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ORIGINAL ARTICLE

Genome-wide significant risk factors for Alzheimer's disease: role in progression to dementia due to Alzheimer's disease among subjects with mild cognitive impairment

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Few data are available concerning the role of risk markers for Alzheimer's disease (AD) in progression to AD dementia among subjects with mild cognitive impairment (MCI). We therefore investigated the role of well-known AD-associated single-nucleotide polymorphism (SNP) in the progression from MCI to AD dementia. Four independent MCI data sets were included in the analysis: (a) the German study on Aging, Cognition and Dementia in primary care patients ($n = 853$); (b) the German Dementia Competence Network ($n = 812$); (c) the Fundació ACE from Barcelona, Spain ($n = 1245$); and (d) the MCI data set of the Amsterdam Dementia Cohort ($n = 306$). The effects of single markers and combined polygenic scores were measured using Cox proportional hazards models and meta-analyses. The clusterin (*CLU*) locus was an independent genetic risk factor for MCI to AD progression (*CLU* rs9331888: hazard ratio (HR) = 1.187 (1.054–1.32); $P = 0.0035$). A polygenic score (PGS1) comprising nine established genome-wide AD risk loci predicted a small effect on the risk of MCI to AD progression in *APOE*-ε4 (apolipoprotein E-ε4) carriers (HR = 1.746 (1.029–2.965); $P = 0.038$). The novel AD loci reported by the International Genomics of Alzheimer's Project were not implicated in MCI to AD dementia progression. SNP-based polygenic risk scores comprising currently available AD genetic markers did not predict MCI to AD progression. We conclude that SNPs in *CLU* are potential markers for MCI to AD progression.

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INTRODUCTION

Alzheimer's disease (AD) is the most common form of neurodegenerative dementia, representing 50–60% of all dementia cases. AD pathology commences years, or even decades, before the appearance of clinical symptoms, and current consensus among scientists is that prevention should be started at an early phase in individuals at increased risk. Patients with mild cognitive impairment (MCI) are at increased risk of developing AD dementia. However, the MCI group is heterogeneous, and wide variation in the annual progression to AD dementia rate has been reported, with estimates ranging from 4 to 31%. In a recent study, which involved the follow-up of 550 MCI subjects for an average of 26.6 months,[1] the present authors found that the majority (45.5%) of those MCI individuals who subsequently developed dementia displayed the AD dementia phenotype. Thus, predicting which MCI cases will actually progress to AD dementia is an important challenge. Several clinical measures and biomarkers have been proposed for this purpose, including neuroimaging, cerebrospinal levels of amyloid-β and phosphorylated and total tau. However, the predictive value of these biomarkers is low.[2,3] Accordingly, research conducted in recent decades has tended to focus on identifying factors that render MCI patients more susceptible to AD dementia.[4] This research is important as the early detection of AD will be essential once an efficacious method of preventing or delaying the disease becomes available.

Individual risk for AD is determined by genetic, environmental and demographic factors, as well as interactions between them. The estimated genetic component of AD, that is, the so-called

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heritability, is as high as 79%. Hence in AD, the majority of pathophysiological pathways are likely to be driven by, or include, genetic determinants. Recent genome-wide association studies (GWAS) and whole-exam sequencing approaches have indeed identified several common and rare low-penetrance risk variants.[5–16]

Within routine clinical practice, the implementation and evaluation of AD risk markers in the prediction of MCI to AD dementia progression is in its inception. To date, the *APOE* (apolipoprotein E) locus is the only marker to have shown a consistent association with MCI to AD progression.[17] For other reported AD genetic markers, studies of MCI to AD dementia progression using single-nucleotide polymorphisms (SNPs), or combinations of SNPs in polygenic scores (PGS), have generated conflicting results.[18,19]

The aim of the present study was to investigate the role of established AD genetic markers in the progression of MCI to AD using follow-up data from four independent MCI data sets ($n = 3216$ subjects).

MATERIALS AND METHODS

Patients

The present cohort comprised MCI patients from Germany, Spain and the Netherlands. These individuals were drawn from the following cohorts: (a) the German study on Aging, Cognition and Dementia in primary care patients (AgeCoDe; $n = 853$);[20] (b) the German Dementia Competence Network (DCN; $n = 812$);[21] (c) the Fundació ACE from Barcelona (ACE, $n = 1245$);[1] and (d) the MCI data sets of the Amsterdam Dementia Cohort (ADC, $n = 306$).[22] Effective sample size varied depending on phenotype analyses and covariation matrices (Table 1). Clinical characteristics, neuropsychological assessment, behavioral and functional scales, and progression to AD dementia rates for each MCI data set are shown in Table 1, Supplementary Table 1 and at http://detritus.fundacioace.com/pgs. The study was approved by the respective ethics committees, and all participants provided written informed consent before inclusion.

DNA extraction, SNP selection and genotyping

DNA from 3216 MCI samples was extracted using commercial methods. SNP selection was based on a review of the literature. Here, only those SNPs in loci identified by GWAS or meta-GWAS efforts were selected. To avoid missing loci, for all of the loci selected for PGS construction, whenever possible, alternative SNPs in linkage disequilibrium were also selected (i.e. linkage disequilibrium proxies). This additional SNP thus served as a backup in the event that the primary selected SNP failed in the sequenom assay. Further details on the references used to select SNPs, the genotyping procedures and genotyping quality control are provided in Supplementary Table 2 and in the *Genotyping procedures* section of the Supplementary Data file. The sequenom technology genotyping methods are described elsewhere.[16]

Statistical analysis

To investigate the influence of genetic markers, demographic factors and PGS on MCI to AD dementia progression, methods from survival analysis were used. For the 40 individual SNPs and the three PGS of interest, hazard ratios (HRs) were calculated using the following three models: (i) crude (model 0); (ii) age- and gender-adjusted (model 1); and (iii) age-, gender-, *APOE*- and education-adjusted (model 2) (for details see Statistical Analysis in the Supplementary Data file). Unless otherwise specified, the subsequent text refers to model 1 only.

PGS construction and evaluation

PGS were calculated in accordance with Purcell *et al.*[23] (for details, see Polygenic Score Construction and Polygenic Score Evaluation in the Supplementary Data file). PGS were constructed using sets of AD-associated loci identified in recent GWAS. Inclusion of SNPs in the PGS was based on definitive evidence of association in large meta-GWAS reported by the International Genomics of Alzheimer's project (IGAP).[15] Since the established association between *APOE* ε4 and AD is also present in our four cohorts, the *APOE* region was excluded from the PGS calculation. PGS1 comprised the nine established AD-associated SNPs reported before publication of the IGAP consortium results (see Supplementary Table 3 and Part A in the Supplementary Data file). PGS2 comprised 9 of the 11 novel AD-associated SNPs identified by IGAP (Supplementary Table 3 and Part B in the Supplementary Data file).[15] PGS3 comprised all SNPs from PGS1 and 2. Each of the three calculated PGS was used as a dose, and the proportional hazards model was employed using the three models applied for the analysis of single SNPs. Meta-analysis techniques were used to estimate the global effects of SNPs and PGS. The meta-analysis was conducted using the standard fixed effect approach implemented in the YAMAS software. YAMAS implements standard fixed

Table 1. Effective sample size and baseline demographics in data sets

	AgeCoDe	*DCN*	*ACE*[a]	*ADC*
Subjects	853	812	1245	316
Detected duplicities	−1	−7	0	0
Detected non-MCI subjects	−46	0	0	0
No/low genotypes	−3	−6	0	−10
No follow-up data[b]	−299	−201	−74	0
No age/sex data	0	−1	0	0
Effective sample size (model 1)	504	597	1171	306
No *APOE*/education data	−4	−157	−1	−23
Effective sample size (model 2)	500	440	1170	283
AD converters (model 1)	209 (41.4%)	76 (12.7%)	395 (33.7%)	110 (35.9%)
AD converters (model 2)	207 (41.4%)	73 (16.5%)	395 (33.8%)	100 (35.3%)
Age (years) (mean)	81.6	66.2	76.0	66.8
Age (years) (s.d.)	4.1	8.9	7.1	7.8
Follow-up time (months) (mean)	43.0	26.9	26.0	27.7
Observational time (months) (s.d.)	25.4	11.2	18.9	17.7
Time to conversion (mean)	38.6	19.2	21.1	27.1
Time to conversion (s.d.)	22.6	8.5	16.0	17.4
Gender (%), female	69.4	43.7	64.6	38.9
APOE-ε4 (%)	25.8	34.2	32.4	52.3
Education % of high education (>3 points in harmonized scores)	13.6	5.6	8.1	15

Abbreviations: ACE, The Fundació ACE from Barcelona; AD, Alzheimer's disease; ADC, Amsterdam Dementia Cohort; AgeCoDe, German study on Aging, Cognition and Dementia in primary care patients; *APOE*, apolipoprotein E; DCN, German Dementia Competence Network; MCI, mild cognitive impairment. [a]ACE, $n = 1245$. [b]Subjects genotyped but without follow-up.

and random-effects meta-analysis, and operates on beta and standard error.[24]

RESULTS

Univariate analyses

The demographic characteristics of the cohorts are summarized in Table 1. The results obtained for each analyzed SNP are shown in Table 2.

In the meta-analysis, the *APOE*-ε4 allele (rs429358 C allele) showed an association with the rate of MCI to AD dementia progression in all cohorts, with a homogeneous effect being observed across data sets (HR = 1.84 (1.64–2.04), heterogeneity index (I^2) = 0, $P = 1.35 \times 10^{-27}$) (Figures 1a and 2a). Interestingly, the relative risk was ~50% of that reported in GWAS.[6–9] Furthermore, the ε4 effect increased with age, reaching its most pronounced effect between 65 and 80 years. In contrast, the *APOE*-ε2 allele conferred a protective effect against MCI to AD dementia progression to other *APOE* genotypes (Figure 1a). As with ε4, the effect of *APOE*-ε2 was dose dependent and homogeneous across data sets. The meta-analysis confirmed the protective effect of *APOE*-ε2 (HR = 0.69 (0.51–0.86), $I^2 = 0$, $P = 0.004$; Table 2). Six MCI subjects carrying the *APOE*-ε2 allele in a homozygous state did not progress to AD dementia during the observational time period.

An additional association signal was observed in SNPs at the *CLU* locus (rs9331888, rs11136000). For these variants, a nominally significant result was obtained in the AgeCoDe cohort, and a consistent trend towards association was observed in the DCN, ACE and ADC cohorts (Table 2). The meta-analysis yielded a significant association for both *CLU* SNPs ($P = 0.003$ and 0.01, respectively). Although rs11136000 showed a heterogeneous HR across the series, the HR for rs9331888 was homogeneous across the four cohorts (Table 2). Association findings for the *CLU* SNPs withstood all adjustments (Table 2, Figure 1b and Supplementary Data files). No major difference in the effect sizes of the *CLU* SNPs was observed following stratification for *APOE* status, gender or age (Table 4 and Figure 2b; $P > 0.71$). Stratification for these variables confirmed the orthogonality of *CLU* markers with key covariates.

Of the remaining SNPs genotyped in the present study, only rs641120 (located at the *SORL1* locus) showed nominal significance with MCI to AD dementia progression (HR = 0.89, $P = 0.043$, model 0). However, this finding did not withstand adjustment.

PGS in MCI to AD dementia progression

The results of the hazard models analysis of PGS are shown in Table 3. In the meta-analysis of PGS1, a trend towards association was observed (HR = 1.31, $P = 0.1$). Interestingly, stratification according to *APOE* genotype revealed a consistently higher effect size for PGS1 in *APOE*-ε4 carriers (Table 4). The meta-analysis of PGS1 showed that the effect in *APOE*-ε4 carriers was nominally significant (HR = 1.74 (1.03–2.97), $P = 0.04$). However, combined analysis revealed no statistically significant interaction between PGS1 and the *APOE* locus in any of the four data sets ($P = 0.14$). In contrast, PGS2 did not contribute to MCI to AD dementia progression. The effect size for PGS2 observed in the meta-analyses indicated a nonsignificant protective effect. This suggests that the accumulation of risk alleles was implicated in protection from MCI to AD dementia progression in the present series.

The analysis of PGS3 yielded an intermediate and nonsignificant result (HR = 1.03, $P = 0.96$). The PGS3 results reflect the findings of PGS1, as biased by the noise from PGS2. No significant interaction was found between PGS3 and age, gender, *APOE*-ε4 status or cohort (Tables 3 and 4).

DISCUSSION

For several years, intensive research has attempted to identify the role of genetic factors in the progression of MCI to AD dementia. To date, however, only the *APOE* locus has shown a consistent association. Elias-Sonnenschein *et al.*[17] performed a meta-analysis of 35 prospective MCI studies, which comprised a total of 6095 subjects. Of these, 1236 individuals progressed to AD dementia within a 2.9-year period of follow-up. For MCI subjects carrying the *APOE*-ε4 allele, the authors reported an odds ratio of 2.29 (1.88–2.80) for progression to AD dementia. The present findings support the hypothesis that the *APOE*-ε4 allele is implicated in MCI to AD dementia progression (HR = 2.20 (1.88–2.53) for subjects carrying *APOE-ε4* allele). However, we cannot exclude the possibility that additional loci around *APOE* may also modulate the age at onset for AD, as has been suggested for *TOMM40*, a gene adjacent to *APOE*.[25,26] Detailed mapping data of the linkage disequilibrium region around *APOE* are now available. These have identified a poly-T length polymorphism in an intron of *TOMM40*. Interestingly, research has demonstrated that the allele distribution of the poly-T polymorphism explains a larger proportion of the observed survival curves of age at onset in AD than is the case for *APOE*-ε4 containing haplotypes alone.[25,26] To confirm the role of *TOMM40* poly-T in AD progression, genotyping of this poly-T is currently being scheduled in our large MCI data set.

In the present study, the MCI to AD dementia progression rate increased continuously with age, whereas the effect of the allele *APOE*-ε4 on AD dementia progression decreased after the age of 80 years (Figure 2a). However, previous research has shown that both the incidence of AD and the AD risk effect of *APOE*-ε4 decrease in the elderly.[27,28] The observation of a reduced association between *APOE*-ε4 and MCI to AD dementia progression is consistent with the survivor effect, as *APOE*-ε4 is a risk factor for both a shorter lifespan and dementia.[29] A plausible hypothesis therefore is that most *APOE*-ε4-carrying MCI patients from the present cohorts had converted to dementia or died at an earlier age, thereby causing an enrichment of survivor *APOE*-ε4 MCI carriers among our elderly MCI subjects. This latter group is protected against the progression risk effect conferred by *APOE*-ε4, and this may have led to the observed reduction in the association between *APOE*-ε4 and progression to AD dementia in the present study. This hypothesis is supported by the fact that a reduced *APOE*-ε4 allele frequency was found within this age group compared with younger individuals (Figure 2a).

Besides *APOE*, no other SNP or PGS combination reached study-wise statistical significance (Bonferroni-corrected *P*-value = 0.00125). However, for some of these markers (i.e. SNPs contributing to PGS1), definitive evidence of association AD has been reported. Hence, the application of Bonferroni correction in this context could be considered overconservative, as our study was based on validated AD susceptibility loci.

The univariate analyses identified a consistent effect on MCI to AD dementia progression for two SNPs (rs11136000 and rs9331888) in the *CLU* gene ($P = 0.0035$). For both SNPs, a small but consistent effect was observed in all four series, as well as in the meta-analysis. The effect sizes and allele directions of both SNPs are consistent with those reported in previous AD case control GWAS.[7] Rodriguez-Rodriguez *et al.*[19] also obtained a significant result for rs11136000 allele T in MCI to AD dementia progression in a small data set. The effect size observed in the Rodriguez-Rodriguez series[9] was inflated compared with both the present data and previous results on the role of *CLU* markers in AD risk.[6,7,15] Nonetheless, the reported confidence interval overlaps with our evaluation. Therefore, the present *CLU* results represent an independent replication of a previous report, and have confirmed, in a much larger sample size, the involvement of *CLU* in MCI to AD dementia progression.

Table 2. Effect of candidate SNPs on conversion of mild cognitive impairment to Alzheimer's disease[a]

Gene	*SNP*	*Chr.*	*Position*	*Minor/major2 meta-analysis*				*AgeCoDe sample*				*DCN sample*			*ACE sample*			*ADC sample*		
				Allele	P-*value*	*HR*	σ*HR*	I^2	P-*value*	*HR*	σ*HR*	P-*value*	*HR*	σ*HR*	P-*value*	*HR*	σ*HR*	P-*value*	*HR*	σ*HR*
ABCA7	Rs3764650	19	1 046 520	G/T	0.2350	0.90	0.08	0.0	0.8280	0.96	0.17	0.3147	0.72	0.23	0.6034	0.94	0.11	0.2485	0.76	0.18
ABCA7	Rs3752246	19	1 056 492	G/C	0.2265	0.90	0.08	27.5	0.4360	0.90	0.12	0.0799	0.64	0.16	0.7572	1.03	0.10	0.1996	0.79	0.15
ADAMST20	Rs7295246	12	43 967 677	G/T	0.4310	1.04	0.05	0.0	0.8770	0.99	0.09	0.2523	1.20	0.19	0.7416	1.02	0.07	0.4502	1.11	0.15
BIN1	Rs7561528	2	127 889 637	A/G	0.5507	1.03	0.06	0.0	0.7590	0.97	0.10	0.1905	1.25	0.22	0.9193	1.01	0.09	0.4831	1.11	0.16
BIN1	Rs744373	2	127 894 615	C/T	0.4857	1.04	0.06	4.0	0.7340	1.04	0.11	0.1277	1.31	0.23	0.5729	0.95	0.09	0.3606	1.15	0.18
CASS4	Rs7274581	20	55 018 260	C/T	0.6657	0.96	0.08	0.0	0.9540	1.01	0.17	0.3610	1.27	0.33	0.4805	0.92	0.11	0.4553	0.82	0.21
CD2AP	Rs10948363	6	47 487 762	G/A	0.6454	0.97	0.06	0.0	0.5560	0.93	0.11	0.9713	0.99	0.18	0.7333	0.97	0.09	0.8185	1.04	0.16
CD33	Rs3865444	19	51 727 962	A/G	0.3575	1.05	0.06	0.0	0.4270	1.09	0.11	0.3488	1.17	0.19	0.9048	0.99	0.09	0.5796	1.08	0.15
CLU	Rs11136000	8	27 464 519	T/C	0.0111	0.87	0.05	0.0	0.0184	0.78	0.08	0.2912	0.84	0.14	0.1411	0.89	0.07	0.8962	1.02	0.15
CLU	Rs9331888	8	27 468 862	C/G	0.0035	1.19	0.07	0.0	0.1380	1.17	0.13	0.7383	1.06	0.18	0.0975	1.16	0.10	0.0210	1.41	0.21
CR1	Rs6656401	1	207 692 049	A/G	0.6741	0.95	0.11	58.1	0.6520	0.95	0.12	0.1433	0.72	0.16	0.0560	1.21	0.12	0.2749	0.82	0.15
CR1	Rs3818361	1	207 784 968	C/T	0.9333	0.99	0.08	19.3	0.6020	0.94	0.12	0.2388	0.78	0.16	0.1693	1.15	0.12	0.6728	0.93	0.16
ECHDC3	Rs7920721	10	11 720 308	G/A	0.4273	1.04	0.05	0.0	0.5920	1.06	0.11	0.4970	0.89	0.15	0.5159	1.05	0.08	0.4677	1.11	0.16
EPHA1	Rs10808026	7	143 099 133	A/C	0.7468	0.98	0.06	0.0	0.7650	0.97	0.11	0.9316	0.98	0.21	0.9940	1.00	0.10	0.7267	0.94	0.17
FERMT2	Rs17125944	14	53 400 629	C/T	0.3620	0.92	0.09	0.0	0.1420	0.77	0.14	0.9712	0.99	0.30	0.9970	1.00	0.15	0.7627	0.94	0.18
FRMD4A	Rs7081208	10	13 991 865	A/G	0.2568	0.90	0.09	53.2	0.3570	1.11	0.12	0.0276	0.65	0.13	0.3280	0.90	0.09	0.2491	0.84	0.12
FRMD4A	Rs17314229	10	14 016 159	T/C	0.7526	1.04	0.11	0.0	0.6330	0.91	0.18	0.9726	0.99	0.30	0.9779	1.00	0.18	0.1650	1.44	0.38
GAB2	Rs2373115	11	78 091 150	T/G	0.5383	0.96	0.07	0.0	0.4200	0.90	0.12	0.4543	0.84	0.20	0.8222	1.02	0.11	0.8089	0.96	0.17
HS3ST1	Rs6448799	4	11 630 049	T/C	0.5308	0.96	0.06	20.1	0.3880	1.10	0.12	0.3730	0.85	0.15	0.0933	0.88	0.07	0.6739	1.07	0.18
INPP5D	Rs35349669	2	234 068 476	T/C	0.9073	1.01	0.07	29.9	0.5260	1.06	0.10	0.2404	0.82	0.14	0.5088	0.95	0.07	0.1509	1.23	0.18
MEF2C	Rs190982	5	88 223 420	G/A	0.1918	1.10	0.08	44.2	0.6150	0.95	0.10	0.4999	1.12	0.19	0.0018	1.26	0.09	0.7074	1.06	0.15
MS4A	Rs4938933	11	60 034 429	C/T	0.3051	0.93	0.06	26.9	0.6660	1.04	0.11	0.6523	1.08	0.18	0.0230	0.83	0.07	0.4229	0.89	0.13
MTHFD1L	Rs11754661	6	151 207 078	A/G	0.8502	0.98	0.11	0.0	0.8420	1.05	0.24	0.9704	0.99	0.29	0.2979	0.84	0.14	0.2580	1.38	0.40
NDUFAF6	Rs7818382	8	96 054 000	T/C	0.1804	1.07	0.05	0.0	0.5010	1.07	0.11	0.5276	1.10	0.16	0.4346	1.06	0.07	0.5178	1.09	0.15
NME8	Rs2718058	7	37 841 534	G/A	0.3797	1.09	0.11	69.0	0.6790	0.96	0.09	0.0196	1.49	0.25	0.2620	0.92	0.07	0.0901	1.29	0.19
None	Rs6678275	1	193 625 233	C/G	0.9538	1.00	0.06	0.0	0.3890	1.11	0.14	0.9359	0.98	0.20	0.2446	0.90	0.09	0.4404	1.13	0.18
PICALM	Rs561655	11	85 800 279	G/A	0.3934	0.95	0.05	0.0	0.3840	0.91	0.10	0.4566	1.13	0.19	0.6238	0.96	0.08	0.4172	0.89	0.13
PICALM	Rs3851179	11	85 868 640	A/G	0.5097	0.96	0.05	0.0	0.8660	0.98	0.10	0.5659	1.10	0.19	0.6135	0.96	0.08	0.2728	0.85	0.13
PILRA	Rs2405442	7	99 971 313	A/G	0.6871	0.97	0.07	27.0	0.2180	0.87	0.10	0.1933	0.79	0.14	0.6401	1.04	0.08	0.3719	1.14	0.16
PILRA	Rs34995835	7	99 990 364	T/G	0.6823	0.98	0.06	0.0	0.3520	0.90	0.10	0.3331	0.84	0.15	0.7368	1.03	0.08	0.7445	1.05	0.16
PTK2B	Rs28834970	8	27 195 121	C/T	0.9757	1.00	0.06	8.6	0.8990	1.01	0.11	0.1338	0.77	0.13	0.9222	1.01	0.07	0.3142	1.16	0.17
SCIMP	Rs7225151	17	5 137 047	A/G	0.1055	1.13	0.08	0.0	0.9920	1.00	0.15	0.9477	0.98	0.25	0.0813	1.19	0.12	0.3362	1.23	0.27
SLC24A4	Rs10498633	14	92 926 952	T/G	0.3628	0.89	0.11	64.5	0.4030	1.10	0.13	0.1515	0.73	0.16	0.7479	1.03	0.10	0.0151	0.62	0.12
SORL1	Rs641120	11	121 380 965	T/C	0.0774	0.91	0.05	0.0	0.3140	0.90	0.09	0.9097	0.98	0.16	0.1913	0.90	0.07	0.4198	0.89	0.13
SORL1	Rs11218343	11	121 435 587	C/T	0.9564	0.99	0.17	37.0	0.2380	1.30	0.29	0.3904	1.31	0.41	0.2513	0.78	0.17	0.2515	0.64	0.25
SORL1	Rs2070045	11	121 448 090	G/T	0.7424	0.98	0.06	0.0	0.6830	0.95	0.11	0.9858	1.00	0.20	0.9881	1.00	0.09	0.7583	0.95	0.15
SPPL2A	Rs8035452	15	51 040 798	C/T	0.6115	0.97	0.06	27.1	0.7820	1.03	0.10	0.2125	1.23	0.20	0.1252	0.89	0.07	0.3613	0.87	0.13
TOMM40	Rs2075650	19	45 395 619	G/A	1.19e − 14	1.62	0.10	0.0	1.02e − 04	1.56	0.18	0.0032	1.67	0.29	1.53e −07	1.76	0.19	0.0022	1.49	0.19
TREML2	Rs9381040	6	41 154 650	T/C	0.7648	0.98	0.08	40.6	0.9700	1.00	0.11	0.0512	0.70	0.13	0.2735	1.09	0.08	0.7930	0.96	0.14
CWPW1	Rs1476679	7	100 004 446	C/T	0.7958	0.99	0.06	0.0	0.4000	0.91	0.10	0.4148	0.86	0.16	0.7532	1.03	0.08	0.6037	1.08	0.16

Abbreviations: ACE, the Fundacio ACE from Barcelona; ADC, Amsterdam Dementia Cohort; AgeCoDe, German study on Aging, Cognition and Dementia in primary care patients; Chr., chromosome; DCN, German Dementia Competence Network; HR, hazard ratio; σHR, hazard ratio standard deviation; I^2, heterogeneity index; SNP, single-nucleotide polymorphism. [a]HRs were calculated with univariate Cox proportional hazard model with adjustment for age and gender (model 1).

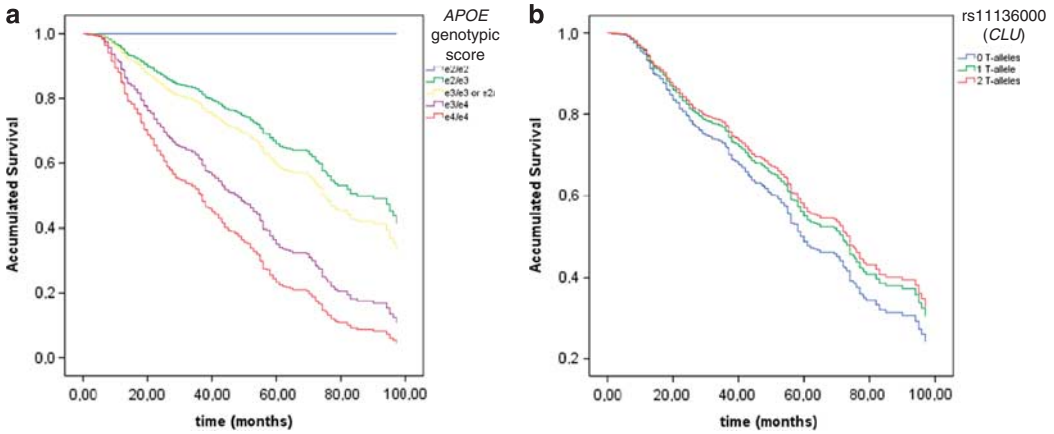

Figure 1. Cox proportional hazard model multivariate dementia-free survival analyses for *APOE* (apolipoprotein E) genotypic score (**a**) and clusterin (*CLU*) rs111360000 (**b**). Hazard ratio meta-analyses were adjusted according to data set, age and gender.

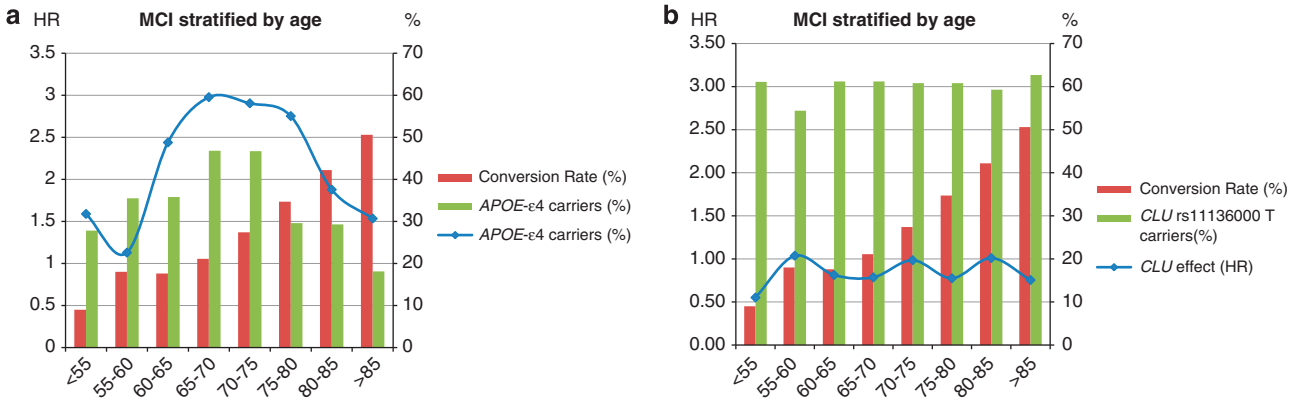

Figure 2. Effect size of the *APOE* (apolipoprotein E) (**a**) and clusterin (*CLU*) (**b**) loci in mild cognitive impairment (MCI) to Alzheimer's disease (AD) dementia progression following stratification for age. *Notes*: Meta-analysis of hazard ratio (HR) for progression to AD dementia in *APOE*-ε4 carriers following stratification for age. The progression rate for each age stratum is shown in the secondary Y2 axis.

Interestingly, other research has shown that the rate of cognitive decline among individuals who were cognitively normal at study baseline, but who subsequently developed MCI or AD, was significantly faster in those carrying the C allele of rs11136000 compared with non-carriers.[30] Furthermore, cognitively normal carriers of the risk allele C of rs11136000 have been reported to show a significant increase in regional cerebral blood flow in brain areas intrinsic to memory processes.[30] Overall, the genetic evidence supports the hypothesis that the *CLU* locus makes an independent contribution to MCI to AD dementia progression.

Along the same lines, the gene product of the *CLU* gene, clusterin/apolipoprotein J, has been proposed as a potential biomarker for AD. In this regard, the plasma concentration of apolipoprotein J has been associated with the severity and speed of disease progression in AD patients, as well as with atrophy of the entorhinal cortex and the hippocampus in AD.[31,32] In the prodromal stages of AD, for example, MCI elevated plasma levels of apoliprotein J have also been associated with lower rate of brain atrophy.[33] This atrophy involved the hippocampus and the entorhinal cortex, that is, brain regions affected in the early stages of AD pathogenesis. Together, these findings suggest that clusterin levels respond in a selective manner along the cascade of events occurring in AD, and that this commences during the prodromal stages. This protective plasma response may modulate, at least in part, the progression of MCI to AD dementia. Our data provide additional support for this hypothesis, as they demonstrate an association between genetic variability in *CLU* and MCI to AD dementia progression. Although the precise molecular mechanism through which genetic variability in *CLU* modulates plasma clusterin levels remains unclear, research suggests the potential involvement of genetic variability in *CLU* in the modulation of gene expression. Hence, *CLU* appears a promising therapeutic target for AD.

The lack of association for most of the investigated SNPs in the present study may suggest that AD susceptibility loci have only small effects in terms of MCI to AD dementia progression risk. If this is the case, the present MCI data sets would have limited statistical power to detect them. Another power-reducing factor may have been the inclusion in MCI subjects who will never develop AD dementia or who will convert to other unrelated forms of dementia. In support of this, the effect sizes of true AD susceptibility genes in the present MCI series were low compared with conventional AD case–control data sets (OR = 3.5 vs HR = 2.2 for *APOE*), and the progression rate for elderly MCI subjects was higher compared with that in the case–control context. An alternative explanation is that the relative risk in GWAS studies was obtained from analysis of progression from healthy control status to AD dementia. In this case, only part of this relative risk was examined in the present study, as our series comprised individuals who were already diagnosed with MCI, many of whom

Table 3. Effect of PGS on conversion from mild cognitive impairment to Alzheimer's disease[a]

PGS	*Meta-analysis*				*AgeCoDe sample*			*DCN sample*			*ACE sample*			*ADC sample*		
	P-*value*	*HR*	*95% CI*	I^2	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*
PGS1	0.139	1.29	(0.86; 1.72)	0.0	0.678	1.15	(0.57; 2.21)	0.504	0.70	(0.25; 1.99)	0.041	1.64	(1.02; 2.44)	0.92	1.05	(0.43; 2.56)
PGS2	0.669	0.89	(0.41; 1.37)	33.1	0.690	0.85	(0.40; 1.84)	0.100	0.34	(0.09; 1.23)	0.667	0.88	(0.47; 1.66)	0.201	2.02	(0.68; 6.00)
PGS3	0.625	1.18	(0.37; 2.00)	31.6	0.895	1.07	(0.40; 2.88)	0.130	0.27	(0.05; 1.46)	0.136	1.81	(0.83; 3.94)	0.489	1.66	(0.40; 6.92)

Abbreviations: ACE, the Fundació ACE from Barcelona; AgeCoDe, German study on Aging, Cognition, and Dementia in primary care patients; CI, confidence interval; DCN, German Dementia Competence; Network; HR, hazard ratio; I^2, heterogeneity index; PGS, polygenic score. [a]HRs were calculated in a univariate Cox proportional hazard model with adjustment for age and gender (model 1).

Table 4. Stratification analysis of candidate SNPs or PGS by the presence of *APOE*-ε4 allele

Marker or SNP polygenic score	*APOE-ε4 carriers HR (95% CI);* P-*value*	*APOE-ε4 non-carriers HR (95% CI);* P-*value*	*Overall HR (95% CI);* P-*value*
CLU rs9331888	1.206 (0.95–1.46); $P=0.081$	1.138 (0.96–1.32); $P=0.112$	1.187 (1.054–1.32); $P=0.0035$
PGS1	1.746 (1.029–2.965); $P=0.038$	1.026 (0.650–1.620); $P=0.912$	1.288 (0.86–1.72); $P=0.139$
PGS2 (new IGAP loci)	0.943 (0.496–1.790); $P=0.857$	0.790 (0.441–1.417); $P=0.428$	0.889 (0.41–1.37); $P=0.668$
PGS3 (all loci)	1.824 (0.805–4.132); $P=0.149$	0.903 (0.433–1.883); $P=0.785$	1.186 (0.37–2.00); $P=0.625$

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; CI, confidence interval; HR, hazard ratio; IGAP, International Genomics of Alzheimer's project; PGS, polygenic scores; SNP, single-nucleotide polymorphism. *Note*: Effect sizes were calculated using Cox proportional models adjusted by cohort, age and gender. *CLU* rs933188 effect size was calculated per each T allele. HRs for PGSs were calculated per each score point. PGS1 comprises nine genome-wide significant AD loci reported in advance of IGAP. PGS2 comprised nine confirmed loci reported by IGAP initiative (Lambert *et al.*[15]). PGS3 included 18 genome-wide loci for AD (PGS1+PGS2). For details on PGS construction see Supplementary Table S2 and Materials and methods.

had not yet converted to AD dementia, yet but would do so in the near future.[1] Hence, 'missing' relative risk in MCI studies may be found when analyzing progression of healthy individuals to MCI or by extending the period of follow-up. Alternatively, the lack of association observed for most SNPs in the present study may suggest that many genuine genetic risk factors for AD exert their pathological effects earlier, that is, during pathological processes that occur during the pre-MCI stages of the disease. This hypothesis would imply that differing genetic factors contribute to AD susceptibility as compared with AD progression. In fact, selecting intermediate phenotypes such as MCI, which are more proximal to a specific event along the causal chain of AD, may capture more variations in the underlying heritable traits and further enhance the statistical power of the study. Interestingly, a number of previous studies selected intermediate AD phenotypes for genetic association analyses, which included neuropsychiatric test measures,[34] magnetic resonance imaging data,[35,36] biomarkers from blood and cerebrospinal fluid[37,38] and direct measurements of AD pathology.[39] Most of the association signals identified by these studies do not overlap with known genetic susceptibility genes.

On the other hand, genetic studies based on longitudinal samples provide new insight into the pathways related to disease progression. A recent GWAS (based on ^{18}F-florbetapir PET) of time-dependent amyloid accumulation in AD implicated the microglial activation-associated gene, *IL1RAP*.[40] Furthermore, the authors also found that *APOE* and *CLU* affect amyloid accumulation, which is consistent with the known effects of these molecules on disease susceptibility. The *IL1RAP* gene was also associated with a greater likelihood of progression from MCI to AD dementia. Interestingly, the interleukin-1 proinflammatory pathway, to which *IL1RAP* belongs, is involved in plaque-associated activation of microglia and amyloid burden.[41] This inflammatory pathway is shared with *CLU* because clusterin modulates neuroinflammation by inhibiting the inflammatory response associated with complement activation.[42] In the case of *APOE*, research has linked the gene product, apoE, to innate inflammatory responses induced via the *TLR4* and interleukin-4 R-receptor pathways.[43] Furthermore, apoE and clusterin cooperate to regulate the clearance and deposition of amyloid-β in the brain.[44] Notably, clusterin and apoE promote the clearance of amyloid-β by interacting with several receptors located on microglia cells, including *TREM2*.[42,45,46] These findings, together with our own, suggest that immune responses and microglial clearance of amyloid-β have a role in disease progression from MCI to AD dementia. Interestingly, a recent study on AD identified a significant association with signals in SNPs located in genes involved in immune response pathways,[47] suggesting a partial overlap between disease progression pathways and those that increase susceptibility to AD.

Previous AD studies have investigated the predictive value of PGS constructed using the effects sizes of multiple SNPs. For example, Verhaaren *et al.*[48] constructed a genetic risk score (GRS) that was similar to the present PGS1. By using this GRS in 5171 non-dementia cases from Rotterdam, the authors demonstrated that although the GRS without *APOE* was associated with the development of AD ($P=0.010$), it provided only a marginal improvement in the prediction of AD dementia beyond that provided by age, sex and *APOE* status (area under the curve: 0.8159 vs 0.8148, respectively). Using a similar strategy, Rodriguez-Rodriguez *et al.*[19] used a GRS based on eight non-*APOE* genetic AD risk variants to study its effect on MCI to AD dementia progression, and on rapid progression from MCI to AD dementia. Although the authors observed no association between GRS and progression risk, they found that AD converters harboring six or more risk alleles progressed twice as rapidly to AD compared with individuals with less than six risk alleles. Thus, the present findings for PGS1 are consistent with these previous studies, and support the hypothesis that the first identified AD susceptibility locus has only a limited role in MCI to AD dementia progression. Interestingly, whereas PGS1 achieved nominal significance in *APOE*-ε4 carriers in the present study, this was not observed by Rodriguez-Rodriguez *et al.*[19] (Table 4). This may have been due to an enrichment of truly prodromal AD within *APOE*-ε4 carriers, who were therefore likely to progress to AD dementia within our

observational time. Nevertheless, this observation with PGS1 suggests that AD susceptibility genes other than *APOE* also contribute to disease progression. However, the predictive value of the PGS1 composite effect for diagnosis is too small to improve prediction, and this precludes its use in routine clinical practice.

The markers included in PGS1 are the best AD-associated SNP set reported to date, as—with the exception of *CD33*—all were reconfirmed in the large replication data set included in the IGAP effort. Many of the SNPs discovered by IGAP only reached GWAS significance during the last round of replication. Consequently, many of these SNPs still await an extensive independent replication effort to confirm genuine loci and remove false positives.[16] The existence of some false positives among the IGAP results cannot be excluded, and this would affect PGS results.

The present study had several limitations. First, the sample size may have been too small to detect certain associations. Unexpectedly, we observed a worsening of PGS risk prediction following the addition of the novel SNPs identified by the IGAP. In fact, a nonsignificant protective effect was observed for PGS2 in our meta-analyses ($P = 0.25$). A possible explanation for this finding is that novel loci included in PGS2 have even smaller effect sizes than the SNPs included in PGS1, in which case our sample would have been too small to detect association. Alternatively, the effect of the IGAP-SNPs may be restricted to very late or early onset AD, or to an undetermined and very specific subgroup of AD patients that was poorly represented in our MCI data sets. Second, only 9 of 11 novel risk loci found by IGAP were represented in PGS2 and PGS3. Unfortunately, the genotyping method failed for rs9271192 at *HLA-DRB5–HLA-DRB1*, and for rs10838725 at *CELF1*, and no additional backup SNPs were available for either locus. Thus, the conclusions drawn for PGS2 and PGS3 should be viewed with caution. Notwithstanding, the small effect sizes of the two markers are unlikely to have made a strong contribution to the overall effect of PGS2 or, more particularly, PGS3. Nevertheless, future efforts are necessary to investigate the potential implications of these missing markers (either by themselves or in combination with other loci) in terms of the progression of MCI to AD dementia.

In summary, the present data support the hypothesis that *CLU* has an independent role in MCI to AD progression. As in previous studies, the data also confirm the role of *APOE* in this process. Furthermore, our longitudinal data suggest that the genetic effect of AD risk factors on MCI progression may be age-dependent. Finally, our findings confirm the poor predictive value of the current genome-wide AD risk loci for MCI to AD dementia progression. Further studies in larger longitudinal MCI samples are now warranted to replicate these data, and to disentangle the genetic factors that influence the progression of MCI to AD dementia. Information on loci acting in the prodromal stages of AD, that is, in patients with MCI, will be of relevance for drug target selection in secondary prevention trials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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