Journal of the Neurological Sciences 368 (2016) 272–276

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Cytokine-related and sodium channel polymorphism as candidate predisposing factors for childhood encephalopathy FIRES/AERRPS

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ARTICLE INFO

Article history:
Received 16 May 2016
Received in revised form 13 July 2016
Accepted 14 July 2016
Available online 16 July 2016

Keywords:
Acute encephalopathy
Association studies in genetics
ILRN
SCN1A
SCN2A

ABSTRACT

Febrile infection-related epilepsy syndrome (FIRES), or acute encephalitis with refractory, repetitive partial seizures (AERRPS), is an epileptic encephalopathy beginning with fever-mediated seizures. The etiology remains unclear. To elucidate the genetic background of FIRES/AERRPS (hereafter FIRES), we recruited 19 Japanese patients, genotyped polymorphisms of the *IL1B*, *IL6*, *IL10*, *TNFA*, *IL1RN*, *SCN1A* and *SCN2A* genes, and compared their frequency between the patients and controls. For *IL1RN*, the frequency of a variable number of tandem repeat (VNTR) allele, RN2, was significantly higher in the patients than in controls ($p = 0.0067$), and A allele at rs4251981 in 5′ upstream of *IL1RN* with borderline significance ($p = 0.015$). Haplotype containing RN2 was associated with an increased risk of FIRES (OR 3.88, 95%CI 1.40–10.8, $p = 0.0057$). For *SCN1A*, no polymorphisms showed a significant association, whereas a missense mutation, R1575C, was found in two patients. For *SCN2A*, the minor allele frequency of G allele at rs1864885 was higher in patients with borderline significance ($p = 0.011$).

We demonstrated the association of *IL1RN* haplotype containing RN2 with FIRES, and showed a possible association of *IL1RN* rs4251981 G > A and *SCN2A* rs1864885 A > G, in Japanese patients. These preliminary findings suggest the involvement of multiple genetic factors in FIRES, which needs to be confirmed by future studies in a larger number of FIRES cases.

1. Introduction

Every year, a small number of previously healthy children are afflicted worldwide by a devastating acute encephalopathy characterized by severe status epilepticus following a febrile infection. This condition has been coined several terms, including febrile infection-related epilepsy syndrome (FIRES) [1,2] and acute encephalitis with refractory, repetitive partial seizures (AERRPS) [3], representing its two aspects, epileptic encephalopathy and brain inflammation, respectively. An accumulating body of evidence points to the close, mutual relationship between epilepsy and inflammation. However, their correlation in the specific context of FIRES remains to be elucidated.

The etiology of FIRES is unknown, but appears to be multi-factorial. In addition to infectious agents [1,2], genetic factors may play a critical role. Candidate genes include *SCN1A* and *SCN2A*, whose mutations are responsible for Dravet syndrome and febrile seizures (FS) [4,5]. Cytokines, as well as their receptors and up- and down-stream factors, could be other promising candidates. Polymorphisms of the *IL1B* and

Abbreviations: FIRES, Febrile infection-related epilepsy syndrome; AERRPS, acute encephalitis with refractory, repetitive partial seizures; *IL1RN*, interleukin 1 receptor antagonist gene; VNTR, variable number of tandem repeats.

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http://dx.doi.org/10.1016/j.jns.2016.07.040
0022-510X/

IL1RN genes are linked to FS [6,7], whereas *IL1RN* polymorphisms are implicated in various neurological disorders including multiple sclerosis [8]. Proinflammatory cytokines and chemokines are increased in the cerebrospinal fluid (CSF) of patients with AERRPS [9]. To elucidate the genetic background of FIRES, we focused on the gene polymorphism of pro-inflammatory cytokines, their related factors, and sodium channels in Japanese patients.

2. Methods

2.1. Subjects

We recruited patients with FIRES from hospitals in Japan during 2008–2013. Diagnostic criteria for FIRES were [1] previously healthy children with status epilepticus, and [2] 2 to 14 days after onset of an acute febrile infection without evidence for CNS infection [10], and those for AERRPS were [1] fever at onset of seizures, [2] focal seizures mainly of the face, [3] status epilepticus with clusters of seizures, [4] marked intractability of seizures, [5] epilepsy in the chronic period, and [6] exclusion of similar disorders [11]. Nineteen Japanese patients, 16 males and 3 females, aged from 1 to 9 years (median, 6 years), met both these criteria and participated in this study (Supplementary Table 1). The male preponderance in our series was consistent with that previously reported for FIRES [12].

2.2. Standard protocol approvals, registrations, and patient consents

The procedures in this study were approved by the University of Tokyo Ethics Committee. Written informed consent was obtained from all guardians of participants participating in the study.

2.2.1. Controls

We screened single nucleotide polymorphisms (SNPs) of the *IL1RN* gene of healthy Japanese adults, consisting of 100 DNA samples extracted from Pharma SNP Consortium (PSC) B cell lines obtained from the National Institute of Biomedical Innovation (Osaka, Japan). We searched the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) in the National Center for Biotechnology Information (NCBI) for the variation frequencies of SNPs.

2.2.2. Procedures

Peripheral blood samples were collected from the patients, and genomic DNA was extracted using standard protocols. SNPs in the promotor region of pro- and anti-inflammatory cytokines (*IL1B*, *IL6*, *IL10*, *TNF*, and *IL1RN*) were genotyped. For gene analysis, we selected cytokines whose polymorphisms are associated with FS, and those whose serum/CSF levels are altered in acute encephalopathy following prolonged FS [13,14]. All exons of *IL1RN* were polymerase chain reaction (PCR) amplified with flanking intronic primers (Supplementary Table 2) and standard PCR conditions. Variable number of tandem repeat (VNTR) polymorphism in intron 2 of *IL1RN* was analyzed, as well as linkage disequilibrium between the VNTR and several tag SNPs in the same haploblock. Tag SNPs were defined as variants with $r^2 > 0.98$ with other SNPs in HapMap-JPT (Tagger, Haploview 3.2). Primer sequences for genotyping of SNPs in cytokine genes are shown in Supplementary Table 2. *SCN1A* and *SCN2A* were PCR amplified with flanking intronic primers and standard PCR conditions [15–17]. PCR products of *SCN1A* and *SCN2A* were sequenced on 310 Genetic Analyzer, 3100 Genetic Analyzer or 3130xl Genetic Analyzer (Life Technologies, Carlsbad, CA, USA). A sequence change was judged as a missense mutation if it had not been reported as a normal variant in database (http://asia.ensembl.org/Homo_sapiens/Transcript/Variationß) and resulted in non-conservative amino acid substitution. Reference sequence of mRNA was based on information available from GenBank (accession number: Human *SCN1A*. NM_001165963.1 and *SCN2A*. NM_001040143). We then screened genotypes of *SCN1A* SNPs reportedly associated with FS (rs3812718 and rs6432860) [18,19] and with drug-resistant epilepsy (rs2298771) [20], intronic *SCN2A* SNP reportedly associated with drug-resistant epilepsy (rs230416) [21] and other 4 SNPs in donor or acceptor sites of intron with sequences conserved among mammalians [22].

2.3. Statistical analysis

Differences in the demographic characteristics of the genotypes between patients (19 cases) and controls were assessed by Pearson χ^2 test and Fisher exact test for categorical data. Goodness-of-fit to the Hardy-Weinberg equilibrium and differences in genotype and allele frequencies between patients and control groups were examined by χ^2 analysis. We estimated the odds ratio (OR) together with the 95% confidence interval (CI) for each allele haplotype frequency with FIRES using Microsoft Office Excel 2010. Significant differences were defined as $p < 0.05$. For multiple comparison, they were considered after Bonferroni's correction.

3. Results

3.1. Clinical information

Supplementary Table 1 presented the clinical information of 19 patients. Family history was unremarkable except for one patient whose maternal cousin had acute encephalopathy (Patient 13). Past history was also unremarkable, except for an episode of simple FS in one patient (Patient 9), mild developmental delay in one (Patient 18) and the use of theophylline prior to onset in one (Patient 15). All the patients had repetitive seizures in the acute period lasting for more than two weeks, and had intellectual deficit and epilepsy in the chronic stage. Patient 2 with an *SCN1A* mutation had previously been reported [17].

3.2. Genotyping of cytokine related genes

Table 1 shows the association of cytokine gene polymorphisms with FIRES. In the second intron of the IL1-RN gene, there is VNTR with an 86 base pair nucleotide sequence as its repeating element (Fig. 1a). RN1 is an allele including four repeats of 86 base pair, which is the major allele in controls and FIRES cases (Fig. 1b). We found a significant association between RN2 (2 repeats) allele of *IL1RN* and FIRES ($p = 0.0067 < 0.05/3$). The minor allele A frequency at *IL1RN* rs4251981 was higher in FIRES than in controls (22.5% vs. 6.6% with $p = 0.015 < 0.05/3$) (Table 1). We observed that in both FIRES and control individuals, the longer VNTR alleles with three or more repeats corresponded completely to the major allele of rs444413, rs451578, rs432014 and rs431726, while the VNTR RN2 allele corresponded to the minor alleles of these SNPs. None of the RN2 carriers had homozygous major allele A genotype at rs3181052, which resulted in three different haplotypes, A, B and C (Fig.1a, c). The haplotype distribution was significantly different between FIRES and the controls ($p = 0.021$). Haplotype C containing RN2 allele was associated with an increased risk of FIRES/AERRPS (OR = 3.88, 95%CI: 1.40–10.7, $p = 0.0057$) (Table 2). We found no mutations in protein coding exons of *IL1RN*. The other SNPs showed no significant difference in the genotypic and allelic distribution between FIRES/AERRPS and controls (Table 1).

3.3. Analysis of SCN1A and SCN2A

We analyzed genetic variations of *SCN1A* and *SCN2A* in patients with FIRES/AERRPS and control subjects. In two patients, Patient 2 and Patient 14, we found the same *SCN1A* mutation, R1575C, which had previously been reported in Rasmussen encephalitis and other types of acute encephalopathy [16,23,24]. Distribution of the polymorphisms in both FIRES and controls met the Hardy-Weinberg equilibrium (Table 1). *SCN1A* SNPs showed no significant difference in the genotypic and allelic distribution between FIRES and controls. Minor allele frequency of