

MISS TULSI PATEL (Orcid ID : 0000-0002-2797-1443)

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

Authors: Tulsi Patel<sup>1</sup>, Keeley J. Brookes<sup>1</sup>, James Turton<sup>1</sup>, Sultan Chaudhury<sup>1</sup>, Tamar Guetta-Baranes<sup>1</sup>, Rita Guerreiro<sup>2,3</sup>, Jose Bras<sup>2,3</sup>, Dena Hernandez<sup>4</sup>, Andrew Singleton<sup>4</sup>, Paul T. Francis<sup>5</sup>, John Hardy<sup>2</sup> and Kevin Morgan<sup>1</sup>

1. Human Genetics Group, University of Nottingham, Nottingham, UK
2. Department of Molecular Neuroscience, Institute of Neurology, University College London, London, UK; UK Dementia Research Institute at UCL (UK DRI), London, UK
3. Department of Medical Sciences, Institute of Biomedicine-iBiMED, University of Aveiro, Aveiro, Portugal
4. Laboratory of Neurogenetics, National Institute of Aging, National Institute of Health, Bethesda, MD, USA
5. Brains for Dementia Research Resource, Wolfson Centre for Age Related Diseases, King's College London, London, UK

Corresponding author: kevin.morgan@nottingham.ac.uk

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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 GWAS 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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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 non-singleton 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 neurologic 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 3,268 variants, sex, age at death and *APOE*  $\epsilon 4$  and  $\epsilon 2$  status as predictors.

### Abbreviations

AD	– Alzheimer’s disease
AUC	– area under curve
BDR	– Brains for Dementia Research
DLB	– Dementia with Lewy bodies
fEOAD	– familial early onset Alzheimer’s disease
FTD	– Frontotemporal dementia
GWAS	– genome-wide association study
LOAD	– late onset Alzheimer’s disease
MAF	– minor allele frequency
NGS	– next generation sequencing
OR	– odds ratio
P	– P-value

PCR – polymerase chain reaction

PD – Parkinson's disease

PRS – polygenic risk score

## 1. 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 (LOAD), with symptoms appearing at 65 years or later. This represents the other 95% of all incidences [4]. Presence of the *APOE*  $\epsilon$ 4 allele is the largest known genetic risk factor for LOAD, with a 2-3 fold increase in risk for carriers and 15 fold for individuals homozygous for the  $\epsilon$ 4 allele [5]. The rare  $\epsilon$ 2 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 5-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*  $\epsilon$ 4 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-characterised brain tissue samples from healthy individuals remains a limiting factor in the study of neurological disorders [15], Brains for Dementia Research (BDR) ([www.brainsfordementiaresearch.org.uk](http://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 as well as 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, standardised cognitive and psychiatric assessment of potential brain donors during life is critical in optimising 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) (*C9orf72*, *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.

## 2. Materials and Methods

### 2.1 BDR samples

The BDR cohort comprised of 132 clinically diagnosed LOAD (age at onset  $\geq 65$  years) cases and 53 cognitively normal controls; all diagnoses were neuropathologically confirmed (Supplementary Table S1). Neuropathological diagnoses were undertaken by experienced neuropathologists within the Brains for Dementia Research network and were based on Thal A $\beta$  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 [24]. Whilst there are no fully established criteria for vascular pathology the VCING criteria were used [25] along with those proposed by Smallwood et al (2012) [26] and Grinberg and Thal (2010) [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*  $\epsilon 4$  carriers were 3-fold higher in cases (64.7%) with almost one fifth (17.3%) being homozygous for the  $\epsilon 4$  allele. In comparison only 24.5% of controls were carriers and no individuals were  $\epsilon 4\epsilon 4$ . The  $\epsilon 2$  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 3-fold frequency observed in control samples correlates with the protective effect of the  $\epsilon 2$  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 includes 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 A $\beta$ -CAA and cerebrovascular atherosclerosis. Detailed clinical and cognitive information on the samples is also available upon request, demonstrating the potential value of the BDR cohort for very detailed analyses in future studies as more extensive genetic data is generated.

### 2.2 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*  $\epsilon$ 2,  $\epsilon$ 3 and  $\epsilon$ 4 alleles using the TaqMan method (Applied Biosystems) to determine *APOE* status.

### **2.3 Exome sequencing library prep**

DNA libraries were hybridised 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 x 100bp paired end read cycles. The Agilent capture library includes 5' and 3' untranslated regions.

### **2.4 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](http://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).

### **2.5 Filtering**

Singleton variants with  $MAF \leq 0.002$  were removed in VCFtools [33]. Coding variants in genes were filtered by annotation with SnpSift [34]. Visualisation 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].

### **2.6 Association analyses**

Quality control filtering was performed on the VCF using VCFtools. Individuals were removed if genotyping rate  $\leq 97\%$ , followed by markers with call rate  $\leq 98\%$ . 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 non-singleton 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*  $\epsilon$ 4 allele count.

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

### **2.7 Polygenic risk scoring**

PRS were generated for BDR samples using PRSice [39]. The International Genomics of Alzheimer's Project (IGAP) summary data was used as the base dataset, collated from 17,008 LOAD cases and 37,154 controls. A region of 500kb 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*  $\epsilon$ 4 and  $\epsilon$ 2 alleles, age, sex and genotypes for the GWAS SNPs.

## **3. Results**

### **3.1 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 non-singleton variants were present in 290 individuals, with a minimum of 2 alleles observed per variant. Filtering to retain only coding mutations resulted in 76,640 variants for exome-wide analysis.

### **3.2 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 5 variants that drive this signal, of which 2 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 non-singleton variants, correcting for age, sex and *APOE*  $\epsilon$ 4 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 signaling 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.

### **3.3 Polygenic risk scoring**

A 500kb 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*  $\epsilon$ 4 allele alone has 71.8% accuracy in discriminating between cases and controls, however this is a poor fit model. Other covariates  $\epsilon$ 2, age, sex and GWAS greatly increase the predictive power 83.0%. In total 3,268 variants were utilised 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  $3.6 \times 10^{-4}$  compared to  $2.7 \times 10^{-4}$  for control samples.

### **3.4 Screening for familial mutations**

Samples were screened for mutations in fEOAD genes to remove any non-sporadic cases. A total of 6 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 5 cases and 1 control sample. *PSEN2* p.S130L (rs63750197) was previously identified as possibly damaging *in silico* with unclear pathogenicity, present in 1 case and 1 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 neurologic 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 1 AD case and 1 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 1 case and control, both also presenting with mild non-amyloid small vessel disease. Samples were heterozygous for both variants.

Previously uncharacterised 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.

### **3.5 Association analyses of known neurologic 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 neurologic 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 supplementary Table S2.

A large proportion of the highly associated variants are synonymous mutations. Four *ABCA7*

variants are present, with 2 suggesting increased risk ( $OR > 1$ ) and 2 showing a protective effect ( $OR < 1$ ). *ABCA7* rs3752234 and rs3752237 are both synonymous mutations that increase risk more than 2-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*  $\epsilon 4$  status. Two of these variants were significant, synonymous variant rs9331939 and rs149859119 (p.S16R), therefore they could be driving the signal in this gene.

#### 4. 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  $\beta$  is synonymous; recent proteomic studies have found that these metalloproteases can cleave APP, affecting A $\beta$  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 amongst the cohort. Using sex, age at death, *APOE*  $\epsilon$ 4 and  $\epsilon$ 2 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 3,268 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 there were no familial EOAD cases amongst the BDR LOAD classified cases. Other neurologic 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 uncharacterised 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 artifact. *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 3 AD case samples which were heterozygotes. This variant can increase risk of developing AD by 2-3 fold [12,47]. DNA was available for these 3 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 4-fold greater frequency will be verified as the BDR sample set increases in size.

Single variant association of all neurologic 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 2 increasing risk and 2 being protective. Synonymous variants rs3752234 and rs3752237 increased AD risk more than 2-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*  $\epsilon 4$  alleles. Both AD and other neurologic 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.

## 5. Conclusion

Although other familial neurologic 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 Information

Supplementary Table S1. Summary data of all case and control samples used for analysis.  
Supplementary Table S2. Variants found in AD-related genes in 129 LOAD cases and 53 controls.

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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).

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**Table 1. Demographics of LOAD cases and cognitively normal samples in the BDR cohort.** 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 ( $\pm$ 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*  $\epsilon$ 4 allele [*APOE*  $\epsilon$ 4+ (%)], *APOE*  $\epsilon$ 4 minor allele frequency [*APOE*  $\epsilon$ 4 MAF], Number and percentage of case or control individuals with two  $\epsilon$ 4 alleles [*APOE*  $\epsilon$ 4 $\epsilon$ 4 (%)].

Centre	N (%)	Mean AAD ( $\pm$ SD)	Females (%)	<i>APOE</i> $\epsilon$ 4+ (%)	<i>APOE</i> $\epsilon$ 4 MAF	<i>APOE</i> $\epsilon$ 4 $\epsilon$ 4 (%)
Bristol	7 (41.2)	86.0 ( $\pm$ 6.1)	4 (57.1)	7 (100.0)	0.43	1 (14.3)
London	29 (58.0)	82.0 ( $\pm$ 7.4)	18 (62.1)	17 (58.6)	0.35	4 (13.8)
Manchester	27 (81.8)	80.8 ( $\pm$ 8.2)	12 (44.4)	18 (66.7)	0.46	7 (25.9)
Newcastle	15 (78.9)	85.1 ( $\pm$ 8.3)	8 (53.3)	10 (66.7)	0.39	2 (13.3)
Oxford	54 (81.8)	83.1 ( $\pm$ 8.9)	28 (51.9)	33 (61.1)	0.4	9 (16.6)
All	132 (71.4)	82.5 ( $\pm$ 8.4)	70 (53.0)	85 (64.4)	0.4	23 (17.3)

(a) Late-onset Alzheimer's disease cases.

Centre	N (%)	Mean AAD ( $\pm$ SD)	Females (%)	APOE $\epsilon$ 4+ (%)	APOE $\epsilon$ 4 MAF	APOE $\epsilon$ 4 $\epsilon$ 4 (%)
Bristol	10 (58.8)	84.5 ( $\pm$ 7.5)	4 (40.0)	6 (60.0)	0.15	0 (0.0)
London	21 (42.0)	83.0 ( $\pm$ 0.0)	11 (52.4)	5 (23.8)	0.12	0 (0.0)
Manchester	6 (18.2)	87.8 ( $\pm$ 5.0)	4 (66.7)	0 (0.0)	0	0 (0.0)
Newcastle	4 (21.1)	86.3 ( $\pm$ 4.6)	1 (25.0)	1 (25.0)	0.13	0 (0.0)
Oxford	12 (18.2)	86.4 ( $\pm$ 7.8)	7 (58.3)	1 (8.3)	0.04	0 (0.0)
All	53 (28.6)	85.9 ( $\pm$ 6.5)	27 (50.9)	13 (24.5)	0.09	0 (0.0)

(b) Cognitively normal control samples.

**Table 2. Burden analysis results for exome-wide analysis.** 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].

Gene	mRNA ID	Position	Number of Variants	P-value
<i>PILRA</i>	NM_013439	7:99971313-99997454	5	3.40E-05
<i>PRSS45</i>	NM_199183	3:46783959-46785453	4	5.92E-05
<i>THNSL2</i>	NM_001244676	2:88470874-88485392	12	1.49E-04
<i>KLK2</i>	NM_001002231	19:51376837-51381777	8	1.96E-04
<i>STOX2</i>	NM_020225	4:184930646-184932631	8	2.42E-04
<i>SEC31A</i>	NM_001077206	4:83740163-83803115	23	3.58E-04
<i>PRSS42</i>	NM_182702	3:46875258-46875258	1	3.81E-04
<i>HAS3</i>	NM_138612	16:69143481-6915 2391	4	4.09E-04
<i>KLRF2</i>	NM_001190765	12:10041364-10048327	5	5.08E-04
<i>SLC22A2</i>	NM_003058	6:160638357-160677614	9	5.48E-04
<i>GRIK2</i>	NM_001166247	6:102134022-102516260	10	5.77E-04
<i>TRDMT1</i>	NM_004412	10:17194026-17243638	12	6.86E-04
<i>FAM136A</i>	NM_032822	2:70528601-70529205	5	7.25E-04
<i>ITGAL</i>	NM_001114380	16:30484308-30522152	13	7.82E-04

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<i>APOC1</i>	NM_001645	19:45419414-45422561	2	8.28E-04
<i>HAS3</i>	NM_001199280	16:69143481-69143816	3	8.52E-04
<i>TMIE</i>	NM_147196	3:46742941-46751229	3	8.84E-04
<i>BCR</i>	NM_004327	22:23523602-23657604	24	8.96E-04
<i>POU4F2</i>	NM_004575	4:147560411-147561971	6	9.54E-04

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**Table 3. Top results shown for exome-wide association analysis of non-singleton variants.** 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*  $\epsilon 4$  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]. \* indicates discrepancy between SIFT and Polyphen2 predictions.

Gene	Variant	Position	Allele	ExAC MAF	MAF (Count)		P-value	Odds Ratio (95% CI)	Consequence	Protein Change	Functional Prediction
					Case	Control					
<i>TCEB3</i>	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 *
<i>AVPR1B</i>	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
<i>PRSS45</i>	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
<i>PRSS45</i>	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
<i>SEC31A</i>	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
<i>ITPR3</i>	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
<i>PCLO</i>	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
<i>CPA1</i>	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
<i>CWF19L2</i>	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

<i>NTN4</i>	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
<i>TMEM260</i>	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
<i>UBR7</i>	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
<i>IGFALS</i>	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
<i>MEP1B</i>	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
<i>LRR8E</i>	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
<i>LRR8E</i>	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
<i>FPR1</i>	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

**Table 4. Predictive accuracy model for 132 AD cases versus 53 controls.** Different predictors were compared for their accuracy in predicting risk of developing AD. *APOE*  $\epsilon 4$  and  $\epsilon 2$  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  $\pm 500$ 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 is a poor fit to the model.

Model	Nagelkerke's $R^2$	Area under ROC curve (AUC)	AUC 95% CI	Hosmer-Lemeshow Test P-value
$\epsilon 4$	0.086	0.718	0.642 – 0.794	0.001
$\epsilon 4 + \epsilon 2$	0.215	0.734	0.660 – 0.809	0.347
$\epsilon 4 + \epsilon 2 + \text{Sex} + \text{Age}$	0.223	0.742	0.668 – 0.816	0.891
$\epsilon 4 + \epsilon 2 + \text{Sex} + \text{Age} + \text{GWAS}$	0.366	0.830	0.770 – 0.891	0.816
$\epsilon 4 + \epsilon 2 + \text{Sex} + \text{Age} + \text{GWAS} + \text{PRS}(P < 0.05)$	0.378	0.838	0.779 – 0.898	0.536

**Table 5. Coding variants found in familial AD genes *APP*, *PSEN1* and *PSEN2* in LOAD cohort.** 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. \* indicates discrepancy between SIFT and Polyphen2 predictions.

Gene	SNP	Position	Allele	ExAC MAF	MAF (Counts)		Consequence	Protein Change	Functional Prediction
					Case	Control			
<i>APP</i>	rs148888161	21:27264121	G>A	0.002	0.007 (2)	0.009 (1)	Synonymous	G708	No functional change
<i>PSEN1</i>	rs17125721	14:73673178	A>G	0.015	0.015 (5)	0.009 (1)	Missense	E318G	Probably damaging *
	rs111405	1:227069677	C>T	0.76	0.189 (50)	0.198 (21)	Synonymous	A23	No functional change
<i>PSEN2</i>	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

**Table 6. Cataloguing pathogenic mutations in other familial neurologic genes.** Variants in genes previously linked to other neurologic 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. \* indicates discrepancy between SIFT and Polyphen2 predictions.

Gene	SNP	Position	Allele	ExAC MAF	MAF (Counts)		Consequence	Protein Change	Functional Prediction	Clinical Significance
					Case	Control				
<i>CHMP2B</i>	chr3:87299112	3:87299112	ATGAC>A	NA	0.003 (1)	0.009 (1)	Frameshift	NA	NA	NA
<i>LRRK2</i>	rs7308720	12:40657700	C>G	0.086	0.078 (24)	0.046 (5)	Missense	N551K	Probably damaging	Not pathogenic
<i>LRRK2</i>	rs33958906	12:40707861	C>T	0.030	0.026 (8)	0.018 (2)	Missense	P1542S	Probably damaging	Not pathogenic
<i>LRRK2</i>	chr12:40697972	12:40697972	A>T	NA	0.003 (1)	0.009 (1)	Missense	L1271P	NA	NA
<i>PARK2</i>	rs149953814	6:161771219	G>A	0.001	0.003 (1)	0.009 (1)	Missense	P246L	Probably damaging	Pathogenic nature unclear
<i>PARK2</i>	rs34424986	6:162206852	G>A	0.002	0.003 (1)	0.009 (1)	Missense	R84W/R 275T	Probably damaging	Pathogenic
<i>PINK1</i>	rs148871409	1:20960385	A>T	0.064	0.059 (18)	0.045 (5)	Missense	Q115P	Probably damaging *	NA
<i>PINK1</i>	rs1043424	1:20977000	A>C	0.297	0.311 (95)	0.255 (28)	Missense	N521T	Tolerated	Not pathogenic
<i>PRNP</i>	rs138688873	20:4680095	229- 252del24	0.019	0.003 (1)	0.009 (1)	In-frame deletion	77-84del	NA	NA
<i>PRNP</i>	rs1799990	20:4680251	A>G	0.308	0.343 (105)	0.382 (42)	Missense	M129V	Tolerated	Risk factor, likely benign

**Table 7. Single-SNP association results for variants in known neurological genes with AD.** Association results for single variants with Alzheimer's disease, corrected for sex, age at death and *APOE*  $\epsilon$ 4 allele count. The variants significant to  $P < 0.05$  are listed here with predicted functional consequence. \* indicates discrepancy between SIFT and Polyphen2 predictions.

Gene	SNP	Position	Allele	ExAC MAF	MAF (Count)		P-value	Odds Ratio (95% CI)	Consequence	Protein Change	Functional Prediction
					Case	Control					
<i>LRRK2</i>	rs35303786	12:40713899	T>C	0.01	0.01 (3)	0.05 (5)	0.018	0.14 (0.03-0.71)	Missense	M1646T	Tolerated
<i>SLC24A4</i>	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
<i>ABCA7</i>	rs3764645	19:1042809	A>G	0.48	0.42 (127)	0.6 (66)	0.001	0.43 (0.26-0.72)	Missense	E188G	Tolerated
<i>ABCA7</i>	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
<i>ABCA7</i>	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
<i>ABCA7</i>	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
<i>CD33</i>	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 *

**Table 8. Burden analysis results for known neurodegenerative genes.** 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.

	Gene	Total Variants	Coding Variants	Number of Variants Tested	P-value
<b>(a) Familial AD genes</b>	<i>APP</i>	10	1	1	0.61
	<i>PSEN1</i>	4	2	2	1.00
	<i>PSEN2</i>	7	4	4	0.83
<b>(b) LOAD genes</b>	<i>ABCA7</i>	48	28	28	0.59
	<i>APOE</i>	4	4	4	0.06
	<i>BIN1</i>	17	6	6	0.25
	<i>CASS4</i>	11	7	7	0.32
	<i>CD2AP</i>	9	4	4	0.58
	<i>CD33</i>	4	4	4	0.77
	<i>CELF1</i>	4	3	3	1.00
	<i>CLU</i>	5	5	5	0.02
	<i>CR1</i>	17	9	9	0.31
	<i>EPHA1</i>	15	10	9	0.14
	<i>FERMT2</i>	7	3	3	0.86
	<i>INPP5D</i>	6	2	2	0.54
	<i>MS4A6A</i>	8	5	5	0.39

	<i>NME8</i>	16	8	8	1.00
	<i>PICALM</i>	11	3	3	0.63
	<i>PTK2B</i>	35	17	16	0.44
	<i>RIN3</i>	11	9	9	1.00
	<i>SLC24A4</i>	22	8	8	0.69
	<i>SORL1</i>	29	16	16	0.05
	<i>TREM2</i>	4	4	3	0.23
	<i>ZCWPW1</i>	9	7	6	0.71
	<i>C9orf72</i>	11	4	4	0.66
	<i>CHMP2B</i>	5	3	3	0.24
	<i>FUS</i>	4	2	2	1.00
	<i>GRN</i>	9	3	3	0.84
<b>(c) Other familial neurologic genes</b>	<i>LRRK2</i>	29	20	20	0.12
	<i>PARK2</i>	13	5	5	0.27
	<i>PARK7</i>	6	1	1	0.33
	<i>PINK1</i>	9	4	4	0.88
	<i>PRNP</i>	7	4	4	0.35
	<i>SNCA</i>	8	2	2	0.15
	<i>VCP</i>	6	2	2	0.34