Title: Genotyping of the Alzheimer's disease GWAS index SNPs in the Brains for Dementia Research (BDR) cohort

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Running Title: Genotyping GWAS variants in BDR

Abstract: 99 words

Main Text: 1721 words

Tables: 3

Abstract:

The Brains for Dementia Research project is a recently established cohort which aims to provide brain tissue for research purposes from neuropathologically defined samples. Here we present the findings from our analysis on the 19 established GWAS index SNPs for Alzheimer's disease, in order to demonstrate if the BDR sample also displays association to these variants. A highly significant association of the *APOE* ε4 allele was identified ($P=3.99x10^{-12}$). Association tests for the 19 GWAS SNPs found that although no SNPs survive multiple testing, nominal significant findings were detected and concordance with the Lambert et al GWAS meta-analysis was observed.

Introduction:

The knowledge on the genetic aetiology of late-onset Alzheimer's disease (LOAD) has been vastly enhanced over the last decade. Whole genome association studies (GWAS), and next-generation sequencing investigations have identified numerous genetic risk variants for the disease in large collaborative samples [1]. The largest GWAS study to date combined the data from four previous GWAS datasets (ADGC, CHARGE, EADI & GERAD) to create the IGAP discovery sample of 17,008 cases and 37,154 controls and imputed over 11 million SNP genotypes for analysis [2]. The addition of data from a replication dataset increased the sample size to 25,580 cases and 48,466 and yielded the now accepted 19 risk loci (excluding the *APOE* locus) for LOAD from GWAS.

The Brains for Dementia Research (BDR) project is a recently established cohort which aims to provide brain tissue for research purposes from neuropathologically defined samples [3]. To date, 600 post-mortem brains have been collected and DNA extracted for the purpose of genetic analysis. Previously we have published the results of our initial exome sequencing project on a sub-sample of the dataset [4], here we present the findings from association

analysis on the full sample set to-date for the established GWAS index SNPs [2], in order to demonstrate if the BDR sample is genetically representative of LOAD.

Methods:

Samples:

The BDR brain cohort currently has a total of 600 samples, with a form of dementia present in 68.9%. The cohort includes 315 LOAD (age at onset >65 years) cases and 149 cognitively normal controls; all diagnoses were neuropathologically confirmed. The division of other dementia diagnoses are shown in Table 1. The average age at death was 82.9 (±8.7) years for LOAD samples. For control individuals, average age at death was 83.6 (±8.7) years. The proportion of females is similar in both groups (49.2% and 47.9% respectively) and neither gender nor age of death were statistically significantly different.

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.

Genotyping:

The NeuroChip [5] is a custom Illumina genotyping array with an extensive genome-wide backbone (n=306,670 variants) and custom content covering 179,467 variants specific to neurological diseases; [6]. There are 284 variants on the NeuroChip that are specific to AD, including 10 of the GWAS index SNPs [2]. The entire BDR sample has been genotyped using this platform, and data pertaining to the 10 GWAS SNPs included on the panel were extracted from the dataset for analysis.

Quality control of the NeuroChip was completed in GenomeStudio (version 2.0, Illumina) and PLINK [7]. A cluster file was used for automatic clustering of all SNPs [5] whilst manual re-clustering was completed for mis-clustered SNPs identified by low GenTrain score, cluster separation score and call frequency. Samples were analysed and removed based on missingness per individual (mind = 0.1), deviation from European ancestry using top 10 principal components analysis, and heterozygosity (±3 standard deviations). Average genotyping rate in remaining individuals equalled 98.6% or the entire chip content.

All 10 of the GWAS index SNPs included on this platform (see Table 2) passed QC and individual genotypes were exported for association analysis in PLINK with an average genotype rate of 96.4%.

For those GWAS index SNPs not included on the NeuroChip panel (n=9, see Table 2), individual SNP genotyping was carried out 'in-house' using KASP assays following standard protocols (LGC, Middlesex), average genotyping rate was 96.2%. Samples were genotyped for APOE ε2, ε3 and ε4 alleles using the TaqMan assay for SNPs rs7412 and rs429358 (Applied Biosystems) to determine *APOE* status, genotype call rate was 99.7%.

Statistical Analysis:

Association analysis was carried out in PLINK v1.09 [7]. APOE genotype was collapsed to test the association of ε2/ε3 versus ε4 alleles. Individual GWAS SNP association analysis was carried out using a logistic regression test correcting for the covariates sex, age at death and APOE ε4 allele count.

Results:

The BDR sample is a growing cohort, with DNA available for over 600 brain samples available for scientific use. The genetic analysis presented here consists of the current path-confirmed diagnosed sample, of 315 AD samples and 149 control samples. The demographics are presented in Table 1. A number of samples were excluded from the analysis due to the dementia being other than AD, including Lewy body dementia, vascular dementia, frontal temporal lobe dementia, mixed dementias and early-onset dementias. Other diagnoses where dementia was present but were comorbid with other pathologies included three cases of Parkinson's disease, three cases of Cerebrovascular disease, two cases of Corticobasal Syndrome, and single cases of Argyrophilic grain disease and PICKS disease. There was also an individual who presented with dementia but for which there was no underlying neuropathology present. There were also a number of control samples where although no dementia symptoms were present, they have been excluded due to other disorders being present, including seven cases of Cerebrovascular disease, six cases of Parkinson's disease, and single cases of Huntingdons' disease, Corticobasal Syndrome, Progressive Supranuclear Palsy, Motor Neuron disease, and CADASIL syndrome.

Table 1: Sample Demographics

Allele frequencies were not shown to deviate from Hardy-Weinberg equilibrium and minor allele frequencies were similar to those observed in the Lambert et al discovery dataset, [2]. One discrepancy arose from a single SNP (rs35349669) associated with the *INPP5D* gene, even though the minor allele frequency was similar to that observed in the Lambert et al study, the minor allele was the opposite to what was expected. In the general population the frequency of T-allele is 21% (1000 genomes), however in the AD-control discovery set investigated by Lambert this minor allele frequency was increased to almost 49%, a similarly high minor allele frequency was also observed in this study (47%) but with the C-allele (Table 2).

Table 2: SNP MAF from dbSNP, Lambert discovery sample, BDR

Logistic regression analyses found that the ε4 allele was highly associated with the AD phenotype ($P = 3.99 \times 10^{-12}$, OR = 3.76 (95%CI 2.59 – 5.46)) as would be expected. Association analysis for the GWAS index SNPs Bonferroni-corrected for multiple testing (n=19 tests, P<0.0026) yielded no significant results. Nominal association was observed for 4 SNPs (Table 3). SNP rs28834970 (*PTK2B*) displayed nominal for significance with a P value of 0.044, whilst strong associations for rs10792832 (*PICALM*), rs35349669 (*INPP5D*) and rs1476679 (*ZWCPW1*) was observed with nominal significance with P values of 0.023, 0.014 and 0.015 respectively. The *PICALM* and *ZWCPW1* associated SNPs both indicated a protective effect with odd ratio of 0.7 and 0.65 respectively in agreeance with what was observed in the Lambert analysis. Whereas the *PTK2B* and *INPP5D* SNPs both increased risk for the development of AD with odds ratios of 1.39 and 1.47 respectively. However as previously noted the association for the *INPP5D* SNP was with the minor allele which is the opposing allele to that observed by Lambert et al. In total 14 out of the 19 SNPs were in concordance with the Lambert et al study with respect to the allele associated and direction of effect size (73.7%).

Table 3: PLINK association results

Discussion:

This study presents data for the association of the established 19 SNP loci associated with LOAD in the newly formed BDR cohort. Although still in its infancy the cohort has collected over 600 path-confirmed brain samples, which have been genetically analysed, with further samples (up to 3000) expected in the next few years. The aim of this study was to investigate if the BDR cohort was representative of other much larger cohorts of LOAD. Using the SNPs

identified in the meta-analysis GWAS study by Lambert et al (2013) we genotyped the BDR sample with the NeuroChip [5] to obtain the GWAS index SNP data and supplemented it with KASP assays for SNPs that were not present on the array. Minor allele frequencies of the 19 SNPs explored were similar to that produced by the discovery sample in the Lambert et al study, indicating that genetically speaking the BDR cohort is representative of other LOAD datasets. The single exception was for rs35349669 (*INPP5D*), where opposing alleles were found in the minor frequencies, despite the T-allele minor frequency in the general population being approximately 21%, in the Lambert el al cohort, the T-allele frequency increased to that of almost 49% (Table 2). The BDR cohort also has a high minor allele frequency for the rs35349669 SNP (47%) however this is with the C-allele. Where samples have high frequencies of the minor allele it is not uncommon to observed 'allele flipping' and may be indicative of subtle variation between the BDR cohort and the Lambert discovery dataset or the difference in sample size [8]. Further to this, this SNP also indicated nominal significant association with the LOAD phenotype in the BDR sample with the C-allele (as opposed to the T-allele in the Lambert study); however, given the current size of the BDR sample this is quite possibly a type 1 error.

Three further SNPs within the BDR cohort were indicative of significance for association rs28834970 (*PTK2B*), rs10792832 (*PICALM*), and rs1476679 (*ZWCPW1*). It is interesting to note that previous analysis of this cohort with whole exome sequencing also indicated association to the *ZWCPW1* gene region with Burden analysis indicating association of the *PILRA* gene which has been shown to be in weak LD ($r^2 = 0.5$) with the *ZWCPW1* GWAS index SNP rs1476679 [4]. Although no SNP displayed significant association after correction for multiple testing, 14/19 SNPs (73.7%) were concordant with the Lambert meta-analysis dataset for allele and direction of effect. Those that were non-concordant were all with the same allele (except rs35349669, *INPP5D*), with effect sizes around 1. Given the small effect sizes of GWAS and the sample size of the current BDR cohort fluctuation around an OR of 1 is expected.

Currently the BDR cohort is underpowered to significantly detect the effect sizes of the established GWAS hits for LOAD. However as the cohort grows, it is envisaged that the data will become increasingly concordant with such studies as the Lambert meta-analysis, given the preliminary data presented here. Genetic data generated form the BDR cohort is publically available upon a data request to BDR and therefore can serve the interests of the research community at large for small-scale projects wanting to investigate the effects of the GWAS hits.

Acknowledgments:

Genotyping was carried out at the UCL Genomics Facility, by G Madhan at UCL Genomics Great Ormond Street Institute of Child Health, London UK.

We would like to gratefully acknowledge all donors and their families for the tissue provided for this study. Human post-mortem tissue was obtained from the South West Dementia Brain Bank, London Neurodegenerative Diseases Brain Bank, Manchester Brain Bank, Newcastle Brain Tissue Resource and Oxford Brain Bank, members of the Brains for Dementia Research (BDR) Network. The BDR is jointly funded by Alzheimer's Research UK and the Alzheimer's Society in association with the Medical Research Council.

We also wish to acknowledge the neuropathologists at each centre and BDR Brain Bank staff for the collection and classification of the samples. Tissue provided by the Newcastle Brain Tissue Resource is funded in part by a grant from the UK Medical Research Council (G0400074), by NIHR Newcastle Biomedical Research Centre and Unit awarded to the Newcastle upon Tyne NHS Foundation Trust and Newcastle University, and by a grant from the Alzheimer’s Society and Alzheimer’s Research UK as part of the Brains for Dementia Research Project. Brains for Dementia Research has ethics approval from London – City and

East NRES committee 08/H0704/128+5 and has deemed all approved requests for tissue to have been approved by the committee. The work presented here was funded by an ARUK Major Project Grant to KM and PTF.

Conflicts of Interest:

The authors declare they have no conflict of interests

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Table 1:

	Bristol	KCL	Manchester	Newcastle	Oxford	TOTAL
Total Sample Number	**55**	**177**	**110**	**71**	**187**	**600**
% Female	45.5%	47.5%	49.1%	38%	48.7%	**46.8%**
Ave Age at Death (SD)	82.7 (9.8)	83.2 (8.2)	80.5 (10.9)	83.6 (10.0)	83.4 (8.7)	**82.7 (9.4)**
# APOE ε4 Positive (%)	43 (78.2%)	93 (52.5%)	66 (60%)	43 (60.6%)	116 (62.0%)	**361 (60.2%)**
With Dementia	**29**	**118**	**80**	**47**	**140**	**414**
% Female	37.9%	44.9%	50.0%	40.4%	49.3%	**46.4%**
Ave Age at Death	81.0 (11.2)	82.8 (7.5)	81.6 (9.6)	82.6 (11.2)	82.8 (8.6)	**82.4 (9.1)**
# APOE ε4 Positive	25 (86.2%)	76 (64.4%)	53 (66.3%)	31 (66.0%)	98 (70.0%)	**283 (68.3%)**
Without Dementia	**24**	**56**	**29**	**18**	**40**	**167**
% Female	50.0%	53.6%	44.8%	38.9%	42.5%	**47.3%**
Ave Age at Death (SD)	84.8 (7.3)	84.1 (9.7)	77.7 (13.9)	85.6 (7.1)	84.3 (8.8)	**83.2 (10.1)**
# APOE ε4 Positive (%)	16 (66.7%)	17 (30.4%)	13 (44.8%)	10 (55.6%)	15 (37.5%)	**71 (42.5%)**
With Alzheimer's Disease	**24**	**96**	**56**	**31**	**108**	**315**
% Female	33.3%	43.8%	53.6%	51.6%	54.6%	**49.2%**
Ave Age at Death (SD)	81.0 (11.0)	83.6 (7.5)	80.3 (9.9)	86.2 (8.5)	83.3 (8.0)	**82.9 (8.7)**
# APOE ε4 Positive (%)	20 (83.3%)	65 (67.7%)	41 (73.2%)	19 (61.3%)	79 (73.1%)	**224 (71.1%)**
Control	**22**	**56**	**24**	**17**	**30**	**149**
% Female	50.0%	53.6%	50.0%	41.2%	46.7%	**47.9%**
Ave Age at Death (SD)	85.4 (6.7)	84.1 (9.7)	79.2 (13.6)	85.9 (7.2)	83.5 (8.1)	**83.6 (8.7)**
# APOE ε4 Positive (%)	15 (68.2%)	17 (30.4%)	9 (37.5)	9 (52.9)	10 (33.3%)	**60 (40.3%)**
Other Dementia's	**5**	**22**	**24**	**16**	**32**	**99**
Vascular	2	2	5	2	5	**16**
LBD	1	8	12	3	13	**37**
FTLD	2	5	3	5	4	**19**
Mixed	0	5	0	6	2	**13**
EOAD	0	1	0	0	2	**3**
Other	0	1	4	0	6	**11**
MCI	2	3	1	6	7	**19**
Other disorders present but no dementia	2	0	5	1	10	**18**

Table 1: Demographic break-down of the BDR, by centre and by diagnosis. Age at the time of death, and % of samples that were female did not significantly differ between groups.

Table 2:

				dbSNP 1000 Genomes		Lambert Discovery Sample		BDR Sample n=464		
GWAS Index SNP	Chr Location	Associated Gene	Major Allele	Minor Allele	MAF	Minor Allele	MAF	Minor Allele	MAF	Genotyping Platform
rs6656401	Chr1:207692049	*CR1*	G	A	0.067	A	0.197	A	0.188	KASP
rs6733839	Chr2:127892810	*BIN1*	C	T	0.395	T	0.409	T	0.432	NeuroChip
rs35349669	Chr2:234068476	*INPP5D*	C	T	0.21	T	0.488	C	0.469	KASP
rs190982	Chr5:88223420	*MEF2C*	A	G	0.22	G	0.408	G	0.421	NeuroChip
rs9271192	Chr6:32578530	*HLA-DRB5*	A	C	0.237	C	0.276	C	0.265	KASP
rs10948363	Chr6:47487762	*CD2AP*	A	G	0.188	G	0.266	G	0.312	KASP
rs1476679	Chr7:100004446	*ZCWPW1*	T	C	0.212	C	0.287	G	0.284	NeuroChip
rs11771145	Chr7:143110762	*EPHA1*	G	A	0.432	A	0.338	A	0.329	NeuroChip
rs2718058	Chr7:37841534	*NME8*	A	G	0.337	G	0.373	G	0.367	NeuroChip
rs28834970	Chr8:27195121	*PTK2B*	T	C	0.316	C	0.366	C	0.403	KASP
rs9331896	Chr8:27467686	*CLU*	T	C	0.383	C	0.379	C	0.364	KASP
rs11218343	Chr11:121435587	*SORL1*	T	C	0.109	C	0.039	C	0.041	KASP
rs10838725	Chr11:47557971	*CELF1*	T	C	0.263	C	0.316	C	0.329	KASP
rs983392	Chr11:59923508	*MS4A6A*	A	G	0.231	G	0.403	G	0.418	KASP
rs10792832	Chr11:85867875	*PICALM*	G	A	0.314	A	0.358	A	0.354	NeuroChip
rs17125944	Chr14:53400629	*FERMT2*	T	C	0.111	C	0.092	G	0.101	NeuroChip
rs10498633	Chr14:92926952	*SLC24A4*	G	T	0.153	T	0.217	T	0.219	NeuroChip
rs4147929	Chr19:1063443	*ABCA7*	G	A	0.175	A	0.190	A	0.209	NeuroChip
rs7274581	Chr20:55018260	*CASS4*	T	C	0.094	C	0.083	C	0.084	NeuroChip

Table 2: Minor allele frequencies (MAF) for each of the 19 GWAS index SNPs. MAFs were obtain from 1000 genomes (dbSNP), the Lambert discovery dataset (Lambert et al 2013) and the current BDR cohort of 315 LOAD and 149 control cases combined. Variations between the LOAD cohorts and that of the 1000 genomes MAFs are clearly apparent, however MAF are similar between the Lambert Discovery dataset and the BDR cohort, indicating that the BDR is genetically representative of previously studied LOAD samples.

Table 3:

		Lambert et al 2013			BDR Sample		
GWAS Index SNP	Associated Gene	Minor Allele	OR (95%CI)	Meta-Analysis P-value	Minor Allele	OR (95%CI)	P-value
rs6656401	*CR1*	A	1.18 (1.14 - 1.28)	5.7×10^{-24}	A	0.92 (0.63 - 1.34)	0.656
rs6733839	*BIN1*	T	1.22 (1.18 - 1.25)	6.9×10^{-44}	T	1.03 (0.77 - 1.39)	0.828
rs35349669	*INPP5D*	T	1.08 (1.05 - 1.15)	3.2×10^{-8}	C	1.47 (1.08 - 2.00)	**0.014**
rs190982	*MEF2C*	G	0.93 (0.90 - 0.95)	3.2×10^{-8}	G	0.96 (0.71 - 1.31)	0.812
rs9271192	*HLA-DRB5*	C	1.11 (1.08 - 1.15)	2.9×10^{-12}	C	0.99 (0.71 - 1.39)	0.955
rs10948363	*CD2AP*	G	1.10 (1.07 - 1.13)	5.2×10^{-11}	G	1.20 (0.86 - 1.66)	0.278
rs1476679	*ZCWPW1*	C	0.91 (0.89 - 0.94)	5.6×10^{-10}	C	0.65 (0.46 - 0.92)	**0.015**
rs11771145	*EPHA1*	A	0.90 (0.88 - 0.93)	1.1×10^{-13}	A	1.00 (0.72 - 1.39)	0.99
rs2718058	*NME8*	G	0.93 (0.90 - 0.95)	4.8×10^{-9}	G	0.85 (0.64 - 1.14)	0.278
rs28834970	*PTK2B*	C	1.10 (1.08 - 1.13)	7.4×10^{-14}	C	1.39 (1.01 - 1.90)	**0.044**
rs9331896	*CLU*	C	0.86 (0.84 - 0.89)	2.8×10^{-25}	C	0.98 (0.72 - 1.32)	0.873
rs11218343	*SORL1*	C	0.77 (0.72 - 0.82)	9.7×10^{-15}	C	0.78 (0.37 - 1.65)	0.52
rs10838725	*CELF1*	C	1.08 (1.05 - 1.11)	1.1×10^{-8}	C	1.04 (0.77 - 1.4)	0.813
rs983392	*MS4A6A*	G	0.90 (0.87 - 0.89)	6.1×10^{-16}	G	0.99 (0.73 - 1.34)	0.951
rs10792832	*PICALM*	A	0.87 (0.85 - 0.89)	9.3×10^{-26}	A	0.70 (0.52 - 0.95)	**0.023**
rs17125944	*FERMT2*	C	1.14 (1.09 - 1.19)	7.9×10^{-9}	C	1.11 (0.67 - 1.85)	0.682
rs10498633	*SLC24A4*	T	0.91 (0.88 - 0.94)	5.5×10^{-9}	T	0.93 (0.64 - 1.35)	0.692
rs4147929	*ABCA7*	A	1.15 (1.11 - 1.19)	1.1×10^{-15}	A	0.95 (0.66 - 1.36)	0.783
rs7274581	*CASS4*	C	0.88 (0.84 - 0.92)	2.5×10^{-8}	C	1.21 (0.69 - 2.1)	0.508

Table 3: Results from PLINK association analysis for the 19 SNPs investigated in the BDR cohort alongside data produced from the Lambert et al meta-analysis dataset (Lambert et al 2013). Logistic regression analysis with correction for sex, age at death and number of APOE Ɛ4 alleles suggest that four SNPs (rs35349669, rs1476679, rs28834970 & rs10792832) display nominal significance for association with the LOAD phenotype (highlighted in red). Multiple test correction with Bonferroni saw no SNPs retain significance (P<0.0026).