LETTER TO JMG

Association of an interleukin 1B gene polymorphism (–511) with Parkinson's disease in Finnish patients

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Alzheimer's disease (AD) and Parkinson's disease (PD) are the two most common neurodegenerative conditions characterised by the presence of abnormal accumulation of proteins and loss of neurones in specific regions of the brain. The aetiology and pathogenesis of AD and PD still remain largely unknown. Evidence has emerged that immune mediated mechanisms may be important in the development of these disorders,[1 2] and cytokine interleukin 1 (IL1) is one of the mediators suggested to be involved.

The IL1 family comprises three proteins, the proinflammatory IL1α and IL1β and their inhibitor IL1 receptor antagonist (IL1Ra), which are encoded by the *IL1A*, *IL1B*, and *IL1RN* genes respectively.[3] Since IL1 may have a role in AD[4–8] and in PD,[9 10] variation in the *IL1A*, *IL1B*, and *IL1RN* genes may be of importance in the development of these disorders. There is already some evidence in support of this, including the postulated association of the *IL1A* (–889) *2/*2 genotype with AD[11] and the influence of the *IL1B* (–511) *1/*1 genotype on age at onset of PD.[12] However, validation in different population groups is needed to determine whether polymorphisms located in the *IL1A*, *IL1B*, and *IL1RN* genes represent actual susceptibility factors for AD and PD. In the present study, our purpose was to investigate whether polymorphisms of the *IL1* cluster genes are associated with the risk of AD or PD in Finnish patients.

MATERIALS AND METHODS

Ethical approval for the investigation was obtained from the local Hospital Ethical Committees. All patients and controls enrolled in the study were from western Finland. The AD group consisted of 92 sporadic late onset (>65 years) patients (63 women, 29 men). Thirty-eight patients (mean age at onset 73.7 years (SD 4.9)) met the clinical diagnostic criteria of the NINCDS-ADRDA Work Group for probable AD,[13] while for 54 patients (59%) (mean age at death 80.5 years (SD 5.5)) the clinical diagnosis had been confirmed neuropathologically using the CERAD criteria.[14] The PD group comprised 52 patients who were all neuropathologically verified (27 women, 25 men; mean age at onset 64.0 years (SD 7.3); mean age at death 76.2 years (SD 6.0)). The pathological diagnosis of PD was based on the loss of pigmented neurones in the substantia nigra with gliosis, the presence of pigment phagocytosis and Lewy bodies, confirming the clinical diagnosis of PD. The 73 controls were cognitively intact elderly subjects with no clinical symptoms or signs of neurological or psychiatric disease (34 women, 39 men; mean age 75.3 years (SD 7.5)). In 59 cases (81%) brain tissue had been obtained at necropsy and verified to be normal based on a thorough neuropathological investigation made according to CERAD recommendations.[14] Amygdala, hippocampus, entorhinal cortex, substantia nigra, and five cortical gyri (medial frontal, rectus, cingulate, angular, medial temporal) were examined for senile plaques and neurofibrillary tangles using the modified Bielschowsky's silver staining method and for Lewy bodies using anti-ubiquitin immunostaining. In addition, other possible neuropathology was identified using haematoxylin-eosin staining. Samples from the striatum, pons, medulla oblongata, and cerebellum were also taken for neuropathological examinations.

Table 1 Genotype and allele frequencies of the *IL1* gene cluster polymorphisms in AD, PD, and control patients

Polymorphism	AD (n=92)	PD (n=52)	Controls (n=73)
IL1A (–889)			
*1/*1	42 (0.46)	28 (0.54)	33 (0.45)
*1/*2	39 (0.42)	20 (0.38)	29 (0.40)
*2/*2	11 (0.12)	4 (0.08)	11 (0.15)
*1	123 (0.67)	76 (0.73)	95 (0.65)
*2	61 (0.32)	28 (0.27)	51 (0.35)
IL1B (–511)			
*1/*1	35 (0.38)	25 (0.48)	24 (0.32)
*1/*2	47 (0.51)	25 (0.48)	30 (0.41)
*2/*2	10 (0.11)	2 (0.04)†	19 (0.26)
*1	117 (0.63)	75 (0.72)	78 (0.53)
*2	67 (0.36)	29 (0.28)	68 (0.47)
IL1RN (VNTR)			
*1/*1	47 (0.51)	28 (0.54)	30 (0.41)
*1/*2	30 (0.33)	19 (0.36)	30 (0.41)
*2/*2	8 (0.09)	0 (0.00)	9 (0.12)
*1/*3	5 (0.05)	3 (0.06)	2 (0.03)
*2/*3	2 (0.02)	1 (0.02)	1 (0.01)
*2/*4	0 (0.00)	1 (0.02)	1 (0.01)
*1	129 (0.70)	78 (0.75)	92 (0.63)
*2	48 (0.26)	21 (0.20)	50 (0.34)
*3	7 (0.04)	4 (0.04)	3 (0.02)
*4	0 (0.00)	1 (0.01)	1 (0.01)

†p=0.001 (*1/*1 + *1/*2 *v* *2/*2).
n, number of subjects genotyped; frequencies shown in parentheses.

Genomic DNA was extracted from whole blood or postmortem brain tissue by standard methods. The single base substitutions at position –889 of *IL1*A and at –511 of *IL1B* as well as the polymorphism of a variable number of tandem repeats (VNTR) in intron 2 of *IL1RN* were genotyped as reported previously.[15–17]

The test for Hardy-Weinberg equilibrium was carried out using Arlequin, version 2.000.[18] The genotype and allele frequencies of the *IL1A*, *IL1B*, and *IL1RN* polymorphisms were compared between cases and controls using the chi-square test. In view of the multiple comparisons, the correction factor n(m-1) (n loci with m alleles each) was applied to correct the significance level ($p<0.002$). The effect of the type of the *IL1* complex gene genotypes on age at onset of PD was analysed using the Kaplan-Meier survival analysis (log rank test statistics).

Abbreviations: AD, Alzheimer's disease; PD, Parkinson's disease

Table 2 Combinations of the *IL1RN* (VNTR) polymorphism with *IL1A* (–889) and *IL1B* (–511) in relation to the presence/absence of allele 2 in AD, PD, and control groups

	IL1A (–889)		*IL1B* (–511)	
IL1RN (VNTR)	*2+, n	*2–, n	*2+, n	*2–, n
AD (n=92)				
*2+	19 (48)	21 (52)	36 (90)	4(10)
*2–	31 (60)	21 (40)	21 (40)	31(60)
PD (n=52)				
*2+	5 (24)	16 (76)	17 (81)	4(19)
*2–	19 (61)	12 (39)	10 (32)	21(68)
Controls (n=73)				
*2+	17 (41)	24 (59)	36 (88)	5(12)
*2–	23 (72)	9 (29)	13 (41)	19(59)

n, number of subjects genotyped; percentages shown in parentheses. *2+, carrier of allele 2; *2–, non-carrier of allele 2.

RESULTS

The distributions of genotypes and allelic frequencies of the *IL1A* (–889), *IL1B* (–511), and *IL1RN* (VNTR) polymorphisms are shown in table 1. No deviations from Hardy-Weinberg equilibrium could be seen in any of the groups of patients and controls studied. The frequency of the combined *IL1B**1/*1 and *1/*2 genotypes, that is, *IL1B**1 carriers, was 0.96 in patients with PD, and significantly higher than the frequency of 0.73 observed in the controls (p=0.001). The relative risk of PD for patients carrying at least one *IL1B**1 was calculated as 8.8 (95% confidence interval 2.0-39.7). The genotype or allele frequencies of the *IL1A* and *IL1RN* polymorphisms did not differ significantly when the patients with PD were compared with the controls. The effect of variation in the genotypes of the *IL1* complex genes on age at onset of PD was also analysed, but no significant differences could be observed between the genotypes of any of the genes (*IL1B* (–511) *1/*1 genotype: mean age at onset 64.7 years (SD 8.4), *1/*2: 63.3 years (SD 6.5), *2/*2: 64.5 years (SD 5.0), p=0.375; data not shown for *IL1A* and *IL1RN*). As regards AD, no significant differences in the frequencies of the *IL1A*, *IL1B*, and *IL1RN* gene polymorphisms could be seen when the patients with AD were compared with the controls.

We also analysed the two locus combinations *IL1RN/IL1A* and *IL1RN/IL1B* in relation to the presence/absence of allele 2. No significant associations between the composite genotypes and AD or PD were found (table 2).

DISCUSSION

In the present study, we found that carrying *IL1B**1 (–511) was significantly associated with PD, indicating that the *IL1B* gene may play a role in the pathogenesis of this disorder. When compared to the statistical significance level required after correction for multiple testing, however, the statistical support for the positive finding remained marginal. Our finding, therefore, needs to be confirmed in independent case-control series (as well as in different populations). We did not observe variation of the *IL1B* genotype to have an effect on age at onset of PD; this in contrast to Nishimura *et al*,[12] who recently reported that Japanese patients with PD carrying the *IL1B* *1/*1 genotype had a significantly earlier disease onset than patients carrying the *2/*2 genotype. Since the frequency of the *2/*2 genotype in our PD patient group was very low, the number of subjects having the *2/*2 genotype remained limited (two out of 52), and therefore further studies with a larger number of Finnish patients are warranted. In accordance with earlier studies,[12 19] we did not find any association between PD and the *IL1A* (–889) or *IL1RN* (VNTR) polymorphisms.

Key points

- We investigated whether polymorphisms of interleukin 1A (*IL1A*), *IL1B*, and IL1 receptor antagonist (*IL1RN*) genes are associated with a risk for Alzheimer's disease (AD) or Parkinson's disease (PD) in Finnish patients.
- The frequency of the combined *IL1B**1/*1 and *1/*2 genotypes was found to be significantly higher in PD patients compared to controls, and the relative risk of PD for patients carrying at least one *IL1B**1 was 8.8 (95% confidence interval 2.0-39.7). The genotype or allele frequencies of the *IL1A* and *IL1RN* polymorphisms did not differ significantly when the PD patients were compared with the controls. No significant differences in the frequencies of the *IL1A*, *IL1B*, or *IL1RN* gene polymorphisms could be found between the AD patients and the controls.
- We conclude that *IL1B* may have a role in the development of PD, whereas the *IL1A*, *IL1B*, and *IL1RN* polymorphisms may not be associated with late onset AD.

The mechanisms whereby *IL1B* (–511) could contribute to the development of PD need to be elucidated. The C to T substitution at position –511 in the promoter region of *IL1B* may regulate the production of IL1β, and indeed the in vitro synthesis capacity of *IL1B* *1/*1 genotype carriers has been found to be lower than that of *1/*2 and *2/*2 carriers.[20] Our finding of *IL1B**1 being associated with PD therefore suggests that the lower synthesis capacity of IL1β could be important in the development of PD. IL1β may thus be a safeguard against the disease as has been suggested by Nishimura *et al*.[12] On the other hand, the level of IL1β in the nigro-striatal regions of the brain has been reported to be increased in PD patients.[9] This increase of the IL1β level in the PD brain could, however, result, for instance, from a compensatory response to neurodegeneration in the course of the disease. It must also be born in mind that *IL1B**1 may not be a real susceptibility factor for PD, but is in linkage disequilibrium with an unknown variant located close to the *IL1B* (–511) polymorphism on chromosome 2.

Possession of the *IL1A* (–889) *2/*2 genotype has been reported to increase the risk of AD.[21 22] Studies in which the association of this polymorphism with the disease was analysed in late and early onset patient groups separately have shown that the *IL1A* gene may be linked with early onset AD,[23 24] but not with the late onset type of the disorder.[23 25–27] Our study supports this latter conclusion. AD patients carrying the *IL1A* *2/*2 genotype have been reported to be some 10 years younger at onset than carriers of the *1/*1 genotype,[23 28] and, instead of being a causative factor for AD, *IL1A* may simply influence the course of the disease. Further, our findings that *IL1B* (–511) and *IL1RN* (VNTR) were not significantly associated with late onset AD are consistent with those of previous investigations.[23 25]

Since *IL1RN**2 has been found to be linked to up regulation of IL1Ra[29] and IL1β[20] proteins, we also analysed the two locus combinations *IL1RN/IL1A* and *IL1RN/IL1B* in relation to the presence/absence of allele 2. None of the combined genotypes of *IL1RN* and *IL1A* or *IL1B*, however, was significantly associated with PD or AD, indicating the absence of coordinately regulated synthesis of IL1 in these disorders.

In summary, we have shown that in Finnish patients, carriage of *IL1B**1 may increase the risk of developing PD, whereas the *IL1A* (–889), *IL1B* (–511), and *IL1RN* (VNTR) polymorphisms may not be significantly associated with late onset AD.

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