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Using CSF biomarkers to replicate genetic associations in Alzheimer's disease

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Abstract

Defining cases and controls on the basis of biomarkers rather than clinical diagnosis may reduce sample sizes required for genetic studies. The aim of this study was to assess whether characterizing case/control status on the basis of cerebrospinal fluid (CSF) profile would increase power to replicate known genetic associations for Alzheimer's disease (AD). Independent of clinical diagnosis, Alzheimer's Disease Neuroimaging Initiative (ADNI) subjects with 2 CSF biomarkers for AD (Aβ1–42 $<$ 192 pg/mL and tau phosphorylated at threonine 181 (p-tau) $>$ 23 pg/mL, "CSF-positive") were compared with those without CSF evidence for AD (Aβ1–42 $>$ 192 pg/mL and 181-phosphorylated tau $<$ 23 pg/mL, "CSF-negative"). Minor allele frequency (MAF) and odds ratios (ORs) between these 2 groups were calculated for 7 single-nucleotide polymorphisms (SNPs) of interest. Two hundred thirty-two individuals were CSF-positive and 94 CSF-negative. There were no differences in age (74.7 $\pm$ 7.2 vs. 75.0 $\pm$ 6.5 years, $p = 0.7$), but significant differences in Mini Mental State Examination (MMSE) (25.9 $\pm$ 2.6 vs. 28.2 $\pm$ 1.7, $p < 0.001$) between the CSF-positive and CSF-negative groups. Significant differences in MAF ($p < 0.05$, uncorrected) were seen for *CR1* (rs1408077; OR, 1.59; 95% confidence interval [CI], 1.01–2.49), *PICALM* (rs541458; OR, 0.68, 95% CI, 0.47–0.98), *TOMM40* (rs2075650; OR, 4.30; 95% CI, 2.61–7.06); and possession of 1 or more *APOE* ε4 alleles (OR, 9.84; 95% CI, 5.48–17.67). These results suggest that using biomarkers of AD pathology to define case and control status may increase power in genetic association studies.

Keywords: Alzheimer's disease; Genome wide association studies; Cerebrospinal fluid

1. Introduction

Until recently, possession of an *APOE* ε4 allele was the only reliably reproducible genetic risk factor for sporadic Alzheimer's disease (AD). Several large genome wide association studies (GWAS) and confirmatory studies have recently demonstrated other risk loci, most notably *PICALM* (Corneveaux et al., 2010; Harold et al., 2009; Jun et al., 2010), *CR1* (Corneveaux et al., 2010; Jun et al., 2010; Lambert et al., 2009), and *CLU* (Corneveaux et al., 2010; Harold et al., 2009; Jun et al., 2010; Lambert et al., 2009). Others including *BIN1* have also been demonstrated in some studies (Biffi et al., 2010; Seshadri et al., 2010). While none of these genes exerts as great a risk as possessing an *APOE* ε4 allele, improved understanding of factors leading to the development of AD may provide insights into disease pathogenesis and allow for identification of novel therapeutic targets. Traditional GWAS require case/control comparisons of many hundreds of individuals. Such individuals are typically distinguished on clinical grounds, with at most a proportion having pathological confirmation of diagnosis (Carrasquillo et al., 2010; Corneveaux et al., 2010; Jun et al., 2010). Given that 30%–40% of individuals living to the tenth decade may develop AD, it is likely that a significant proportion of "healthy" controls have a genetic tendency to develop AD that has not manifested clinically. Similarly, even in the most experienced hands, a clinical diagnosis of AD is associated with a significant misdiagnosis rate. Cerebrospinal fluid measures of Aβ1–42, tau, and tau phos-

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phorylated at threonine 181 (p-tau) are emerging as important biomarkers for AD, and are beginning to be utilized as quantitative traits for GWAS (Cruchaga et al., 2010; Han et al., 2010; Kim et al., 2011). The aim of this study was to test the hypothesis that basing case/control distinctions on cerebrospinal fluid (CSF) findings rather than clinical diagnosis would improve the power to confirm existing GWAS findings.

2. Methods

2.1. Subjects

All subjects were drawn from the Alzheimer's Disease Neuroimaging Initiative (ADNI), a multicenter public/private funded longitudinal study investigating adult subjects with AD, amnestic mild cognitive impairment (MCI), and normal cognition. Participants undergo baseline and periodic clinical and neuropsychometric assessments and serial magnetic resonance imaging (MRI). Approximately 60% have CSF, and a subset positron-emission tomography (PET) imaging. Details are available at www.adni-info.org, with data downloadable from www.loni.ucla.edu/ADNI/. Written informed consent was obtained, as approved by the Institutional Review Board at each of the participating centers.

2.1. Cerebrospinal fluid (CSF)

Details of the CSF analysis and quality control measures have previously been published (Shaw et al., 2009). In brief, for all individuals with CSF available for analysis, measures of total tau, tau phosphorylated at threonine 181 (p-tau) and Aβ1–42 were performed centrally using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX, USA) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium) immunoassay kit-based reagents.

2.2. Genetics

Details of the genotyping methods have previously been described (Saykin et al., 2010). Individual-level genotype data including *APOE* genotype were downloaded from the UCLA Laboratory of Neuroimaging (LONI) ADNI database. Based on the results of prior GWAS analyses, data for 7 single-nucleotide polymorphisms (SNPs) of interest were extracted: rs3818361 and rs1408077 (*CR1*); rs11136000 (*CLU*); rs744373 (*BIN1*); rs3851179 and rs541458 (*PICALM*); and rs2075650 (*TOMM40*).

2.3. Statistical approach and patient selection

A previous CSF study from a group of patients with autopsy confirmed AD analyzed using identical methodology to that employed in ADNI showed that a CSF Aβ1–42 cut off of 192 pg/mL had 96% sensitivity and 77% specificity for distinguishing AD from controls; and that a CSF p-tau cut off of 23 pg/mL had 68% sensitivity and 73% specificity (Shaw et al., 2009). This entire cohort irrespective of diagnosis at baseline was separated into 3 groups: (1) those with both low CSF Aβ1–42 (<192 pg/mL) and high p-tau (>23 pg/mL) — "CSF positive"; (2) those with both high CSF Aβ1–42 (>192 pg/mL) and low p-tau (<23 pg/mL) — "CSF negative"; and (3) and those not fulfilling criteria for either "CSF positive" or "CSF negative".

To enrich the study into those cases, only the groups most likely to have AD pathology (CSF positive) and those least likely to have AD pathology (CSF negative) were included in the genetic analysis, with the remainder being excluded. For each of these 2 groups minor allele frequency for each SNP was established and odds ratios comparing the CSF positive and CSF negative groups were calculated. All analyses were performed in Stata 10 (StataCorp, TX, USA).

3. Results

A total of 412 subjects with CSF results were available for analysis. Of these, 114 were classified clinically as controls, 196 as MCI, and 102 as AD. On the basis of the predefined CSF cut offs, 232 individuals were classified as CSF-positive, 94 as CSF-negative, with the remaining 86 being excluded from the analysis (Fig. 1). Eighty-four of 102 (82.4%) of the total AD group, 125/196 (63.8%) of the total MCI group, and 23/114 (20.2%) of the control group were classified as CSF-positive; 4/102 (3.9%) of the total AD group, 38/196 (19.4%) of the total MCI group, and 52/114 (45.6%) of the total control group were classified as CSF-negative.

Demographic details of the groups classified as CSF-positive or CSF-negative and those excluded from the anal-

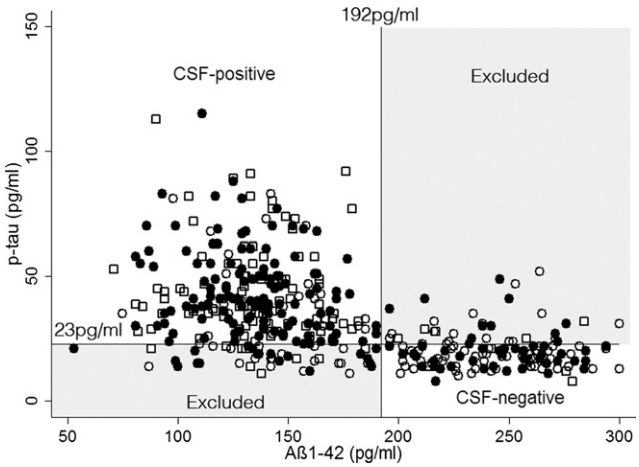

Figure 1. Baseline cerebrospinal fluid (CSF) Aβ1–42 is plotted against baseline CSF tau phosphorylated at threonine 181 (p-tau). Alzheimer's disease (AD) cut offs for Aβ1–42 (192 pg/mL) and p-tau (23 pg/mL) are shown. Individuals classified clinically as AD are shown as open squares; mild cognitive impairment (MCI) as filled circles; and controls as open circles. CSF positive individuals are those in the upper left quadrant; CSF negative individuals in the lower right quadrant; and the remainder — excluded from the analysis — in the shaded upper right and lower left quadrants.

ysis are shown in Supplementary Table 1. The CSF-positive group comprised 9.9% classified clinically as controls, 53.9% as MCI, and 36.2% as AD. The CSF-negative group comprised 55.3% classified clinically as controls, 40.4% as MCI, and 4.3% as AD. Comparing the CSF-positive and CSF-negative groups there were no significant differences in age (74.7 ± 7.2 vs. 75.0 ± 6.3 years, $p = 0.7$), but there were significant differences in Mini Mental State Examination (MMSE) (25.9 ± 2.6 vs. 28.2 ± 1.7, $p < 0.001$).

Minor allele frequencies and odds ratios for each SNP comparing the CSF-positive and CSF-negative groups are shown in Table 1, alongside previously reported odds ratios from case/control studies. Significant differences in minor allele frequency at the $p < 0.05$ level (uncorrected) were seen for *CR1* (rs1408077), *PICALM* (rs541458), *TOMM40* (rs2075650), and *APOE* E4. Alternative SNPs for *CR1* (rs3818361) and *PICALM* (rs3851179) showed directionally similar effects but failed to reach significance. For all SNPs tested bar rs744373, the direction of association was the same as has previously been reported in other GWAS studies.

4. Discussion

This study, assigning case or controls status on the basis of CSF biomarkers, provides further confirmatory evidence that *CR1*, *PICALM*, *TOMM40*, and *APOE* E4 are risk factors for the development of AD pathology. This was possible using just over 300 subjects, an order of magnitude fewer than used in traditional GWAS studies. These findings suggest that confirmatory or exploratory genetic analyses based on biomarker evidence of AD pathology may have increased power to detect case/control differences, and may therefore be possible using smaller sample sizes.

While due to the small sample size confidence intervals were large, the minor alleles of *CR1*, *PICALM*, *TOMM40,* and *APOE* E4 were associated with greater odds ratios than have previously been suggested in many other GWAS, significantly so in the case of *APOE* E4. Thus odds ratios were for *CR1* (rs1408077) 1.59, *PICALM* (rs541458) 0.68, *TOMM40* (rs2075650) 4.30, and *APOE* E4 vs. E3 8.32, with meta-analyses of previous studies reporting odds ratios of 1.13, 0.88, 2.79, and 3.68 respectively (Bertram et al., 2007). A previous confirmatory GWAS study using 740 of the ADNI cohort and employing a logistic regression model across clinical diagnosis groups reported significant, but smaller effects of *APOE* E4 (odds ratio [OR], 2.07) and *CR1* (rs1408077) (OR, 1.27), and no effect of *PICALM* (Biffi et al., 2010). These differences are likely to reflect the difficulties of relying on clinical diagnosis: in keeping with previous reports (De Meyer et al., 2010; Shaw et al., 2009) of all the controls available for analysis, ~20% would have been classified as CSF-positive; and ~19% of the MCI group and ~4% of the AD group as CSF-negative. Basing the analysis on patients with a CSF AD profile and those without, independent of clinical diagnosis, might explain the larger odds ratios; and while considerable caution is required given the small numbers in the study and the wide confidence intervals, this suggests that these haplotypes may confer larger risks of developing AD pathology than have previously been described.

Compared with results from formal GWAS, there was a directionally similar but nonsignificant association for *CLU*. This is likely to an issue of insufficient power. Based on case/control minor allele frequencies from the Alzgene meta-analysis, 232 cases and 94 controls would have 99% and 85% power (5% level) to detect differences in *APOE* ε4 and *TOMM40* respectively, but only 5%–7% power for *CLU*, *CR1*, *BIN1,* or *PICALM*. Based on these estimates, the chance of detecting significance for both *CR1* and *PICALM* in this sample is <1/400, providing further support for the hypothesis that better group separation may be achievable by basing diagnosis on disease biomarkers than clinical diagnosis.

Table 1
Associations of SNP minor alleles and *APOE*4 are shown, comparing CSF-positive and CSF-negative groups. Previously reported case/control meta-analysis results are shown for comparison

SNP	Gene	*p*	OR (95% CI)	CSF-positive		CSF-negative		Alzgene (Bertram et al., 2007), OR (95% CI)	Jun et al. (2010)[a], OR (95% CI)
				n =	With minor allele, %	*n* =	With minor allele, %		
rs3818361	*CR1*	0.12	1.41 (0.91–2.17)	232	23.1	94	17.6	1.14 (1.08–1.20)	1.14 (1.07–1.22)
rs1408077	*CR1*	0.04	1.59 (1.01–2.49)	225	22.4	94	15.4	1.13 (1.06–1.20)	1.14 (1.07–1.22)
rs11136000	*CLU*	0.87	0.97 (0.69–1.37)	232	39.2	94	39.9	0.88 (0.86–0.91)	0.91 (0.85–0.96)
rs744373	*BIN1*	0.36	0.87 (0.60–1.26)	229	28.6	92	31.5	1.15 (1.10–1.20)	—
rs3851179	*PICALM*	0.29	0.82 (0.58–1.18)	232	30.8	94	35.1	0.88 (0.85–0.91)	0.89 (0.84–0.94)
rs541458	*PICALM*	0.04	0.68 (0.47–0.98)	232	26.1	94	34.4	0.88 (0.85–0.91)	0.88 (0.83–0.93)
rs2075650	*TOMM40*	<0.001	4.30 (2.61–7.06)	232	33.8	94	10.6	2.79 (2.38–3.27)	—
	APOE E4 vs. no E4	<0.001	9.84 (5.48–17.67)	232	42.2	94	6.9	—	—
	APOE E4 vs. E3	<0.001	8.32 (4.61–15.01)	220	43.4	77	8.4	3.68 (3.30–4.11)	—

Key: CI, confidence interval; CSF, cerebrospinal fluid; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a] Unadjusted meta-analysis of 5935 cases and 7034 controls (includes 286 cases and 195 controls from ADNI).

There are a number of important caveats that need to be considered in relation to this study. Assigning case/control status neither on the basis of cognition nor on evidence of neurodegeneration means that the genetic risks identified can only truly be associated with the development of CSF signatures of AD and not of AD itself. Nonetheless, these findings which accord closely with previous literature, suggest that employing endophenotypic traits may be a useful means of providing confirmatory and exploratory GWAS studies in neurodegenerative diseases. The use of any CSF cut off is inevitably associated with a degree of inaccuracy, and standardization of CSF measurement is important if similar, predefined cut offs are to be used in other studies. This study is not a formal GWAS, but was designed to replicate known genetic risk factors as a proof-of-concept for the use of an enrichment strategy. As such, and to allow comparisons with other such studies and the Alzgene meta-analytic data, uncorrected p values are presented. Applying a strict Bonferonni correction results in an adjusted statistical significance level of $p = 0.00625$, at which level only the *TOMM40* and *APOE* genes remain significant. This is likely to reflect the much higher risk factor conferred by these 2 genes. Determination of genes with relatively small influences may however also aid in our understanding of the pathogenesis of neurodegenerative diseases, and while use of endophenotypes to enrich case/control studies may increase power to determine genetic associations, this does not negate the fact that large sample sizes will be required to determine small effects.

There is increasing realization that a substantial proportion of apparently normal older individuals may be in the prodromal stage of AD (Schott et al., 2010). Presuming these individuals are also likely to harbor risk variants, GWAS studies assuming that do not take this into account risk missing potential genetic associations, or underestimating the effects of identified genes. Using biomarkers to define cases and controls, or as quantitative traits, may increase the power of studies to detect genetic influences: indeed during the revision of this paper, a formal GWAS study based on the CSF data from the ADNI cohort was published (Kim et al., 2011). The findings reported here require replication in larger cohorts of patients with CSF, and in subjects stratified on the basis of other biomarkers including amyloid positron-emission tomography (PET) imaging.

Disclosure statement

The author reports no conflicts of interest.

The ADNI study underwent ethical review, and written informed consent was obtained for participation in these studies, as approved by the Institutional Review Board at each of the participating centers.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2011.02.008.

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Supplementary Table 1
Demographics of the CSF-positive, CSF-negative, and excluded groups

	CSF-positive (Aβ1–42 < 192 + p-tau > 23)	CSF-negative (Aβ1–42 > 192 + p-tau < 23)	Excluded (neither fulfilling criteria for CSF-positive or CSF-negative)
n	232	94	86
Age, mean years (95% CI)	74.7 (73.9–75.7)	75.0 (73.7–76.3)	75.7 (74.1–77.2)
Male, %	59.5	60.6	61.6
MMSE, mean score (95% CI)	25.9 (25.5–26.2)	28.2 (27.9–28.6)	27.2 (26.6–27.7)
Aβ1–42, mean pg/mL (95% CI)	134.7 (131.4–138.0)	244.9 (239.5–250.3)	182.5 (170.4–194.6) (n = 85)
p-tau, mean pg/mL (95% CI)	45.2 (43.0–47.5)	16.7 (16.0–17.5)	23.3 (21.4–25.1)
Total tau, mean pg/mL (95% CI)	127.2 (120.0–134.8) (n = 229)	55.8 (52.6–59.1) (n = 93)	68.4 (62.3–74.5) (n = 84)
Clinical diagnosis per CSF-group, *n* (group %)			
Control	23 (9.9)	52 (55.3)	39 (45.4)
MCI	125 (53.9)	38 (40.4)	33 (38.4)
AD	84 (36.2)	4 (4.3)	14 (16.3)

Key: AD, Alzheimer's disease; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; p-tau, tau phosphorylated at threonine 181.