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Complement receptor 1 coding variant p.Ser1610Thr in Alzheimer's disease and related endophenotypes

Caroline Van Cauwenberghe [a,b], Karolien Bettens [a,b], Sebastiaan Engelborghs [b,c], Mathieu Vandenbulcke [d,e], Jasper Van Dongen [a,b], Steven Vermeulen [a,b], Rik Vandenberghe [e,f], Peter P. De Deyn [b,c,g], Christine Van Broeckhoven [a,b], Kristel Sleegers [a,b,*]

[a] Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB, Antwerp, Belgium
[b] Institute Born-Bunge, University of Antwerp, Antwerp, Belgium
[c] Department of Neurology and Memory Clinic, Hospital Network Antwerp, Middelheim and Hoge Beuken, Antwerp, Belgium
[d] Department of Psychiatry and Memory Clinic, University of Leuven, Leuven, Belgium
[e] University Hospitals Leuven Gasthuisberg, Leuven, Belgium
[f] Laboratory for Cognitive Neurology, Department of Neurology, University of Leuven, Leuven, Belgium
[g] Department of Neurology and Alzheimer Research Center, University Medical Center, Groningen, the Netherlands

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ABSTRACT

We previously described an intragenic functional copy number variation (CNV) in complement receptor 1 (*CR1*) that is associated with Alzheimer disease (AD) risk. A recent study, however, reported a rare *CR1* coding variant p.Ser1610Thr (rs4844609) associated with AD susceptibility, explaining the effect of genome wide association (GWA) top single nucleotide polymorphism rs6656401. We assessed the role of the Ser1610Thr variant in AD pathogenesis and the effect on AD-related endophenotypes in a Flanders-Belgian cohort. We evaluated whether this rare variant rather than the *CR1* CNV could explain the association of *CR1* in our population. The Ser1610Thr variant was not associated with AD, memory impairment, total tau, amyloid β_{1-42} or tau phosphorylated at threonine 181 levels. It did not explain (part of) the association of genome wide association top single-nucleotide polymorphisms rs3818361/rs6656401, nor of the *CR1* CNV, with AD in our cohort, whereas the *CR1* CNV and rs3818361/rs6656401 represented the same association signal. These findings question a role for the Ser1610Thr variant in AD risk and related endophenotypes, and reaffirm our previous observation that the *CR1* CNV could be the true functional risk factor explaining the association between *CR1* and AD.

1. Introduction

Genome wide association (GWA) studies have led to considerable progress in understanding the complex genetics of Alzheimer's disease (AD), by identifying at least 9 novel risk loci (Carrasquillo et al., 2010; Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Naj et al., 2011; Seshadri et al., 2010). In the wake of these studies, the next challenge lies in determining which genetic variants at these loci truly affect disease susceptibility. Identification of the true susceptibility alleles will be key in understanding the molecular mechanisms through which risk genes are involved in AD pathogenesis. Moreover, epidemiological estimates of novel risk genes such as population attributable fraction, and genetic risk profiling become more accurate when the true underlying genetic risk factors are known. Molecular reclassification of AD, which is an etiologically heterogeneous disorder, will improve (early) diagnosis, streamline clinical trials in drug development, and aid future clinical decision-making.

For 1 of the GWA risk genes for AD, complement receptor 1 (*CR1*), we previously described an intragenic copy number variation (CNV) that could explain the GWA signal (Brouwers et al., 2012). This CNV translates into 2 major isoforms of the complement receptor, CR1-F and CR1-S, because of duplication of a low copy repeat (LCR). The CNV has functional repercussions, because it results in a variable number of C3b/C4b and cofactor activity binding sites at the receptor, which are important in the complement cascade (Brouwers et al., 2012; Khera and Das, 2009; Krych-Goldberg and Atkinson, 2001), but might also have more direct implications for AD. Fibrillar amyloid beta (Aβ) peptides are known to activate the complement cascade and become bound to C3b. CR1 induces clearance or phagocytosis of these C3b-opsonized particles

* Corresponding author at: Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB, University of Antwerp - CDE, Universiteitsplein 1, B-2610, Antwerp, Belgium. Tel.: +32 32651032; fax: +32 32651112.
E-mail address: kristel.sleegers@molgen.vib-ua.be (K. Sleegers).

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(Bradt et al., 1998; Rogers et al., 1992, 2006; Webster et al., 1997). The mode of action remains unclear, but the first exploration in brain samples suggests that the CR1-S isoform is expressed at lower protein levels than CR1-F and therefore likely linked with increased complement activation. Additionally, both isoforms show a different pattern of CR1 distribution in neurons, which could indicate that CR1-S and CR1-F isoforms are differentially processed in neurons (Hazrati et al., 2012).

Keenan and colleagues, however, described a rare coding variant rs4844609 in *CR1*, located outside the CNV region, resulting in a serine to threonine change at position 1610 (p.Ser1610Thr) with a minor allele frequency (MAF) of 0.02. This coding variant was associated with AD susceptibility, a composite score of episodic memory decline, and increased neuritic pathology in a study cohort primarily consisting of non-Hispanic Caucasian individuals. Moreover, their results suggested that this rare variant could account for the effect of the index GWA single nucleotide polymorphism (SNP) rs6656401 on episodic memory decline (Keenan et al., 2012).

In this study we assessed the association of the *CR1* Ser1610Thr variant with AD in an extended Flanders-Belgian AD study cohort, part of which was previously included in the replication stage of GWA studies (Lambert et al., 2009), and showed a trend toward association for *CR1* top SNP rs6656401 (odds ratio [OR], 1.24; 95% confidence interval [CI], 0.99–1.24; $p = 5.6 \times 10^{-2}$) (rs3818361 (OR, 1.05; 95% CI, 0.84–1.48; $p = 6.8 \times 10^{-1}$)). Further, we assessed the association of the Ser1610Thr variant with memory impairment and cerebrospinal fluid (CSF) biomarker levels. We evaluated whether the Ser1610Thr coding variant rather than the intragenic *CR1* CNV could account for the known effect of the GWA SNPs on AD.

2. Methods

2.1. Study cohort

The Flanders-Belgian study cohort consisted of 1276 AD patients (mean age at onset 74.4 ± 8.8 years, 65.7% women) and 1128 healthy control individuals (mean age at inclusion 65.0 ± 13.7 years, 56.1% women).

Most of the AD cohort was ascertained at the Memory Clinic of the ZNA Middelheim and Hoge Beuken, Antwerp, Belgium (PPDD and SE) as part of a prospective study of neurodegenerative and vascular dementia in Flanders, the Dutch-speaking region of Belgium (Engelborghs et al., 2003, 2006). Consensus diagnosis of possible and probable AD was given by at least 2 neurologists based on the National Institute of Neurological and Communication Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria (McKhann et al., 1984). Another subset of patients was collected at the Memory Clinic of the University Hospitals of Leuven, Gasthuisberg, Leuven, Belgium (RV and MV) as part of a prospective study on the molecular genetics of cognitive impairment using the same clinical assessments and biosampling schemes (Bettens et al., 2010). Each AD patient underwent a neuropsychological examination and structural and/or functional neuroimaging (Bettens et al., 2010). For a subset of patients ($n = 298$; mean age at onset 79.9 ± 7.5 years, 62.4% women), CSF levels of Aβ peptide ($A\beta_{1-42}$), total tau (T-tau), and tau phosphorylated at threonine 181 (P-tau_{181P}) were available as part of the diagnostic work-up, determined with commercially available single parameter enzyme-linked immunosorbent assay kits (Innogenetics, Ghent, Belgium). For 94 patients, autopsy confirmed a definite diagnosis of AD.

The control cohort consisted of unrelated individuals, without neurological or psychiatric antecedents or neurological complaints and without organic disease involving the central nervous system, examined at the Memory Clinic of ZNA Middelheim and Hoge Beuken, Antwerp, Belgium, and the memory clinic at the University Hospitals of Leuven, Gasthuisberg, Leuven, Belgium. Additional community control individuals were included after interview concerning medical and family history.

Memory impairment was assessed in all AD patients and control individuals using the Mini Mental State Examination (MMSE) (control cohort MMSE score >24) (Folstein et al., 1975). Different cognitive domains (orientation, recall, attention, concentration, memory, and language) were examined and a composite score has been assigned as MMSE value.

2.2. Genotyping

Rs3818361 was selected for genotyping in our cohort based on significance in a previous meta-analysis study (Hollingworth et al., 2011). SNP genotyping of the Ser1610Thr variant and rs3818361 was performed using MassARRAY using iPLEX Gold chemistry (Sequenom, Hamburg, Germany), followed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry. Polymerase chain reaction and extension primers were designed using MassARRAY Assay Design software v3.0.2.0 (Sequenom). Genotypes were called automatically using MassARRAY Typer software v4.0 (Sequenom) and were visually inspected by 2 researchers blinded to disease status. The genotype success rate was 96.3% for Ser1610Thr and 98.7% for rs3818361. Rs6656401 genotype data were available on a subset of 1052 patients and 469 control subjects because genotyping (with a success rate of 98.3%) had been performed as part of the GWA replication study (Lambert et al., 2009). *APOE* ε4 genotype assay and *CR1* CNV data were previously described (Brouwers et al., 2006, 2012).

2.3. Statistical analysis

Genotype frequencies of the Ser1610Thr variant and rs3818361 were compared between AD patients and healthy control individuals using χ^2 statistics. To determine the effect of the Ser1610Thr variant and rs3818361 on AD risk, ORs (and 95% CI) were calculated in a logistic regression model, adjusted for sex, age at onset (age at inclusion for control individuals), and *APOE* ε4 status.

Deviations from Hardy–Weinberg equilibrium of the genotyped variants Ser1610Thr and rs3818361 were assessed using an exact Hardy–Weinberg equilibrium test (www.pharmgat.org/IIPGA2/Bioinformatics/exacthweform).

The effect of the Ser1610Thr variant on AD-related endophenotypes, including memory impairment and CSF biomarkers, was assessed in a univariate analysis of variance. MMSE data were used to assess memory impairment; disease duration, and age at time of MMSE testing were retained in the model as covariates. Possible covariates affecting CSF biomarker levels (age and disease duration at time of lumbar puncture, *APOE* ε4 genotype, and sex) were evaluated and retained in the analysis. For $A\beta_{1-42}$, disease duration at time of lumbar puncture and *APOE* ε4 status were retained in the analysis of variance; for T-tau, age at time of lumbar puncture, and for P-tau_{181P} *APOE* ε4 status significantly affected the model. T-tau and P-tau_{181P} were $\log_{10}$ transformed to approximate normality before analysis of variance.

Conditional logistic regression was used to assess which variations and/or *CR1* CNV best captured the association identified in the GWA study. ORs (and 95% CI) were calculated in a logistic regression model, adjusted for sex, age at onset (age at inclusion for control individuals), and *APOE* ε4 status.

The full study population had 98% power, assuming an additive model of association and the effect size of the discovery cohort reported by Keenan and colleagues, to detect association with a rare variant with MAF of 0.02 (Keenan et al., 2012; Purcell et al., 2003). The subsets for whom data on MMSE or CSF biomarker levels