Heterogeneous effects of genetic risk for Alzheimer’s disease on the phenome

Hei Man Wu, Alison M. Goate and Paul F. O’Reilly

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Here we report how four major forms of Alzheimer’s disease (AD) genetic risk—APOE-ε4, APOE-ε2, polygenic risk and familial risk—are associated with 273 traits in ~500,000 individuals in the UK Biobank. The traits cover blood biochemistry and cell traits, metabolic and general health, psychosocial health, and cognitive function. The difference in the profile of traits associated with the different forms of AD risk is striking and may contribute to heterogenous presentation of the disease. However, we also identify traits significantly associated with multiple forms of AD genetic risk, as well as traits showing significant changes across ages in those at high risk of AD, which may point to their potential roles in AD etiology. Finally, we highlight how survivor effects, in particular those relating to shared risks of cardiovascular disease and AD, can generate associations that may mislead interpretation in epidemiological AD studies. The UK Biobank provides a unique opportunity to powerfully compare the effects of different forms of AD genetic risk on the phenotype in the same cohort.

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BACKGROUND
Genetic risk for Alzheimer’s disease (AD) takes several forms, including: Mendelian, rare, polygenic, APOE and family risk [1]. It is important to investigate the impact that these different forms of AD risk have on the array of biomarkers, traits and clinical outcomes comprising the ‘phenome’ for two major reasons. (1) Heterogeneity in the presentation and pathophysiology of AD has been widely reported [2–4]. A part of this heterogeneity may be caused by the different effects that different risk factors have on the phenotype. (2) Growing evidence suggests that modifiable factors, such as exercise, diet and alcohol consumption, contribute substantially to the risk of late-onset Alzheimer’s Disease (LOAD) [5, 6]. Any modifiable risk factor, or active biological compound on which it acts, will have a genetic component. This genetic component must also be a genetic component of AD itself, since it initiates a path towards a higher risk of AD. Therefore, one strategy for identifying candidate modifiable risk factors is to investigate the downstream effects of genetic risk for AD [6]. While such an approach will highlight many traits due to (horizontal) pleiotropic effects on the trait and AD, it will produce a long list of mediation candidates. In combination with evidence from other sources, these candidates can then be prioritized for further study, which may ultimately lead to the identification of targets for intervention.

The APOE gene contains risk alleles for LOAD that are among the largest common genetic effects on any disease, with homozygous APOE-ε4 carriers ~12 times more likely to develop the disease [7, 8]. While the precise mechanism of APOE-ε4’s role in the pathogenesis of AD is unknown, APOE has reported roles in B-amyloid clearance, lipid homeostasis and cholesterol transport [7, 9]. In contrast, APOE-ε2 has apparent protective effects against AD. As well as reduced incidence of LOAD, APOE-ε2 is associated with increased cognitive reserve [10, 11], reduced cortical thinning and lower amyloid-β accumulation [11]. Outside the APOE gene, the genetic effects on LOAD are thought to be largely polygenic: 21 significant loci were identified in the latest GWAS of LOAD [12], while the estimated $\text{Pr}_\text{LOAD}^2$ is 7.1%, and after removal of the APOE region is 6.4% [12]. Polygenic Risk Scores (PRS), which combine the effects of many risk alleles genome-wide to provide a proxy of polygenic risk to a disease [13], have been widely used in AD research, finding associations with AD clinical diagnosis [14, 15], disease progression [16], cognitive impairment [15, 16], and educational attainment in children [17]. An alternative proxy for the genetic risk of AD is family history of AD, which reflects both common and rare genetic risk variants, risk variants for early and late onset AD, as well as environmental risks. In recent years, GWAS performed on family history of AD [18] or “AD-by-proxy” [19], based on parental AD/dementia status and age, has been demonstrated as a powerful way of identifying risk variants for AD.

In this study, we report the associations between AD genetic risk and a range of traits in the UK Biobank, some of which may mediate risk for AD. We examine four major forms of AD genetic risk: (1) APOE-ε4, the strongest known genetic factor for LOAD, which is also associated with early-onset AD [20], (2) APOE-ε2, a protective allele of AD, (3) Polygenic risk, using the AD PRS (with APOE excluded) as a proxy, and (4) Familial risk, according to the parental history of AD. A key advantage of this study is the unique opportunity that the UK Biobank provides to systematically and powerfully compare the effects of these four forms of genetic risk across a large number of traits, in the same cohort. We utilize the rich data resources of the UK Biobank to investigate hundreds of traits relating to education, cognitive function, lifestyle and environment, psychosocial factors, health and medical history, and physical and biological measures. Our findings highlight...
substantial heterogeneity in the profile of traits associated with different forms of AD genetic risk and the phenotype.

METHODS

Study population

This study used the UK Biobank (UKB) data [21], which is a large prospective multi-ancestry cohort study of ~500,000 participants (European ancestry samples, n = 472,725; non-European ancestry samples, n = 27,034), aged 40–69 years when baseline measures were taken during recruitment across the UK between 2006 and 2010. We used the genotype and phenotype data generated from the UKB.

Dementia cases including AD, vascular dementia, frontotemporal dementia and all-cause dementia were removed from analyses to limit the downstream effects of the disease on observed trait values. Cases were identified as having a primary/secondary diagnosis from hospital records or primary/secondary cause of death from the death registry, using ICD-10 codes in categories F01-F99 (mental and behavioural disorder chapter) or G00-G09 (disease of the nervous system chapter). Participants who self-reported having dementia/Alzheimer’s/cognitive impairment (F20002) were also excluded. A total of 1532 dementia cases were identified and excluded from the dataset.

Genetic risk factors

APOE risk: APOE contains three common forms of the “ε allele” associated with AD risk: ε2, ε3 and ε4. The ε4 allele is the strongest common genetic risk factor for LOAD, while ε2 is associated with substantially reduced risk relative to the ε3 allele, which typically considered the reference allele in association testing. Which of the three APOE ε alleles (or haplotypes) an individual has on each chromosome is defined by their alleles at two missense SNPs, rs7412 and rs429358, and APOE ε genotypes are the pair of these an individual has on their two chromosomal strands (e.g. ε2/ε4, ε3/ε3). The two SNPs defining the allele were directly genotyped in the UK Biobank (Hardy-Weinberg P > 0.05; genotyping rate > 80%), but do not pass standard GWAS quality control (QC), which typically requires genotyping rate > 95%. However, in this study, these two SNPs were retained to define the APOE ε genotype given their known importance to AD risk. The concordance between the genotype calls of the two SNPs from the genotyping array and from whole-exome sequencing among 34,453 individuals of the first phase of whole-exome sequencing in the UK Biobank is extremely high (rs7412: 99.93%; rs429358: 99.96%).

We investigated the dosage effects of the ε4 and ε2 alleles relative to the ε3 allele. Specifically, to test the effect of APOE-ε4 on traits we considered an additive genetic model with ε3/ε3 (n = 242,205) coded as 0, ε3/ε4 heterozygotes (n = 97,137) as 1, and ε4/ε4 (n = 5,693) coded as 2. To test the effect of APOE-ε2 on traits we coded ε3/ε3 as 0, ε3/ε2 heterozygotes (n = 501,999) as 1, and ε2/ε2 homozygotes (n = 2,329) as 2. In common with other AD studies [22, 23], participants with ε2/ε4 were excluded to avoid their effects being conflated. A total of 401,563 individuals with complete genotype data at rs7412 and rs429358 to determine APOE alleles were available for analysis.

Polygenic risk: PRSice-2 [24] was used to construct polygenic risk scores (PRS) using the latest GWAS of LOAD [12] (n = 94,437) as base data, and the UK Biobank sample as target data. Due to restricted permissions associated with using the latest GWAS of LOAD [12] to predict education and intelligence outcomes, an earlier GWAS of LOAD [25] was used as base data for predicting education and intelligence with the latest GWAS of LOAD [12] to predict education and intelligence factors, available in the UK Biobank. Supplementary Note 1 contains details about data processing and coding. For traits with 10 or more unique values, we removed outliers >6 standard deviations from the mean and applied a rank-based inverse normal transformation to all.

Statistical analysis

We first tested for associations between the AD genetic risk factors and all 273 traits, using linear and logistic regression for continuous and binary traits, respectively. All models were adjusted by age, sex⁵, sex, an age-sex interaction term, socioeconomic status based on Townsend deprivation index, assessment centre, and, for the genetic analyses, genotyping batch and the first 12 principal components of ancestry. We additionally adjusted for APOE genotype when assessing familial risk as a genetic risk factor, to account for the effect of APOE in familial risk. Models for which the blood biochemistry genotype when assessing familial risk as a genetic risk factor, to account for the effect of APOE in familial risk. Models for which the blood biochemistry outcomes, an earlier GWAS of LOAD [25] was used as base data for predicting education and intelligence factors, available in the UK Biobank. Supplementary Note 1 contains details about data processing and coding. For traits with 10 or more unique values, we removed outliers >6 standard deviations from the mean and applied a rank-based inverse normal transformation to all.

To investigate potential confounding by cardiovascular disease (CVD), stratified analyses were performed by grouping the individuals into cases and controls in each of the following categories: statin use (F20003), self-reported CVD (i.e. heart attack, angina, stroke or high blood pressure: F6150), and having a biological parent with high blood pressure, stroke, or heart disease (F20107, F20110, F20110). Given that the CVD stratified analyses showed a substantial difference in APOE-ε4 effects between CVD cases and controls for a subset of traits, in particular metabolic health traits, in our primary analyses (Fig 1) we additionally included adjusting for these factors (i.e. statin use, self-reported CVD condition, family history of CVD), and a healthy lifestyle score, as
covariates. Briefly, the healthy lifestyle score [28] is a composite score consisting of smoking, physical activity, diet and alcohol consumption. The purpose of adjusting for the healthy lifestyle score was to account for the potential ascertainment of healthier lifestyle and genetics among living individuals of middle or older age with higher risk for CVD due to APOE-ε4.

A conservative Bonferroni correction, assuming independence of all tests, was used to control for multiple testing in the analyses described above. A P-value threshold of 4.5 × 10^{-5} was used (0.05/273(number of traits)*4(number of predictors)) for inferring statistical significance. The correlation between the sets of results among the four forms of AD genetic risk is assessed by Kendall’s rank correlation test, in each of the six trait categories separately. A P-value threshold of 0.00139 was used (0.05/6(number of categories)*6(number of pairwise correlations among the AD predictors)) for inferring significance. For the 98 traits with significant associations (P < 4.5 × 10^{-5}) in the final model, we then tested for interactions between the AD genetic risk factor and age. To investigate the sex-specific genetic effects, we also performed the analysis stratified by sex and tested for differences between their results using t-tests. A P-value threshold of 6.4 × 10^{-5} was used (0.05/98(number of traits)*4(number of predictors)*2(age and sex interaction tests)) for inferring significance.

RESULTS

In this study, we examined associations between four forms of AD genetic risk and 273 traits across the UK Biobank. Standard linear and logistic regression analyses were performed, controlling for covariates including age, sex, genetic principal components, parental age, CVD factors and lifestyle score (see Methods). Parental age, CVD factors and lifestyle score were only added to our model after observing the effects of potential confounding in our initial analyses (see below).

These analyses identified significant associations (P < 4.5 × 10^{-5}) between APOE-ε4 and 51 traits, APOE-ε2 and 42 traits, polygenic risk and 16 traits, and familial risk and 27 traits (Fig. 1, Table 1, Supplementary Table 1). While the four forms of AD genetic risk inevitably have a different power to identify significant associations with the traits, each is sufficiently powered to detect multiple traits and the broad profile of associations is noticeably different across the four forms. APOE-ε4 dosage is primarily associated with blood biochemistry traits, metabolic health and cognitive function, while APOE-ε2 dosage shows the opposite associations for most of these traits, although not with cognitive function. Both polygenic (excluding APOE) and familial risk (controlling for APOE genotype) provide proxies for genome-wide genetic risk outside the APOE region. While AD PRS is associated with a range of traits across the categories of blood biochemistry, blood cell traits, metabolic health and general health, familial risk for AD is primarily associated with psychosocial health.

In order to provide context to these results, we performed the PRS and familial risk analyses in relation to Parkinson’s disease, major depressive disorder, diabetes, and height, each of which showed a markedly different profile of associations with the 273

![Fig. 1 Associations between APOE-ε4, APOE-ε2, polygenic risk (AD PRS) and AD familial risk and 273 traits in the UK Biobank](image-url)

These association analyses between the genetic risk factors and UK Biobank traits (described in the “Methods” section) were controlled for age, age^2, sex, age*sex interaction term, socioeconomic status based on Townsend deprivation index, genotyping batch, assessment centre, and the first 12 principal components of ancestry, self-reported CVD, statin use, parental CVD and lifestyle score in the models. The grey dotted lines correspond to the P-value significance threshold (P < 4.5 × 10^{-5}; see Methods). The Y-axis has been truncated to show all associated traits to improve visualization, with extreme results shown in the grey area with inverted triangles.
These analyses correspond to the 30 most significant associations from Fig. 1, in which association analyses between the genetic risk factors and UK Biobank traits (described in the "Methods" section) were controlled for age, sex, age*sex interaction term, socioeconomic status based on Townsend deprivation index, genotyping batch, assessment centre, and the first 12 principal components of ancestry, self-reported CVD, statin use, parental CVD and lifestyle score in the models. P-values shown in bold indicate a significant (P < 4.5 × 10⁻⁴; see Methods). See Supplementary Table 1 for full association results corresponding to all 273 traits.

RDW Red blood cell erythrocyte distribution width; Illness injury...* Illness, injury, bereavement, stress in last 2 years; retic. reticulocyte; HL High light scatter; Freq. of tiredness...* Frequency of tiredness/lethargy in last 2 weeks; Seen GP for nerves...* Seen doctor (GP) for nerves, anxiety, tension or depression; Freq. of depressed...* Frequency of depressed mood in last 2 weeks; Non-cancer illnesses* Number of self-reported non-cancer illnesses; Diabetes* Diabetes diagnosed by doctor; Substitution Test* Symbol Digit Substitution Test.

| Table 1. Details of the 30 most significant associations between APOE-e4, APOE-e2, polygenic risk (AD PRS) and AD familial risk and traits in the UK Biobank. |
|---|---|---|---|---|---|---|---|---|---|
| Phenotype | Category | APOE-e4 | | | | | | | |
| | | Est (SE) | P | r² | Est (SE) | P | r² | Est (SE) | P | r² |
| Apolipoprotein B | Blood biochemistry | -0.24 (0.0039) | 0.025 | -0.069 (0.0055) | 0.016 | 9.5 × 10⁻⁴ | 0.028 | -0.03 (0.82) | 0.0 | 2.9 × 10⁻⁷ |
| C reactive protein | Blood biochemistry | -0.26 (0.0041) | 0.02 | 0.039 (0.0057) | 0.02 | 5.8 × 10⁻⁵ | 0.01 | -0.089 (0.018) | 0.01 | 3.3 × 10⁻⁵ |
| Cholesterol | Blood biochemistry | -0.17 (0.0039) | 0.013 | -0.042 (0.0055) | 0.015 | 5.3 × 10⁻⁴ | 0.025 | 0.048 (0.8) | 0.0 | 1.1 × 10⁻⁵ |
| LDL direct | Blood biochemistry | 0.19 (0.0039) | 0.015 | -0.054 (0.0055) | 0.015 | 1.9 × 10⁻⁴ | 0.048 | 0.011 (0.8) | 0.0 | 5.4 × 10⁻⁸ |
| Apolipoprotein A | Blood biochemistry | -0.092 (0.0039) | 0.024 | 0.099 (0.0055) | 0.026 | 3.2 × 10⁻⁴ | 0.96 | 0.57 (0.79) | 0.07 | 2.6 × 10⁻⁶ |
| Triglycerides | Blood biochemistry | 0.073 (0.004) | 0.12 | 0.056 (0.0056) | 0.12 | 0.28 | 0.011 | 0.0011 | 0.81 | 2.8 × 10⁻⁶ |
| HDL cholesterol | Blood biochemistry | -0.064 (0.0038) | 0.05 | 0.054 (0.0053) | 0.05 | 1.4 × 10⁻⁵ | 0.001 | 0.51 (0.77) | 0.051 | 2.0 × 10⁻⁶ |
| RDW | Blood cell traits | -0.005 (0.0045) | 0.01 | 0.1 (0.0063) | 0.01 | 3.5 × 10⁻⁵ | 0.003 | 0.0011 | 0.88 | 1.4 × 10⁻⁶ |
| Illness, injury...* | Psychosocial health | -0.015 (0.0086) | 0.09 | 0.0011 | 0.005 | 0.9 | 0.022 | 0.14 | 0.059 | 2.1 × 10⁻⁵ |
| Direct bilirubin | Blood biochemistry | -0.029 (0.0044) | 0.01 | 0.081 (0.006) | 0.01 | 1.8 × 10⁻⁵ | 0.001 | 0.0011 | 0.81 | 5.9 × 10⁻⁷ |
| Immature retic. fraction | Blood cell traits | -0.023 (0.0046) | 0.07 | 0.076 (0.0064) | 0.07 | 3.7 × 10⁻⁵ | 0.052 | 0.3 | 0.9 | 1.5 × 10⁻⁴ |
| HL retic. percentage | Blood cell traits | 0.0066 (0.0046) | 0.13 | 0.071 (0.0064) | 0.13 | 2.2 × 10⁻⁵ | 0.076 | 0.41 | 6.6 × 10⁻⁶ | 1.0 × 10⁻⁴ |
| HL retic. count | Blood cell traits | -0.0172 (0.0046) | 0.12 | 0.1 | 0.0054 (0.0056) | 0.1 | 8.5 × 10⁻⁵ | 0.051 | 0.4 | 1.3 × 10⁻⁶ |
| Gycerated haemoglobin | Blood biochemistry | -0.037 (0.0041) | 0.17 | 0.077 (0.0057) | 0.17 | 3.5 × 10⁻⁷ | 0.0091 | 0.07 | 0.32 | 1.1 × 10⁻⁶ |
| Seen GP for nerves...* | Psychosocial health | 0.0014 (0.0092) | 0.66 | 0.0042 (0.0013) | 0.66 | 10.0 | 0.014 | 0.044 | 0.07 | 3.4 × 10⁻⁷ |
| Freq. of depressed...* | Psychosocial health | 0.0027 (0.0024) | 0.27 | 0.0022 (0.0034) | 0.27 | 1.5 × 10⁻⁵ | 0.0046 | 0.19 | 0.08 | 1.5 × 10⁻⁵ |
| Non-cancer illnesses...* | General health | -0.001 (0.0032) | 0.6 | 0.0059 | 0.011 | 0.0043 | 0.01 | 0.066 (0.0052) | 0.066 | 1.4 × 10⁻⁴ |
| Retic. percentage | Blood biochemistry | 0.0039 (0.0046) | 0.12 | -0.055 (0.0064) | 0.12 | 2.3 × 10⁻⁴ | 0.09 | 3.8 | 0.19 | 6.5 × 10⁻⁴ |
| Creatinine | Blood biochemistry | -0.0026 (0.0034) | 0.9 | 0.0013 | 0.024 | 7.2 × 10⁻⁴ | 0.13 | 12.0 (0.08) | 0.05 | 1.4 × 10⁻⁴ |
| Retic. count | Blood cell traits | 0.0037 (0.0046) | 0.42 | 0.02 | 0.005 (0.0064) | 0.42 | 4.3 × 10⁻⁴ | 0.079 | 4.5 | 3.9 × 10⁻⁸ | 1.4 × 10⁻⁴ |
| Alkaline phosphatase | Blood biochemistry | -0.031 (0.0041) | 0.17 | 0.025 (0.0057) | 0.17 | 8.5 × 10⁻⁵ | 0.036 | 2.2 | 0.0061 | 1.3 × 10⁻⁵ |
| Diabetes* | Metabolic health | -0.053 (0.0071) | 0.15 | -0.0066 (0.0098) | 0.15 | 8.7 × 10⁻⁴ | 0.022 | 0.82 | 0.0061 | 1.3 × 10⁻⁵ |
| Substitution Test* | Cognitive function | 0.016 (0.0044) | 0.11 | -0.011 | 0.0019 | 1.6 × 10⁻⁴ | 0.011 | 0.0008 | 0.11 | 1.6 × 10⁻⁵ |
| SHBG | Blood biochemistry | 0.028 (0.0038) | 0.11 | 0.0085 (0.0054) | 0.11 | 8.7 × 10⁻⁴ | 0.036 | 0.014 | 0.51 | 2.2 × 10⁻⁴ |
| Insomnia | General health | -0.0094 (0.003) | 0.016 | 0.009 (0.0042) | 0.016 | 2.9 × 10⁻⁴ | 0.0035 | 0.6 | 0.013 | 1.3 × 10⁻⁴ |
| Urea | Blood biochemistry | -0.029 (0.004) | 0.02 | 0.014 | 0.0056 | 0.02 | 7.9 × 10⁻⁴ | 0.04 | 1.4 (0.81) | 0.078 | 1.2 × 10⁻⁵ |
traits tested (Supplementary Figs. 1 and 2), highlighting the specificity of the AD results and providing reassurance that they are not due to artifact or cohort effects.

We examined the rank correlation between the results of Fig. 1 generated by the different forms of AD genetic risk (Fig. 2) to quantify the level of convergence or divergence of AD genetic risk. A significant negative correlation between the association results of APOE-ε4 and APOE-ε2 for metabolic health traits (Kendall's τ = −0.4; \( P = 5.3 \times 10^{-5} \)) was observed, suggesting the antagonistic effect of the APOE alleles on the metabolism of fats in the body. Apart from this expected result, we also observed a significant negative correlation between APOE-ε2 and familial risk among blood cell traits (\( P = 2.9 \times 10^{-5} \)). There were also negative correlations of borderline significance between APOE-ε4 and APOE-ε2 for blood biochemistry, and between APOE-ε4 and familial risk for metabolic health traits. There were no significant correlations between polygenic and familial risk in any of the phenotypic categories, despite both constituting proxies of genome-wide (non-APOE) genetic risk for AD. We repeated the analyses using only those individuals with both PRS and familial risk data (\( n = 286,427 \)), and re-examined the correlation between the two (Supplementary Fig. 3). Again, no significant correlations were observed between the two and there was minimal difference in the results.

**Trait associations with APOE-ε4 and APOE-ε2**

The strongest associations between any of the AD genetic risk factors and the 273 traits tested are between the APOE-ε4 and APOE-ε2 dosages and the blood lipid levels (Table 1). APOE-ε4 dosage is highly significantly associated with higher levels of "bad cholesterol", including APO-B, LDL, total cholesterol, and triglycerides, and lower levels of "good cholesterol", including APO-A and HDL. Consistent with this, APOE-ε4 carriers are more likely to take cholesterol-lowering medication. The reverse trends are observed for APOE-ε2 dosage, which is associated with significantly lower "bad cholesterol" and higher "good cholesterol" traits. The impact that APOE-ε2 dosage has on these cholesterol levels is striking, explaining ~9% overall variance in APO-B, ~6% variance in LDL and ~4% variance in total cholesterol, despite the ε2 allele being of low frequency (allele frequency 6.8% in the UK Biobank). Notable exceptions to these opposite trends in association between APOE-ε4 and APOE-ε2 and the lipid traits are observed for triglycerides, in which there is a highly significant positive association with APOE-ε4 and APOE-ε2, and decreased levels of lipoprotein A with APOE-ε4 and APOE-ε2 dosage. Given that APOE-ε4 increases the risk of AD, while APOE-ε2 reduces AD risk, these results suggest that triglycerides and lipoprotein A may not play a direct role in AD pathology.

As expected, APOE-ε4 is associated with an increased risk of cognitive impairments (Fig. 1, Supplementary Table 1). APOE-ε4 carriers show cognitive decline across several cognitive tests, for example, taking longer to complete the Trail Marking Test and making fewer correct matches in the Digit symbol substitution test. No significant associations were observed between APOE-ε2 and cognitive function, which may reflect a smaller AD protective effect offered by the ε2 allele relative to the AD risk of the ε4 allele [30]. An example of a trait that APOE-ε4 and APOE-ε2 have effects on that are particularly consistent with their effects on AD is alanine aminotransferase (APOE-ε4 effect = −0.04; APOE-ε2 effect = 0.018; see Supplementary Table 1), which adds some support to its recently reported association with AD [31].

In addition to its effect on blood lipids, APOE-ε2 dosage is strongly associated with reduced levels of reticulocyte in blood, including immature reticulocyte fraction, high light scatter reticulocyte percentage, high light scatter reticulocyte count, reticulocyte percentage, and reticulocyte count (see Table 1).

**Fig. 2 Correlations between the trait association results of different forms of AD genetic risk.** The value reported in each cell is the rank correlation coefficient (Kendall’s τ) between the genetic-trait association results, illustrated in Fig. 1, of the corresponding forms of genetic risk. All the results are adjusted for age, age², sex, age-sex interaction term, socioeconomic status based on Townsend deprivation index, genotyping batch, assessment centre, and the first 12 principal components of ancestry, self-reported CVD, statin use, parental CVD and lifestyle score (see Methods). Correlations of nominal significance (\( P < 0.01 \)) are highlighted by a box, while those that are significant after correction for multiple testing (\( P < 0.0014 \); see Methods) are highlighted by a bold box.

APOE-ε4 is associated with reduced immature reticulocyte fraction, but not with the other reticulocyte-related traits.

Furthermore, APOE-ε4 dosage is strongly associated with lower levels of C-reactive protein (CRP), a lower incidence of diabetes and reduced regular use of aspirin. While the associations with diabetes and aspirin are consistent with the lower level of CRP found in APOE-ε4 carriers, their effects are unexpected in that diabetes [32, 33] and high levels of CRP [34, 35] have been reported as associated with an elevated risk of neurodegenerative diseases. The opposite directions of effect are observed in relation to APOE-ε2 carriers, who have higher levels of CRP and higher incidence of diabetes, but only a nominal increase in aspirin use.

APOE-ε4 is also a known risk factor for CVD [36]. To replicate this finding, we examined the effect of APOE on CVD in the UK Biobank using logistic regression, adjusting for the standard covariates and parental age. We observed a significantly higher risk of heart attack in APOE-ε4 carriers, but no associations were observed for angina, stroke and high blood pressure. Conversely, the ε2 allele appears to decrease the risk of heart attack and angina, but not in stroke or high blood pressure. In our initial analyses we observed multiple examples of the different forms of genetic risk for AD associated with positive health outcomes, including healthier dietary intake, lower BMI, increased lung function and even increased height. These effects were also observed for APOE-ε4 dosage. To investigate these unexpected results, we re-evaluated the APOE-ε4 associations with metabolic traits, stratifying individuals according to their CVD status in terms of (i) statin use, (ii) self-reported CVD condition, and (iii) family history of CVD. Overall, there was a marked reduction in the APOE-ε4 effect on these factors among those individuals who were ‘controls’ for CVD (Fig. 3). For example, APOE-ε4 dosage is strongly associated with decreased BMI in statin users but this effect is largely ameliorated (two-sided t-test \( P \)-value = \( 3.1 \times 10^{-5} \)) in statin non-users, while APOE-ε4 dosage is associated with reduced red meat intake in individuals with a parent with CVD (\( P = 1.5 \times 10^{-11} \)) but not in those with no parent with CVD (\( P = 0.99 \)). Possible explanations for these results are (1) collider bias caused by stratifying by CVD risk, (2) positive lifestyle changes made by APOE-ε4 carriers who are made
traits, metabolic health and general health (Fig. 1, Table 1), likely to start insulin treatment within the transferase and diabetes. Individuals with higher AD PRS are less including reticulocyte percentage and count, Aspartate amino- (~1%). The only traits showing some evidence for shared effects high genetic risk according to present AD PRS, albeit current AD largely restricted to individuals with APOE-

metabolism plays an important role in AD pathology, then this is evidence of association with the lipids. This indicates that if lipid effects associated with higher longevity if the AD studies used in the late onset of the disease. Thus, individuals whose parents have higher longevity than random members of the population, given

the other forms of AD risk are associated with is markedly different (Fig. 1). Familial risk is mainly associated with psychosocial factors, which appear to be directly linked with caring for a parent with Alzheimer’s and/or anxiety over suffering from AD themselves in the future. For example, the strongest association is with experience of illness, injury, bereavement, the stress in the last 2 years (P = 1.4 × 10⁻⁵), followed by higher neuroticism score, increased tiredness in last two weeks, increased depressed mood in last two weeks, seen doctor for nerves or depression and reduced family relationship satisfaction. In addition, individuals who have or had parents with AD report a higher number of non-cancer illnesses. However, given the broad definition of non-cancer illness, which includes cardiovascular, respiratory and neurological disease, it is not possible to determine whether this is likely a consequence of the negative impact on their psychosocial health or due to other effects of having increased risk of AD.

The absence of associations between familial risk and the traits that the other forms of AD risk are associated with is likely a combination of the low convergence of effects between APOE effects and AD risk factors genome-wide, indicated by the results of AD PRS, and the greater heterogeneity of familial risk of AD, which incorporates rare genetic and all environmental factors. Accounting for the survivor effect is critical when using familial risk as a predictor. In an initial analysis that we performed without controlling for parental age, we observed that individuals with positive AD family history appeared to be healthier or of higher socio-economic position across a range of traits, including having significantly lower diastolic blood pressure, lower TV watching and higher fluid intelligence. Individuals with LOAD generally have higher longevity than random members of the population, given the late onset of the disease. Thus, individuals whose parents have LOAD on average have parents with relatively high longevity, which is associated with a favourable genetic profile, socioeconomic advantage, and better health factors, all of which are inherited by offspring through a combination of the environment and genetics. However, once parental age is accounted for as a covariate then these associations are ameliorated or eliminated (Fig. 4); for example, diastolic blood pressure (Unadjusted: P = 1.7 × 10⁻⁵; Adjusted: P = 0.026), and fluid intelligence score (Unadjusted: P = 3.9 × 10⁻⁻⁷; Adjusted: P = 0.078). The effects of APOE-e4, APOE-e2 and polygenic risk remain unaffected by the parental age adjustment.

**Trait associations with polygenic risk**

Polygenic risk, captured here by the AD PRS, is associated (P < 4.5 × 10⁻⁵) with 16 traits across blood biochemistry, blood cell traits, metabolic health and general health (Fig. 1, Table 1, Supplementary Table 1). Despite the highly significant associations between the APOE alleles and the lipid traits, the AD PRS shows no evidence of association with the lipids. This indicates that if lipid metabolism plays an important role in AD pathology, then this is largely restricted to individuals with APOE-e4 rather than with high genetic risk according to present AD PRS, albeit current AD PRS (without APOE) explain only a tiny fraction of variance in AD (~1%). The only traits showing some evidence for shared effects across APOE and polygenic risk are several reticulocyte measures, including reticulocyte percentage and count, Aspartate aminotransferase and diabetes. Individuals with higher AD PRS are less likely to start insulin treatment within the first year of diagnosis of diabetes. Although the higher prevalence of insulin abnormalities and insulin resistance are reported in patients with AD [37, 38], how AD genetic risk impacts the clinical profiles for diabetic patients, leading to reduced or delayed initialization of insulin therapy determined by their physicians, is unclear.

AD PRS is associated with higher standing height, higher calcium levels and improved lung function, as measured by increased forced expiratory volume in one second (FEV1). This is in conflict with some previous epidemiological studies. Increased adult height has reported associations with better cognitive performance and reduced risk of AD [39–41], which may relate to increased cognitive reserve associated with the larger head circumference or better nutrition leading to greater height and reduced AD risk [39]. Moreover, individuals with impaired lung function have been reported to have a higher risk of cognitive decline and dementia [42, 43]. One potential explanation for these unexpected findings is that the AD PRS may include genetic effects associated with higher longevity if the AD studies used in the latest AD GWAS [9] included older cases than controls. While most AD studies age-match cases and controls, not all are able to, which allows the possibility of a systematic difference in longevity between cases and controls. To account for the longevity effect—a possible confounder in our results of AD PRS—we repeated the analyses adjusting for longevity PRS [44] as a covariate. However, the difference in the results was minimal (Supplementary Fig. 4), which may be due to the low predictive power of the present longevity PRS (R² of 0.19%).

In terms of metabolic health, individuals with higher AD PRS are associated with more lean body mass, having higher basal (resting) metabolic rate, arm fat-free mass, trunk fat-free mass and trunk predicted mass. In contrast, APOE-e4 carriers are associated with lower levels of lean body mass. However, the trait variance explained by the PRS associations is extremely small and so should be considered with caution.

**Trait associations with familial risk**

Familial risk for AD, as defined by number of parents with AD, controlled for parental age, is significantly associated (P < 4.5 × 10⁻⁵) with 27 of the 273 traits. The profile of traits that familial risk and the other forms of AD risk are associated with is markedly different (Fig. 1, Table 1). Familial risk is mainly associated with psychosocial factors, which appear to be directly linked with caring for a parent with Alzheimer’s and/or anxiety over suffering from AD themselves in the future. For example, the strongest association is with experience of illness, injury, bereavement, the stress in the last 2 years (P = 1.4 × 10⁻⁵), followed by higher neuroticism score, increased tiredness in last two weeks, increased depressed mood in last two weeks, seen doctor for nerves or depression and reduced family relationship satisfaction. In addition, individuals who have or had parents with AD report a higher number of non-cancer illnesses. However, given the broad definition of non-cancer illness, which includes cardiovascular, respiratory and neurological disease, it is not possible to determine whether this is likely a consequence of the negative impact on their psychosocial health or due to other effects of having increased risk of AD.

Finally, we investigated how the effects of AD genetic risk factors on traits varied across age. We also investigated age effects stratified by sex and tested for sex differences (see Methods). These are not individual-level longitudinal analyses, since all participants only
provide data at a single time-point, and so the results may be influenced by generational effects. However, if generational effects on a trait are small relative to age effects, then these results should provide a good proxy to longitudinal analyses.

Regression results using the sex-combined sample show a significant interaction ($P < 8 \times 10^{-5}$; see Methods) between the APOE-$e4$ effect and age for 22 traits, including 4 blood lipid traits, Urate, Alanine aminotransferase, 15 metabolic traits relating to adiposity, and the Symbol Digit Substitution Test (Fig. S; Supplementary Table 2). Assuming that there are not strong generational effects, then individuals with APOE-$e4$ appear to have increasingly lower body fat across age (BMI: interaction $P = 4.4 \times 10^{-10}$; Body fat %: interaction $P = 2.6 \times 10^{-8}$), increasingly lower alanine aminotransferase (interaction $P = 8.3 \times 10^{-5}$) and increasing cognitive decline as measured by the Symbol Digit Substitution Test (interaction $P = 9.7 \times 10^{-8}$). These data indicate that cognition function can start to deteriorate from age 40 to 50 under the influence of APOE-$e4$. This analysis, therefore, highlights, in addition to cognitive decline, adiposity and alanine aminotransferase as potentially having close links with AD pathology given the timing of the effect of APOE-$e4$ on them. This adds evidence to a longitudinal study that reported an increasing negative association of APOE-$e4$ with BMI across age in a prospectively measured cohort [45], as well as a recent study that implicated alanine aminotransferase in AD diagnosis and pathophysiology [31].

APOE-$e2$, which is associated with reduced LDL and cholesterol during the ages 40–50 in these data, is associated with increasing LDL and cholesterol with age (LDL: interaction $P = 3.8 \times 10^{-6}$; Cholesterol: interaction $P = 1.1 \times 10^{-5}$). It should be noted that the interactions with age observed here in relation to the APOE variants may reflect the survivor effect (discussed in the previous section). For example, the elevated risk of CVD among APOE-$e4$ carriers may be sufficient that they need to have markedly better metabolic health to survive mid-life.

No significant interaction effects were observed for AD PRS. Significant interactions between the effects of familial risk and age were observed for illness, injury, bereavement, the stress in the last 2 years, and frequency of depressed mood. Both factors had an apparently decreasing effect with age. Note that familial risk with AD is associated with these stress/mood factors in all age groups, but with a reducing effect across age, which may reflect a greater burden for relatively younger carers and for those with a younger parent with AD.

There were no significant differences in interaction effects between males and females for these analyses.

**Fig. 4 Effect of parental age adjustment in different forms of AD genetic risk.** Estimated effects (standardized) of familial risk, APOE-$e4$, APOE-$e2$, polygenic risk on selected traits using the primary analysis model (Fig. 1, see Methods), with or without adjustment of parental age. Points show effect size estimates and whiskers show 95% confidence intervals.

**DISCUSSION**

This study is the most powerful systematic comparison of how the major forms of AD genetic risk affect the phenotype so far. We were able to make direct comparisons between the effects of forms of AD risk, such as APOE-$e2$ and familial risk, which can only be powerfully studied together in exceptionally large cohorts.

The largest associations were between the APOE alleles and lipid metabolism. APOE-$e2$ explains $\sim$4%–6% and $\sim$9% variance in cholesterol, LDL and Apolipoprotein-B (APO-B), respectively, while APOE-$e4$ explains 1–2% variance in each. These are striking effects for single common variants. There is already substantial evidence for a role of lipid metabolism [46, 47], and its effects, such as atherosclerosis [48], on Alzheimer’s pathophysiology. However, there is no evidence in these data for an association between polygenic or familial risk and lipid traits. While the relatively low power of the polygenic and familial risk factors mean that convergence of effects on lipid metabolism and AD across a subset of genetic loci cannot be ruled out, it is clear that polygenic and familial risk should be separated out from APOE effects in AD research into lipid metabolism to avoid conflating their effects.

APOE-$e4$ had its largest impact on any single trait on CRP—a marker of low-grade inflammation—explaining $\sim$2% variance in CRP. However, as observed elsewhere [49], APOE-$e4$ dosage is associated with reduced CRP, despite the fact that low-grade inflammation is an established risk factor for CVD [50, 51] and AD [34, 35]. This apparent paradox may be explained by the potentially divergent processes leading to AD from APOE-$e4$ and from the genetic risk of AD across the rest of the genome.

We identified 98 traits significantly associated with at least one of the four forms of AD risk. While likely most of these traits are associated with AD genetic risk due to (horizontal) pleiotropic effects, the possibility that they may be mediators between AD genetic risk and AD can be considered broadly in terms of their convergence across different forms of AD, the consistency of APOE-$e4$/APOE-$e2$ trait and AD effects, and their changing effects with age. Traits highlighted due to several of these factors include: C reactive protein (CRP), BMI, diabetes, alanine aminotransferase and reticulocyte blood cell measures. For example, alanine aminotransferase was highlighted due to the fact that APOE-$e4$ has a threefold higher, but opposing effect, on it as APOE-$e2$, consistent with APOE effects on AD, and due to the significant interaction between the APOE-$e4$ effect on it and age (Fig. 5). This adds further evidence to the reported associations between alanine aminotransferase and AD [31].

Despite some evidence of convergent effects on traits among different genetic risk factors for AD, the overall pattern was of contrasting profiles of associations between the APOE variants, polygenic risk, and familial risk. Much of this is likely caused by the relatively low power of the PRS and familial risk factors, and that genetic risk variants for any disease also often have pleiotropic effects on a range of other traits. However, part of this heterogeneity of effects on traits may reflect heterogeneity in the disease etiology itself, and so these findings warrant further interrogation. Some of the associations can be more easily explained, such as familial AD risk being associated with factors relating to caring for a parent with Alzheimer’s, but these are still important to account for in AD research and in the clinical care of Alzheimer’s patients. Our study also re-iterated the importance of accounting for the survivor effect in AD research, in relation to both the overlap between CVD and AD risk factors (namely APOE-$e4$ here) and the fact that individuals with late-onset AD will typically have had an otherwise healthy profile given their longevity.

All of the associations reported in this study were investigated univariately in terms of traits, are based on cross-sectional observational data, and are analysed in a large sample with the power to detect very small but potentially unimportant effects, and so all results should be considered exploratory in nature.

We expect our study to act as a useful resource to aid in understanding the effects that different forms of AD genetic risk have
on the phenotype. We hope that our results provide an ideal starting point for further observational interrogation of AD aetiology, including replicating these analyses in other populations, and that insights gained here provide leads for experimental studies that may be able to shed light on those causal mechanisms that both underlie these associations and lead to AD.

**CODE AVAILABILITY**

Analytic code to define outcomes and regression models for this work is available upon request.

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