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Whole-exome sequencing of the BDR cohort: evidence to support the role of the *PILRA* gene in Alzheimer's disease

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Abstract

Aim—Late-onset Alzheimer's disease (LOAD) accounts for 95% of all Alzheimer's cases and is genetically complex in nature. Overlapping clinical and neuropathological features between AD, FTD and Parkinson's disease highlight the potential role of genetic pleiotropy across diseases. Recent genome-wide association studies (GWASs) have uncovered 20 new loci for AD risk; however, these exhibit small effect sizes. Using NGS, here we perform association analyses using exome-wide and candidate-gene-driven approaches.

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There are no conflicts of interest.

Author Contributions

Tulsi Patel – data generation, analysis, manuscript preparation. Keeley J. Brookes – data collection, critiqued manuscript. James Turton – analysis, critiqued manuscript. Sultan Chaudhury – analysis, critiqued manuscript. Tamar Guetta-Baranes – sample preparation, critiqued manuscript. Rita Guerreiro – data generation, critiqued manuscript. Jose Bras – data generation, critiqued manuscript. Dena Hernandez – data generation, critiqued manuscript. Andrew Singleton – data generation, critiqued manuscript. Paul T. Francis – sample contribution, critiqued manuscript. John Hardy – study design, study supervision and management, critiqued manuscript. Kevin Morgan – study design, study supervision and management, data generation, analysis, manuscript preparation.

Approval

Written informed consent was obtained for all individuals and approved by the appropriate institutional review boards (BDR ethics number 08/H0704/128 + 5).

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Summary data of all case and control samples used for analysis.

Table S2. Variants found in AD-related genes in 129 LOAD cases and 53 controls.

Methods—Whole-exome sequencing was performed on 132 AD cases and 53 control samples. Exome-wide single-variant association and gene burden tests were performed for 76 640 nonsingleton variants. Samples were also screened for known causative mutations in familial genes in AD and other dementias. Single-variant association and burden analysis was also carried out on variants in known AD and other neurological dementia genes.

Results—Tentative single-variant and burden associations were seen in several genes with kinase and protease activity. Exome-wide burden analysis also revealed significant burden of variants in *PILRA* ($P = 3.4 \times 10^{-5}$), which has previously been linked to AD via GWAS, hit *ZCWPW1*. Screening for causative mutations in familial AD and other dementia genes revealed no pathogenic variants. Variants identified in *ABCA7*, *SLC24A4*, *CD33* and *LRRK2* were nominally associated with disease ($P < 0.05$) but did not withstand correction for multiple testing. *APOE* ($P = 0.02$) and *CLU* ($P = 0.04$) variants showed significant burden on AD.

Conclusions—In addition, polygenic risk scores (PRS) were able to distinguish between cases and controls with 83.8% accuracy using 3268 variants, sex, age at death and *APOE* ε4 and ε2 status as predictors.

Keywords

Alzheimer’s disease; burden analysis; polygenic risk score; Whole-exome sequencing

Introduction

Alzheimer’s disease (AD) is the most common form of dementia, affecting over 850 000 people in the UK alone, a number expected to rise to 1 million by 2025 [1]. There are two forms distinguished by the age when symptoms first appear. In the early-onset familial form (fEOAD), symptoms appear before 65 years of age; however, this only accounts for about 5% of cases [2]. Mutations in the familial genes *APP*, *PSEN1* and *PSEN2* are rare but highly penetrant. Individuals with these mutations are almost certain to develop fEOAD [3]. The majority of cases are sporadic in nature and classified as late-onset Alzheimer’s disease (LOAD), with symptoms appearing at 65 years or later. This represents the other 95% of all incidences [4]. Presence of the *APOE* ε4 allele is the largest known genetic risk factor for LOAD, with a two- to three-fold increase in risk for carriers and 15-fold for individuals homozygous for the ε4 allele [5]. The rare ε2 allele confers a protective effect and appears to reduce AD risk by up to 40%.

Genome-wide association studies (GWAS) have identified 20 risk variants associated with LOAD [6–9]; this has implicated several new pathways in AD, such as endocytic processing, inflammation and cholesterol transport [8]. Although highly replicable in Caucasian groups, these effects have been difficult to replicate in other populations [10]. These common variants also exert only small effects on disease risk, which does not account for much of the missing heritability in AD. It is likely that low-frequency variants, not detected by GWAS, could have greater effect sizes and therefore explain more of the heritable component.

Advances in next-generation sequencing (NGS) in recent years have allowed exomes and entire genomes to be explored at single-base level. The exome accounts for approximately

1% of the human genome, yet it harbours almost 85% of known mutations underlying disease-related traits [11]. Therefore, rare mutations can be identified using this technology. Whole-exome sequencing (WES) has identified a rare variant in the *TREM2* gene, R47H, associated with a five-fold increase in AD risk [12]. These studies have also found rare causative variants in *CLU* and *SORL1* that were overlooked by GWAS [13]. By identifying the genetic variants of individuals, WES has the potential to uncover more rare variants associated with AD risk.

There are several overlapping clinical and neuropathological features across different dementias. For example, dementia with Lewy bodies (DLB) shares clinical features with AD and Parkinson's disease (PD), often resulting in misdiagnosis. PD and DLB are both synucleinopathies presenting with alpha-synuclein deposits in the brain, whereas *APOE* ε4 increases risk of disease in AD and DLB [14]. This suggests that genetic risk factors may contribute to more than one disease, known as genetic pleiotropy, whereby a gene or DNA variant can influence multiple phenotypes.

Clinically well-characterized brain tissue samples from healthy individuals remain a limiting factor in the study of neurological disorders [15]; Brains for Dementia Research (BDR) (www.brainsfordementiaresearch.org.uk) is a network of six leading UK brain banks (jointly funded by Alzheimer's Research UK and Alzheimer's Society), specifically created to address the shortages of high-quality brain tissue samples from healthy individuals and those with dementia. This project is a planned brain donation programme with over 3000 participants, aged 65 years and above, with and without the diagnosis of dementia. Regular, standardized cognitive and psychiatric assessment of potential brain donors during life is critical in optimizing the value of brain tissue for research [16,17].

We performed single-variant and burden analysis on coding variants to identify significant associations with LOAD. We also report on screening of 132 LOAD patients from the Brains for Dementia Research (BDR) resource with the aim to identify causative or predicted pathogenic coding variants in 40 selected genes. Of these, 16 are associated with familial forms of neurodegeneration, including fEOAD (*APP*, *PSEN1* and *PSEN2*), frontotemporal dementia (FTD) and Amyotrophic lateral sclerosis (ALS) (*C9or72*, *CHMP2B*, *FUS*, *GRN*, *MAPT*, *TARDBP* and *VCP*), PD (*LRRK2*, *PARK2*, *PARK7*, *PINK1* and *SNCA*) and Prion disease (*PRNP*). The remaining genes were selected from AD GWAS and NGS (20 GWAS, *APOE* and *TREM2*).

Polygenic risk scores (PRS) have been increasingly used to investigate the effect of multiple genetic variants on disease traits. It is based on the notion that many variants with small effects will not be detectable at genome-wide significance; however, collectively they may have a strong effect [18]. PRS were generated to examine the association between multiple genetic markers and their collective effects on LOAD.

Materials and Methods

BDR samples

The BDR cohort comprised 132 clinically diagnosed LOAD (age at onset ≥65 years) cases and 53 cognitively normal controls; all diagnoses were neuropathologically confirmed (Table S1). Neuropathological diagnoses were undertaken by experienced neuropathologists within the Brains for Dementia Research network and were based on Thal Aβ phases [19], neurofibrillary tangle Braak stages [20], Consortium to Establish a Registry for Alzheimer's disease (CERAD) criteria for AD [21] which are all combined in the National Institute on Aging – Alzheimer's Association guidelines [22], together with the Newcastle/McKeith criteria for Lewy body disease [23] and for FTLD-TDP as described by Mackenzie et al [24]. While there are no fully established criteria for vascular pathology, the VCING criteria were used [25] along with those proposed by Smallwood et al [26] and Grinberg and Thal [27].

Demographics of case and control samples for each centre are shown in Table 1. The average age at death was 82.5 years (range 65–101 years) for LOAD samples. For control individuals, average age at death was 85.9 years (range 58–104 years). The proportion of females between cases and controls were similar, accounting for around 50% of the total sample size. *APOE* ε4 carriers were three-fold higher in cases (64.7%) with almost one fifth (17.3%) being homozygous for the ε4 allele. In comparison, only 24.5% of controls were carriers and no individuals were ε4ε4. The ε2 allele was present in 10 control samples (18.9%) and 8 cases (6.2%), with both a case and control sample being homozygous for the allele. The higher three-fold frequency observed in control samples correlates with the protective effect of the ε2 allele. All samples in the BDR cohort used for analysis were classified as AD or control by neuropathology.

The BDR has a number of neuropathological features available for more definitive analysis of genotype–phenotype correlation [28]. Data include the CERAD scale, Braak tangle and Lewy body staging, with simplified measures (present/absent; mild/moderate/severe) of small vessel disease, deposition of TDP-43 protein, arteriolar Aβ-CAA and cerebrovascular atherosclerosis. Detailed clinical and cognitive information on the samples is also available on request, demonstrating the potential value of the BDR cohort for very detailed analyses in future studies as more extensive genetic data are generated.

DNA extraction

DNA was extracted from brain tissue using standard phenol–chloroform procedures. Samples were analysed on the Agilent TapeStation and quantified using the Nanodrop 3300 spectrometer to ensure high concentration and quality material was obtained. Samples were genotyped for *APOE* ε2, ε3 and ε4 alleles using the TaqMan method (Applied Biosystems) to determine *APOE* status.

Exome-sequencing library prep

DNA libraries were hybridized to exome-capture probes with Agilent SureSelect Human All Exon Kit V4 for Illumina GA (Agilent Technologies) as per manufacturer's protocol.

Exome-enriched libraries were sequenced on the Illumina HiSeq 2500 using 2 × 100 bp paired end read cycles. The Agilent capture library includes 5′ and 3′ untranslated regions.

Bioinformatics

Paired-end sequence reads were aligned to the human reference genome build 19 (UCSC hg19) using Burrows-Wheeler Aligner [29]. Format conversion, indexing and removal of PCR duplicates were performed with Picard (www.picard.sourceforge.net/index.shtml). The Genome Analysis Toolkit was used for recalibration of base quality scores, realignment around indels and variant calling [30]. Variants were annotated using ANNOVAR [31], and Variant Effect Predictor [32] predicted SIFT and Polyphen2 scores of protein coding variants. Consistency between SIFT and Polyphen2 predictions and the databases allowed more reliable classification. Variants were also checked against established databases (dbSNP v.149, 1000 Genomes Project and Exome Variant Server).

Filtering

Singleton variants with MAF ≤ 0.002 were removed in VCFtools [33]. Coding variants in genes were filtered by annotation with SnpSift [34]. Visualization of variants was performed, when necessary, using Integrative Genomics Viewer [35]. Individuals with a calculated age at onset below 65 years were removed and samples were screened for causative mutations in fEOAD genes *APP*, *PSEN1* and *PSEN2* to ensure only sporadic cases were used for analyses. Pathogenicity of variants was determined using pathogenic status in AD&FTD and PD mutation databases [36].

Association analyses

Quality control filtering was performed on the VCF using VCFtools. Individuals were removed if genotyping rate ≤97%, followed by markers with call rate ≤98%. Markers with significant deviation ($P < 0.001$) from the Hardy–Weinberg equilibrium in control individuals were removed. After removing samples and markers failing quality control, 290 individuals remained with 76 640 nonsingleton variants in coding regions. The average genotyping rate was 99.9%.

Plink files were imported to PLINK-SEQ [37] (https://atgu.mgh.harvard.edu/plinkseq/). Single-variant association was performed on samples using a logistic regression test correcting for the covariates sex, age at death and *APOE* ε4 allele count.

Gene-based association for genes of interest was calculated in R using a SKAT-O [38] burden test. The Calpha test was used for exome-wide analysis, and the SKAT-O test was used for selected genes.

Polygenic risk scoring

PRS were generated for BDR samples using PRSice [39]. The International Genomics of Alzheimer's Project (IGAP) summary data were used as the base dataset, collated from 17 008 LOAD cases and 37 154 controls. A region of 500 kb around the *APOE* locus was excluded from the analysis. The best-fit model with the greatest predictive accuracy was computed using area under the curve (AUC) in SPSS. Additional predictor variables

included were the number of *APOE* ε4 and ε2 alleles, age, sex and genotypes for the GWAS SNPs.

Results

Overview of data

Exome sequencing was performed on a total of 292 individuals. The final cohort consisted of 132 LOAD cases and 53 control samples after quality control filtering. A total of 157 217 nonsingleton variants were present in 290 individuals, with a minimum of two alleles observed per variant. Filtering to retain only coding mutations resulted in 76 640 variants for exome-wide analysis.

Exome-wide analyses

Burden analysis using a C-alpha test highlighted some nominally significant gene associations with AD, shown in Table 2. *PILRA* and *PRSS45* are just below the Bonferroni-corrected threshold ($P = 2 \times 10^{-6}$) at $P = 3.4 \times 10^{-5}$ and $P = 5.9 \times 10^{-5}$, respectively. *PILRA* has previously been linked to AD through *ZCWPW1*, which was highlighted by the GWAS meta-analysis [9]. Five variants in *PILRA* contribute to the effect: intronic variants rs7792525, rs190071731 and rs148891131, synonymous mutation rs2405442 and missense variant p.S279L (rs34266222). GWAS SNP rs1476679 is in weak LD with rs2405442 (R^2=0.50). This mutation is tolerated as predicted by SIFT and Polyphen2. The other genes have not previously been linked to AD. *PRSS45* contains five variants that drive this signal, of which two were highly associated with disease (Table 3); therefore, it is possible that these SNPs are contributing to the signal.

Exome-wide association analysis was performed on the nonsingleton variants, correcting for age, sex and *APOE* ε4 status of individuals. As expected, *APOE* SNP rs429358 showed the most significant association prior to adjusting for covariates ($P = 7.2 \times 10^{-9}$, OR = 6.5 [3.2–13.1]). There were no significant associations at the genome-wide threshold ($P = 5 \times 10^{-8}$) or at the suggestive threshold ($P = 1 \times 10^{-5}$) after correction. However, due to low sample numbers, we do not have the power to detect any association at that level. Some tentative associations were observed and are shown in Table 3.

Several of the genes encompassing variants are involved in signalling pathways, including serine proteases *PRSS42* and *PRSS45* and inositol triphosphate receptor *ITPR3*. SIFT and Polyphen2 predictions indicate that most mutations are benign or tolerated. However, missense mutations *TMEM260* p.A245S/T (rs17776256) and *AVPR1B* p.K65N (rs35369693) were predicted to be probably damaging by both software. Both variants are more frequent in control samples, signifying a protective effect. *MEP1B* is a metalloprotease recently implicated in *APP* cleavage and has been implicated in inflammation. The synonymous variant p.S537 (rs173032) has an odds ratio of 3.1 (1.6–5.9) and has a significantly greater frequency in cases than controls, inferring an association with AD. Two missense mutations in *PRSS45* are associated with protection against AD, p.I190L (rs58830807) and P130Q (rs58943210); however, both are predicted to be benign. None of the genes aside from *MEP1B* have been directly linked to dementia.

Polygenic risk scoring

A 500-kb region around the *APOE* gene containing 227 variants was excluded from the analysis to identify effects independent of *APOE*. The predictive accuracy of each tested model is given in Table 4, denoted by area under the curve (AUC), with 95% confidence intervals. The *APOE* ε4 allele alone has 71.8% accuracy in discriminating between cases and controls; however, this is a poor fit model. Other covariates ε2, age, sex and GWAS greatly increase the predictive power 83.0%. In total, 3268 variants were utilized to score risk of developing AD using the best-fit model, which had a predictive accuracy of 83.8% when combined with all covariates as predictors. The addition of PRS only increased this accuracy by 0.8%, which is similar to improvements seen in other studies. There is overlap between scores for both groups; however, on average, scores were higher for cases. Mean scores for AD cases were 3.6×10^{-4} compared with 2.7×10^{-4} for control samples.

Screening for familial mutations

Samples were screened for mutations in fEOAD genes to remove any nonsporadic cases. A total of six coding variants were found in *APP* (1), *PSEN1* (1) and *PSEN2* (4), shown in Table 5. All individuals were heterozygous for the variants listed and mutations were synonymous except two identified as missense. *PSEN1* p.E318G (rs17125721) was classified as a risk modifier but not pathogenic, found in five cases and one control sample. *PSEN2* p.S130L (rs63750197) was previously identified as possibly damaging *in silico* with unclear pathogenicity, present in one case and one control. Both SNPs were predicted to be deleterious depending on the transcript. However, no causative or fully penetrant pathogenic mutations were observed in these genes, confirming that these samples are representative of sporadic AD.

Other known neurological genes were also screened for potential pathogenic mutations to identify genetic overlap between sporadic LOAD and other neurodegenerative diseases (Table 6). Mutations in Parkinson's genes *LRRK2*, *PARK2* and *PINK1* appear to have some possibly damaging consequences on the proteins. *PARK2* p.R275W (rs34424986) is very rare and present in one AD case and one control sample with mild cerebral amyloid angiopathy and presence of an unspecified dementia. SIFT/Polyphen2 predictions both indicate a potentially deleterious effect of this mutation. Variant p.P246L (rs149953814) was also found in one case and control, both also presenting with mild nonamyloid small vessel disease. Samples were heterozygous for both variants.

Previously uncharacterized mutations were found in *CHMP2B* and *LRRK2*. The frameshift variant in *CHMP2B* was seen in a healthy control and an individual with AD. The *LRRK2* mutation was heterozygous in a sample of each phenotype and results in a p.L1271P change. *PRNP* p.M129V (rs1799990) has previously been implicated as a risk factor for prion disease; here, however, it is observed in an equal number of case and controls, suggesting no effect in AD.

Association analyses of known neurological genes

Following on from this, genes were selected based on whether they had been linked to AD or other neurological diseases. Direct functionality was inferred from data filtered for coding

variants only. A total of 76 640 variants were annotated as coding mutations, with 219 variants in 35 of the selected genes. Association and burden analyses were performed as before on the subset of variants. No coding variants were identified in AD genes *HLA-DRB1*, *HLA-DRB5* and *MEF2C* or the other neurological genes *MAPT* and *TARDBP*.

We performed single-variant association on all 219 variants in the selected genes. No variants reached genome-wide or suggestive levels of significance as identified by a logistic regression test with correction for covariates. The most significant associations ($P < 0.05$) are listed in Table 7. Results for all 219 variants are shown in Table S2.

A large proportion of the highly associated variants are synonymous mutations. Four *ABCA7* variants are present, with two suggesting increased risk (OR>1) and two showing a protective effect (OR<1). *ABCA7* rs3752234 and rs3752237 are both synonymous mutations that increase risk more than two-fold. *SLC24A4* synonymous SNP (rs7144273) also showed strong effects in the risk direction (OR = 1.63, $P = 0.018$).

The majority of variants appear to be exhibiting a protective effect as indicated by the odds ratios, as they were observed more frequently in control samples. *LRRK2* p.M1646T (rs35303786) missense is predicted as benign; however, it is found in a greater frequency in control samples (OR = 0.14, $P = 0.018$). We calculated gene-based burden using a SKAT-O test to provide greater statistical power than that of a single-marker test (Table 8). Both sets of familial genes did not appear to exhibit any burden on LOAD. Burden analysis revealed two significant associations; *APOE* and *CLU* were the only genes to reach significance ($P \leq 0.05$). However, they would not pass Bonferroni correction ($P = 0.0014$). Five variants in *CLU* contributed to the effect seen, which was corrected for age, sex and *APOE* ε4 status. Two of these variants were significant, synonymous variant rs9331939 and rs149859119 (p.S16R); therefore, they could be driving the signal in this gene.

Discussion

In this study, we initially investigated genetic association with LOAD using an exome-wide approach. Although the analyses did not find any significant associations when corrected for multiple testing, the sample size only provides enough power to detect common variant (MAF > 5%) associations with an effect size above 2.2 with 80% certainty. Nonetheless, single-variant analysis highlighted some interesting tentative associations which may merit further exploration.

Burden analysis revealed a tentative association with *PILRA*, an inhibitory immunoglobulin receptor involved in regulating signal transduction in the immune system. This gene has previously been linked to AD via its interaction with paired activation receptor *PILRB* and GWAS hit *ZCWPW1*. It is expressed on myeloid cells and works with *PILRB*, which also associates with *DAP12* and *TREM2* [40]. *PILRA* SNP rs2405442 is in weak LD ($r^2 = 0.5$) with GWAS SNP rs1476679, suggesting this signal is likely to be independent of the GWAS association. *ZCWPW1* locus SNP rs1476679 was nominally associated with reduced *PILRA* levels [41]. This suggests a potential role for the gene in AD, highlighting the need for further investigation.

Many of the remaining genes on the burden list are enzymes with serine/threonine activity or serine proteases, such as *PRSS45*, *BCR*, *KLK2* and *THNSL2*. Efficient breakdown of proteins is important as impairments in this can lead to the buildup of misfolded proteins. Dysfunction of the amyloid protein degradation pathway has been implicated in AD. None of these genes have been previously linked to AD. However, in combination, enzymes regulating protein function and breakdown could play a greater role in disease and this too warrants further exploration.

Multiple *PRSS45* variants were observed, with two missense mutations found to be associated with AD, exerting a protective effect. However, functional predictions indicate that both polymorphisms are benign. This gene encodes a serine protease, part of a group of enzymes that cleave peptide bonds. *PRSS45* SNPs were also highly associated when tested in burden. Missense variants *TMEM260* p.A245S and *AVPR1B* p.K65N were both predicted to be damaging to the protein *in silico* and found more frequently in control samples. The function of *TMEM260* is not clearly understood, whereas *AVPR1B* is a vasopressin receptor located in the anterior pituitary gland that stimulates ACTH release. *AVPR1B* SNP rs35369693 has been linked to mood disorders and found more frequently in affected females [42]. The mutation in *MEP1B*, known as meprin β, is synonymous; recent proteomic studies have found that these metalloproteases can cleave APP, affecting Aβ levels [43,44]. While these associations are tentative, examination of other larger datasets could be worthwhile.

PRS generated for individuals showed that, on average, scores were significantly higher in LOAD cases than controls, despite an overlap among the cohort. Using sex, age at death, *APOE* ε4 and ε2 allele counts and GWAS SNP genotypes as variables for prediction, the model was able to distinguish cases and control with 83.8% accuracy. A total of 3268 variants were used to predict disease risk. The presence of controls with high-risk scores suggests that these individuals may have gone on to develop AD had they lived longer. The utility of PRS has already been demonstrated in AD, with individuals' genetic risk profiles able to predict disease susceptibility with more than 80% accuracy [45]. However, there were controls with high PRS and no phenotypic changes indicative of dementia and also cases with low PRS. Although PRS can identify more of the genetic component of AD, this shows that there is still unexplained missing heritability.

Mutations in familial AD genes, *APP*, *PSEN1* and *PSEN2,* are rare but highly penetrant. Screening of these genes revealed no pathogenic variants and samples harbouring mutations were heterozygotes, confirming that there were no familial EOAD cases among the BDR LOAD-classified cases. Other neurological familial genes were also screened for pathogenic mutations linked to related dementias. No known causative mutations were identified; however, *PARK2* p.R275W was predicted to be deleterious and has unknown pathogenicity in the PD mutation database. It produces an unusual distribution of parkin with large cytoplasmic and nuclear inclusions [46]. The variant was present in one case and control sample, however, which suggests that it is likely benign and not pathogenic in nature. Previously uncharacterized mutations were identified in *CHMP2B* and *LRRK2*, with a frameshift variant in *CHMP2B* and a missense variant in *LRRK2*. The frameshift variant was only seen in one control sample, suggesting that it could be a sequencing artefact.

LRRK2 p.L1271P is present in a case and control sample so does not appear to segregate with disease. *PRNP* p.M129V has been highlighted as a risk factor for prion disease but appears not to be having any effect in AD.

TREM2 mutation p.R47H was observed in three AD case samples which were heterozygotes. This variant can increase risk of developing AD by two- to three-fold [12,47]. DNA was available for these three samples and Sanger sequencing confirmed the presence of the variant in these subjects. No control samples harboured this variant. However, given the documented frequency of R47H (MAF = 0.002), this cohort appears to have a greater MAF of 0.008. This four-fold greater frequency will be verified as the BDR sample set increases in size.

Single-variant association of all neurological gene variants revealed several synonymous mutations to be nominally associated with AD at $P \leq 0.05$. The majority of variants exerted effects in the protective direction with greater frequency in controls than case samples. Four *ABCA7* variants were significantly associated at $P < 0.05$ with two increasing risk and two being protective. Synonymous variants rs3752234 and rs3752237 increased AD risk more than four-fold, which is contradictory to previous findings [48,49], where the effects were protective. Conversely, rs4147915 and missense mutation rs3764645 p.E188G are protective. *ABCA7* p.E188G is predicted as tolerated and previously shown to have no effect on disease risk [48]. These findings need to be validated as the sample size increases.

Missense variant *LRRK2* p.M1646T was associated with protection against AD, but the amino acid substitution is predicted to be tolerated. However, the mutation is known to increase risk of developing PD [50]. *LRRK2* mutations have previously been linked to AD with PD risk variant p.R1628P found in greater frequencies in AD cases than controls [51]. The variant increased apoptosis and cell death in transfected human cell lines. Therefore, it is likely that genetic pleiotropy possibly occurs across several neurodegenerative diseases. *LRRK2* is involved in autophagy and recycling proteins in the retrograde trafficking pathway. Mutations in this protein are associated with dendrite shortening in neurons, a possible cause of motor symptoms in PD [46]. With some shared clinical features, mutations in *LRRK2* could also affect the autophagy process in AD.

Gene-based burden analysis in SKAT-O allowed adjustment to correct for the effect of age, sex and number of *APOE* ε4 alleles. Both AD and other neurological familial genes did not exhibit any burden on LOAD. *APOE* and *CLU* were significant to $P < 0.05$ but did not pass Bonferroni correction. Only SNP p.S16R in *CLU* was significantly associated in single-variant testing, indicating that this is driving the signal.

Conclusion

Although other familial neurological genes did not show any burden on LOAD, an individual missense variant in *LRRK2* was tentatively associated; preliminary exploration of the data has indicated that genetic pleiotropy is likely to play a role in diseases with overlapping features. *LRRK2* is involved in PD, yet few studies have investigated its role in other dementias.

Exome-wide analysis has revealed a significant burden of *PILRA* variants on AD. Previous studies have identified a possible link with AD via GWAS hit *ZCWPW1* and paired receptor *PILRB* which associates with *DAP12* and *TREM2*. *PILRA* and *PILRB* function may be co-regulated, and therefore, further investigation should involve looking at both genes in AD.

Limitations in power have made it difficult to find many significant associations, but with ongoing data collection, the sample size will increase to address this issue. However, using an exome-sequencing approach, it has been possible to detect rare variants with greater effect sizes, which previous GWAS did not permit.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographics of LOAD cases and cognitively normal samples in the BDR cohort

Centre	*N (%)*	*Mean AAD (±SD)*	*Females (%)*	*APOE* ε4 + (%)	*APOE* ε4 MAF	*APOE* ε4ε4 (%)
(a) Late-onset Alzheimer's disease cases						
Bristol	7 (41.2)	86.0 (±6.1)	4 (57.1)	7 (100.0)	0.43	1 (14.3)
London	29 (58.0)	82.0 (±7.4)	18 (62.1)	17 (58.6)	0.35	4 (13.8)
Manchester	27 (81.8)	80.8 (±8.2)	12 (44.4)	18 (66.7)	0.46	7 (25.9)
Newcastle	15 (78.9)	85.1 (±8.3)	8 (53.3)	10 (66.7)	0.39	2 (13.3)
Oxford	54 (81.8)	83.1 (±8.9)	28 (51.9)	33 (61.1)	0.4	9 (16.6)
All	132 (71.4)	82.5 (±8.4)	70 (53.0)	85 (64.4)	0.4	23 (17.3)
(b) Cognitively normal control samples.						
Bristol	10 (58.8)	84.5 (±7.5)	4 (40.0)	6 (60.0)	0.15	0 (0.0)
London	21 (42.0)	83.0 (±0.0)	11 (52.4)	5 (23.8)	0.12	0 (0.0)
Manchester	6 (18.2)	87.8 (±5.0)	4 (66.7)	0 (0.0)	0	0 (0.0)
Newcastle	4 (21.1)	86.3 (±4.6)	1 (25.0)	1 (25.0)	0.13	0 (0.0)
Oxford	12 (18.2)	86.4 (±7.8)	7 (58.3)	1 (8.3)	0.04	0 (0.0)
All	53 (28.6)	85.9 (±6.5)	27 (50.9)	13 (24.5)	0.09	0 (0.0)

The cohort contains individuals from multiple centres. Sample demographics are divided into (a) LOAD cases and (b) cognitively normal controls for each centre and overall. Information provided includes number and percentage of case or control individuals per centre [N (%)], mean age at death with standard deviation [Mean AAD (±SD)], number and percentage of case or control females per centre [Females (%)], number and percentages of case or control individuals harbouring at least one *APOE* ε4 allele [*APOE* ε4 + (%)], *APOE* ε4 minor allele frequency [*APOE* ε4 MAF], number and percentage of case or control individuals with two ε4 alleles [*APOE* ε4ε4 (%)].

Table 2

Burden analysis results for exome-wide analysis

Gene	*mRNA ID*	*Position*	*Number of variants*	*P*-value
PILRA	NM_013439	7:99971313-99997454	5	3.40E-05
PRSS45	NM_199183	3:46783959-46785453	4	5.92E-05
THNSL2	NM_001244676	2:88470874-88485392	12	1.49E-04
KLK2	NM_001002231	19:51376837-51381777	8	1.96E-04
STOX2	NM_020225	4:184930646-184932631	8	2.42E-04
SEC31A	NM_001077206	4:83740163-83803115	23	3.58E-04
PRSS42	NM_182702	3:46875258-46875258	1	3.81E-04
HAS3	NM_138612	16:69143481-6915 2391	4	4.09E-04
KLRF2	NM_001190765	12:10041364-10048327	5	5.08E-04
SLC22A2	NM_003058	6:160638357-160677614	9	5.48E-04
GRIK2	NM_001166247	6:102134022-102516260	10	5.77E-04
TRDMT1	NM_004412	10:17194026-17243638	12	6.86E-04
FAM136A	NM_032822	2:70528601-70529205	5	7.25E-04
ITGAL	NM_001114380	16:30484308-30522152	13	7.82E-04
APOC1	NM_001645	19:45419414-45422561	2	8.28E-04
HAS3	NM_001199280	16:69143481-69143816	3	8.52E-04
TMIE	NM_147196	3:46742941-46751229	3	8.84E-04
BCR	NM_004327	22:23523602-23657604	24	8.96E-04
POU4F2	NM_004575	4:147560411-147561971	6	9.54E-04

Burden analysis of all genes was performed using a C-alpha test without correction for covariates. The results shown are significant to $P < 0.001$. Information includes gene name [Gene], mRNA [mRNA ID], chromosomal position in genome build hg19 [Position], number of variants contributing to the signal [Number of Variants], significance [*P*-value].

Table 3

Top results shown for exome-wide association analysis of nonsingleton variants

Gene	Variant	Position	Allele	ExAC MAF	MAF (Count)		P-value	Odds ratio (95% CI)	Consequence	Protein change	Functional prediction
					Case	Control					
TCEB3	rs2235541	1:24077451	C>T	0.09	0.06 (15)	0.15 (16)	2.44E-04	0.15 (0.06–0.42)	Missense	T145M	Deleterious *
AVPR1B	rs35369693	1:206224635	G>C	0.04	0.05 (13)	0.16 (17)	8.44E-04	0.21 (0.09–0.53)	Missense	K65N	Probably damaging
PRSS45	rs58830807	3:46783959	T>C	0.18	0.09 (23)	0.24 (25)	1.41E-04	0.21 (0.1–0.47)	Missense	I190V	Tolerated
PRSS45	rs58943210	3:46784467	G>A	0.18	0.09 (23)	0.23 (24)	2.67E-04	0.23 (0.1–0.5)	Missense	P130L	Tolerated
SEC31A	rs10025654	4:83795806	C>T	0.51	0.4 (105)	0.58 (62)	5.29E-04	0.38 (0.22–0.66)	Synonymous	R199	No functional change
ITPR3	rs35506178	6:33658780	C>T	0.04	0.03 (7)	0.08 (9)	8.50E-04	0.12 (0.03–0.41)	Synonymous	A2373	No functional change
PCLO	rs2877	7:82764425	G>C	0.75	0.26 (69)	0.44 (47)	4.88E-04	0.36 (0.2–0.64)	Missense	S814T	Benign
CPA1	rs968404	7:130022041	C>T	0.13	0.14 (38)	0.25 (26)	4.67E-04	0.26 (0.12–0.55)	Synonymous	Y158	No functional change
CWF19L2	rs659040	11:107299631	G>A	0.16	0.15 (40)	0.32 (34)	2.28E-04	0.27 (0.13–0.54)	Missense	H443Y	Tolerated
NTN4	rs17851048	12:96077312	C>T	0.22	0.2 (53)	0.41 (43)	2.18E-04	0.31 (0.17–0.58)	Synonymous	A452	No functional change
TMEM260	rs17776256	14:57075920	G>T	0.12	0.08 (20)	0.18 (19)	4.96E-04	0.21 (0.09–0.5)	Missense	A245S/T	Probably damaging
UBR7	rs2286653	14:93673655	G>A	0.15	0.05 (12)	0.16 (17)	6.21E-04	0.17 (0.06–0.47)	Missense	A7T	Tolerated
IGFALS	rs17559	16:1841033	G>A	0.15	0.07 (18)	0.2 (21)	8.34E-04	0.26 (0.12–0.57)	Synonymous	Y500	No functional change
MEP1B	rs173032	18:29795076	C>T	0.68	0.41 (107)	0.25 (27)	6.48E-04	3.08 (1.61–5.89)	Synonymous	S537	No functional change
LRRC8E	rs2042919	19:7963949	A>G	0.23	0.25 (67)	0.36 (38)	6.45E-04	0.32 (0.17–0.62)	Missense	E181G	Tolerated
LRRC8E	rs3745382	19:7964727	G>A	0.21	0.26 (68)	0.36 (38)	6.60E-04	0.32 (0.17–0.62)	Synonymous	E440	No functional change
FPR1	rs5030878	19:52250216	G>A	0.81	0.19 (50)	0.35 (37)	4.46E-04	0.32 (0.17–0.61)	Missense	I11T	Tolerated

Logistic regression results are shown for variants associated with AD ($P < 0.001$). Significance values were adjusted for the covariates sex, age at death and number of *APOE* ε4 alleles. Information includes gene containing the variant [Gene], variant RSID [Variant], position in hg19 [Position], allelic change [Allele], MAF in ExAC database [ExAC MAF], MAF and counts in cases and controls [MAF (Count)], significance of association [P-value], size of effect with 95% confidence intervals [odds ratio (95% CI)], coding consequence [Consequence], amino acid change in protein [Protein Change], predicted consequence of change using SIFT and Polyphen2 [Functional Prediction].

* Discrepancy between SIFT and Polyphen2 predictions.

Table 4

Predictive accuracy model for 132 AD cases versus 53 controls

Model	*Nagelkerke's R^2*	*Area under ROC curve (AUC)*	*AUC 95% CI*	*Hosmer–Lemeshow test P*-value
ε4	0.086	0.718	0.642–0.794	0.001
ε4 + ε2	0.215	0.734	0.660–0.809	0.347
ε4 + ε2 + Sex + Age	0.223	0.742	0.668–0.816	0.891
ε4 + ε2 + Sex + Age + GWAS	0.366	0.830	0.770–0.891	0.816
ε4 + ε2 + Sex + Age + GWAS + PRS (P < 0.05)	0.378	0.838	0.779–0.898	0.536

Different predictors were compared for their accuracy in predicting risk of developing AD. *APOE* ε4 and ε2 alleles, sex, age at death and genotypes for 19 of the GWAS SNPs were used as predictors. PRS were constructed using independent variants associated with AD at a threshold of $P < 0.05$, excluding the *APOE* region ±500 kb and the GWAS variants. Nagelkerke's R^2 expresses the proportion of variance explained by the model, with the largest R^2 value indicating the best model. Area under the receiver operating characteristic curve (AUC) is a measure of predictive accuracy, which quantifies the overall ability to discriminate between case and control individuals. The Hosmer–Lemeshow statistic is a goodness-of-fit test for risk prediction models, with a significant result indicating that the data are a poor fit to the model.

Table 5

Coding variants found in familial AD genes *APP*, *PSEN1* and *PSEN2* in LOAD cohort

Gene	SNP	Position	Allele	ExAC MAF	MAF (Counts)		Consequence	Protein change	Functional prediction
					Case	Control			
APP	rs148888161	21:27264121	G>A	0.002	0.007 (2)	0.009 (1)	Synonymous	G708	No functional change
PSEN1	rs17125721	14:73673178	A>G	0.015	0.015 (5)	0.009 (1)	Missense	E318G	Probably damaging *
PSEN2	rs11405	1:227069677	C>T	0.76	0.189 (50)	0.198 (21)	Synonymous	A23	No functional change
	rs1046240	1:227071525	T>C	0.49	0.439 (116)	0.396 (42)	Synonymous	H87	No functional change
	rs63750197	1:227073271	C>T	0.0006	0.004 (1)	0.009 (1)	Missense	S130L	Possibly damaging *
	rs61730652	1:227076671	T>C	0.013	0.004 (1)	0 (0)	Synonymous	S236	No functional change

Coding variants present in BDR samples in familial AD genes *APP* (1), *PSEN1* (1) and *PSEN2* (4) are catalogued here with a prediction of the functional consequences of each mutation.

* Discrepancy between SIFT and Polyphen2 predictions.

Table 6

Cataloguing pathogenic mutations in other familial neurological genes

Gene	SNP	Position	Allele	ExAC MAF	MAF (Counts)		Consequence	Protein change	Functional prediction	Clinical significance
					Case	Control				
CHMP2B	chr3:87299112	3:87299112	ATGAC>A	NA	0.003 (1)	0.009 (1)	Frameshift	NA	NA	NA
LRRK2	rs7308720	12:40657700	C>G	0.086	0.078 (24)	0.046 (5)	Missense	N551K	Probably damaging	Not pathogenic
LRRK2	rs33958906	12:40707861	C>T	0.030	0.026 (8)	0.018 (2)	Missense	P1542S	Probably damaging	Not pathogenic
LRRK2	chr12:40697972	12:40697972	A>T	NA	0.003 (1)	0.009 (1)	Missense	L1271P	NA	NA
PARK2	rs149953814	6:161771219	G>A	0.001	0.003 (1)	0.009 (1)	Missense	P246L	Probably damaging	Pathogenic nature unclear
PARK2	rs34424986	6:162206852	G>A	0.002	0.003 (1)	0.009 (1)	Missense	R84W/R275T	Probably damaging	Pathogenic
PINK1	rs148871409	1:20960385	A>T	0.064	0.059 (18)	0.045 (5)	Missense	Q115P	Probably damaging *	NA
PINK1	rs1043424	1:20977000	A>C	0.297	0.311 (95)	0.255 (28)	Missense	N521T	Tolerated	Not pathogenic
PRNP	rs138688873	20:4680095	229–252del24	0.019	0.003 (1)	0.009 (1)	In-frame deletion	77–84del	NA	NA
PRNP	rs1799990	20:4680251	A>G	0.308	0.343 (105)	0.382 (42)	Missense	M129V	Tolerated	Risk factor, likely benign

Variants in genes previously linked to other neurological disorders were investigated for known pathogenic mutations. Only mutations causing an amino acid change are listed here; therefore, synonymous mutations were excluded. Clinical significance was determined from information available online from AD&FTD and PD mutation databases. ‘NA’ indicates that no functional or clinical information or MAF was available.

* Discrepancy between SIFT and Polyphen2 predictions.

Table 7

Single-SNP association results for variants in known neurological genes with AD

Gene	SNP	Position	Allele	ExAC MAF	MAF (Count)		P-value	Odds ratio (95% CI)	Consequence	Protein change	Functional prediction
					Case	Control					
LRRK2	rs35303786	12:40713899	T>C	0.01	0.01 (3)	0.05 (5)	0.018	0.14 (0.03–0.71)	Missense	M1646T	Tolerated
SLC24A4	rs7144273	14:92920371	T>C	0.50	0.5 (153)	0.39 (43)	0.044	1.63 (1.01–2.63)	Synonymous	P319	No functional change
ABCA7	rs3764645	19:1042809	A>G	0.48	0.42 (127)	0.6 (66)	0.001	0.43 (0.26–0.72)	Missense	E188G	Tolerated
ABCA7	rs3752234	19:1047002	G>A	0.55	0.51 (157)	0.39 (43)	0.004	2.06 (1.25–3.4)	Synonymous	A608	No functional change
ABCA7	rs3752237	19:1047161	G>A	0.68	0.46 (142)	0.29 (32)	0.001	2.42 (1.46–4.02)	Synonymous	G617	No functional change
ABCA7	rs4147915	19:1049305	C>A	0.18	0.11 (34)	0.2 (22)	0.010	0.4 (0.2–0.8)	Synonymous	V807	No functional change
CD33	rs35112940	19:51738917	G>A	0.16	0.17 (53)	0.25 (28)	0.033	0.53 (0.3–0.95)	Missense	G304R	Possibly damaging *

Association results for single variants with Alzheimer's disease, corrected for sex, age at death and *APOE* ε4 allele count. The variants significant to $P < 0.05$ are listed here with predicted functional consequence.

* Discrepancy between SIFT and Polyphen2 predictions.

Table 8

Burden analysis results for known neurodegenerative genes

	Gene	Total variants	Coding variants	Number of variants tested	P-value
(a) Familial AD genes	*APP*	10	1	1	0.61
	PSEN1	4	2	2	1.00
	PSEN2	7	4	4	0.83
(b) LOAD genes	*ABCA7*	48	28	28	0.59
	APOE	4	4	4	0.06
	BIN1	17	6	6	0.25
	CASS4	11	7	7	0.32
	CD2AP	9	4	4	0.58
	CD33	4	4	4	0.77
	CELF1	4	3	3	1.00
	CLU	5	5	5	0.02
	CR1	17	9	9	0.31
	EPHA1	15	10	9	0.14
	FERMT2	7	3	3	0.86
	INPP5D	6	2	2	0.54
	MS4A6A	8	5	5	0.39
	NME8	16	8	8	1.00
	PICALM	11	3	3	0.63
	PTK2B	35	17	16	0.44
	RIN3	11	9	9	1.00
	SLC24A4	22	8	8	0.69
	SORL1	29	16	16	0.05
	TREM2	4	4	3	0.23
	ZCWPW1	9	7	6	0.71
(c) Other familial neurological genes	*C9orf72*	11	4	4	0.66
	CHMP2B	5	3	3	0.24
	FUS	4	2	2	1.00

Gene	Total variants	Coding variants	Number of variants tested	*P*-value
GRN	9	3	3	0.84
LRRK2	29	20	20	0.12
PARK2	13	5	5	0.27
PARK7	6	1	1	0.33
PINK1	9	4	4	0.88
PRNP	7	4	4	0.35
SNCA	8	2	2	0.15
VCP	6	2	2	0.34

Burden analysis results from SKAT-O are shown here for (a) Familial AD genes; (b) LOAD-specific genes from GWAS and NGS studies; (c) Familial genes in other neurodegenerative diseases. The total numbers of markers within genes are shown, together with number of coding variants and the number of markers used for burden testing. The *P*-value signifies the association of multiple markers with AD. SKAT-O accounts for markers that show effects in both risk and protective directions.

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