Poststroke Fatigue: Hints to a Biological Mechanism

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Background: Poststroke fatigue (PSF) is common, but the biological basis of this fatigue is unknown. We explored the possibility that PSF is related to systemic inflammation by investigating polymorphisms in 2 genes that affect the immune response. *Methods:* In a substudy of a larger trial that evaluated the role of the immune response on stroke outcome, fatigue was assessed at 30, 90, 180, and 365 days after ischemic stroke using the Fatigue Assessment Scale. Subjects were genotyped for 3 single nucleotide polymorphisms, one in the interleukin-1 receptor antagonist gene (*IL1RN*; rs4251961, a T/C substitution) and two in the in toll-like receptor-4 (*TLR4*) gene (1063 A/G [Asp299Gly] rs4986790 and 1363 C/T [Thr399Ile] rs4986791). *Results:* Of the 39 participants, 22 (56%) endorsed fatigue during the study. The degree of fatigue was remarkably constant over time and independent of stroke outcome. The C allele of the rs4251961 single nucleotide polymorphism (SNP) in *IL1RN* was associated with self-reported fatigue ($P = .03$), whereas the cosegregating polymorphisms in *TLR4* were associated with lower levels of fatigue ($P = .04$). *Conclusions:* SNPs in 2 genes with opposing effects on inflammatory immune responses were significantly, but differentially, associated with PSF. These findings suggest a direct link between immune signaling dysregulation and PSF. **Key Words:** Poststroke fatigue—inflammation—polymorphisms—*IL1RN*—*TLR4*.

Poststroke fatigue (PSF) is common after stroke and adversely affects quality of life.[1,2] Despite the high prevalence of PSF and its negative impact on recovery, the biological underpinnings of PSF are unknown. A potential role for inflammation in the genesis of PSF has been considered.[3-5] We hypothesized that if inflammation contributes to PSF, single nucleotide polymorphisms (SNPs) in genes that affect the immune response may be associated with PSF. The SNP rs4251961 is located in the promoter region of *IL1RN*; its C allele is associated with lower circulating concentrations of the gene product (interleukin, IL-1ra) and higher concentrations of proinflammatory cytokines.[6,7] In the toll-like receptor-4 (*TLR4*) gene, 2 functional polymorphisms, 1063 A/G (Asp299Gly; rs4986790) and 1363 C/T (Thr399Ile; rs4986791), are described. In Caucasians, these 2 SNPs cosegregate such that it is more common for them to occur together rather than independently.[8] These *TLR4* SNPs result in altered TLR4 proteins with decreased responsiveness to TLR4 ligands.[8,9] Given their opposing effects on systemic inflammatory responses, we hypothesized

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that the *IL1RN* SNP and the 2 cosegregating *TLR4* SNPs would be associated with different rates of PSF.

Methods

Research Subjects

The parent–patient population is described elsewhere.[10] Briefly, patients with ischemic stroke admitted to Harborview Medical Center from September 2005 through May 2009 who were at least 18 years of age were enrolled within 72 hours of symptom onset. Individuals with ongoing therapy for malignancy, known history of human immunodeficiency virus, hepatitis B or C, history of brain tumor, anemia (hematocrit <35 on admission), and those taking immunomodulatory drugs were excluded. All study procedures were approved by the University of Washington Institutional Review Board.

Clinical Data

Clinical and demographic data were collected on all subjects. Stroke severity was determined by the National Institutes of Health Stroke Scale score. Outcome was assessed by the modified Rankin Scale (mRS) score. Total infarct volume on initial diffusion-weighted magnetic resonance imaging was calculated by the ABC/2 method.[11] Subjects were asked about fatigue by the study nurse using the Fatigue Assessment Scale (FAS), a well characterized scale for assessing PSF.[12] Approval to administer the FAS was obtained approximately 30 months after study onset. This article includes data from the 39 subjects who provided FAS data at one or more time points. Subjects were also asked if they felt sad or blue at these same time points.

Genotyping

DNA was extracted from blood plasma samples using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) per manufacturer's protocols. For all 3 of the SNPs examined, genotyping was carried out using TaqMan SNP Genotyping Assay Sets and Master Mix (Applied Biosystems, Carlsbad, CA). In brief, 2 ng of sample DNA was genotyped per manufacturer's protocols on StepOnePlus Real-Time PCR (polymerase chain reaction) System (Applied Biosystems) under the following cycling conditions: 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute. An allelic discrimination plot was then generated using StepOne Software, v2.0 (Applied Biosystems). Target SNP reference identification numbers were rs4986790 and rs4986791 for the 2 TLR4 SNPs and rs4251961 for the IL1RN SNP. All samples were processed in triplicate. Reproducibility of the genotyping method was confirmed as described.[10] In brief, plasma-based PCR genotyping method was confirmed by carrying out identical PCR-based genotyping on DNA extracted from isolated leukocytes in a subset (n = 42) of patients. In these 42 patients, there was 100% concordance between the plasma-based and leukocyte-based samples. Genotype distributions for all 3 SNPs did not differ significantly from Hardy–Weinberg equilibrium (not shown).

Statistics

Descriptive data for continuous variables are presented as mean and standard deviation or median and interquartile range and compared using *t* tests for normally distributed data and the Mann–Whitney *U* test for non–normally distributed data. Data for categorical variables are presented as percentages and compared using the linear-by-linear association. Good outcome was defined as mRS less than 2. Patients were categorized based on the highest observed FAS score using previously defined cut points: 10-21 = not fatigued, 22-34 = fatigued, and 35-50 = very fatigued.[13] Significance was set at *P* less than .05.

Results

Individual FAS scores over time are shown in Figure 1. Median FAS scores did not differ over time and were similar among those with good outcome (mRS <2) and those without. Among our 39 participants, 17 (44%) did not endorse fatigue (FAS, 10-21) at any time point after stroke, 14 (36%) had fatigue (FAS, 22-34) at one or more time points, and 8 (20%) felt extremely fatigued (FAS, 35-50) at one or more time points in the year after stroke. The clinical characteristics of these subjects are shown in Table 1. In this cohort, there was no relationship between fatigue and infarct volume, infarct location, or infarct

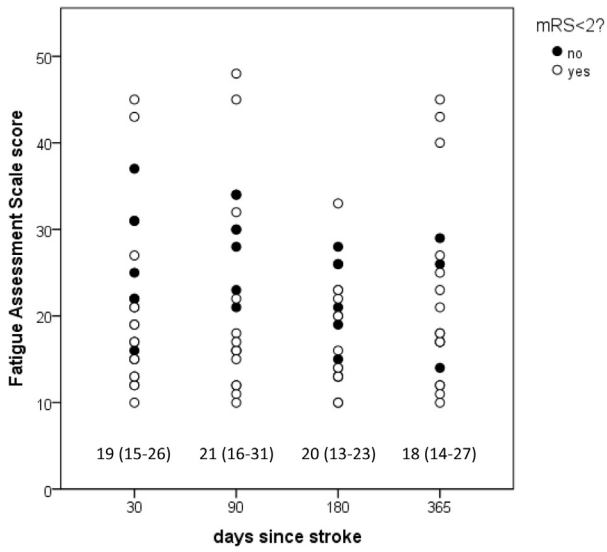

Figure 1. *Dot plot of individual patient Fatigue Assessment Scale (FAS) scores at each time point after stroke. Patients with good outcome (modified Rankin Scale; mRS <2) are displayed with open circles, those with mRS are displayed with closed circles. Not every subject contributed data at each time point. The median (interquartile range) FAS for the entire cohort at each time point is displayed along the x axis.*