Inflammatory Cytokine Gene Polymorphisms and Increased Risk of Parkinson Disease

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Background: The proinflammatory cytokines tumor necrosis factor α (TNF-α) and IL-1β (interleukin 1β) have a role in neuroinflammation, and functional polymorphisms in the TNF-α and IL-1β genes may affect susceptibility to Parkinson disease (PD).

Objective: To investigate whether functional DNA polymorphisms of the TNF-α and IL-1β genes affect the risk of PD.

Design: Population-based case-control study.

Setting: Three rural California counties (Fresno, Tulare, and Kern).

Participants: Two hundred eighty-nine incident idiopathic PD cases and 269 population control subjects, marginally matched by age, sex, and race/ethnicity.

Main Outcome Measures: Genotypes of IL-1β-511 and TNF-α-308.

Results: We observed a greater than 2-fold increased risk of PD among carriers of the homozygous variant genotype of IL-1β-511 (odds ratio [OR], 2.26; 95% confidence interval [CI], 1.27-4.02) and the homozygous variant genotype of TNF-α-308 (OR, 2.49; 95% CI, 0.90-6.85) and an almost 3-fold increased risk among carriers of the homozygous variant genotype for either or both polymorphisms (OR, 2.92; 95% CI, 1.66-5.16).

Conclusions: A smaller magnitude of PD risk increase among carriers of the heterozygous genotype for either or both polymorphisms suggests a gene-dosing effect (OR, 1.45; 95% CI, 0.97-2.16; P<.001 for trend). Results were not sensitive to exclusion of all nonwhite subjects or to adjustment for nonsteroidal anti-inflammatory drug use or smoking.

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on PD risk is modified by environmental exposures such as smoking or nonsteroidal anti-inflammatory drug (NSAID) use. To the best of our knowledge, we present results for the first population-based case-control study of either polymorphism in a non-Asian population; our study is also the first to assess the effect of both polymorphisms combined and in conjunction with environmental exposures on PD risk. We tried to limit misclassification of disease by using a movement disorder specialist (J.M.B.) to assess and confirm all clinical diagnoses of PD in our sample.

METHODS

SUBJECTS

A population-based approach was used to recruit 289 patients with incident idiopathic PD enrolled within 3 years of first diagnosis and 269 control subjects from 3 rural California counties (Fresno, Tulare, and Kern) between January 1, 2001, and April 1, 2006. Population control subjects were randomly selected from Medicare records or from residential parcels sampled from a shape file of the tricounty area. Controls were marginally matched to cases by age, sex, and race/ethnicity.

A University of California, Los Angeles (UCLA) movement disorder specialist (J.M.B.) confirmed a diagnosis of clinically probable or possible PD if patients met the following criteria: (1) manifestation of at least 2 of the characteristics of resting tremor, bradykinesia, or cogwheel rigidity, at least 1 of which is resting tremor or bradykinesia; (2) no suggestion of parkinsonian syndrome due to trauma, brain tumor, infection, cerebrovascular disease, or other known neurological disease and no past treatment with dopamine-blocking or dopamine-depleting agents; (3) no atypical features such as prominent oculomotor palsy, cerebellar signs, vocal cord paresis, severe orthostatic hypotension, pyramidal signs, amyotrophy, or limb apraxia; (4) asymmetric onset; and (5) if treatment with levodopa had been initiated, symptomatic improvement after treatment. Probable cases met criteria 1 through 5. Possible cases exhibited at least 1 characteristic from criterion 1 and fulfilled criteria described in 2 and 3. Although sometimes included under criterion 1, postural reflex impairment was excluded as a criterion because it usually occurs late in PD and may typically occur early in other parkinsonian disorders (ie, multiple system atrophy and vascular parkinsonism).15

All participants provided blood or buccal samples for genotyping. Interviewees blinded to the case-control status of interviewees collected demographic and risk factor data through telephone interviews. Our medical questionnaire included questions regarding lifetime aspirin and nonaspirin NSAID use. All participants provided informed consent, and the study was approved by the UCLA ethics committee.

LABORATORY SAMPLES

Blood and buccal samples were stored and processed at the UCLA Biologic Specimen Core Facility. We genotyped the IL-1β-511 promoter polymorphism (rs16944), which exhibits a C (allele 1) to a T (allele 2) substitution at position 511, and the TNF-α-308 promoter polymorphism (rs1800629), which results from a G (allele 1) to an A (allele 2) transition at nucleotide 308. The IL-1β polymorphism was detected by polymerase chain reaction using the primers F5’GAGCCTATTCGACGGGTGGC3’ and R5’CTTCTGGGGCTACTGACTGAT3’. Both products were analyzed via pyrosequencing.

STATISTICAL ANALYSIS

All analyses were performed using SAS 8.0 software (SAS Institute Inc, Cary, NC). We assessed Hardy-Weinberg equilibrium using a χ² test. We compared the genotype frequencies of cases and controls and examined gene-dosing effects by comparing carriers of homozygous variant and heterozygous genotypes with carriers of the homozygous wild-type genotype. Stratified analysis was used to assess associations in subgroups by sex, age at diagnosis (≤ 60 vs >60 years), ever having smoked regularly (≥1 year), pack-years of smoking (0, >0 to <19, or ≥19 pack-years), and regular aspirin and nonaspirin NSAID use (≥2 tablets/wk for ≥1 month). We used logistic regression analysis with indicator variables for each genotype to obtain odds ratios (ORs) adjusted for sex, race/ethnicity, age at diagnosis (continuous), and pack-years of smoking (0, >0 to <10, ≥10 to <40, or ≥40 pack-years) for genotypes at each locus. We repeated some analyses using a log-additive model that relies on counts of allele frequency and assumes that disease risk increases in a log-linear fashion with allele frequency.

We also grouped together (1) carriers of a homozygous variant genotype for either or both polymorphisms and (2) carriers of a heterozygous genotype but no homozygous variant genotype for either or both polymorphisms and compared PD risk in these groups with PD risk among carriers of a homozygous wild-type genotype for both polymorphisms. Because of the potential for population stratification in our mixed-ethnicity population, we repeated the analyses restricting the population to white participants only (235 cases and 220 controls).

The total population accrued was primarily white (81.4%) and included Hispanics (9.1%), Native Americans (4.7%), Asians (2.7%), and black subjects (2.1%). The median age of cases at diagnosis was 70.1 years. Table 1 gives demographic characteristics of the study population. We controlled for the variables in Table 1 in our model independent of whether cases and controls differed at the univariate level because these factors could be confounders in a more complex multivariate manner. We also controlled for age, sex, and race/ethnicity to account for frequency matching on these variables. Cases and controls were found to be in Hardy-Weinberg equilibrium for both studied polymorphisms (range, P = .13 to P = .86).

Table 2 gives results for the IL-1β-511 and TNF-α-308 gene polymorphisms for the study population. The homozygous variant (2/2 genotype) of the IL-1β-511 polymorphism was more frequent in cases than in controls (16.6% vs 9.3%, respectively). Compared with carriers of the homozygous wild-type genotype, carriers of the homozygous variant genotype exhibited a greater than 2-fold (OR, 2.26; 95% confidence interval [CI], 1.27-4.02) increased risk of PD. This strong positive association was not seen in participants who were heterozygous for the variant allele (OR, 1.19; 95% CI, 0.82-1.72). However, when using a log-additive (allele count) model, we noted an increased risk for homozygous variant and heterozygous IL-1β-511 carriers (OR, 1.96; 95% CI, 1.16-3.29 for homozygotes; and OR, 1.40; 95% CI, 1.08-1.81 for heterozygotes). Adjusting for the TNF-α-308 poly-

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morphpism did not change our effect estimates. Similar effects were noted when restricting analyses to white participants (OR, 2.12; 95% CI, 1.11-4.07 for homozygotes; and OR, 1.08; 95% CI, 0.73-1.61 for heterozygotes). Population prevalences among the white controls only were 45.7% for the homozygous wild-type genotype, 46.5% for the heterozygous genotype, and 7.8% for the homozygous variant genotype.

The homozygous variant (2/2 genotype) of the TNF-α-308 polymorphism was also more frequent in cases than in controls (4.5% vs 2.2%, respectively) (Table 2). Accordingly, our data suggested an increased PD risk among carriers of the homozygous variant genotype (OR, 2.49; 95% CI, 0.90-6.85), with similar results when restricting our analyses to white subjects (OR, 2.72; 95% CI, 0.93-8.00). Any association between PD risk and the heterozygous genotype was at best weak and was consistent with no association (OR, 1.14; 95% CI, 0.76-1.72). The observed increase in PD risk was weaker among carriers of the homozygous variant genotype but was stronger among carriers of the heterozygous genotype in a log-additive (allele count) model (OR, 1.68; 95% CI, 0.87-3.25 for homozygotes; and OR, 1.30; 95% CI, 0.93-1.80 for heterozygotes). Adjusting for the IL-1β-511 polymorphism did not change our effect estimates. Population prevalences among the white controls were 73.0% for the homozygous wild-type genotype, 24.7% for the heterozygous genotype, and 2.3% for the homozygous variant genotype.

Using stratified analysis, we observed no effect measure modification for either polymorphism by age at diagnosis, ever having smoked regularly, pack-years of smoking, or aspirin and nonaspirin NSAID use. Table 3 gives the results when assessing IL-1β-511 and TNF-α-308 variant alleles together for the study population. We found that carriers of a homozygous variant genotype for either or both polymorphisms exhibited an almost 3-fold (OR, 2.92; 95% CI, 1.66-5.16) increase in PD risk compared with those carrying a homozygous wild-type genotype for both polymorphisms. Furthermore, carriers of a heterozygous genotype but no homozygous variant genotype for either or both polymorphisms exhibited a 1.45-fold (OR, 1.45; 95% CI, 0.97-2.16) greater PD risk than those carrying a homozygous wild-type genotype for both polymorphisms, suggesting a gene-dosing effect (P<.001 for trend). We observed similar results when restricting our analyses to white subjects only (OR, 2.85; 95% CI, 1.52-5.35 for homozygotes; and OR, 1.36; 95% CI, 0.88-2.08 for heterozygotes; P=.001 for trend). Again, we observed no difference in effect estimates when stratifying by sex, age at diagnosis, ever having smoked regularly, pack-years of smoking, or aspirin and nonaspirin NSAID use. Results were not sensitive to controlling for aspirin and nonaspirin NSAID use or pack-years of smoking in our regression models.

The proteins encoded by the IL-1β and TNF-α genes are proinflammatory cytokines that actively regulate a broad spectrum of biological processes, most notably neuro-
The polymorphisms IL-1β-511 and TNF-α-308 are located in the promoter regions of their respective genes, and each is reported to result in increased gene expression levels.11,12

The greater than 2-fold increased risk of PD observed in carriers of the IL-1β-511 homozygous variant genotype is consistent with the notion that the variant allele increases gene expression, as overexpression of IL-1β is hypothesized to lead to nigral degeneration by increasing susceptibility of dopaminergic neuronal cells to toxins.12,13 Findings from 2 studies12,13 among 3 previous studies12-14 conducted among white populations suggested an increased risk of PD in carriers of the variant allele, in accord with our results. However, the estimated effect size among carriers of the homozygous variant genotype varied widely. McGeer et al13 reported a 9-fold (OR, 9.78; 95% CI, 2.70-35.45) increased risk, while Schulte et al12 suggested only a moderate increase in risk (OR, 1.45; 95% CI, 0.87-2.42 for homozygotes; and OR, 1.55; 95% CI, 1.08-2.22 for heterozygotes).12,13 Neither study used a population-based design. In addition, the study by McGeer et al13 drew from a small sample, as did the Finnish study14 reporting a decreased PD risk.

Tumor necrosis factor α has been shown to induce pathological changes in neuronal cells and is up-regulated in PD.6,21 Furthermore, TNF-α immunoreactive glial cells have been identified in the substantia nigra of patients with PD.7 Accordingly, our finding of an increased risk among carriers of the homozygous variant of the TNF-α-308 genotype is consistent with reports indicating that the variant allele of the TNF-α-308 polymorphism is a stronger transcriptional activator than the more common variant 1 allele.15 Two previous studies15,16 evaluating the relationship between TNF-α-308 and PD did not report risk increases. A small study by Ross et al15 observed no allele or genotype association between TNF-α-308 and PD but was likely statistically underpowered because of the rarity of the homozygous variant genotype. Krüger et al16 observed an increased risk only in carriers of the heterozygous genotype (OR, 1.53; 95% CI, 0.98-2.37), but it is unclear whether the control genotype frequency is representative of white populations because the patients with PD were a highly selected sample drawn from a tertiary care center.

Given that IL-1β and TNF-α are both involved in neuroinflammation and evidence exists that they may biologically interact, we also assessed the statistical independence of each gene polymorphism’s association with PD risk. Indeed, IL-1 has biologically been shown to induce the expression of TNF-α.22 Although IL-1 and TNF-α act on distinct cell surface receptors, they share common signaling mechanisms, some of which have been identified in the central nervous system and relate to neurodegeneration.22 Both TNF-α and IL-1 reportedly activate the transcription factor nuclear factor κB in brain cells, particularly in glia, thereby implicating both genes in oxidative stress–induced apoptosis, which might be a possible mechanism for rendering neurons susceptible to inflammatory cytokines.23

If each gene polymorphism’s association with PD was statistically independent, in logistic regression models we would expect to see a log-additive increase in PD risk in carriers of both homozygous variant genotypes. Unfortunately, we were unable to separately assess carriers of both homozygous variant genotypes because we identified no more than 2 such individuals. However, among carriers of at least 1 homozygous variant genotype, we noted an almost 3-fold increased risk of PD (Table 3) (ie, the point estimates were only slightly larger than those for homozygous carriers of each polymorphism alone). Given our results, one could speculate that IL-1β and TNF-α promoter variants act in the same biological pathway and trigger similar biological responses, such that high gene expression due to one or the other polymorphism achieves the same goal, while adding a second homozygous variant polymorphism will not increase PD risk further. Examining both genes together represents a relevant inquiry because 68% of our control population carried at least 1 variant allele of either or both polymorphisms, indicating that most individuals in a population carry variant polymorphisms that may increase PD risk and susceptibility.

We observed no effect measure modification by smoking or NSAID use, despite biological evidence linking inflammation to smoking and NSAID use, with smoking triggering acute inflammatory responses and NSAID use preventing cyclooxygenase-2 up-regulation (ie, the opposite effect of TNF-α and IL-1β inducing cyclooxygenase-2 expression).25,26 However, these subgroup analyses were limited by small sample sizes. It is also possible that, although carrying the variant genotype of the polymorphisms makes the carrier increasingly vulnerable to external insults that trigger neuroinflammation, smoking or NSAID use might not have occurred concomitantly with the relevant insult. Controlling for both factors did not affect our results for these polymorphisms.

Our study contributes to a growing body of evidence suggesting that proinflammatory cytokines exert their effect through complex and interacting biological pathways rather than independently and, more generally, that neuro-inflammation has an important role in idiopathic PD. Further population-based studies are warranted to corroborate these novel findings. An understanding of proinflammatory cytokine biology will allow for better-informed epidemiological studies in which subjects can be grouped into relevant biological inflammatory response categories. An improved understanding of the relationship between neuroinflammation and PD might help identify patients at high risk for this neurodegenerative disorder and offer insight into preventative intervention.

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