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ORIGINAL ARTICLE

Follow-up of loci from the International Genomics of Alzheimer's Disease Project identifies *TRIP4* as a novel susceptibility gene

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To follow-up loci discovered by the International Genomics of Alzheimer's Disease Project, we attempted independent replication of 19 single nucleotide polymorphisms (SNPs) in a large Spanish sample (Fundació ACE data set; 1808 patients and 2564 controls). Our results corroborate association with four SNPs located in the genes *INPP5D, MEF2C, ZCWPW1* and *FERMT2,* respectively. Of these, ZCWPW1 was the only SNP to withstand correction for multiple testing ($P = 0.000655$). Furthermore, we identify *TRIP4* (rs74615166) as a novel genome-wide significant locus for Alzheimer's disease risk (odds ratio = 1.31; confidence interval 95% (1.19–1.44); $P = 9.74 \times 10^{-9}$).

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INTRODUCTION

Alzheimer's disease (AD) is a complex multifactorial neuropsychiatric disorder whose etiology involves both environmental and genetic factors. The major genetic risk factor for AD is the apolipoprotein E4 (*APOE4*) allele.[1] For 17 years after its discovery, this remained the only confirmed genetic risk factor for the disorder. However, subsequent meta-analyses of genome-wide association studies identified further genetic risk loci. These include signals close to, or within, candidate genes such as *CLU,*[2,3] *PICALM,*[2] *CR1,*[3] *BIN1,*[4] *ABCA7,*[5] *EPHA1,*[5,6] *CD33,*[5,6] CD2AP[5,6] and *ATP5H/KCTD2,*[7] as well as the *MS4A* gene cluster.[5,6,8]

The International Genomics of Alzheimer's Disease Project (IGAP) is the largest genetic epidemiology investigation of AD risk to date. In 2013, the IGAP reported a mega meta-analysis, which was divided into a discovery step (stage 1) and a replication step (stage 2). This mega meta-analysis comprised 74 046 samples, including those of 25 580 AD cases, and identified 11 new loci, thus doubling the number of genome-wide significant loci reported for AD.[9] The analysis also identified 13 suggestive loci. These findings may serve as the starting point for novel discoveries in future AD genomics studies.

Four of the 11 genome-wide significant loci in the IGAP analyses reached significance in stage 1 (rs8093731 *DSG2*; rs28834970 *PTK2B*; rs11218343 *SORL1*; rs10498633 *SLC24A4*). The remaining seven only reached genome-wide significance in stage 2—that is, after the inclusion of the replication sample (rs35349669 *INPP5D*;

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Table 1. Results for the 19 IGAP SNPs in the Fundació ACE data set

SNP	Chr.	Base pair	Maj/min allele	Locus	IGAP status	OR (IGAP)	MAF (IGAP)	OR (F.ACE)	MAF (F.ACE)	P-value (F.ACE)	OR (com)	P-value (com)	Het*
rs8093731	18	29088958	C/T	*DSG2*	NL-ST 1	0.73 (0.62–0.86)	0.017	0.728 (0.486–1.090)	0.011	0.1217	0.7292	3.02×10^{-5}	0.0006
rs28834970	8	27195121	T/C	*PTK2B*	NL-ST 1	1.10 (1.08–1.13)	0.366	0.975 (0.893–1.065)	0.372	0.571	1.0936	2.39×10^{-12}	0.026
rs11218343	11	121435587	T/C	*SORL1*	NL-ST 1	0.77 (0.72–0.82)	0.039	0.864 (0.678–1.099)	0.035	0.233	0.7757	6.91×10^{-15}	0.6301
rs10498633	14	92926952	G/T	*SLC24A4*	NL-ST 1	0.91 (0.88–0.94)	0.217	0.922 (0.827–1.028)	0.191	0.1418	0.9107	1.99×10^{-9}	0.6781
rs7274581	20	55018260	T/C	*CASS4*	NL-ST 2	0.88 (0.84–0.92)	0.083	1.017 (0.882–1.173)	0.098	0.8153	0.8888	1.75×10^{-7}	0.1372
rs35349669	2	234068476	C/T	*INPP5D*	NL-ST 2	1.08 (1.05–1.11)	0.488	1.104 (1.014–1.203)	0.439	0.02314	1.0807	2.59×10^{-9}	0.5807
rs2718058	7	37841534	A/G	*NME8*	NL-ST 2	0.93 (0.90–0.95)	0.373	1.081 (0.992–1.178)	0.418	0.1201	0.9368	2.41×10^{-7}	0.0044
rs190982	5	88223420	A/G	*MEF2C*	NL-ST 2	0.93 (0.90–0.95)	0.408	0.885 (0.811–0.966)	0.388	0.006285	0.9232	1.18×10^{-9}	0.5718
rs17125944	14	53400629	T/C	*FERMT2*	NL-ST 2	1.14 (1.09–1.19)	0.092	1.238 (1.036–1.478)	0.060	0.01851	1.1470	6.71×10^{-10}	0.5585
rs1476679	7	100004446	T/C	*ZCWPW1*	NL-ST 2	0.91 (0.89–0.94)	0.287	0.846 (0.769–0.932)	0.271	0.000655	0.9147	5.04×10^{-12}	0.1727
rs9381040	6	41154650	C/T	*TREML2*	SUG	0.93 (0.91–0.96)	0.297	0.991 (0.901–1.089)	0.277	0.8446	0.9365	1.30×10^{-6}	0.2321
rs8035452	15	51040798	T/C	*SPPL2A*	SUG	0.93 (0.91–0.96)	0.339	1.102 (1.009–1.204)	0.362	0.03098	0.9455	1.99×10^{-5}	0.001
rs7920721	10	11720308	A/G	*ECHDC3*	SUG	1.07 (1.04–1.10)	0.387	1.049 (0.962–1.145)	0.395	0.2778	1.0696	1.68×10^{-7}	0.8768
rs7818382	8	96054000	C/T	*NDUFAF6*	SUG	1.07 (1.04–1.10)	0.469	1.003 (0.921–1.093)	0.455	0.9405	1.0657	2.48×10^{-7}	0.3428
rs74615166	15	64725490	T/C	*TRIP4*	SUG	1.29 (1.17–1.42)	0.02	1.519 (1.148–2.012)	0.023	0.003265	1.3102	9.74×10^{-9}	0.1357
rs7295246	12	43967677	T/G	*ADAMST20*	SUG	1.07 (1.04–1.10)	0.406	1.044 (0.958–1.139)	0.399	0.3253	1.0693	2.23×10^{-7}	0.764
rs7225151	17	5137047	G/A	*SCIMP*	SUG	1.10 (1.06–1.15)	0.121	0.952 (0.839–1.081)	0.129	0.4475	1.0898	3.06×10^{-6}	0.0751
rs6678275	1	193625233	G/C	*None*	SUG	1.09 (1.05–1.13)	0.169	0.948 (0.849–1.059)	0.180	0.3419	1.0775	4.21×10^{-6}	0.0444
rs6448799	4	11630049	C/T	*HS3ST1*	SUG	1.08 (1.05–1.11)	0.300	0.994 (0.905–1.091)	0.293	0.9006	1.0729	2.70×10^{-7}	0.247

Abbreviations: Chr, chromosome; F.ACE, Fundació ACE data set; IGAP, International Genomics of Alzheimer's Disease Project; MAF, minor allele frequency; Maj/Min allele: major and minor allele; NL-ST 1, new locus in stage 1 of IGAP study; NL-ST 2, new locus in stage 2 of IGAP study; OR, odds ratio; SNP, single nucleotide polymorphism; SUG, suggestive locus. Het*: *P*-value Brelow-day test.

rs190982 *MEF2C*; rs2718058 *NME8*; rs1476679 *ZCWPW1*; rs10838725 *CELF1*; rs17125944 *FERMT2*, and rs7274581 *CASS4*). Replication of the IGAP findings in an independent series is therefore warranted. The present report describes the follow-up of 10 novel and nine suggestive IGAP loci using subjects drawn from the Spanish Fundació ACE cohort.[7]

MATERIALS AND METHODS

Patients and controls

The present study involved 4372 individuals. These included 1 808 patients with a possible or probable diagnosis of AD, as assigned by a neurologist,[8,10] and 2564 unrelated healthy controls from the Spanish general population who were selected from the Neocodex bio-bank.[11] The

AD cases were recruited consecutively from three centers: Barcelona ($n = 1627$); Madrid ($n = 161$); and Murcia ($n = 20$). None of these AD patients had been included in the IGAP replication analyses. To avoid the issue of population stratification, all cases and controls were of Spanish ancestry, which was defined as a history of two generations of registered Spanish ancestors. The demographic characteristics of the Fundació ACE participants are described elsewhere.[7] Written informed consent was obtained from all participants, or from their legal representatives when necessary. The study was approved by the respective ethics committees, and was performed in accordance with the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association.

DNA isolation and genotyping

DNA was extracted using 'Magnapure' technology (Roche Diagnostics, Mannheim, Germany). Twenty-five single nucleotide length polymorphisms (SNPs) with an AD-association risk of below $P < 10^{-6}$ in the IGAP consortium study were selected for replication. The primer molecules for the multiplex reaction were designed using the Assay Design Suite tool (www.mysequenom.com, Sequenom, San Diego, CA, USA). Assay designs were successful for 21 of the 25 selected variants. Four SNPs (rs72807343 (SQSTM1); rs9271192 (HLA-DRB5/HLA-DRB1); rs2337406 (IGH@); and chr17:61,538,148 (ACE)) were rejected during this phase due to technical problems. Primer sequences and assay conditions for the genotyped SNPs are available upon request.

Quality control

A total of 1808 AD patients and 2564 controls were genotyped for 21 SNPs using Sequenom's Mass Array System (Sequenom) and iPlex Gold reagents in accordance with the manufacturer's instructions. Only SNPs with a call rate of ⩾95% and a Hardy–Weinberg equilibrium *P*-value of >0.01 in the whole data set were included in the subsequent analyses (Supplementary Table 1). All SNP major and minor alleles and allelic frequencies obtained in the Fundació ACE data set were fully consistent with those reported by the IGAP consortium (Table 1). The overall conversion rate was 96.7%. The SNPs rs10751667 (*AP2A2*) and rs10838725 (*CELF1*) failed quality control and were excluded from the statistical analyses. The 19 successfully genotyped SNPs and their status in the IGAP analyses (that is, genome-wide association studies significant or suggestive) are specified in Table 1.

Statistical analysis

Genetic association analyses and calculation of allelic frequencies and Hardy–Weinberg equilibrium were conducted using the online tool at the TUM Helmholtz Center (Munich, Germany; http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Briefly, tests adapted from those of Sasieni[12] were used. Age- and sex-adjusted binary logistic regression analyses were performed using SPSS 15.0 software (SPSS, Chicago, IL, USA). In addition, Mantel–Haenzsel-stratified analyses were conducted according to gender and the presence or absence of the *APOE*-ε4 allele (Supplementary Tables 2 and 3, respectively). Breslow-day tests were conducted to measure the significance of SNP × *APOE* and SNP × gender interactions. All SNP calculations were double-checked using PLINK or INTERSNP software.[13,14] Meta-analyses were conducted using the PLINK software. All results were doubled-checked using Ken Rothman's Episheet spreadsheet and PLINK (for details see http://pngu.mgh.harvard.edu/~purcell/plink/; http://krothman.hostbyet2.com/episheet.xls). Power calculations were performed using Episheet.

RESULTS AND DISCUSSION

In the present replication effort, a nominally significant signal ($P < 0.05$) was detected for six of the 19 investigated SNPs: rs35349669 at *INPP5D* ($P = 0.023$); rs190982 at *MEF2C* ($P = 0.0062$); rs1476679 at *ZCWPW1* ($P = 0.00065$); rs17125944 at *FERMT2* ($P = 0.018$); rs8035452 at *SPPL2A* ($P = 0.031$); and rs74615166 at *TRIP4* ($P = 0.0032$) (Table 1). Of these, rs1476679 at the *ZCWPW1* locus, which had shown genome-wide significance in stage 2 of the IGAP analyses, was the only SNP to withstand correction for multiple testing ($P = 0.000655$). However, the observed inflation factor for χ^2 was $\lambda = 4.7$. As in genome-wide analyses, the λ-value was computed as the median of the χ^2-test statistics obtained for the 19 investigated SNPs. Under the null hypothesis of no association, the expected λ value is 1 in the absence of true association. In our study, however, the λ value is 4.7, which is a strong sign of an overall increased degree of significant associations for the SNPs investigated in the Spanish cohort. In the present context, the λ value is considered to indicate the overall degree of positive associations for a small set of SNPs, in contrast to the genome-wide setting, where it is used as an indicator of residual inflation caused by spurious association. Thus, the present observations in our study are unlikely to represent chance findings. Furthermore, four of these five nominally significant association signals displayed the same effect direction as that reported by the IGAP (Table 1). The exception was the marker rs8035452. The IGAP reported this as a suggestive signal. However, an effect in the opposite direction was found in the Fundació ACE data set. This observation might reflect a lack of power in our data set to detect this signal. Alternatively, the original finding may represent a false-positive.

No significant association was found for three of the four genome-wide significant loci detected during IGAP Stage 1 (rs11218343 at *SORL1;* rs10498633 at *SLC24A4;* rs8093731 at *DSG2*). However, since the effect sizes and directions of these three loci were fully consistent with those reported by the IGAP, our failure to replicate them may have been attributable to a lack of power.

In total, seven of the 10 investigated genome-wide significant loci from the IGAP displayed a consistent effect in the present data set. The non-consistent effects observed for the *NME8*, *PTKB2,* and *CASS4* signals may have been attributable to a lack of power. It should be noted that, although our series may appear underpowered compared with the IGAP data set, the present study had on average a power of 45% to detect each of the genome-wide association studies significant signals reported by the IGAP.

The results of the *APOE* and gender-adjusted stratified analyses suggested that for most of the 19 investigated SNPs, *APOE* status and gender had little impact on effect size or the association results (Supplementary Table 3). Interestingly, nominal *P*-values for *APOE* interaction were obtained for two nonsignificant SNPs in our series (rs7295246 *ADAMST20;* and rs7225151 *SCIMP*). Both loci were reported as being suggestive by the IGAP. The results of the present stratification analyses support the hypothesis that these two loci represent susceptibility factors in only a fraction of AD patients, and that their effects are dependent upon the APOE-ε4 genotype. This observation may facilitate determination of their role in AD development in future studies (Supplementary Table 3).

Of the nine suggestive loci proposed by the IGAP, only one SNP was significant in the present analyses (Table 1). The statistically significant signal was obtained for rs74615166 at the thyroid receptor interacting protein gene 4, *TRIP4,* locus (odd ratio = 1.519 (1.148–2.012), $P = 0.0032$). This variant had a minor allele frequency of 0.02 in both the IGAP and the Fundació ACE (Table 1). Interestingly, a larger effect size was observed in the Fundació ACE data set than in the IGAP. However, an advantage of the present analyses was that this SNP was genotyped directly, whereas the IGAP had to rely in part on imputed genotypes. Since imputation for rarer variants is more difficult, this might explain why a stronger effect was observed in the present cohort. The present findings for the nine suggestive IGAP signals may indicate that these loci have a weaker effect on AD risk than the genome-wide significant SNPs. As a direct consequence, the power to detect them using our data set is relatively low (33% on average for suggestive signals). However, our data set had a >99.9% power to detect at least one suggestive locus (0.33),[9] thus explaining the results for the *TRIP4* locus.

Meta-analysis of the present results with the IGAP meta-analysis data identified *TRIP4* as a novel genome-wide significant locus. The new susceptibility AD SNP is located within the eleventh intron of *TRIP4* (15q22.31; rs74615166; OR = 1.31 (1.17–1.42), $P = 9.74 \times 10^{-9}$; Table 1). According to publicly available databases (genome.cse.ucsc.edu), *TRIP4* is highly expressed in the immune system and has been detected in various tissues, including the

brain. Research suggests that *TRIP4* is a component of the nuclear receptor-coupled co-activation machinery that enables or disables DNA transcription.[15] A homolog of the *TRIP4* gene in *Caenorhabditis elegans* showed elevated transcript levels in aged or starved adults, which suggests that TRIP4 has a role in cellular maintenance or survival.[16] The TRIP class of proteins show thyroid hormone-dependent interaction with their receptors, and the association between *TRIP4* and AD risk may partly explain previous findings of an association between low thyroid-stimulating hormone levels in clinically euthyroid subjects and increased AD risk.[17] TRIP proteins show a similar ligand-dependent interaction with the retinoid X receptor. This is of interest, since a recent AD mouse model study reported that administration of the retinoid X receptor agonist bexarotene resulted, within hours, in enhanced clearance of soluble Aβ.[18] Besides *TRIP4,* the linkage disequilibrium block that contains rs74615166 includes several other candidate genes, such as *CSNK1G1*. *CSNK1G1* is a member of the CK-1 family, and its gene product has been implicated in the amyloid cascade.[19]

A major limitation of the present study was the lack of power to confirm all true associations. Therefore, our negative results cannot be interpreted as confirmation of a lack of association for the respective SNPs, which remain putative susceptibility loci for AD. A fraction of the suggestive SNPs reported by the IGAP may be genuine, and these SNPs warrant further investigation.

The results obtained for *TRIP4* underscore the importance of follow-up and comprehensive replication of consortia results. Further genotyping and re-sequencing efforts to investigate *TRIP4* and the other IGAP loci are underway in order to elucidate the role of *TRIP4* in AD risk and corroborate further genuine signals. Further studies are now warranted to identify the functional mechanism underlying the association between *TRIP4* and AD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Study concept and design: A Ramirez, WM, MB, MMN, FJ, MMB, A Ruiz, SH, TB. Acquisition of data**:** A Ruiz, SH, TB, IH, MB, A Ramirez, FJ, MMN, WM, CB, JCB, DH, HW, MT, RM, JLH, MAP-V, LAF, GDS, AG, RS, VC, LJL, CvD, SS. Contribution of samples**:** AM, MR-R, OS-G, AE, MA, JLA, AL, SR, LV, MB, MAD, CA, MS-R, LT, OR, GO, IH, RM, JLH, MAP-V, LAF, GDS, CB, JCB, DH, AG, RS, VC, LJL, CvD, SS. Data analysis**:** MMN, A Ramirez, A Ruiz, SH, TB, ML, AL, CB, JCB, DH, HW, MT, JB, IH. Statistical analysis and interpretation**:** MMN, A Ramirez, A Ruiz, SH, TB, ML, AL. Drafting of the manuscript**:** MMN, MB, A Ramirez, FJ, MMB, A Ruiz, SH, TB.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)