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Replication of *BIN1* association with Alzheimer's disease and evaluation of genetic interactions

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Abstract

The most recent late-onset Alzheimer's disease (LOAD) genome-wide association study revealed genome-wide significant association of two new loci: rs744373 near *BIN1* ($p=1.6\times10^{-11}$) and rs597668 near *EXOC3L2*/*BLOC1S3*/*MARK4* ($p=6.5\times10^{-9}$). We have genotyped these variants in a large (3,287 LOAD, 4,396 controls), independent dataset comprising eleven case-control series from the USA and Europe. We performed meta-analyses of the association of these variants with LOAD and also tested for association using logistic regression adjusted by age-at-diagnosis, sex and *APOE ε4* status. Meta-analysis results showed no evidence of series heterogeneity and logistic regression analysis successfully replicated the association of *BIN1* (rs744373) with LOAD with an odds ratio (OR=1.17, $p=1.1\times10^{-4}$) comparable to that previously reported (OR=1.15). The variant near *EXOC3L2* (rs597668) showed only suggestive association with LOAD (p=0.09) after correcting for the presence of the *APOE* ε4 allele. Addition of our follow-up data to the results previously reported increased the strength of evidence for association with *BIN1* (11,825 LOAD, 32,570 controls, rs744373 Fisher combined $p=3.8\times10^{-20}$). We also tested for epistatic interaction between these variants and *APOE*ε4 as well as with the previously replicated LOAD GWAS genes (*CLU*: rs11136000, *CR1*; rs3818361, and *PICALM*: rs3851179). No significant interactions between these genes were detected. In summary, we provide additional evidence for the variant near *BIN1* (rs744373) as a LOAD risk modifier, but our results indicate that the effect of *EXOC3L2* independent of *APOE* ε4 should be studied further.

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Keywords

Alzheimer Disease; Late Onset; Heterogeneity; Meta-Analysis; Case-Control Studies

Introduction

Despite strong evidence from twin studies [1, 2] that there is a substantial genetic component to late-onset Alzheimer's disease (LOAD), few variants have shown consistent replication across independent studies besides the *apolipoprotein E* (*APOE*) alleles. In our recent study [3] we successfully replicated the association of variants in three genes (*CLU*: rs11136000, *CR1*; rs3818361, and *PICALM*: rs3851179) initially identified by two large GWAS [4,5]. Combined analysis of all available data (13,574 AD cases, 21,173 controls) resulted in remarkable Fisher combined p-values (*CLU*: $p=2.7\times10^{-24}$, *CR1*: $p=1.8\times10^{-16}$, *PICALM*: $p=3.5\times10^{-15}$), providing the strongest evidence to-date for LOAD genes other than *APOE* and highlighting the utility of large GWAS for identification of novel genetic loci.

In a recently published study, Seshadri *et al.* [6] performed a three-stage analysis of previously published LOAD GWAS [4,5,7,8] and new data from the CHARGE consortium. In this 3 stage meta-analysis (8,371 LOAD cases and 26,965 controls), the strongest associations observed (after *APOE*, *CLU*, and *PICALM*) were for two variants, rs744373 residing near *BIN1* on chromosome 2 (OR=1.15, $p=1.6\times10^{-11}$) and rs597668 on chromosome 19 near *EXOC3L2* (OR=1.17, $p=6.5\times10^{-9}$). In the Seshadri *et al.* study, both *BIN1* (OR=1.17, p=0.02) and *EXO3CL2* (OR=1.26, p=0.01) associations were successfully replicated in an independent Spanish sample (1,140 LOAD cases and 1,209 controls). Lambert *et al.* [9] have replicated the association of *BIN1* and *EXO3CL2* with the risk of AD in 3 European populations (rs744373: OR=1.26, 95% CI [1.15–1.38], $p=2.9\times10^{-7}$; rs597668: OR=1.19, 95% CI [1.06–1.32], $p=2.0\times10^{-3}$), although the association with *EXO3CL2* did not appear to be independent from *APOE*. Lee *et al.* also found significant association with variants in *BIN1* in a Caribbean Hispanic LOAD cohort [10]. Furthermore, Biffi *et al.* have also reported an association between a variant in *BIN1* and two AD-related neuroimaging measures, namely entorhinal cortex thickness and temporal pole cortex thickness [11].

We have analyzed the *BIN1* and *EXOC3L2* associations in our large, independent case-control series (3,287 LOAD cases and 4,396 controls). We report successful replication of the association observed for the variant near *BIN1* (rs744373), and provide a notable Fisher combined p-value of 3.8×10^{-20} for all available data (12,798 AD cases and 32,570 controls). However, the association with *EXOC3L2* was no longer significant after adjusting for *APOE* genotype.

Materials and Methods

Ethics statement

Approval was obtained from the ethics committee or institutional review board of each institution responsible for the ascertainment and collection of samples. Written informed consent was obtained for all individuals that participated in this study.

Case-control subjects

Samples used in this study do not overlap with those included in the Seshadri *et al.* [6] publication. The Mayo case-control series consisted of Caucasian subjects from the United States ascertained at the Mayo Clinic Jacksonville, Mayo Clinic Rochester, or through the

Mayo Clinic Brain Bank. Additional Caucasian subjects from the United States were obtained through the National Cell Repository for Alzheimer's Disease (NCRAD), and European Caucasian subjects were obtained from Norway [12], Poland [13], and from six research institutes in the United Kingdom that are part of the Alzheimer's Research Trust Network (ART). The ART samples used in this follow-up study and those employed in the original GWAS publication by Seshadri *et al.,* [6], were collected by members of the Alzheimer's Research Trust consortium, thus similar subject/sample ascertainment methodologies were followed. Sample identifiers used in the two studies were compared, and samples that overlapped were eliminated prior to our data analysis. The ART series included here are from Bristol, Leeds, Manchester, Nottingham, Oxford and Southampton. Since the Manchester cohort only consisted of LOAD cases, the Manchester cases were combined with subjects in the Nottingham series.

Genotyping

All genotyping was performed at the Mayo Clinic in Jacksonville using TaqMan® SNP Genotyping Assays in an ABI PRISM® 7900HT Sequence Detection System with 384-Well Block Module from Applied Biosystems, California, USA. The genotype data was analyzed using the SDS software version 2.2.3 (Applied Biosystems, California, USA).

Statistical Analyses

Meta-analysis of allelic association and Breslow-Day tests were performed using StatsDirect v2.5.8 software. Meta-analyses were performed using the results from each individual case-control series. Summary ORs and 95% CI were calculated using the DerSimonian and Laird (1986) [14] random-effects model. Breslow-Day tests were used to test for heterogeneity between populations. PLINK software [15] (http://pngu.mgh.harvard.edu/purcell/plink/) was used to perform logistic regression analysis under an additive model adjusting for age-at-diagnosis, sex and presence of *APOE* ε4 as covariates. Since genotype counts were not reported for series included in the Seshadri *et al.* study, we employed a Fisher combined test to combine p-values across series. All 15 pair-wise combinations of the variants in *BIN1*, *EXOC3L2*, *APOE* ε4, *CLU*, *CR1* and *PICALM* were tested for epistatic interactions using PLINK. Both the "-- epistasis" as well as "--logistic --interaction" commands were implemented. Since the test for epistasis does not allow the use of covariates, the logistic regression interaction tests were run using an additive model and included covariates for sex, age at diagnosis and presence or absence of the *APOE*ε4 allele.

Results

In an effort to replicate the results of Seshadri *et al.*, we genotyped rs744373 near *BIN1* and rs597668 near *EXOC3L2* in our independent case-control series from four North American and seven European Caucasian series. Detailed information about these samples as well as genotype and allele counts is shown in Table 1.

As shown in Figure 1, meta-analysis of allelic association in these eleven series revealed significant pooled ORs (DerSimonian-Laird random effects model) for both *BIN1* (OR=1.15, p=0.02) and *EXOC3L2* (OR=1.16, p=0.01), thus successfully replicating the associations reported by Seshadri *et al.*, (*BIN1*: OR=1.15, *EXOC3L2*: OR=1.17) with comparable ORs. Breslow-Day tests provided no significant evidence that the ORs for *BIN1* or *EXOC3L2* were heterogeneous among our series (both p>0.09), but the I^2 values estimating the percentage of variation due to heterogeneity across studies were 38.8% (95% CI 0%–68.5%) and 24% (95% CI 0%–62.2%) respectively, indicating the presence of some heterogeneity within these series. Regardless of any potential underlying heterogeneity, the associations with these two variants remained significant. To adjust for important covariates

(age-at-diagnosis/entry, sex and *APOE ε4* status), we included these covariates in logistic regression analyses of *BIN1* and *EXOC3L2*. The results for individual series and all series combined are shown in Table 2. The association with the *BIN1* variant (rs744373) in our series (Mayo follow-up) remained highly significant when these covariates were included (OR=1.17, $p=1.1\times10^{-4}$) and addition of our data to that previously published (Mayo/Seshadri) increased the strength of evidence for the *BIN1* variant ($p=3.8\times10^{-20}$) as a LOAD risk modifier. However, the OR for the *EXOC3L2* variant (rs597668) diminished in our series and no longer achieved significance (OR=1.08, p=0.09). Thus in our series the association of rs597668 with LOAD appeared to be due, in large part, to linkage disequilibrium between the minor allele of rs597668 and the *APOE* ε4 allele, which has a large effect (OR=4.83, 95% CI 4.41–5.29) and highly significant association with LOAD ($p=1.7\times10^{-248}$) and is located 296.9 kb proximal to rs597668.

In order to determine whether there are significant pair-wise interactions among the variants in *BIN1*, *EXOC3L2*, the three other newly discovered LOAD genes (*CLU*, *CR1*, *PICALM*) and the powerful *APOE*ε4 allele, we tested all fifteen pairs formed by these six variants for epistatic interaction in our large series of 3,287 LOAD and 4,396 controls. None of these interactions showed significance after correction for the fifteen tests performed. However, nominal significance was observed for the interaction between *CR1* and *APOE*ε4 (p=0.03).

Discussion

Our results for rs744373 near *BIN1* were in excellent agreement with those previously reported, and a p-value of 3.8×10^{-20} that was obtained when our results were combined with these, provide compelling evidence that rs744373 is associated with LOAD. In our previous replication study [3], the combined p-values of our follow-up results and those previously reported were 2.7×10^{-24}, 1.8×10^{-16}, and 3.5×10^{-15} for variants in *CLU* (rs11136000), *CR1* (rs3818361), and *PICALM* (rs3851179) respectively. Thus the large, case-control series that have been assembled in the last few years to perform genome-wide association studies have unequivocally identified four new LOAD loci.

Meta analysis of rs597668 near *EXOC3L2* in the eleven series that comprised our follow-up study, where there was no adjustment for linkage disequilibrium with *APOE* alleles, showed significant (p=0.01) association with an OR of 1.16 (95% CI 1.03–1.30) as compared to an OR of 1.15 (95% CI 1.11–1.20) in the larger stage 3 meta-analysis of Seshadri *et al.*, where association was highly significant ($p=1.6\times10^{-11}$). To determine if the effect of rs597668 was independent from the effect of the *APOE* ε4 allele, Seshadri *et al.*, analyzed this variant adjusting for the presence of the *APOE* ε4 allele in their stage 1 dataset (CHARGE, TGen and Mayo GWAS) and noted that the OR declined from 1.18 (95% CI 1.08–1.24) with a p-value of 3.9×10^{-4} to an OR of 1.10 (95% CI 1.00–1.16) with a p-value of 0.05. When we adjusted for *APOE* ε4 (Table 2) in the same way as Seshadri *et al.*, the OR was reduced to 1.08 (95% CI 0.99–1.19) and the association was no longer significant (p=0.09). In the recently published study by Lambert *et al.* the authors also reported that the association observed with the *EXOC3L2* variant does not seem to be independent of the effect conferred by *APOE* [9]. Overall, these results indicate that rs597668 shows only weak, marginally significant association with LOAD that is independent of linkage disequilibrium with *APOE* alleles.

Finally, we analyzed pair-wise epistatic interaction in the fifteen pairs formed by the significant SNPs in *BIN1*, *EXOC3L2*, *CLU*, *CR1*, *PICALM*, and the *APOE ε4* allele. Although none of the tests yielded significant results after correction for multiple tests, one interaction (*CR1* * *APOE*ε4) was suggestively significant. It is reassuring that both approaches, epistasis (OR=1.26, unadjusted p=0.038) and the test for interaction by logistic

regression with covariates (OR=1.28, unadjusted p=0.031), yielded similar results. The other fourteen pair-wise interactions had ORs that ranged from 0.89 to 1.11 and p-values that ranged from 0.13 to 0.99. In a recently published article an interaction between *PICALM* (rs3851179) and *APOE*ε4 was reported (interaction OR=0.84, unadjusted p=0.0068), but not between *CR1* (rs3818361) and *APOE*ε4 (interaction OR=1.01, unadjusted p=0.28) [16]. In our study, the test for interaction between *PICALM* and *APOE*ε4 was not significant (OR=0.97, unadjusted p=0.74); however, it is possible that our sample was not of sufficient size to detect the interaction previously reported. Thus, in our large replication series, none of the 10 pairs formed by the newly discovered LOAD weak susceptibility alleles in *BIN1*, *EXOC3L2 CLU*, *CR1*, and *PICALM* showed even nominally significant association. The only nominally significant epistatic interactions reported to date are with the powerful *APOE ε4* allele; the *CR1* * *APOE*ε4 interaction reported here and the *PICALM* * *APOE*ε4 interaction reported previously. These two interactions were both weak and neither replicated. Further investigation of epistatic interaction in additional large, independent studies will be important, but the available data suggest that there may be little or no pair-wise interaction among the LOAD susceptibility alleles that are now well-established.

After more than a decade in which no consensus could be reached for any of the many variants that were studied in smaller case-control series, the identification of these new LOAD loci represents substantial progress. All of the new variants that have been discovered have effect sizes far less than that of *APOE* ε4, so they have little value for improving predictive models for risk of incident AD in the general population [6]. Thus the value of the newly discovered loci is likely to come from investigation of the mechanism(s) by which they modify risk of LOAD. The effect of a naturally occurring variant on risk of LOAD can be far less than the effect of a potent drug acting through the same mechanism, so there is reason to be optimistic that understanding the mechanism(s) involved can lead to new approaches to effective therapy.

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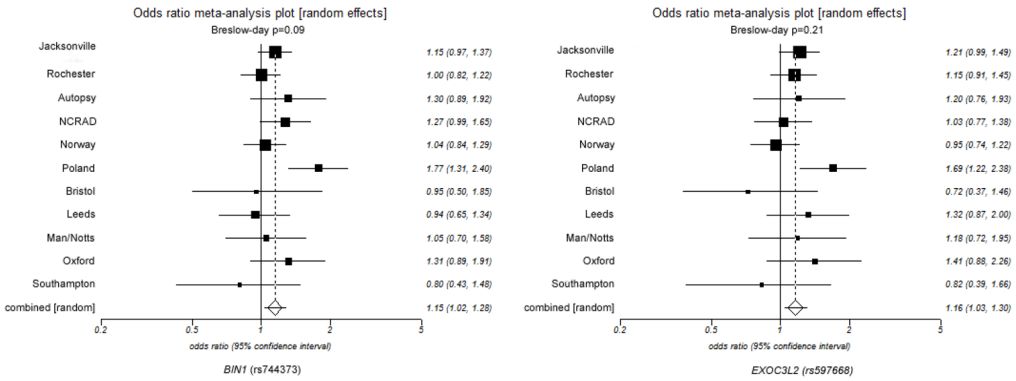

Figure 1. Forest plots for meta-analysis of *BIN1* (rs744373) and *EXOC3L2* (rs597668) in our eleven case-control series

ORs (boxes) and 95% CI (whiskers) are plotted for each population and shown on the right of each plot. Combined OR is the overall OR calculated by the meta-analysis using a random effects model. P-values are provided for the combined ORs and Breslow-Day tests of heterogeneity.

Table 1

Details of samples used in this study and genotype counts.

										BIN1 (rs744373)		*EXOC3L2* (rs597668)	
	Number of samples			Mean Age (SD)		% Female		% ε4+		AA/AG/GG	AA/AG/GG	TT/TC/CC	TT/TC/CC
Series	AD	CON	Total	AD	CON	AD	CON	AD	CON	AD	CON	AD	CON
Jacksonville	502	967	1,469	80.1 (6.5)	81.6 (7.6)	62.2	56.3	60.4	21.8	238/201/48	508/358/83	332/150/18	684/246/29
Rochester	316	1,644	1,960	85.7 (4.5)	80.3 (5.2)	62.0	54.6	42.1	22.5	164/124/22	851/658/110	215/81/15	1142/436/39
Autopsy	311	101	412	87.3 (4.8)	85.9 (4.3)	67.5	52.5	60.8	14.9	138/128/30	54/32/9	205/92/7	73/24/3
NCRAD	700	209	909	75.2 (6.8)	78.3 (8.9)	64.7	61.7	78.6	16.3	324/298/68	105/87/10	460/209/29	138/64/7
Norway	344	551	895	80.2 (7.3)	75.4 (7.3)	70.1	59.7	62.8	24.0	164/141/35	280/208/62	224/102/12	351/175/18
Poland	479	184	663	76.7 (4.8)	73.0 (5.9)	66.2	77.2	56.6	19.0	225/192/51	112/62/6	273/169/28	128/51/3
Bristol	205	40	245	76.9 (7.3)	75.8 (6.4)	58.0	55.0	54.1	42.5	69/59/7	15/16/1	141/56/3	23/13/1
Leeds	113	276	389	75.1 (6.4)	76.9 (6.2)	50.4	49.3	65.5	22.5	63/36/14	137/109/26	72/36/5	190/81/4
Man/Notts	183	89	272	75.8 (9.4)	73.1 (8.3)	57.9	38.2	60.7	21.3	71/83/19	35/41/8	111/60/6	57/29/1
Oxford	98	205	303	73.0 (7.2)	77.2 (8.0)	49.0	57.1	59.2	25.4	43/41/14	102/83/18	64/28/5	149/50/5
Southampton	36	130	166	81.2 (6.5)	76.3 (6.3)	66.7	48.5	44.4	24.6	17/16/2	56/59/13	24/11/1	79/44/5
Total	3,287	4,396	7,683	79.0 (7.6)	79.1 (7.1)	63.4	56.1	61.8	22.3	1516/1319/310	2255/1713/346	2121/994/129	3014/1213/115

The number of LOAD patients (AD) and controls (CON), mean age-at-diagnosis, percentage that are female, percentage that possess at least one copy of the *APOE*ε4 allele and genotype counts for *BIN1* (rs744373) and *EXOC3L2* (rs597668) variants are given for each individual series. Mean age is given as age at diagnosis/entry with the standard deviation (SD) from the mean in parentheses. None of the samples comprising the Jacksonville, Rochester and autopsy-confirmed Mayo Clinic series, or those from the United Kingdom (ART) series comprising the Bristol, Leeds, Mann-Notts (Manchester & Nottingham), Oxford and Southampton, which were included in this follow-up study overlap with those used in the Seshadri *et al.* GWAS. The NCRAD, Norway and Polish series have not been included in LOAD GWAS.

Table 2

Association of *BIN1* and *EXOC3L2* with LOAD in the initial study and Mayo follow-up series.

	N[a]		MAF[b]		Association test	
Study	**Cases**	**Controls**	**Cases**	**Controls**	**OR (95% CI)**	**p-value**
***BIN1*-rs744373-G (minor) allele**						
Seshadri GWAS stage 3 meta-analysis[c]	8,371	26,965			1.15 (1.11–1.20)	1.6×10^{-11}
CHARGE (CHS, FHS, Rotterdam, AGES)	1,367	12,904			1.07 (0.98–1.17)	1.3×10^{-1}
TGen	829	536			1.29 (0.82–1.57)	1.1×10^{-2}
Mayo GWAS	810	1,202			1.23 (1.06–1.42)	6.9×10^{-3}
EADI1	2,032	5,328			1.15 (1.06–1.25)	5.7×10^{-4}
GERAD1	3,333	6,995			1.17 (1.09–1.25)	3.2×10^{-6}
Seshadri follow-up	1,140	1,209	0.30	0.27	1.17 (1.03–1.33)	2.0×10^{-2}
Mayo follow-up[d]	3,158	4,363	0.31	0.28	1.17 (1.08–1.26)	1.1×10^{-4}
Jacksonville	487	949	0.30	0.28	1.14 (0.95–1.37)	1.4×10^{-1}
Rochester	310	1,619	0.27	0.27	0.97 (0.78–1.20)	7.6×10^{-1}
Autopsy	296	95	0.32	0.26	1.33 (0.88–2.00)	1.7×10^{-1}
NCRAD	690	202	0.31	0.26	1.37 (1.01–1.86)	4.2×10^{-2}
Norway	340	550	0.31	0.30	1.06 (0.84–1.33)	6.5×10^{-1}
Poland	468	180	0.31	0.21	1.82 (1.31–2.53)	3.4×10^{-4}
ART	554	719	0.31	0.31	1.07 (0.89–1.29)	4.5×10^{-1}
Mayo/Seshadri[e]	11,683	32,488				3.8×10^{-20}
***EXOC3L2*-rs597668-C (minor) allele**						
Seshadri GWAS stage 3 meta-analysis[c]	8,371	26,965			1.17 (1.11–1.23)	6.5×10^{-9}
CHARGE (CHS, FHS, Rotterdam, AGES)	1,367	12,904			1.16 (1.04–1.31)	1.1×10^{-2}
TGen	829	536			1.00 (1.54–0.66)	9.9×10^{-1}
Mayo GWAS	810	1,202			1.23 (1.47–1.04)	1.7×10^{-2}
EADI1	2,032	5,328			1.19 (1.07–1.32)	1.1×10^{-3}
GERAD1	3,333	6,995			1.16 (1.08–1.25)	5.2×10^{-5}
Seshadri follow-up	1,140	1,209	0.13	0.11	1.26 (1.05–1.51)	1.0×10^{-2}

	N[a]		MAF[b]		Association test	
Study	**Cases**	**Controls**	**Cases**	**Controls**	**OR (95% CI)**	**p-value**
Mayo follow-up[d]	3,258	4,392	0.19	0.16	1.08 (0.99–1.19)	8.9×10^{-2}
Jacksonville	500	959	0.19	0.16	1.01 (0.81–1.25)	9.7×10^{-1}
Rochester	311	1,617	0.18	0.16	1.27 (0.99–1.63)	5.9×10^{-2}
Autopsy	304	100	0.17	0.15	1.04 (0.63–1.72)	8.8×10^{-1}
NCRAD	698	209	0.19	0.19	0.86 (0.62–1.21)	3.9×10^{-1}
Norway	338	544	0.19	0.19	0.87 (0.65–1.15)	3.3×10^{-1}
Poland	470	182	0.24	0.16	1.41 (0.99–2.02)	5.7×10^{-2}
ART	623	731	0.19	0.17	0.98 (0.79–1.22)	8.5×10^{-1}
Mayo/Seshadri[e]	11,782	32,516				8.8×10^{-15}

Abbreviations: MAF, minor allele frequency; OR, odds ratio for the minor allele; 95% CI, 95% confidence interval

[a]The numbers shown for the series in the Seshadri *et al.* study refer to the complete set analyzed. The numbers for the Mayo follow-up data refer to the number of samples successfully genotyped.

[b]MAFs were not reported for LOAD and control groups in the Seshadri *et al.* study.

[c]Stage 3 results in the Seshadri *et al.* study obtained by meta-analysis, in contrast to the results from the individual GWAS studies (CHARGE, TGen, Mayo, EADI1 and GERAD1) that were obtained by logistic regression adjusted for age and sex.

[d]The results shown here for the Mayo follow-up dataset combined and for the subseries were obtained using logistic regression adjusted for age, sex and *APOE ε4* status. The Mayo follow-up dataset reported here is independent of that which was incorporated in the GWAS reported by Seshadri *et al.* The results for each of the Mayo follow-up subseries (Jacksonville, Rochester, Autopsy-confirmed, NCRAD, Norway, Poland and ART) are listed immediately below the results for the Mayo follow-up dataset combined.

[e]Indicates Fisher's combined p-value for each individual GWAS in the Seshadri *et al.* study (CHARGE, TGen, Mayo, EADI1, GERAD1) and the Seshadri *et al.* and Mayo independent follow-up series.