in the gut (Fig. S2 Table S8). Additionally, predicted levels of 5alpha-androstan-3beta,17beta-diol disulfate were identified to be positively associated

with UBA1066 sp900317515 levels, and predicted UBA1066 sp900317515 abundances were negatively associated with AD risk. These results were consistent with the negative association between predicted levels of 5alpha-androstan-3beta,17beta-diol disulfate and AD risk (Fig. S2).

Discussion

This is the first study with a large sample size to systematically evaluate the associations between genetically predicted blood metabolite abundances and AD risk using comprehensive genetic prediction models as instruments. Overall, we identified 14 metabolites that were significantly associated with AD risk. We compared our results with those of a previous study [54]. A total of four metabolites (epiandrosterone sulfate, 5alpha-androstan-3beta,17beta-diol disulfate, sphingomyelin, and glutamine) were identified to have a causal effect on AD in the previous study [54]. Three of these metabolites were available in the current study, with the exception of sphingomyelin. Importantly, our findings revealed nominal p-values < 0.05 for all the three metabolites with consistent effect directions, indicating the reliability of our results (Table S9). Combining with information of gut microbiota, we identified five consistent directions of effect for the microbiome-metabolite-AD risk pathway and two consistent directions of effect for the metabolite-microbiome-AD risk pathway.

The ‘amyloid hypothesis’ proposed amyloid-beta (Aβ) as the central event in the pathological process of the AD [55], and Aβ was an important signature for the identification of AD [56]. In the current study, we identified one metabolite, 3-(3-hydroxyphenyl)propionate sulfate, which might be positively associated with AD risk by regulating the amount of Aβ. The 3-(3-hydroxyphenyl)propionate sulfate showed a highly significant association with AD risk at p-value = 1.45×10^{-17} (Table 1). The organic anion transporter 1 (OAT1) was reported to be involved in the process of AD. When crossed OAT1 knockout mice with the tg2576 (AD model), the heterozygous OAT1-deficient tg2576 mice showed impaired learning and memory, as well as higher soluble Aβ amount compared with age-matched control tg2576 mice [57]. Knockout of the homologous gene organic anion transporter 3 (OAT3) showed significantly increased levels of the 3-(3-hydroxyphenyl)propionate sulfate compared with wild type with more than 8-fold change (p-value ≤ 0.05) [58]. In our results, the predicted 3-(3-hydroxyphenyl)propionate sulfate levels in the blood were positively associated with AD risk. We speculate that the deficiency of OAT genes could simulate the production of the 3-(3-hydroxyphenyl)propionate sulfate in AD mice, resulting in Aβ accumulation, and thus accelerating the process of AD.

In the current study, we identified five dipeptides, including phenylalanylserine (Phe-Ser), leucylalanine (Lue-Ala), alpha-glutamyltyrosine, alpha-glutamylglycine, and gamma-glutamylglutamine, to be associated with AD risk (Table 1). The Phe-Ser, Lue-Ala, alpha-glutamyltyrosine, and alpha-glutamylglycine were identified to be associated with the angiotensin-converting enzyme (*ACE*) gene in previous studies [23, 59, 60]. *ACE* was reported to be associated with AD [61] through regulating the blood pressure [62–64]. We speculate that the amount of Phe-Ser, Lue-Ala, alpha-glutamyltyrosine, and alpha-glutamylglycine may be associated with AD risk by regulating blood pressure. Additionally,

the observed inverse associations between predicted levels of gamma-glutamylglutamine (Z-score = −3.14, *p*-value = 1.67×10^{-3}) and alpha-glutamyltyrosine (Z-score = −4.80, *p*-value = 1.61×10^{-6}) with risk of AD were consistent with findings of previous studies [7, 8].

Several epidemiologic studies show that sex affects the incidence, development, and clinical manifestation of AD, resulting in a higher risk of AD in females [65–68]. In the Framingham Heart Study, a total of 2,611 (1,061 men and 1,550 women) cognitively intact subjects were involved to study the development of AD during one's lifetime. The result showed that the remaining lifetime risk of AD and developing any dementing illness was higher in females than in males. The remaining lifetime risk of AD for a 65-year-old female was 12%, compared with only 6.3% for a male [69]. In our result, we identified four androgen-related metabolites, 5alpha-androstan-3beta,17beta-diol disulfate (Z-score = −4.28, *p*-value = 1.88×10^{-5}), 5alpha-androstan-3alpha,17beta-diol monosulfate (1) (Z-score = −3.72, *p*-value = 2.00×10^{-4}), androsterone sulfate (Z-score = −4.33, *p*-value = 1.46×10^{-5}), and epiandrosterone sulfate (Z-score = −4.24, *p*-value = 2.27×10^{-5}) to be inversely associated with risk of AD. In previous studies, the plasma dehydroepiandrosterone (DHEA) concentrations were reported to be significantly lower in AD patients compared with controls [70, 71]. The DHEA could be converted to androstenedione (AAD) or testosterone (T) by a series of reactions involving the activity of 17β-hydroxysteroid dehydrogenase (17β-HSD) and 3β-hydroxysteroid dehydrogenase (3β-HSD). Then, AAD or T is converted to androstenedione (AAD) and 5α-dihydrotestosterone (DHT) with the steroid 5α-reductase and 5α-reductase. DHT could be converted to 5α-androstan-3α,17β-diol or 5α-androstan-3β,17β-diol with the activity of 3β-HSD, from which the two metabolites (5α-androstan-3α,17β-diol monosulfate (1) and 5α-androstan-3β,17β-diol disulfate) associated with AD risk are derived (Fig. 3). Similarly, the other two metabolites (androsterone sulfate and epiandrosterone sulfate) negatively associated with AD risk are also derived from androsterone (A) and epiandrosterone (EpiA), respectively (Fig. 3). Notably, these two metabolites were previously reported to be significantly lower in AD patients [72], which was the same effect direction as in the current study. Overall, we identified four metabolites in the DHEA metabolism pathway associated with AD risk.

Two metabolites (succinylcarnitine and arabonate/xylonate) were also detected to be negatively associated with AD risk. The succinylcarnitine is involved in the energy super pathway, while the arabonate/xylonate participates in the carbohydrate super pathway. Based on findings of previous studies, the dysfunctional glucose metabolism in AD patients resulted in a lack of ATP in the brain. Then the cognitive function was impaired due to the lack of energy which led to synaptic dysfunction and neuronal death result [73]. Succinylcarnitine was identified to be negatively (Z-score = −3.71, *p*-value = 2.96×10^{-4}) associated with risk of AD, which was consistent with findings of a previous study [74]. Both succinylcarnitine and arabonate/xylonate are involved in glucose or energy metabolism, indicating their potential link with etiology of AD. Notably, metabolomics was considered the type of markers that is the closest to phenotype compared with other types of 'omics' markers [75]. A previous study elucidated how the identified associated metabolites could be used for drug development treating the diseases of interest [76]. This study involved ten cohorts from Biobanking and BioMolecular Resources Research Infrastructure of the Netherlands (BBMRI-NL) to test the drug-metabolite associations. Researchers could

explore the candidate drugs by combining the drug-metabolite association and metabolite-disease association studies [76]. Due to relatively limited information of the drug-metabolite atlas, we are not able to find any drug-metabolite associations for the 14 metabolites identified in the current study, unfortunately. Future more comprehensive databases that are expected to be available will help us identify novel candidate drugs for AD through the identified metabolites A previous study reported that glutamine supplementation might be of interest in preventing or delaying the degenerative diseases of aging [77], which provides new insight into potential utility of the identified associated metabolites for AD. Among the 14 associated metabolites, 13 metabolites (except for 3-(3-hydroxyphenyl)propionate sulfate), showed negative associations with AD, suggesting that these metabolites may also be used as supplementation for preventing or delaying AD, if there are more supporting evidence from future research.

In the current study, we used data of 881 subjects to develop comprehensive genetic prediction models for metabolite levels in blood. The GWAS summary statistics from the largest AD study to date, which includes 39,106 clinically diagnosed AD cases, 46,828 proxy-ADD cases, and 401,577 controls, were used for the association analyses between metabolites and AD risk. The design of using genetic instruments could decrease the selection bias and reverse causation, which usually arise in traditional epidemiological design [33, 34]. Our study provides an improved power in detecting the metabolite-AD associations through leveraging comprehensive metabolite genetic prediction models compared with the design of simply using individual metabolite quantitative trait loci as instruments, due to higher proportions of metabolite levels that can be captured by the strategy of using multiple potentially informative SNPs.

We further applied another innovational design involving microbiome features to further understand the detected associations of metabolites-AD risk. As the first attempt of its type, our work uncovers novel metabolites and microbiome features potentially playing an important role in AD etiology, which warrants further investigation.

On the other hand, there are also some potential limitations of our study. First, we can’t eliminate potential false-positive discoveries, which can be caused by correlated metabolite levels among subjects, correlated genetically predicted metabolite levels, and shared genetic variants, although we carefully adjusted for available covariates. The *in vivo* and *in vitro* experiments are required to confirm the potential effect of the associated metabolites on AD risk. Second, some metabolites were not evaluated for their associations with AD risk due to a lack of associated variants at our pre-defined threshold as potential predictors for model development. Novel strategy that can cover more metabolites will be needed in the future study for examining additional metabolites. Thirdly, although we adjusted for sex during model building, most subjects of TwinsUK used in metabolite model building are females. In the AD GWAS both females and males were included. Further studies are needed to validate the identified metabolites associated with AD risk. Lastly, consistent with our goal of characterizing genetically predicted metabolite level-AD risk associations, in the current work we only focused on the genetically regulated components of metabolites levels, which are just a proportion of the overall variance of metabolite levels that can be captured in studies directly measuring the metabolite levels in AD cases and controls. Future

work evaluating the directly measured metabolite levels in a well-designed prospective study setting would be useful to further characterize AD related metabolites.

In summary, in this large-scale study, we identified fourteen metabolites in blood, involving amino acid, peptide, lipid, energy, and carbohydrate super pathways, to be associated with AD risk. The 3-(3-hydroxyphenyl)propionate sulfate is positively associated with AD risk and the other 13 metabolites are inversely associated with AD risk. We further observed that five microbial showed associations with AD risk through interactions with associated metabolites in blood. Our study provides substantial new information to improve our understanding of the interaction of blood metabolites and gut microbiome with the risk of AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Individual level genotype and metabolite were available from Twins UK scientific community.

Complete summary statistics of Alzheimer's disease was downloaded from the NHGRI-EBI GWAS Catalog (https://www.ebi.ac.uk/gwas/) under accession no. GCST90027158.

Complete summary statistics of microbial taxa could be downloaded from the NHGRI-EBI GWAS Catalog (https://www.ebi.ac.uk/gwas/) from accession GCST90032172 to GCST90032644. The scripts and prediction models of the 14 metabolites significantly associated with AD risk are available at https://github.com/Arthur1021/Metabolites-prediction-models. The genetic prediction models for other metabolites will be deposited to be publicly available upon paper publication.

References

1. Collaborators GBD 2019 DF. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. Lancet Public Health. 2022. 2022.
2. Mosconi L, Berti V, Glodzik L, Pupi A, De Santi S, de Leon MJ. Pre-clinical detection of Alzheimer's disease using FDG-PET, with or without amyloid imaging. Journal of Alzheimer's Disease. 2010;20:843–854.

3. Mosconi L Glucose metabolism in normal aging and Alzheimer's disease: methodological and physiological considerations for PET studies. Clin Transl Imaging. 2013;1:217–233.
4. Kuehn BM. In Alzheimer Research, Glucose Metabolism Moves to Center Stage. JAMA. 2020;323:297–299. [PubMed: 31913419]
5. Demarest TG, Varma VR, Estrada D, Babbar M, Basu S, Mahajan UV, et al. Biological sex and DNA repair deficiency drive Alzheimer's disease via systemic metabolic remodeling and brain mitochondrial dysfunction. Acta Neuropathol. 2020;140:25–47. [PubMed: 32333098]
6. Liang Y, Xie S, He Y, Xu M, Qiao X, Zhu Y, et al. Kynurenine Pathway Metabolites as Biomarkers in Alzheimer's Disease. Dis Markers. 2022;2022.
7. Abidin FNZ, Wells HRR, Altmann A, Dawson SJ. Hearing difficulty is linked to Alzheimer's disease by common genetic vulnerability, not shared genetic architecture. NPJ Aging Mech Dis. 2021;7:1–8. [PubMed: 33398019]
8. Gu J Detecting genetic similarity between complex human traits by exploring their common molecular mechanism. University of California, San Francisco; 2019.
9. Joshi P, Perni M, Limbocker R, Mannini B, Casford S, Chia S, et al. Two human metabolites rescue a C. elegans model of Alzheimer's disease via a cytosolic unfolded protein response. Commun Biol. 2021;4:843. [PubMed: 34234268]
10. Boldyrev AA, Aldini G, Derave W. Physiology and pathophysiology of carnosine. Physiol Rev. 2013. 2013.
11. Pocivavsek A, Notarangelo FM, Wu H-Q, Bruno JP, Schwarcz R. Astrocytes as pharmacological targets in the treatment of schizophrenia: focus on kynurenic acid. Handb Behav Neurosci, vol. 23, Elsevier; 2016. p. 423–443.
12. Luo S, Feofanova EV, Tin A, Tung S, Rhee EP, Coresh J, et al. Genome-wide association study of serum metabolites in the African American Study of Kidney Disease and Hypertension. Kidney Int. 2021;100:430–439. [PubMed: 33838163]
13. Bar N, Korem T, Weissbrod O, Zeevi D, Rothschild D, Leviatan S, et al. A reference map of potential determinants for the human serum metabolome. Nature. 2020;588:135–140. [PubMed: 33177712]
14. Chadeau-Hyam M, Ebbels TMD, Brown IJ, Chan Q, Stamler J, Huang CC, et al. Metabolic profiling and the metabolome-wide association study: significance level for biomarker identification. J Proteome Res. 2010;9:4620–4627. [PubMed: 20701291]
15. Varma VR, Oommen AM, Varma S, Casanova R, An Y, Andrews RM, et al. Brain and blood metabolite signatures of pathology and progression in Alzheimer disease: A targeted metabolomics study. PLoS Med. 2018;15:e1002482. [PubMed: 29370177]
16. Casanova R, Varma S, Simpson B, Kim M, An Y, Saldana S, et al. Blood metabolite markers of preclinical Alzheimer's disease in two longitudinally followed cohorts of older individuals. Alzheimer's & Dementia. 2016;12:815–822.
17. Lord J, Jermy B, Green R, Wong A, Xu J, Legido-Quigley C, et al. Mendelian randomization identifies blood metabolites previously linked to midlife cognition as causal candidates in Alzheimer's disease. Proceedings of the National Academy of Sciences. 2021;118.
18. Huo Z, Yu L, Yang J, Zhu Y, Bennett DA, Zhao J. Brain and blood metabolome for Alzheimer's dementia: findings from a targeted metabolomics analysis. Neurobiol Aging. 2020;86:123–133. [PubMed: 31785839]
19. Liu J, Amin N, Sproviero W, Arnold M, Batra R, Bonnechere B, et al. Longitudinal analysis of UK Biobank participants suggests age and APOE-dependent alterations of energy metabolism in development of dementia. MedRxiv. 2022:2022.02.25.22271530.
20. van der Lee SJ, Teunissen CE, Pool R, Shipley MJ, Teumer A, Chouraki V, et al. Circulating metabolites and general cognitive ability and dementia: Evidence from 11 cohort studies. Alzheimer's & Dementia. 2018;14:707–722.
21. Shin S-Y, Fauman EB, Petersen A-K, Krumsiek J, Santos R, Huang J, et al. An atlas of genetic influences on human blood metabolites. Nat Genet. 2014;46:543–550. [PubMed: 24816252]
22. Draisma HHM, Pool R, Kobl M, Jansen R, Petersen A-K, Vaarhorst AAM, et al. Genome-wide association study identifies novel genetic variants contributing to variation in blood metabolite levels. Nat Commun. 2015;6:7208. [PubMed: 26068415]

23. Long T, Hicks M, Yu HC, Biggs WH, Kirkness EF, Menni C, et al. Whole-genome sequencing identifies common-to-rare variants associated with human blood metabolites. Nat Genet. 2017;49:568–578. [PubMed: 28263315]
24. Yang Y, Wu L, Shu X-O, Cai Q, Shu X, Li B, et al. Genetically predicted levels of DNA methylation biomarkers and breast cancer risk: data from 228 951 women of European descent. JNCI: Journal of the National Cancer Institute. 2020;112:295–304. [PubMed: 31143935]
25. Jiang C, Li G, Huang P, Liu Z, Zhao B. The gut microbiota and Alzheimer's disease. Journal of Alzheimer's Disease. 2017;58:1–15.
26. Moayyeri A, Hammond CJ, Hart DJ, Spector TD. The UK adult twin registry (twinsUK resource). Twin Research and Human Genetics. 2013;16:144–149. [PubMed: 23088889]
27. McCaw ZR, Lane JM, Saxena R, Redline S, Lin X. Operating characteristics of the rank-based inverse normal transformation for quantitative trait analysis in genome-wide association studies. Biometrics. 2020;76:1262–1272. [PubMed: 31883270]
28. Patterson N, Price AL, Reich D. Population structure and eigenanalysis. PLoS Genet. 2006;2:2074–2093.
29. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. Nat Genet. 2016;48:1284–1287. [PubMed: 27571263]
30. Gusev A, Ko A, Shi H, Bhatia G, Chung W, Penninx BWJH, et al. Integrative approaches for large-scale transcriptome-wide association studies. Nat Genet. 2016;48:245–252. [PubMed: 26854917]
31. Sun Y, Zhu J, Zhou D, Canchi S, Wu C, Cox NJ, et al. A transcriptome-wide association study of Alzheimer's disease using prediction models of relevant tissues identifies novel candidate susceptibility genes. Genome Med. 2021;13:1–11. [PubMed: 33397400]
32. Liu D, Zhu J, Zhao T, Sharapov S, Tiys E, Wu L. Associations Between Genetically Predicted Plasma N-Glycans and Prostate Cancer Risk: Analysis of Over 140,000 European Descendants. Pharmgenomics Pers Med. 2021;14:1211. [PubMed: 34588798]
33. Zhu J, Yang Y, Kisiel JB, Mahoney DW, Michaud DS, Guo X, et al. Integrating genome and methylome data to identify candidate DNA methylation biomarkers for pancreatic cancer risk. Cancer Epidemiology and Prevention Biomarkers. 2021;30:2079–2087.
34. Zhu J, O'mara TA, Liu D, Setiawan VW, Glubb D, Spurdle AB, et al. Associations between genetically predicted circulating protein concentrations and endometrial cancer risk. Cancers (Basel). 2021;13:2088. [PubMed: 33925895]
35. Liu D, Zhou D, Sun Y, Zhu J, Ghoneim D, Wu C, et al. A transcriptome-wide association study identifies candidate susceptibility genes for pancreatic cancer risk. Cancer Res. 2020;80:4346–4354. [PubMed: 32907841]
36. Bhattacharya A, Li Y, Love MI. MOSTWAS: multi-omic strategies for transcriptome-wide association studies. PLoS Genet. 2021;17:e1009398. [PubMed: 33684137]
37. Bellenguez C, Küçükali F, Jansen IE, Kleineidam L, Moreno-Grau S, Amin N, et al. New insights into the genetic etiology of Alzheimer's disease and related dementias. Nat Genet. 2022:1–25. [PubMed: 35022602]
38. Zhu Z, Zhang F, Hu H, Bakshi A, Robinson MR, Powell JE, et al. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. Nat Genet. 2016;48:481–487. [PubMed: 27019110]
39. Ge T, Chen C-Y, Ni Y, Feng Y-CA, Smoller JW. Polygenic prediction via Bayesian regression and continuous shrinkage priors. Nat Commun. 2019;10:1776. [PubMed: 30992449]
40. Hemani G, Tilling K, Davey Smith G. Orienting the causal relationship between imprecisely measured traits using GWAS summary data. PLoS Genet. 2017;13:e1007081. [PubMed: 29149188]
41. Wu C, Wu L, Wang J, Lin L, Li Y, Lu Q, et al. Systematic identification of risk factors and drug repurposing options for Alzheimer's disease. Alzheimer's & Dementia: Translational Research & Clinical Interventions. 2021;7:e12148.
42. Zhu J, Wu C, Wu L. Associations Between Genetically Predicted Protein Levels and COVID-19 Severity. J Infect Dis. 2021;223:19–22. [PubMed: 33083826]

43. Ghoneim DH, Zhu J, Zheng W, Long J, Murff HJ, Ye F, et al. Mendelian Randomization Analysis of n-6 Polyunsaturated Fatty Acid Levels and Pancreatic Cancer Risk. Cancer Epidemiology, Biomarkers & Prevention. 2020;29:2735–2739.
44. Lawlor DA. Commentary: Two-sample Mendelian randomization: opportunities and challenges. Int J Epidemiol. 2016;45:908–915. [PubMed: 27427429]
45. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife. 2018;7:e34408. [PubMed: 29846171]
46. Qin Y, Havulinna AS, Liu Y, Jousilahti P, Ritchie SC, Tokolyi A, et al. Combined effects of host genetics and diet on human gut microbiota and incident disease in a single population cohort. Nat Genet. 2022;54:134–142. [PubMed: 35115689]
47. Yin X, Chan LS, Bose D, Jackson AU, VandeHaar P, Locke AE, et al. Genome-wide association studies of metabolites in Finnish men identify disease-relevant loci. Nat Commun. 2022;13:1644. [PubMed: 35347128]
48. Jaladanki SK, Elmas A, Malave GS, Huang K. Genetic dependency of Alzheimer's disease-associated genes across cells and tissue types. Sci Rep. 2021;11:12107. [PubMed: 34103633]
49. Potjewyd FM, Annor-Gyamfi JK, Aubé J, Chu S, Conlon IL, Frankowski KJ, et al. Use of AD Informer Set compounds to explore validity of novel targets in Alzheimer's disease pathology. Alzheimer's & Dementia: Translational Research & Clinical Interventions. 2022;8:e12253.
50. Esteban-Martos A, Brokate-Llanos AM, Real LM, Melgar-Locatelli S, de Rojas I, Castro-Zavala A, et al. A Functional Pipeline of Genome-Wide Association Data Leads to Midostaurin as a Repurposed Drug for Alzheimer’s Disease. Int J Mol Sci. 2023;24.
51. Proitsi P, Lee SH, Lunnon K, Keohane A, Powell J, Troakes C, et al. Alzheimer's disease susceptibility variants in the MS4A6A gene are associated with altered levels of MS4A6A expression in blood. Neurobiol Aging. 2014;35:279–290. [PubMed: 24064185]
52. He Z, Le Guen Y, Liu L, Lee J, Ma S, Yang AC, et al. Genome-wide analysis of common and rare variants via multiple knockoffs at biobank scale, with an application to Alzheimer disease genetics. The American Journal of Human Genetics. 2021;108:2336–2353. [PubMed: 34767756]
53. Mahajan UV, Varma VR, Griswold ME, Blackshear CT, An Y, Oommen AM, et al. Dysregulation of multiple metabolic networks related to brain transmethylation and polyamine pathways in Alzheimer disease: A targeted metabolomic and transcriptomic study. PLoS Med. 2020;17.
54. Sun L, Guo D, Jia Y, Shi M, Yang P, Wang Y, et al. Association between human blood metabolome and the risk of Alzheimer's disease. Ann Neurol. 2022;92:756–767. [PubMed: 35899678]
55. Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci. 1991;12:383–388. [PubMed: 1763432]
56. Hampel H, Hardy J, Blennow K, Chen C, Perry G, Kim SH, et al. The Amyloid-β Pathway in Alzheimer's Disease. Mol Psychiatry. 2021;26:5481–5503. [PubMed: 34456336]
57. Wu X, Zhang J, Liu H, Mian Y, Liang B, Xie H, et al. Organic Anion Transporter 1 Deficiency Accelerates Learning and Memory Impairment in tg2576 Mice by Damaging Dendritic Spine Morphology and Activity. Journal of Molecular Neuroscience. 2015;56:730–738. [PubMed: 25725783]
58. Bush KT, Wu W, Lun C, Nigam SK. The drug transporter OAT3 (SLC22A8) and endogenous metabolite communication via the gut–liver– kidney axis. Journal of Biological Chemistry. 2017;292:15789–15803. [PubMed: 28765282]
59. Krege JH, John SWM, Langenbach LL, Hodgin JB, Hagaman JR, Bachman ES, et al. Male-female differences in fertility and blood pressure in ACE-deficient mice. Nature. 1995;375:146–148. [PubMed: 7753170]
60. de Vries PS, Yu B, Feofanova EV, Metcalf GA, Brown MR, Zeighami AL, et al. Whole-genome sequencing study of serum peptide levels: The Atherosclerosis Risk in Communities study. Hum Mol Genet. 2017;26:3442–3450. [PubMed: 28854705]
61. Altmaier E, Menni C, Heier M, Meisinger C, Thorand B, Quell J, et al. The pharmacogenetic footprint of ACE inhibition: A population-based metabolomics study. PLoS One. 2016;11:e0153163–e0153163. [PubMed: 27120469]

62. Feldstein CA. Association between chronic blood pressure changes and development of Alzheimer's disease. Journal of Alzheimer's Disease. 2012;32:753–763.
63. Koronyo-Hamaoui M, Shah K, Koronyo Y, Bernstein E, Giani JF, Janjulia T, et al. ACE overexpression in myelomonocytic cells: Effect on a mouse model of Alzheimer's disease. Curr Hypertens Rep. 2014;16:1–9.
64. Gregson J, Qizilbash N, Iwagami M, Douglas I, Johnson M, Pearce N, et al. Blood pressure and risk of dementia and its subtypes: a historical cohort study with long-term follow-up in 2.6 million people. Eur J Neurol. 2019;26:1479–1486. [PubMed: 31233665]
65. Kim S, Kim MJ, Kim S, Kang HS, Lim SW, Myung W, et al. Gender differences in risk factors for transition from mild cognitive impairment to Alzheimer's disease: A CREDOS study. Compr Psychiatry. 2015;62:114–122. [PubMed: 26343475]
66. Podcasy JL, Epperson CN. Considering sex and gender in Alzheimer disease and other dementias. Dialogues Clin Neurosci. 2022. 2022.
67. Rahman A, Jackson H, Hristov H, Isaacson RS, Saif N, Shetty T, et al. Sex and Gender Driven Modifiers of Alzheimer's: The Role for Estrogenic Control Across Age, Race, Medical, and Lifestyle Risks. Frontiers in Aging Neuroscience. 2019;11.
68. LaPlume AA, McKetton L, Anderson ND, Troyer AK. Sex differences and modifiable dementia risk factors synergistically influence memory over the adult lifespan. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring. 2022;14:e12301.
69. Seshadri S, Wolf PA, Beiser A, Au R, McNulty K, White R, et al. Lifetime risk of dementia and Alzheimer's disease: the impact of mortality on risk estimates in the Framingham Study. Neurology. 1997;49:1498–1504. [PubMed: 9409336]
70. Aldred S, Mecocci P. Decreased dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) concentrations in plasma of Alzheimer's disease (AD) patients. Arch Gerontol Geriatr. 2010;51:e16–e18. [PubMed: 19665809]
71. Scassellati C, Galoforo AC, Esposito C, Ciani M, Ricevuti G, Bonvicini C. Promising Intervention Approaches to Potentially Resolve Neuroinflammation And Steroid Hormones Alterations in Alzheimer's Disease and Its Neuropsychiatric Symptoms. Aging Dis. 2021;12:1337–1357. [PubMed: 34341712]
72. Vaňková M, Hill M, Velíková M, Včelák J, Vacínová G, Dvořáková K, et al. Preliminary evidence of altered steroidogenesis in women with Alzheimer's disease: Have the patients "OLDER" adrenal zona reticularis? J Steroid Biochem Mol Biol. 2016;158:157–177. [PubMed: 26704533]
73. Butterfield DA, Halliwell B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. Nat Rev Neurosci. 2019;20:148–160. [PubMed: 30737462]
74. Dong Y, Brewer GJ. Global metabolic shifts in age and Alzheimer's disease mouse brains pivot at NAD+/NADH redox sites. Journal of Alzheimer's Disease. 2019;71:119–140.
75. Patti GJ, Yanes O, Siuzdak G. Metabolomics: the apogee of the omic triology NIH Public Access. Nat Rev Mol Cell Biol. 2012;13:263–269. [PubMed: 22436749]
76. Liu J, Lahousse L, Nivard MG, Bot M, Chen L, van Klinken JB, et al. Integration of epidemiologic, pharmacologic, genetic and gut microbiome data in a drug–metabolite atlas. Nat Med. 2020;26:110–117. [PubMed: 31932804]
77. Chen J, Herrup K. Glutamine as a potential neuroprotectant in Alzheimer's disease. Diet and Nutrition in Dementia and Cognitive Decline, Elsevier; 2015. p. 761–771.
78. Ishizaki F, Nishiyama T, Kawasaki T, Miyashiro Y, Hara N, Takizawa I, et al. Androgen deprivation promotes intratumoral synthesis of dihydrotestosterone from androgen metabolites in prostate cancer. Sci Rep. 2013;3:1528. [PubMed: 23524847]
79. Kihel L El. Oxidative metabolism of dehydroepiandrosterone (DHEA) and biologically active oxygenated metabolites of DHEA and epiandrosterone (EpiA) – Recent reports. Steroids. 2012;77:10–26. [PubMed: 22037250]
80. Ando T, Nishiyama T, Takizawa I, Ishizaki F, Miyashiro Y, Takeda K, et al. Dihydrotestosterone synthesis pathways from inactive androgen 5α-androstane-3β,17β-diol in prostate cancer cells: Inhibition of intratumoural 3β-hydroxysteroid dehydrogenase activities by abiraterone. Sci Rep. 2016;6:32198. [PubMed: 27561382]

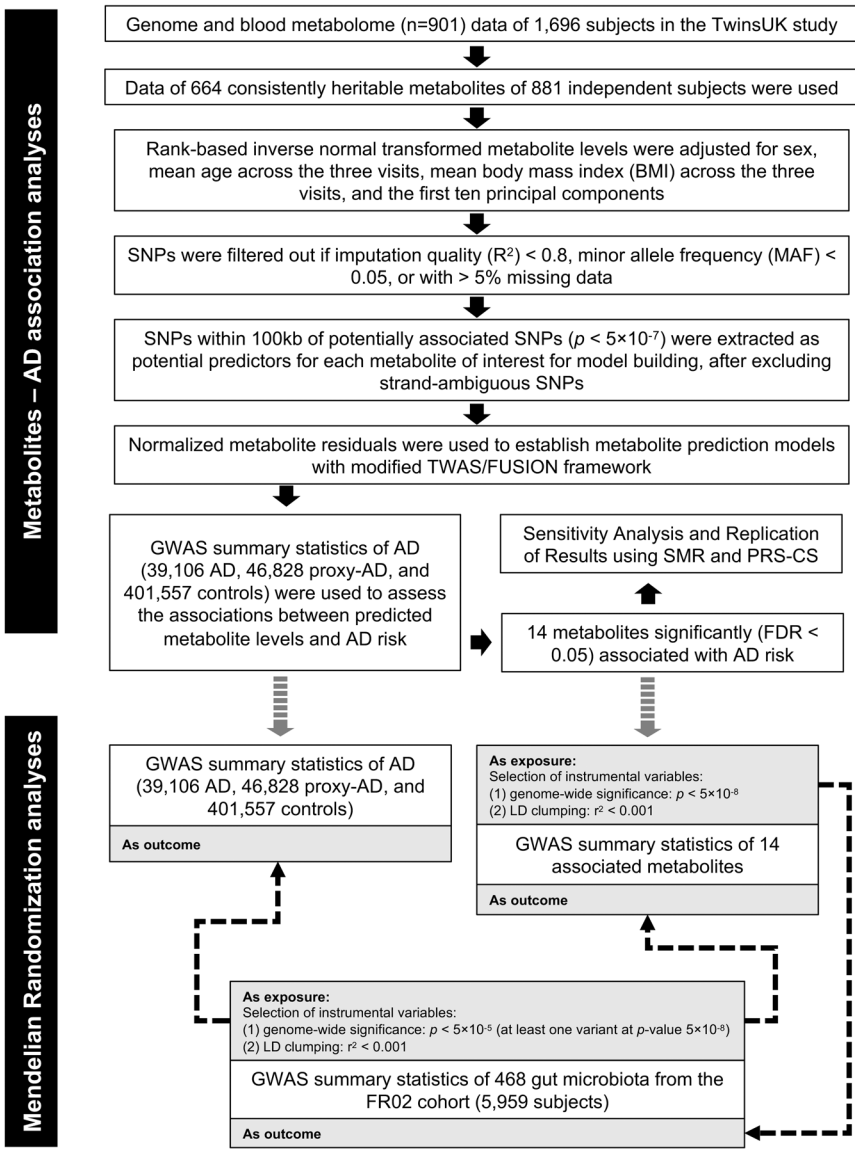

Fig. 1.
The overall design of this study.

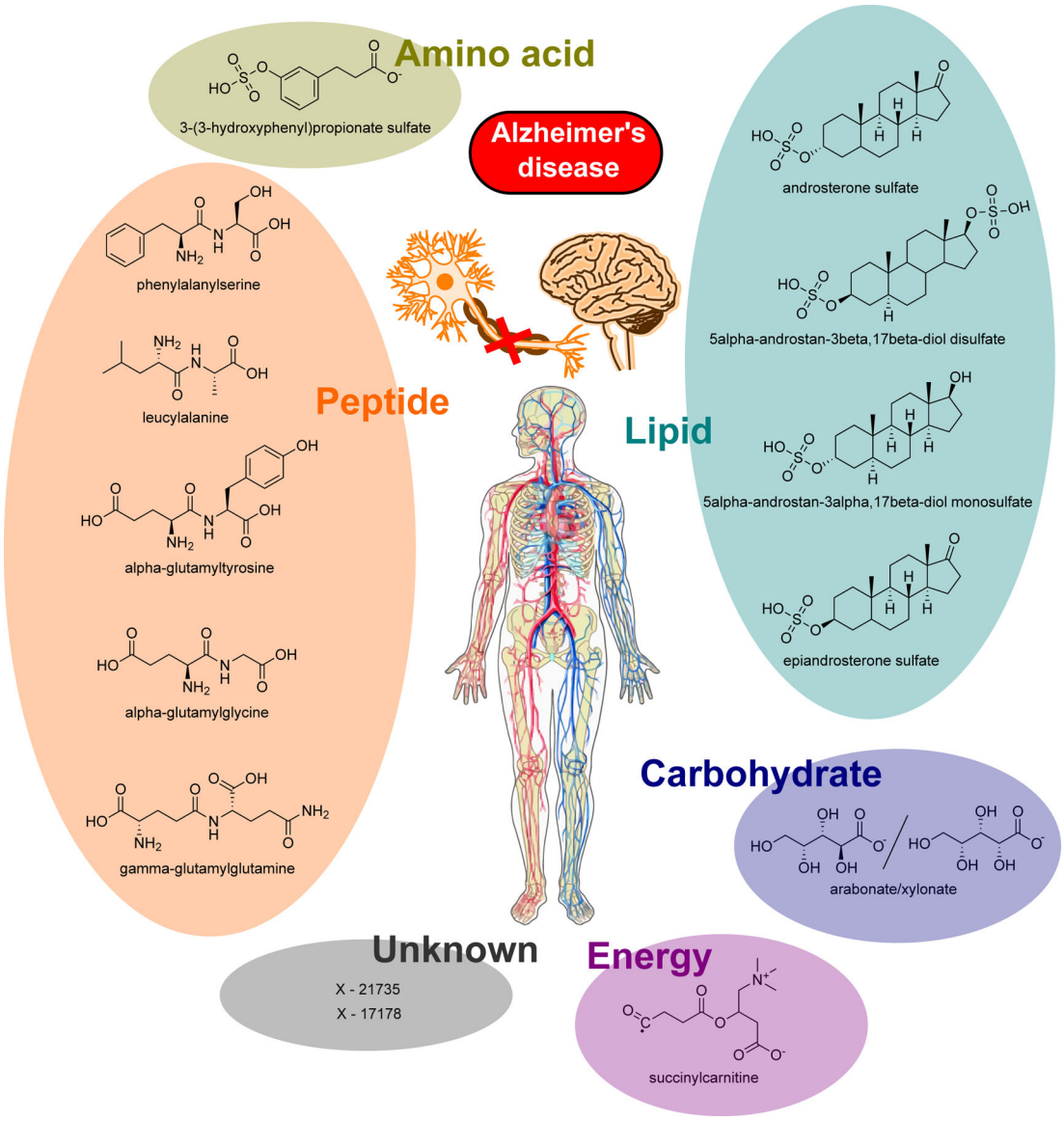

Fig. 2.

Fourteen metabolites whose genetically predicted levels in blood are associated with AD risk.

The blood metabolites involved in amino acid, peptide, lipid, carbohydrate, energy, and unknown super pathways are shown in olive, orange, teal, navy blue, patriarch, and grey ellipses, respectively. The figure is generated by ChemDraw software.

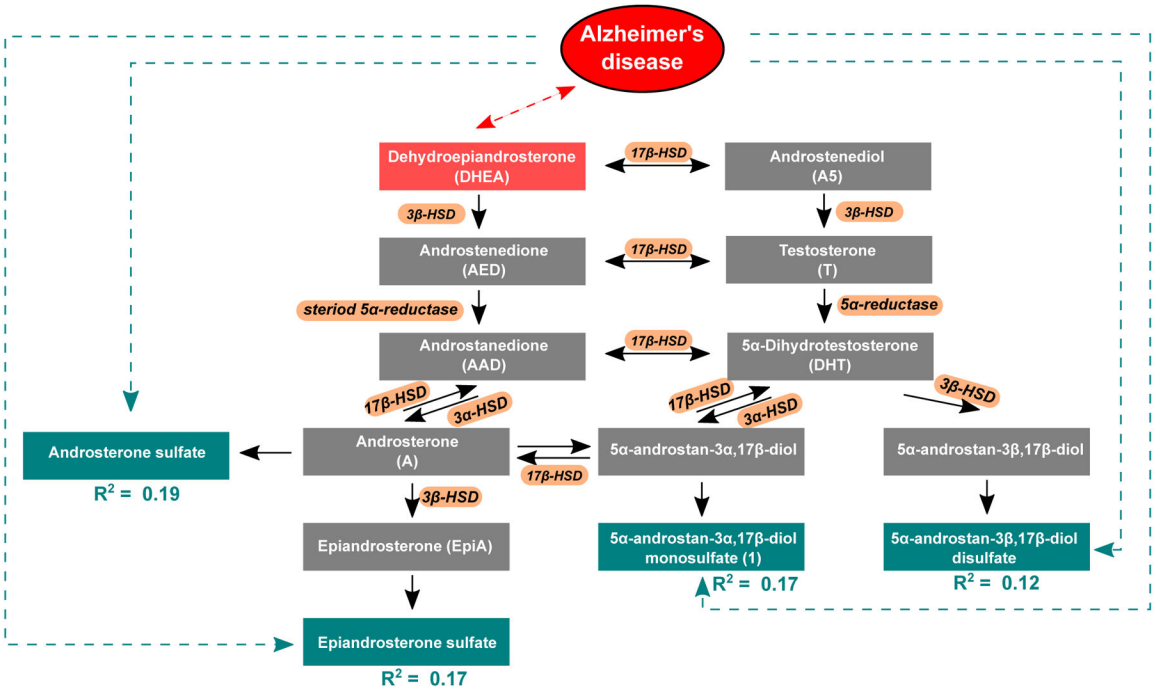

Fig. 3.

Selected aspects of dehydroepiandrosterone (DHEA) metabolism.

The reported metabolites associated with AD, intermediate metabolites in the DHEA metabolism pathway, and metabolites identified in the current study are shown in red box, grey boxes, and teal boxes, respectively. The catalytic enzyme in each reaction is shown in orange rounded boxes. Dark straight lines with arrows indicate the direction of reactions. Dashed lines with arrows show the association between metabolites and AD risk. R^2 represents the proportion of metabolite level variance explained by the genetic instruments. This pathway is reviewed in [78–80].

Table 1

Genetically predicted metabolite abundances in blood associated with AD risk.

Metabolon ID	Name	Super pathway	Sub pathway	No. of predicting SNPs in model	Modeling method	Model internal cross validation R^2	Z score	P-value	FDR
M45415	3-(3-hydroxyphenyl)propionate sulfate	Amino Acid	Phenylalanine and Tyrosine Metabolism	13	lasso	0.02	8.52	1.62×10^{-17}	6.79×10^{-15}
M40016	phenylalanylserine	Peptide	Dipeptide	1	top1	0.05	−6.70	2.15×10^{-11}	4.50×10^{-9}
M40010	leucylalanine	Peptide	Dipeptide	40	enet	0.08	−5.84	5.16×10^{-9}	7.21×10^{-7}
M40033	alpha-glutamyltyrosine	Peptide	Dipeptide	1,187	blup	0.03	−4.80	1.61×10^{-6}	1.69×10^{-4}
M46406	X - 17178	Unknown	Unknown	56	enet	0.14	−4.49	7.16×10^{-6}	6.00×10^{-4}
M41530	alpha-glutamylglycine	Peptide	Dipeptide	7	lasso	0.04	−4.34	1.41×10^{-5}	8.74×10^{-4}
M31591	androsterone sulfate	Lipid	Steroid	46	enet	0.19	−4.33	1.46×10^{-5}	8.74×10^{-4}
M37190	5alpha-androstan-3beta,17beta-diol disulfate	Lipid	Steroid	9	lasso	0.12	−4.28	1.88×10^{-5}	9.86×10^{-4}
M33973	epiandrosterone sulfate	Lipid	Steroid	38	enet	0.17	−4.24	2.27×10^{-5}	1.06×10^{-3}
M37186	5alpha-androstan-3alpha,17beta-diol monosulfate (1)	Lipid	Steroid	18	enet	0.17	−3.72	2.00×10^{-4}	7.85×10^{-3}
M37058	succinylcarnitine	Energy	TCA Cycle	18	lasso	0.05	−3.71	2.96×10^{-4}	7.85×10^{-3}
M48255	arabonate/xylonate	Carbohydrate	Pentose Phosphate Pathway	1	top1	0.03	−3.60	3.23×10^{-4}	0.01
M46904	X - 21735	Unknown	Unknown	21	enet	0.07	−3.27	1.07×10^{-3}	0.03
M02730	gamma-glutamylglutamine	Peptide	Gamma-glutamyl Amino Acid	229	blup	0.02	−3.14	1.67×10^{-3}	0.05

lasso: LASSO; top1: top SNPs; enet: Elastic Net; blup: Best Linear Unbiased Predictor

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