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Published

2008

Journal Title

Human Molecular Genetics

DOI

10.1093/hmg/ddn183

Association study of the *NEDD9* gene with the risk of developing Alzheimer's and Parkinson's disease

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ABSTRACT

Alzheimer's disease (AD) and Parkinson's disease (PD), the two most common neurodegenerative disorders in the elderly, have been hypothesized to share genetic determinants. Recently, Li *et al*. proposed that a variant in the *NEDD9* gene may be one of these common genetic factors. We attempted to confirm this initial observation by conducting an equivalent analysis in terms of pathologies and sample size. We genotyped the *NEDD9* rs760678 SNP in three independent AD case-control studies (*N*=3,176) and two independent PD cases-controls studies (*N*=1,855). However, we failed to detect an association of this SNP with the risk of developing AD or PD, in any of these populations. In conclusion, these data indicate that the rs760678 SNP of the *NEDD9* gene is at best a weak genetic determinant of AD or PD.

INTRODUCTION

Alzheimer's disease (AD) and Parkinson's disease (PD) are the main causes of neurodegenerative disorders in the elderly. Both pathologies are multifactorial resulting from the complex interactions between environmental and genetics factors. AD shows strong evidence for genes contributing to disease susceptibility (between 60-80%) (1) whereas the genetic component of PD appears less important (between 20-30%) (2). Interestingly, familial aggregation of these two disorders has been observed, suggesting a common genetic cause (3,4). This hypothesis has been reinforced by the observation that similar clinical and pathological characteristics can be found in AD and PD patients. For instance, some PD patients develop dementia and some AD patients have Lewy bodies (5,6).

The characterisation of common causative genetic factors may be useful to better define physiopathological processes in both diseases. To date, several genes or loci of interest have been proposed. The ε4 allele of the apoliporotein E (APOE) gene is a major genetic risk factor in AD has been associated with an increased risk of developing PD in some studies. However, this observation was suspected to potentially result from an increase in dementia in PD cases bearing the ε4 allele (7). As yet unknown genes in common to AD and PD may lie on chromosomes 6 and 10. Loci in these chromosomes were reported to contain gene(s) that modify age at onset of AD and PD (8). One gene on chromosome 10, the glutathione S-transferase, omega-1, received particular attention but contradictory findings in these diseases were reported (9-11).

Recently, the *NEDD9* (Neural precursor cell Expressed, Developmentally Down-regulated) gene has been reported as a potential common candidate gene for both AD and PD (12). From 4,692 putative functional SNPs in 3,664 genes, the authors focused on the rs760678 SNP located in a region containing clusters of TATA- and GATA- binding motifs within *NEDD9* gene. They found that the major CC genotype was associated with an increased risk of developing AD and PD in their cohorts (respectively, OR=1.38 [1.20-1.59] and OR=1.31 [1.05-1.62]). Since *NEDD9* is involved in the formation of neurite-like membrane extensions and neurite outgrowth (13,14), the authors suggested that a differential expression of *NEDD9*, may affect the reservoir of neurons and synapses in the brain and influence neuronal degeneration under stressful conditions.

We attempted to replicate the association of the rs760678 SNP with the risk of developing both AD and PD. We developed an comparable analysis to the initial report in terms of pathologies and sample size using three independent AD case-control studies (*N*=3,176) and two independent PD cases-controls studies (*N*=1,855).

RESULTS

The association between the rs760678 SNP and the risk of developing AD or PD was assessed in three AD and two independent PD case-control studies (Table 2). The genotype distributions were in Hardy-Weinberg equilibrium in all the controls and cases populations.

In contrast to the initial report (12), we did not observe an association of the rs760678 SNP with AD or PD risk in any of our populations. No deleterious impact of the rs760678 CC genotype was found on the risk of developing AD or PD (respectively, OR=0.92, p=0.26; OR=1.00; p=0.99, in the combined populations adjusted for center, Table 3). These results were furthermore similar when adjusted for age and gender for each population studied and independent of the *APOE* status in AD (data not shown). Finally, no association between the rs760678 SNP and age at onset of AD or PD was observed (data not shown).

We next combined Li et al's data with our own (Figure 1). The rs760678 CC genotype was still weakly associated with AD (OR=1.14, 95% CI [1.03-1.26], p=0.01) but not with PD (OR=1.10, 95% CI [0.97-1.27], p=0.13).

DISCUSSION

Based on analyses of 4,692 putative functional SNPs in 3,664 genes, Li *et al*. reported that the *NEDD9* gene may be a new candidate genetic risk factor for AD and PD (12). The CC genotype of the rs760678 SNP within this gene was associated with an increased risk of developing AD (N=3,521; OR=1.38 $p=5.4x10^{-6}$) and PD (N=1,464; OR=1.31 p=0.01). To further explore this observation, we assessed the association of this SNP with AD in three independent cases-controls populations (N=3,176) and with PD in two independent cases-controls populations (N=1,855). The combined cohorts had 99% and 82% power to detect OR of 1.38 and 1.31 for AD and PD respectively (assuming an α level of 0.05). Conversely to the initial report of Li *et al*., we were unable to detect an association even after combination of cohorts to generate an equivalent numbers of cases and controls in AD and PD studies (respectively, OR=0.92, p=0.26 and OR=1.00, p=0.99, in the combined populations).

Even if it has been argued that European population stratification does not represent a significant source of bias in epidemiological studies, recent SNP studies have highlighted significant patterns of structure within Europe along a north-south axis (15). The analyses showed consistent clustering of the Mediterranean populations from other European populations which appear to be more similar (15). Since we mainly studied populations from the North of France and the UK, the genetic structures of the populations analyzed in ours and Li et al's reports are likely to be similar. However, we cannot exclude that a slight variation in LD could affect the levels of associations observed in the different independent populations. The discrepancy between ours and Li et al's findings maybe due to the presence of a different polymorphism being responsible for risk that is in linkage disequilibrium (LD) with the rs760678 variant. However, Li et al. have already explored this possibility to some degree by examining the LD profile in the HapMap data set (www.hapmap.org) upstream and down stream of rs760678, a region encompassing over a 500 kb region. Only seven SNPs, over 20.7 kb, share $r^2 > 0.1$ with rs760678. Supplementary analyses performed by Li *et al*. indicated that the rs760678 SNP alone explained the observed associations in their different populations (12).

In this report, we restricted our analysis to the rs760678 SNPs because a repeat association with precisely the same variant in independent samples is the gold standard for the replication approach (16). Our data do not support the claim that the rs760678 SNP in the *NEDD9* gene is a genetic determinant of AD or PD. However, it is important to note that only SNPs referenced in the HapMap data set were analysed in the initial study. It is still possible that unknown SNPs potentially in LD with the rs760678 variant exist. Li *et al*. sequenced all the *NEDD9* gene exons in 40 LOAD cases and only characterized one rare non-synonymous variant (12). However, it is well established that cell-type specific regulatory transcriptional sequences may be located within introns (17,18) and in remote sequences. Only systematic and ambitious efforts in sequencing and genotyping (even rare variants) in combination with replication in large independent populations will help determine whether the *NEDD9* gene is a genetic determinant of AD and PD or not.

MATERIALS AND METHODS

Subjects

The main characteristics of the different populations are described in Table 1. Written informed consent for participation was given by all subjects, or a caregiver, legal guardian, or other proxy where patients had substantial cognitive impairment. The study protocols for all populations were reviewed and approved by the appropriate institutional review boards of each country.

Alzheimer case-control populations.

LILLE case-control study (19). All samples were Caucasian from the north of France (AD cases n=770, controls n=659). Clinical diagnosis of probable AD was established according to the DSM-III-R and NINCDS-ADRDA criteria. Caucasian controls were defined as subjects without DMS-III-R dementia criteria and with integrity of their cognitive functions (MMS>25). Presence of family history of dementia was considered as a criterion of exclusion. Controls were recruited in retirement homes or from electoral rolls (altruistic volunteers).

ROUEN case-control study (19). All subjects were Caucasian from the West of France. (AD cases n=739, controls n=691). Clinical diagnosis of probable AD was established according to the DSM-III-R and NINCDS-ADRDA criteria. Control subjects (mainly spouses of patients) were required to have a MMSE score above 28.

BIRMINGHAM case-control study (19). All samples were Caucasian from Greater Birmingham (AD cases n=416, controls n=167). Clinical diagnosis of probable AD was established according to the DSM-III-R and NINCDS-ADRDA criteria. Control subjects were assessed using either DSM-III-R questionnaire or had a MMSE score above 28.

The APOE distribution of all the AD case-control study are indicated in Table S1 (see supplementary material).

Parkinson case-control populations.

AUSTARLIAN case-control study (20). Prevalent PD cases (n=722) were recruited at the Movement Disorders clinic at Princess Alexandra Hospital. The diagnosis of probable or definite PD was made when the subject had a combination of three of the following features: resting tremor, rigidity, bradykinesia, postural instability; or two of these features with asymmetry in tremor, rigidity or bradykinesia. All subjects were examined by a Movement Disorders Neurologist (n=462 controls).

TERRE case-control study (20). Participants were recruited through a French health insurance organisation, the Mutualité Sociale Agricole (MSA), which is responsible for the reimbursement of health related expenses to workers in the agricultural area (PD cases n=204, controls n=467). PD cases were recruited among subjects submitting their first application to benefit from the free health care coverage for PD. The diagnosis of PD was established using standard criteria after examination by a neurologist or, when such an examination was not possible, using information provided by the patient's neurologist. Controls were recruited among MSA affiliates. A maximum of three controls were matched with each case for age (±2 years), sex, and region of residency at the time of the study.

Genotyping

The rs760678 SNP was genotyped by *Nla* III digestion following PCR amplification using 5'-AACAGGGGCACCCTTATCAT-3' and 5'-GGGCGATTTTGTTGTATTCC-3' oligonucleotides. Ninety individuals were randomly selected for direct sequencing and no discrepancies were observed (see supplementary material, Figure S1).

Statistical analysis

The SAS software release 8.02 was used for statistical analyses (SAS Institute, Cary, NC). To study the impact of the SNP (rs760678) on AD an PD, an univariate analysis was first performed using Pearson's χ^2 test. Before pooled analyses, homogeneity between populations was tested using Breslow-day computation (21). The impact of the rs760678 SNP on PD or AD was then estimated by multiple logistic regression models adjusted for age, gender and centre when necessary. Meta-analysis were performed using the RevMan 5.0 software and Mantel-Haentzel, fixed ORs were estimated for overall effect.

ACKNOLEDGEMENTS:

Julien Chapuis and Frédéric Moisan were supported by a PhD fellowship from the French Ministry of Research. This work was funded by INSERM and the Pasteur Institute of Lille. The TERRE study is supported by Agence Nationale de la Recherche (ANR, Santé-environnement 2005), Agence française de sécurité sanitaire de l'environnement et du travail (AFSSET, Santé-environnement 2006), France Parkinson (2005), and Inserm.

Conflict of Interest statement: The authors declare that the have no conflicting interests.

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Legend to Figure

Figure 1. Association of the rs760678 C allele and CC genotype with the risk of developing AD in the Li's study, our study and the combined one.

Table 1. Main characteristics of the different AD and PD case-control populations (respectively **A** and **B**).

A

	Lille		Rouen		Birmingham	
	AD cases	controls	AD cases	controls	AD cases	controls
n	770	659	739	691	416	167
Mean age	73.0 ± 8.4	73.1 ± 8.5	64.9 ± 9.8	66.3 ± 10.6	74.8 ± 7.1	71.2 ± 7.3
Mean age at onset	69.5 ± 7.4	-	64.9 ± 9.8	-	n.a	-
% of men	36	36	38	46	43	38

B

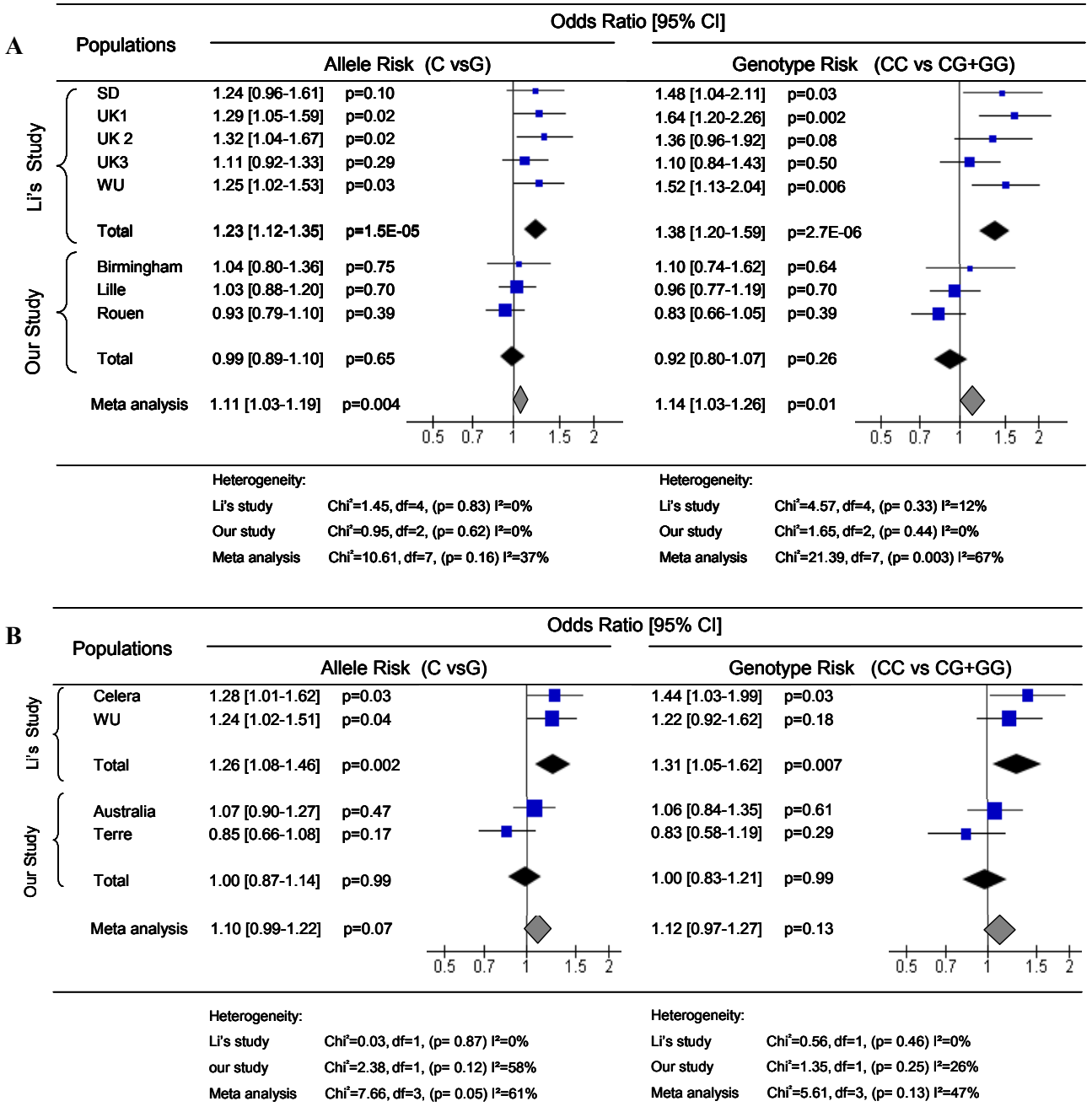
	Terre		Australia	
	PD cases	controls	AD cases	controls
n	209	488	740	468
Mean age	68.0 ± 19.0	68.0 ± 21.5	72.6 ± 10.7	70.5 ± 11.5
% of men	57	59	60	38

Table 2. Genotype distribution in the different AD and PD case-control populations

rs760678	**Allele distribution** freq. (*n*)					**Genotype distribution** freq. (*n*)						
	C		**G**		***P*[1]**	**CC**		**CG**		**GG**		***P*[1]**
Alzheimer												
Lille												
Controls *(638)*	0.614	*(784)*	0.386	*(492)*	*P*=0.70	0.393	*(251)*	0.442	*(282)*	0.165	*(105)*	*P*=0.31
Cases *(749)*	0.621	*(931)*	0.379	*(567)*		0.383	*(287)*	0.477	*(357)*	0.140	*(105)*	
Rouen												
Controls *(584)*	0.616	*(719)*	0.384	*(449)*	*P*=0.39	0.380	*(222)*	0.471	*(275)*	0.149	*(87)*	*P*=0.19
Cases *(643)*	0.599	*(770)*	0.401	*(516)*		0.337	*(217)*	0.522	*(336)*	0.140	*(90)*	
Brimingham												
Controls *(154)*	0.571	*(176)*	0.429	*(132)*	*P*=0.75	0.344	*(53)*	0.454	*(70)*	0.201	*(31)*	*P*=0.88
Cases *(408)*	0.582	*(475)*	0.418	*(341)*		0.365	*(149)*	0.434	*(177)*	0.201	*(82)*	
Whole												
Controls *(1,376)*	0.610	*(1,679)*	0.390	*(1,073)*	*P*=0.65	0.382	*(526)*	0.456	*(627)*	0.162	*(223)*	*P*=0.30
Cases *(1,800)*	0.604	*(2,176)*	0.396	*(1,424)*		0.363	*(653)*	0.483	*(870)*	0.154	*(277)*	
Parkinson												
Terre												
Controls *(467)*	0.623	*(582)*	0.377	*(352)*	*P*=0.17	0.381	*(178)*	0.484	*(226)*	0.135	*(63)*	*P*=0.36
Cases *(204)*	0.583	*(238)*	0.417	*(170)*		0.338	*(69)*	0.490	*(100)*	0.172	*(35)*	
Australia												
Controls *(462)*	0.612	*(566)*	0.388	*(358)*	*P*=0.47	0.374	*(173)*	0.476	*(220)*	0.149	*(69)*	*P*=0.74
Cases *(722)*	0.627	*(906)*	0.373	*(538)*		0.389	*(281)*	0.476	*(344)*	0.134	*(97)*	
Whole												
Controls *(929)*	0.618	*(1,148)*	0.382	*(710)*	P=0.99	0.378	*(351)*	0.480	*(446)*	0.142	*(132)*	*P*=1.00
Cases *(926)*	0.618	*(1,144)*	0.382	*(708)*		0.378	*(350)*	0.480	*(444)*	0.142	*(132)*	

[1] Pearson's χ^2 test for respectively allele and genotype distribution

Figure 1


A
Populations
Odds Ratio [95% CI]
Allele Risk (C vsG)
Genotype Risk (CC vs CG+GG)
Li's Study
SD 1.24 [0.96-1.61] p=0.10 1.48 [1.04-2.11] p=0.03
UK1 1.29 [1.05-1.59] p=0.02 1.64 [1.20-2.26] p=0.002
UK 2 1.32 [1.04-1.67] p=0.02 1.36 [0.96-1.92] p=0.08
UK3 1.11 [0.92-1.33] p=0.29 1.10 [0.84-1.43] p=0.50
WU 1.25 [1.02-1.53] p=0.03 1.52 [1.13-2.04] p=0.006
Total 1.23 [1.12-1.35] p=1.5E-05 1.38 [1.20-1.59] p=2.7E-06
Our Study
Birmingham 1.04 [0.80-1.36] p=0.75 1.10 [0.74-1.62] p=0.64
Lille 1.03 [0.88-1.20] p=0.70 0.96 [0.77-1.19] p=0.70
Rouen 0.93 [0.79-1.10] p=0.39 0.83 [0.66-1.05] p=0.39
Total 0.99 [0.89-1.10] p=0.65 0.92 [0.80-1.07] p=0.26
Meta analysis 1.11 [1.03-1.19] p=0.004 1.14 [1.03-1.26] p=0.01
0.5 0.7 1 1.5 2
Heterogeneity:
Li's study Chi²=1.45, df=4, (p= 0.83) I²=0%
Our study Chi²=0.95, df=2, (p= 0.62) I²=0%
Meta analysis Chi²=10.61, df=7, (p= 0.16) I²=37%
Heterogeneity:
Li's study Chi²=4.57, df=4, (p= 0.33) I²=12%
Our study Chi²=1.65, df=2, (p= 0.44) I²=0%
Meta analysis Chi²=21.39, df=7, (p= 0.003) I²=67%
B
Populations
Odds Ratio [95% CI]
Allele Risk (C vsG)
Genotype Risk (CC vs CG+GG)
Li's Study
Celera 1.28 [1.01-1.62] p=0.03 1.44 [1.03-1.99] p=0.03
WU 1.24 [1.02-1.51] p=0.04 1.22 [0.92-1.62] p=0.18
Total 1.26 [1.08-1.46] p=0.002 1.31 [1.05-1.62] p=0.007
Our Study
Australia 1.07 [0.90-1.27] p=0.47 1.06 [0.84-1.35] p=0.61
Terre 0.85 [0.66-1.08] p=0.17 0.83 [0.58-1.19] p=0.29
Total 1.00 [0.87-1.14] p=0.99 1.00 [0.83-1.21] p=0.99
Meta analysis 1.10 [0.99-1.22] p=0.07 1.12 [0.97-1.27] p=0.13
0.5 0.7 1 1.5 2
Heterogeneity:
Li's study Chi²=0.03, df=1, (p= 0.87) I²=0%
our study Chi²=2.38, df=1, (p= 0.12) I²=58%
Meta analysis Chi²=7.66, df=3, (p= 0.05) I²=61%
Heterogeneity:
Li's study Chi²=0.56, df=1, (p= 0.46) I²=0%
Our study Chi²=1.35, df=1, (p= 0.25) I²=26%
Meta analysis Chi²=5.61, df=3, (p= 0.13) I²=47%