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SHORT REPORT

Closing the case of APOE in multiple sclerosis: no association with disease risk in over 29 000 subjects

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ABSTRACT

Background Single nucleotide polymorphisms (SNPs) rs429358 (ε4) and rs7412 (ε2), both invoking changes in the amino-acid sequence of the apolipoprotein E (APOE) gene, have previously been tested for association with multiple sclerosis (MS) risk. However, none of these studies was sufficiently powered to detect modest effect sizes at acceptable type-I error rates. As both SNPs are only imperfectly captured on commonly used microarray genotyping platforms, their evaluation in the context of genome-wide association studies has been hindered until recently.

Methods We genotyped 12 740 subjects hitherto not studied for their APOE status, imputed raw genotype data from 8739 subjects from five independent genome-wide association studies datasets using the most recent high-resolution reference panels, and extracted genotype data for 8265 subjects from previous candidate gene assessments.

Results Despite sufficient power to detect associations at genome-wide significance thresholds across a range of ORs, our analyses did not support a role of rs429358 or rs7412 on MS susceptibility. This included meta-analyses of the combined data across 13 913 MS cases and 15 831 controls (OR=0.95, p=0.259, and OR 1.07, p=0.0569, for rs429358 and rs7412, respectively).

Conclusion Given the large sample size of our analyses, it is unlikely that the two APOE missense SNPs studied here exert any relevant effects on MS susceptibility.

INTRODUCTION

Multiple sclerosis (MS), the most common chronic inflammatory disease of the central nervous system, is likely caused by interplay of environmental and genetic factors. Recent genome-wide association studies (GWAS) have identified almost 60 putative risk loci apart from a well-established association with the human leukocyte antigen region on chromosome 6p21 (eg. refs.1–5). Despite this recent progress, it has been estimated that approximately 80% or more of the genetic variance remains unexplained by the currently known loci.5 The failure to decipher the full genetic spectrum of MS susceptibility may in part be due to incomplete coverage of the genome by the available GWAS arrays. Along these lines, two single nucleotide polymorphisms (SNPs) frequently tested for association with MS risk in the pre-GWAS era, that is, rs429358 (a.k.a. ‘ε4’) and rs7412 (‘ε2’), in encoding apolipoprotein E (APOE), are absent from most GWAS genotyping platforms. Thus, these two APOE SNPs could not be directly assessed in previous MS GWAS and GWAS meta-analyses. The most recent release of whole-genome sequence data from the 1000 Genomes Project now makes it possible to impute genotypes at both sites for data from most GWAS genotyping platforms.

The investigation of APOE, the single most important risk locus for Alzheimer’s disease, in MS has been motivated by reports of genetic linkage to the APOE-containing region on chromosome 19q15 (eg. ref.6) as well as by its functional role in lipid transport, immunoregulation, neuroplasticity and repair mechanisms.7 However, APOE association studies in MS have yielded mostly negative results to date with some studies reporting significant effects while most others were unable to confirm these associations. These inconsistencies can at least in part be attributed to small sample sizes of the individual datasets. As a matter of fact, none of the previously performed APOE association studies in MS included more
than 450 cases (see supplementary table S1 for an overview of study-specific sample sizes). Even two previous meta-analyses combining published data\(^6\,7\) were limited in power to detect modest effects (ie, ORs at or below 1.2) at acceptable type-I error rates, eg, at a genome-wide significance threshold (typically defined as \(\alpha=5\times10^{-8}\)).

To provide a more conclusive assessment of the potential association between the two most commonly studied \(A\)PO\(E\) SNPs and MS susceptibility in populations of European descent, we performed a large-scale association study of rs429358 and rs7412 using MS risk as outcome. To this end, we collected and genotyped 12,740 subjects hitherto not studied for their \(A\)PO\(E\) status, imputed raw genotype data from 8,759 subjects from five independent GWAS datasets using the most recent high-resolution reference panels, and extracted genotype data for 8,265 subjects from previous candidate gene assessments to arrive at an overall samples size of up to 15,915 MS cases and 15,831 controls.

**SUBJECTS AND METHODS**

The following section only provides a brief summary of the methods applied to our study. A more detailed description can be found in the supplementary material.

**Literature search and data abstraction**

Genetic association studies investigating the role of \(A\)PO\(E\) on MS susceptibility in populations of European descent were identified by a search of NCBI’s PubMed database and via inspecting cross-references in related publications. Only independent case-control studies investigating the association of \(A\)PO\(E\) rs429358 and/or rs7412 with MS risk that were published in peer-reviewed journals in English and available until 1st July 2012 were considered. Next, demographic details and genotype summary data were extracted from eligible publications. Hardy-Weinberg equilibrium (HWE) testing in controls and association analyses of rs429358 and rs7412 per dataset were performed using Pearson’s \(\chi^2\) test implemented in R language.

**GWAS analysis**

We applied for and obtained raw genotypes for three previously published GWAS, below referred to as ‘International Multiple Sclerosis Genetics Consortium (IMSGC)’,\(^1\) ‘GeneMSA’,\(^8\) and ‘ANZgene’.\(^2\) Following pre-imputation QC filtering, IMSGC comprised 926 MS and 2,519 control samples, GeneMSA 986 MS and 920 control samples (across three datasets from the US, Switzerland and the Netherlands), and ANZgene 1,618 MS and 1988 control samples. Imputation of the 19q13 region including the uncovered SNPs rs429358 and rs7412 was based on ‘1000GP_hg19-Jun2011_Phase1’ data panels using the IMPUTE programme V2.0 (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html). To estimate sample-specific additive ORs, association analysis was performed using frequentist and expectation maximization models adjusting for sex, population stratification as well as genotype uncertainty during the imputation process.

**Direct genotyping**

**Subjects:** This arm of the study included 6,741 MS cases and 5,999 healthy controls of self-reported European descent from Germany, France and Spain that were not previously assessed for rs429358 and rs7412.

**Genotyping:** Genotypes in all German samples were determined by Sanger sequencing using the BigDye terminator v3.1 sequencing kit, resolved on an ABI3750 XL genetic analyser (Applied Biosystems). Raw sequences were analysed with SeqMan II V8.0.2 (DNAStar). The French and the Spanish samples were genotyped using allelic discrimination assays based on TaqMan chemistry (Applied Biosystems).

**Association analysis:** HWE in controls was tested using Pearson’s \(\chi^2\) as implemented in PLINK V1.07 (http://pngu.mgh.harvard.edu/purcell/plink/). Logistic regression without and with adjustment for age and/or sex (where available in >90% of subjects) was performed in PLINK using an additive transmission model.

**Meta-analysis**

Meta-analyses were based on random-effects models including all published and newly generated datasets. In addition, we performed meta-analyses after stratification for published and newly generated datasets. Sensitivity analyses were performed after exclusion of datasets in which control subjects showed HWE violations (\(p<0.05\)). Between-study heterogeneity was quantified using the \(I^2\) metric. Evidence for small-study effects, which can be indicative of publication or selective reporting biases, was assessed using a modified regression test.\(^8\) All analyses were performed in R, using packages ‘HardyWeinberg’, V1.4 and ‘meta’, V2.16. All reported meta-analysis p values are two-tailed. Statistical significance was defined at a genomewide level (\(p<5\times10^{-8}\)), while a trend for association was set to \(p<1\times10^{-4}\).

**RESULTS**

Overall, our study comprised 13,913 cases and 15,831 controls across 29 individual datasets for rs429358, and 13,202 cases and 15,258 controls across 26 datasets for rs7412 (see supplementary figure S1 and tables S1–S3 for details). In the combined analyses, we had approximately 99% and 72% power to detect an OR of 1.20 at a genome-wide significance threshold (\(\alpha=5\times10^{-8}\)) for rs429358 and rs7412, respectively. In total, our study exceeded the above mentioned recent meta-analyses\(^6\,7\) across 5,831\(^\circ\) and 7,706 Caucasian subjects\(^7\) by \(\sim23,900\) and 22,000 subjects, respectively.

Meta-analyses of rs429358 and rs7412 across all datasets did not reveal significant evidence for association and yielded effect size estimates close to the null (rs429358: OR=0.95, p=0.259, rs7412: OR 1.07, p=0.0569, figure 1) with moderate and no evidence for heterogeneity, respectively (rs429358: \(I^2=55\), rs7412: \(I^2=0\)). Stratified analyses testing on published, GWAS, and newly genotyped datasets for rs429358 yielded similar results (figure 1 and see supplementary table S4). There was no evidence for small-study effects, as a measure of potential publication bias, for the published data on rs429358 (\(p=0.942\)). For rs7412, meta-analysis results across published datasets yielded a relatively pronounced OR of 1.19 (\(p=0.0263\)), and no evidence for heterogeneity (\(I^2=0\), see supplementary table S4). However, in this stratum the regression test also showed significant evidence for small-study effects (\(p=0.0047\)), which could indicate the presence of publication or selective reporting bias. In this context, it is of note that two publications reported data on rs429358 but not rs7412 (see supplementary table S1), although those data had been generated in the respective studies. Along these lines, analysis of rs7412 stratified for GWAS and newly genotyped datasets showed only non-significant effect estimates close to the null (OR=0.96, \(p=0.579\), and OR=1.04, \(p=0.616\), respectively; figure 1 and see supplementary table S4). Accordingly, combining all available
data for rs7412 led to an overall non-significant meta-analysis result for rs7412 (OR 1.07, p=0.0569; figure 1).

**DISCUSSION**

Upon combining genotype data from more than 29,000 individuals we failed to detect any noteworthy genetic effects of two commonly studied missense SNPs in APOE on MS risk. Given its sample size and inherent statistical power, our study compellingly suggests that even modest effects of rs429358 and rs7412 on MS risk are unlikely. The fact that previous studies yielded only inconclusive results can most likely be attributed to a lack in power, a problem overcome by the present analysis.

Despite the large sample size, we cannot exclude that APOE rs429358 and rs7412 exhibit smaller genetic effects than assumed here. For instance, the smallest effect size estimate to reach genome-wide significance in the recent MS GWAS was 1.09. Given the allele frequencies at APOE rs429358 and rs7412, this would require a total of 88,000 and 140,000 subjects, respectively, to yield ~80% power to detect such effect sizes at a genome-wide significance threshold. While our study lacked power to detect such minor effects, we do not even observe a trend for association in the present datasets making the existence of any role of the investigated APOE SNPs highly unlikely.

Another limitation of our study pertains to the lack of effectively controlling for hidden population substructure in a substantial number of datasets. However, the results of our GWAS meta-analyses, which were adjusted for these covariates on the association with disease risk. Furthermore, we only assessed the potential association of MS with the functional variants rs429358 and rs7412. Thus, we cannot exclude that other variants in this region, which are in weak or no linkage disequilibrium with those two SNPs, exert an effect on MS risk. However, this is unlikely given the collectively negative results in recent MS GWAS and GWAS meta-analyses, that have captured a substantial fraction of the genetic variation at this locus, except for SNPs rs429358 and rs7412 which could not be effectively imputed until very recently. Finally, our study was restricted to assessing the influence of the two APOE SNPs on MS susceptibility only. A possible influence of these SNPs on other MS-relevant traits, such as disease progression or MRI changes, cannot be excluded.

Studies investigating the association of APOE and these outcomes in MS are numerous and typically show contradicting results, possibly due to small sample sizes. Despite these caveats, it is interesting to note that the largest studies on APOE as predictor of MS severity, cognition, or brain atrophy published to date do not suggest any noteworthy effects (see ref. 11 as example and for an overview of other related studies).

In summary, our study, which combines de novo genotyping results from multiple populations, imputed GWAS data, and systematically collated evidence from the literature does not support a role of the two most commonly studied SNPs in APOE in modifying susceptibility for MS in populations of European descent.

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**Figure 1** Forest plots of random effects meta-analyses of datasets assessing the association between APOE SNPs rs429358 and rs7412 and MS risk in populations of European descent. The x-axis depicts the OR. Study-specific ORs (black squares) and 95% CIs (lines) were calculated for each included dataset. The summary ORs and 95% CIs (grey diamonds) were calculated combining all datasets and after stratification for published datasets, imputed GWAS datasets, and newly genotyped datasets as indicated. Exclusion of studies violating Hardy-Weinberg equilibrium in controls did not substantially change the meta-analysis results (data not shown). References to the published datasets listed in this figure can be found in the e-References. ANZgene, Australia and New Zealand Multiple Sclerosis Genetics Consortium; APOE, apolipoprotein E; CH, Switzerland; GWAS, genome-wide association studies; IMSGC, International Multiple Sclerosis Genetics Consortium; MS, multiple sclerosis; NL, Netherlands; SNPs, single nucleotide polymorphisms.
We are grateful to the subjects participating in this study. We would like to thank investigators from the International Multiple Sclerosis Genetics Consortium and from the GeneMSA project for making their GWAS data available via the dbGaP platform. We acknowledge use of the cohort of the Genetics Consortium and from the GeneMSA project for making their GWAS data available via the dbGaP platform. We acknowledge use of the cohort of the CRB-REFGENSEP and thank ICM, CIC Pitié-Salpêtrière, Généthon and REFGENSEP for their help and support.

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**Contributors** Study design: CML, LB (Berlin), FZ Literature searches and data extraction: CML, ML, EM Acquirement of data: CML, DA, VD, AA (Granada), RA, PB, AW, LAG, FL, OE GI, AA (Bilbao), SH, ICR, HE CC, ET, FP, PC, ANGere Consortium, TD, ES-T, LB (Stockholm), HHK, S-CL, UL, AC, H-PH, OA, PL, TK, CK, JTE, UKZ, BE, FM, FZ. Performed the experiments: CML, B-IMMS, AA (Granada), M40, ALL, CC, FM. Data analysis: CML, TL, JTR, SG, KV, FM, EU, LB (Berlin) Interpretation of results: CML, EU, LB (Berlin), FZ Writing of the manuscript: CML, TL, LB (Berlin), FZ with help of all co-authors.

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**Competing interests** Dr LA Gerdes reports to have received travel expenses and personal compensation from Merck Serono, Teva Pharmaceutical Industries, Bayer Schering Pharma, Novartis, and Biogen Idec. Dr T Kumpfel reports to have received travel expenses and personal compensation from Bayer Schering Pharmacy, Teva, Merck-Serono, Novartis, Sanofi-Aventis and Biogen-Idec as well as grant support from Bayer-Schering AG. None of the other authors reports any disclosures.

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Complex traits


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