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SHORT REPORT

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

Christina M Lill,[1,2] Tian Liu,[2,3] Brit-Maren M Schjeide,[2] Johannes T Roehr,[2] Denis A Akkad,[4] Vincent Damotte,[5] Antonio Alcina,[6] Miguel A Ortiz,[7] Rafa Arroyo,[8] Aitzkoa Lopez de Lapuente,[9] Paul Blaschke,[10] Alexander Winkelmann,[10] Lisa-Ann Gerdes,[11] Felix Luessi,[1] Oscar Fernandez,[12] Guillermo Izquierdo,[13] Alfredo Antigüedad,[14] Sabine Hoffjan,[4] Isabelle Cournu-Rebeix,[5,15] Silvana Gromöller,[2] Hans Faber,[16] Maria Liebsch,[2] Esther Meissner,[2] Coralie Chanvillard,[17] Emmanuel Touze,[18] Fernando Pico,[19] Philippe Corcia,[20] ANZgene Consortium,† Thomas Dörner,[21] Elisabeth Steinhagen-Thiessen,[22] Lars Bäckman,[23] Hauke R Heekeren,[3,24] Shu-Chen Li,[3] Ulman Lindenberger,[3] Andrew Chan,[25] Hans-Peter Hartung,[26] Orhan Aktas,[26] Peter Lohse,[27] Tania Kümpfel,[11] Christian Kubisch,[28] Joerg T Epplen,[4] Uwe K Zettl,[10] Bertrand Fontaine,[5,15] Koen Vandenbroeck,[9,29] Fuencisla Matesanz,[6] Elena Urcelay,[7] Lars Bertram,[2] Frauke Zipp,[1]

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For numbered affiliations see end of article

Correspondence to
Professor Frauke Zipp, Rhine Main Neuroscience Network (rmn2), Johannes Gutenberg University Center Mainz, Neurology Department, Langenbeckstr. 1, Mainz 55131, Germany; frauke.zipp@unimedizin-mainz.de

†A list of all co-authors for the ANZgene consortium can be found at the end of this manuscript

LB and FZ contributed equally

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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.[1–3]). Despite this recent progress, it has been estimated that approximately 80% or more of the genetic variance remains unexplained by the currently known loci.[3] 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 *APOE* (encoding apolipoprotein E), 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 19q13 (eg, ref.[4]) as well as by its functional role in lipid transport, immunoregulation, neuroplasticity and repair mechanisms.[5] 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-1 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 *APOE* 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 *APOE* status, imputed raw genotype data from 8739 subjects from five independent GWAS datasets using the most recent high-resolution reference panels, and extracted genotype data for 8265 subjects from previous candidate gene assessments to arrive at an overall samples size of up to 13 913 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 *APOE* 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 *APOE* 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 χ^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 quality control filtering, IMSGC comprised 926 MS and 2319 control samples, GeneMSA 986 MS and 902 control samples (across three datasets from the US, Switzerland and the Netherlands), and ANZgene 1618 MS and 1988 control samples. Imputation of the 19q13 region including the uncovered SNPs rs429358 and rs7412 was based on '1000GP_hg19_Jun2011_PhaseI' data panels using the IMPUTE programme V.2.0 (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 6741 MS cases and 5999 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 ABI3730XL genetic analyser (Applied Biosystems). Raw sequences were analysed with SeqMan II V.8.0.2 (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 χ^2 as implemented in PLINK V.1.07 (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', V.1.4 and 'rmeta', V.2.16. All reported meta-analysis p values are two-tailed. Statistical significance was defined at a genome-wide 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 5831[6] and 7706 Caucasian subjects[7] by ~23 900 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: $I^2=35$, 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

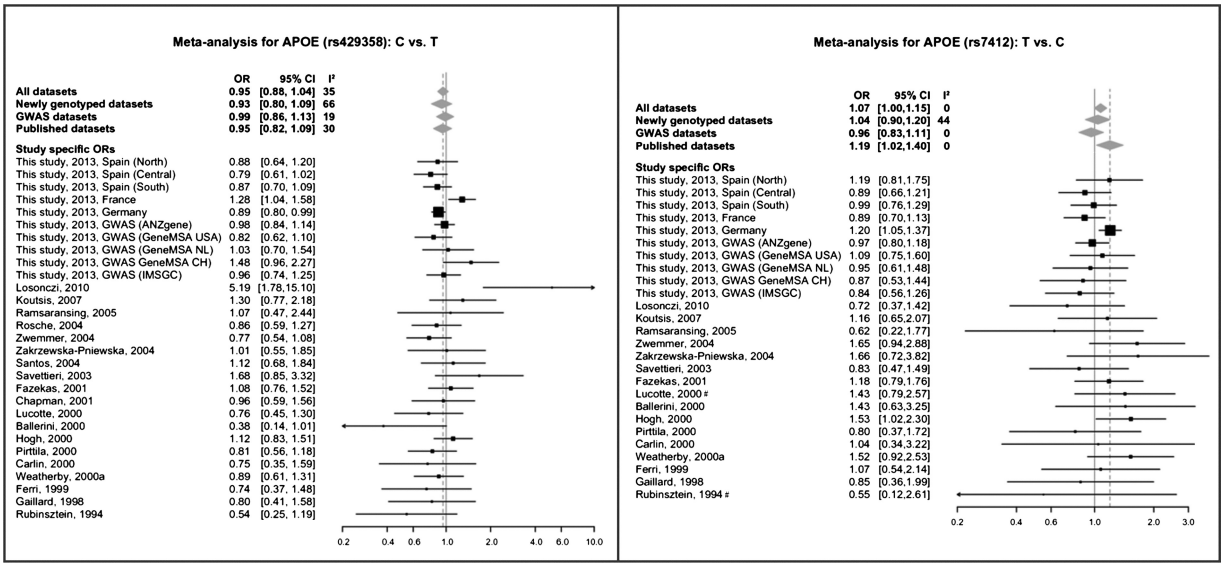

Figure 1 Forest plots of random effects meta-analyses of datasets assessing the association between *APOE* SNPs rs429358 and rs7412 and MS risk in populations of European descent. The x-axis depicts the OR. Study-specific ORs (black squares) and 95% CIs (CIs, lines) were calculated for each included 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 ($p<0.05$, labelled with '#') 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 supplementary 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.

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 exert smaller genetic effects than assumed here. For instance, the smallest effect size estimate to reach genome-wide significance in the recent MS GWAS[3] 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 population substructure, yielded results that were very similar in comparison to the meta-analysis results of the newly genotyped datasets. Similarly, due to the lack of access to individual-level genotype and covariate data, we were not able to adjust the association results from the published datasets for age at examination and sex. However, the results from other datasets of our study, where inclusion of age and sex as covariates in the association analysis was possible, do not suggest an appreciable effect of 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[1 2] and GWAS meta-analyses,[9 10] 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.

Author affiliations
[1]Department of Neurology, Focus Program Translational Neuroscience, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany
[2]Department of Vertebrate Genomics, Max Planck Institute for Molecular Genetics, Berlin, Germany
[3]Max Planck Institute for Human Development, Berlin, Germany
[4]Department of Human Genetics, Ruhr University, Bochum, Germany
[5]INSERM-CNRS-UPMC-ICM, UMR 975-7225, Institut du Cerveau et de la Moelle épinière

[6]Institution Parasitología y Biomedicina 'López Neyra', CSIC, Granada, Spain
[7]Inmunología, Hospital Clínico San Carlos, Instituto de Investigación Sanitaria San Carlos (IdISSC), Madrid, Spain
[8]Multiple Sclerosis Unit, Neurology Department, Hospital Clínico San Carlos, Instituto de Investigación Sanitaria San Carlos (IdISSC), Madrid, Spain
[9]Neurogenomiks Laboratory, Department of Neuroscience, University of the Basque Country UPV/EHU, Leioa, Spain
[10]Department of Neurology, University of Rostock, Rostock, Germany
[11]Institute for Clinical Neuroimmunology, Ludwig Maximilian University, Munich, Germany
[12]Instituto de Neurociencias Clinicas, Hospital Universitario Carlos Haya, Málaga, Spain
[13]Unidad de Esclerosis Múltiple, Hospital Virgen Macarena, Sevilla, Spain
[14]Servicio de Neurología, Hospital de Basurto, Bilbao, Spain
[15]Department of Neurology, Hopital Pitié-Salpêtrière, Paris, France
[16]Section of Neurology, Max Planck Institute for Psychiatry, Munich, Germany
[17]Experimental and Clinical Research Center, a joint cooperation between the Charité Medical Faculty and the Max-Delbrück Center for Molecular Medicine, Berlin, Germany
[18]Department of Neurology, Hôpital Sainte-Anne, Paris, France
[19]Department of Neurology, Centre Hospitalier de Versailles, Le Chesnay, France
[20]Department of Neurology, Centre Hospitalier Regional Universitaire, Tours, France
[21]Department of Medicine, Rheumatology, and Clinical Immunology, Charité University Medicine, Berlin, Germany
[22]Interdisciplinary Metabolic Center, Lipids Clinic, Charité University Medicine, Berlin, Germany
[23]Aging Research Center, Karolinska Institute, Stockholm, Sweden
[24]Department of Education and Psychology, Free University, Berlin, Germany
[25]Department of Neurology, St. Josef-Hospital, Ruhr-University, Bochum, Germany
[26]Department of Neurology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany
[27]Department of Clinical Chemistry, Ludwig Maximilian University, Munich, Germany
[28]Institute of Human Genetics, University of Ulm, Ulm, Germany
[29]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

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Collaborators Co-authors of the Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene) Melanie Bahlo[1], David R Booth[2], Simon A Broadley[3,4], Matthew A Brown[5], Brian L Browning[6], Sharon R Browning[7], Helmut Butzkueven[8,9], William M Carroll[10,11], Mathew B Cox[12,13], Caron Chapman[14], Glynnis Clarke[15], Patrick Danoy[5], Karen Drysdale[16], Judith Field[17], Simon J Foote[16], Judith M Greer[18], Lyn R Griffiths[19], Johanna Hadler[5], Cathy J Jensen[17], Laura J Johnson[17], Allan G Kermode[10,11], Robert N Heard[2], Trevor J Kilpatrick[17,20,21], Jeanette Lechner-Scott[12, 22], Mark Marriott[21], Deborah Mason[23], Pablo Moscato[12,13], Michael P Pender[24,25], Victoria M Perreau[20], Justin P Rubio[17], Rodney J Scott[12,13,22], Mark Slee[26], Jim Stankovich[18], Graeme J Stewart[2], Lotfi Tajouri[19], Bruce V Taylor[18], James Wiley[27], Ella J Wilkins[17]

(1) The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

(2) The Westmead Millenium Institute, Westmead, New South Wales, Australia

(3) School of Medicine, Griffith University, Queensland, Australia

(4) Department of Neurology, Gold Coast Hospital, Queensland, Australia

(5) The University of Queensland Diamantina Institute, Princess Alexandra Hospital, University of Queensland, Brisbane, Queensland, Australia

(6) Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, Washington, USA

(7) Department of Biostatistics, University of Washington, Seattle, Washington, USA

(8) Department of Medicine, Melbourne Brain Centre at the Royal Melbourne Hospital, University of Melbourne, Australia

(9) Department of Neurology, Box Hill Hospital, Monash University, Australia

(10) Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia

(11) Australian Neuromuscular Research Institute, Nedlands, West Australia, Australia

(12) Hunter Medical Research Institute, Newcastle, New South Wales, Australia

(13) Centre for Bioinformatics, Biomarker Discovery and Information-based Medicine, University of Newcastle, New South Wales, Australia

(14) Barwon Health, Geelong, Victoria, Australia

(15) Christchurch School of Medicine & Health Sciences, University of Otago, New Zealand

(16) Menzies Research Institute, University of Tasmania, Hobart, Tasmania, Australia

(17) The Howard Florey Institute, University of Melbourne, Victoria, Australia

(18) UQ Centre for Clinical Research, University of Queensland, Queensland, Australia

(19) Genomics Research Centre, Griffith University, Queensland, Australia

(20) Centre for Neuroscience, University of Melbourne, Victoria, Australia

(21) Royal Melbourne Hospital, Parkville, Victoria, Australia

(22) John Hunter Hospital, Hunter New England Health Service, Newcastle, New South Wales, Australia

(23) Canterbury District Health Board, Christchurch, New Zealand

(24) School of Medicine, University of Queensland, Queensland, Australia

(25) Department of Neurology, Royal Brisbane and Women's Hospital, Queensland, Australia

(26) School of Medicine, Department of Neurology, Flinders University, Bedford Park, Adelaide, South Australia, Australia

(27) Department of Medicine, Nepean Hospital, Penrith, New South Wales, Australia

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, L-AG, FL, OF, GI, AA (Bilbao), SH, IC-R, HF, CC, ET, FP, PC, ANZgene Consortium, TD, ES-T, LB (Stockholm), HRH, S-CL, UL, AC, H-PH, OA, PL, TK, CK, JTE, UKZ, BF, FM, FZ. Performed the experiments: CML, B-MMS, AA (Granada), MAO, 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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