Journal reading

Interleukin-10 is associated with resistance to febrile seizures: Genetic association and experimental animal studies
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Background-1

- Febrile seizures (FS) are the most common form of convulsions, occurring in 2–5% of infants in Europe and North America, and 6–9% in Japan.
- The etiology of FS is considered to be multifactorial and heterogeneous.
- The importance of genetic factors in the occurrence of FS has long been recognized, on the basis of family studies, twin studies, and complex segregation analysis.

Background-genetic

- In large families or families of probands with multiple FS, the febrile seizure susceptibility trait is inherited in an autosomal dominant manner with reduced penetrance.
- In contrast, the inheritance appears to be polygenic or multifactorial in small families, sporadic cases, or families of probands with a single febrile seizure, which account for a large proportion of FS (Rich et al., 1987; Kugler & Johnson, 1998).
- Linkage analysis, mainly of large families, revealed seven putative febrile seizure loci, chromosomes 8q, 19p, 2q, 5q, 6q, 18p, and 3p, which are at least partly associated with epilepsy or febrile seizures.
- Mutations of the sodium channel (SCN1A, 2q24.3; SCN1B, 19q13.1) and gamma-aminobutyric acid receptor (GABRG2, 5q31.1–33.1) genes, which cause fever-related epileptic syndromes, were identified in only a small number of patients with FS.

Background-cytokine

- FS are mostly provoked by infections.
- A proinflammatory cytokine, interleukin (IL)-1β, plays an important role in host-defense responses during infections and acts as an endogenous pyrogen.
- Therefore, there have been many reports on the possible contribution of IL-1β to the pathogenesis or pathophysiology of FS with respect to the plasma or cerebrospinal fluid level, IL-1β production by peripheral blood mononuclear cells, genetic polymorphic markers, and experimental FS with an immune rodent model.
- Our previous case-control study of 229 Japanese FS patients demonstrated that the -511C/T polymorphism of the IL1B gene was associated with the development of simple FS of sporadic occurrence.

Background-cytokine

- The balance between proinflammatory (IL-1, tumor necrosis factor [TNF]-α, IL-8, and IL-6) and antiinflammatory (IL-1 receptor antagonist and IL-10) cytokines influences the regulation of infections and could, therefore, play a role in the pathogenesis of FS.
- However, there are few general views about the contribution of these cytokines to the pathogenesis of FS based on investigations using clinical samples.

AIM

- In the present study, to determine whether pro- and antiinflammatory cytokine genes are responsible for susceptibility to FS, we have performed an association study on functional single-nucleotide polymorphisms (SNPs) of promoter regions of the IL6, IL8, IL10, and TNFA genes in patients with FS and controls.
- Because the haplotype of the IL10 gene showed a significant and positive association with FS, we further examined the in vivo role of IL-10 in hypertermia induced seizure models.
Subjects, Materials, and Methods

- Association study
- The study population consisted of 249 unrelated patients with FS, comprising 186 simple (105 males and 81 females) and 63 complex FS (34 males and 29 females) patients, living in the northern Kyushu area of Japan, and 225 normal 6- to 9-year-old children (114 males and 111 females) in the same area. All 229 patients and 158 controls in our previous study were included.
- The patients visited the Kyushu University Hospital and related hospitals and clinics from January 1999 to October 2000. Most of the precipitating infectious diseases were upper respiratory tract infections and influenza.

Definition - febrile seizure-1

- Convulsion associated with a temperature of higher than 38.0°C not caused by an intracranial infection.
  1. Negative family history of epilepsy,
  2. Negative past history of any disease with potential to cause brain damage
  3. Age at the first FS between 6 months and 6 years of age
  4. Duration of convulsions less than 20 min,
  5. Pattern of convulsions: generalized, bilateral symmetrical, or lacking focal symptoms,
  6. No clustering of frequent convulsions within a short period,
  7. Postictal phase: uneventful and complete recovery without sequelae (e.g., long-lasting disturbance of consciousness, hemiplegia, aphasia, and dementia),
  8. Interictally, neither obvious neurologic nor mental defects.

Definition - febrile seizure-2

- FS not in accord with any one of the above eight items were considered as being of the complex type.
- Family histories were obtained through pediatricians’ interviews with the parents.
- The ages at the initial seizure and blood sampling for all FS, simple FS, and complex FS are shown in Table 1.

Table 1. Clinical data for patients with all FS, simple FS, and complex FS

<table>
<thead>
<tr>
<th></th>
<th>All FS (N = 249)</th>
<th>Simple FS (N = 186)</th>
<th>Complex FS (N = 63)</th>
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<tbody>
<tr>
<td>Sex (M/F)</td>
<td>139/110</td>
<td>105/81</td>
<td>34/29</td>
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<td>Age at initial FS (months) Median</td>
<td>21</td>
<td>23</td>
<td>17</td>
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<tr>
<td>Range</td>
<td>5–81</td>
<td>6–60</td>
<td>5–81</td>
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<tr>
<td>Age at blood sampling (months) Median</td>
<td>33</td>
<td>34</td>
<td>31</td>
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<tr>
<td>Range</td>
<td>6–192</td>
<td>6–146</td>
<td>7–192</td>
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</tbody>
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<p>| Table 2. Genotype and allele frequencies of TNFA, II, II, II, and II promoter polymorphisms in patients with FS and controls. |
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<table>
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<tr>
<th>SNP</th>
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<td>All FS</td>
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</table>

METHOD

- DNA extraction ➔ Genomic DNA was extracted from peripheral blood using a MagExtractor.
- Selection of SNPs ➔ We selected functional and disease-associated SNPs in the promoter region of the following inflammatory cytokine genes;
  - IL6 -572C/G (rs1800796),
  - IL8 -251A/T (rs4073),
  - IL10 -592A/C (rs1800872), and
  - TNFA -1037T/C (rs1799724) or
  - Haplotype analysis, IL10 -1082A/G (rs 1800896) and -819T/C (rs 1800871) .
Discussion-1

- In the present study, we first demonstrated an association between the IL10 gene and resistance to FS.
- The current data suggest that the IL10-592C allele and -1082A/-819C/-592C (ACC) haplotype, which have been reported to be associated with increased production of IL-10, may confer resistance to FS.
- In addition, our hyperthermia-induced seizure models showed that administration of IL-10 increased the seizure threshold temperature.
- Taken together, these results imply that IL-10 is genetically associated with FS and acts as an attenuating factor for them.

Discussion-2

- IL-10 is a multifunctional antiinflammatory cytokine produced by monocytes, macrophages, B cells, T cells, and microglias, and inhibits the production of proinflammatory cytokines including TNF-α, IL-1, IL-6, and IL-8 (Moore et al., 2003).
- Straussberg et al. (2001) have shown increased lipopolysaccharide-induced IL-10 production by peripheral blood mononuclear cells from children with a history of FS.
- Virta et al. (2002b) have reported that no difference in plasma IL-10 level was found between children with and without FS.
- Therefore, little is known about the involvement of IL-10 in the pathogenesis of FS.

Limitation

- IL10 promoter genotype/haplotype was significantly related to simple FS but not complex FS, as in the case of the IL1B promoter genotype (Kira et al., 2005).
- On the other hand, it is suggested that IL-1B contributes to long-lasting hyperexcitability and excitotoxicity underlying prolonged and repetitive FS, which have been closely linked to the development of hippocampal epilepsy (Dube et al., 2000, 2005).
- Because IL-10 has a neuroprotective effect against glutamate-induced or hypoxic–ischemic neuronal cell death (Grilli et al., 2000; Mesples et al., 2003), further investigation is needed to determine whether IL-10 could play a role in the pathophysiology of complex FS, as represented by prolonged and repetitive FS.
- Including small sample sizes,
- Inappropriate selection of controls,
- Population stratification,
- And inappropriate stringent significance threshold.
- In the present genetic study, potential bias caused by population stratification can be minimized by sampling and matching cases and controls from the same source population and geographic region.
- However, we cannot exclude the possibility of false-positive results caused by the limitation of statistical power, since the association detected between IL10 gene and FS is only weakly significant (p = 0.014 for the C allele and 0.013 for the ACC haplotype).
- Studies with thousands of samples are needed to validate this finding.
Conclusion

• the -592C allele and ACC haplotype in the promoter region of the IL10 gene are significantly associated with resistance to FS.
• In experimental hyperthermic seizures in immature rodent models, IL-10 plays an anticonvulsant role.
• It is possible that IL-1b contributes to the susceptibility to FS, whereas IL-10 confers resistance to FS.

THANK YOU FOR YOUR LISTENING~