Original Article

Genome-wide identification of febrile seizure and related epilepsy phenotype loci

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Abstract

Febrile seizure (FS), autosomal dominant epilepsy with FS plus (ADEFS+), and severe myoclonic epilepsy of infancy (SMEI) are all triggered by high fever at the onset of disorder, but are clinically distinct from each other. Although various identified gene mutations account for approximately 15% of families with ADEFS+ and 80% of patients with SMEI, the responsible gene for FS is essentially un-

known. The present study was conducted to identify the loci associated with the risk of these 3 disorders. Among 154 patients (77 with SMEI, 34 with ADEFS+ and 43 with FS), we found 21, 24 and 27 novel susceptibility loci associated with the three diseases, respectively. This is the first phase of a two-part study on the genetic risk profile for fever-related seizure disorders.

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Introduction

The last decade has witnessed rapid advances in elucidating the role of genetic factors in epilepsy. Several families exhibiting epilepsy transmitted in a Mendelian manner have been found to have mutations in single genes, and they are dominantly inherited and most of them code for ion channels [1, 2]. However, only a few candidate genes for febrile seizures (FS) have been reported, and so far identified mutations only account for approximately 15% of families with autosomal dominant epilepsy with FS+ [ADEFS+ or generalized epilepsy with febrile seizure plus (GEFS+)], while mutations in GABA receptor genes (*GABRA1*, *GABRD*) and sodium channel gene (*SCN1A*) have been found in approximately 80% of patients with severe myoclonic epilepsy of infancy (*SMEI*) [3, 4].

Febrile seizures are defined as convulsions occurring in infancy or childhood, usually between 3 months and 5 years of age, which are associated with fever but not with evidence of intracranial infection or a defined cause. FS account for the majority of childhood seizure disorders, affecting 2 to 8% of children before 5 years of age in Western countries and even more in some countries including Japan [5-8]. Patients with ADEFS+ (GEFS+) have classic FS, FS that persist beyond 5 years of age, and subsequent afebrile seizures including absences. We previously proposed the term ADEFS+ instead of GEFS+ because GEFS+ clearly includes both generalized and partial epilepsy [9]. *SMEI* is an intractable epileptic syndrome with onset in the first year of life.

The initial manifestation is a prolonged febrile, tonic-clonic, generalized or unilateral seizure, and later the children manifest focal seizures and myoclonia, and develop ataxia and psychomotor delay within 2 years from the onset [10]. The term ADEFS+ is used here instead of GEFS+ in this article, because of a wide variation of additional febrile seizures not necessarily limited to generalized epilepsy, thus ADEFS+ is more appropriate term than GEFS+ for this spectrum of epilepsy phenotypes [9].

This is the first of a two-part report of our genome-wide identification of epilepsy loci determined by micro-satellite markers. This study will be followed by a second analysis with substantially larger number of subjects with these three phenotypes together with new controls to be analyzed by high-density oligonucleotide micro-arrays [11] to exclude false-positive loci and to identify mutations of candidate genes.

2. Materials and methods

2.1. DNA pool construction and typing

The blood samples were obtained from 93 controls, 43 FS (female 15, male 28), 34 ADEFS+ (female 13, male 21) and 77 *SMEI* (female 49, male 28) patients who were registered at the Epilepsy Genetic Study Group, Japan, and affiliated hospitals. The mean $\pm$ SD age at examination was 6.02 ± 5.32 years (range 1-29) for FS, 10.73 ± 8.62 (2-34 range) for ADEFS+ and 6.82 ± 7.74 (range 1-26) for *SMEI*. A written informed consent to participate was obtained from each subject. Clinical

information obtained by epileptologists included proband's familial history, seizure type, age of seizure onset, past history of FS, electroencephalographic (EEG) findings, and brain computerized tomography (CT) and/or magnetic resonance imaging (MRI) if available. Further detailed clinical characteristics of these subjects will be reported separately in the second phase study. High-molecular-weight genomic DNA was extracted from the peripheral white blood cells of the subjects according to standard protocols. We performed a genome-wide case-control analysis in the 93 controls, 77 SMEI patients, 34 ADEFS+ patients and 43 FS patients using 811 microsatellite markers from the ABI PRISM Linkage Mapping Set HD-5 (Applied Biosystems) to cover the entire chromosome with an average interval of 4.6 cM. We analyzed PCR products on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) [12]. We determined all allele sizes using the GeneScan (version 3.1.2 ABI) and GENE-MAPPER programs (Applied Biosystems). This study was approved by the ethical committees of Hirosaki University and participating institutions for this study.

2.2. Statistical analysis

To calculate p-value, we used the χ^2 -test on $2 \times m$ contingency tables for each locus, where m refers to the number of marker alleles observed in a population, and the Fisher's exact test on 2×2 contingency tables for each of the individual allele. The simple 'allelic' rather than 'genotypic' association was used in 2×2 contingency tables for microsatellite markers.

Fisher's exact test on 2×2 contingency tables was conducted for individual alleles and the alleles of each marker which did not show significant association were considered to be pseudo-positive [13]. We corrected the P-values using Bonferroni's correction (corrected P-value: Pc-value = P-value $\times$ total number of the contingency tables). Pc-value < 0.05 was considered statistically significant. These statistical analyses were carried out using the language and environment for statistical computing, R version 2.4, an open-source statistical package.

3. RESULTS

3.1. Microsatellite markers with significant association

In the screening using 811 microsatellite markers, we found a significant association (Pc-value < 0.05) for 21 (SMEI), 24 (ADEFS+) and 29 (FS) markers as assessed by the χ^2 -test on $2 \times m$ contingency tables. Markers revealed to have a significant association with FS, ADEFS+ or SMEI are listed in Table 1. The column of significant allele size and allele frequency show these values of which these allele has the lowest P-value in the locus. Pseudo-positive markers were removed.

As a result, we identified 21 loci for SMEI, 24 loci for ADEFS+ and 29 loci for FS.

Locus 5q14-15 in which *MASS1* is located [14], locus 21q22 [15] previously reported to be a FS-related locus, and locus 2q24 in which *SCN1A* and *SCN2A* are located and reported to be a SMEI- and ADEFS+- related locus [16-

34] were found in all three phenotype groups. Additionally, several loci reported for other epilepsy phenotypes such as juvenile myoclonic epilepsy (JME), benign familial neonatal convulsions (BFNC) and autosomal dominant partial epilepsy with auditory features (ADPEAF) [23, 35-38] were also found to be significant.

Figure 1 is the Venn diagram showing the number of loci associated with each phenotype. Fourteen loci were associated with all three phenotypes (Area VII). Five (Area I), 3 (Area II) and 2 (Area III) loci were specifically associated with FS, ADEFS+ and SMEI, respectively. Six loci were associated with both FS and ADEFS+ patient groups (Area IV), one locus was associated with both ADEFS+ and SMEI (Area V), and four loci were associated with both FS and SMEI (Area VI).

4. Discussion

4.1 Loci significantly associated with FS, ADEFS+ and SMEI

We found 29, 24 and 21 loci associated with FS, ADEFS+ and SMEI, respectively (Figure 1). Novel susceptibility genes for FS, ADEFS+ and SMEI may exist in these loci. Fourteen loci common to all phenotypes may play a role in epileptogenesis [39], while genes located in loci belonging to Area I (5), Area II (3) and Area III (2) of Figure 1 may be one of the causative factors of FS, ADEFS+ and SMEI phenotypes. Genes located in the loci (Areas IV and V) identified in two phenotypes may be responsible for intermediate phenotypes between FS and ADEFS+ or SMEI and ADEFS+. Clinically these three phenotypes are distinct from each other, but the three phenotypes are considered to be a

Figure. 1. Venn diagram showing number of loci associated with each phenotype

Table 1. List of markers that show significant association with FS, ADEFS+ and SMEI

Phenotypes	Markers	Locus	Number of alleles		Significant allele size	Allele frequency		P-value	2×n	2×n**
			All alleles	Positive alleles		Control	Case			
FS	D1S423	1q44	4	2	51	0.00	0.28	2.71e-10	2.20e-07	6.92e-07
	D2S347	2q19.4	15	6	30	0.00	0.53	2.20e-16	1.78e-13	2.20e-16
	D2S2241	2q23	16	5	46	0.05	0.53	2.20e-16	1.78e-13	4.77e-16
	D3S3697	3p14	22	4	61	0.00	0.07	3.59e-06	2.91e-03	3.02e-03
	D4S2986	4q24	6	2	58	0.00	0.02	1.30e-07	1.05e-04	3.13e-01
	D5S428	5q14	17	5	47	0.06	0.19	2.20e-16	1.78e-13	2.88e-04
	D5S630	5p15.2	30	7	52	0.01	0.19	3.44e-06	2.79e-03	1.72e-04
	D6S1575	6p12	23	15	50	0.00	0.28	2.20e-16	1.78e-13	2.61e-10
	D6S1671	6p12	31	17	48	0.00	0.14	2.20e-16	1.78e-13	8.09e-11
	D6S300	6p15.1	14	8	56	0.00	0.58	7.58e-09	6.15e-06	2.20e-16
	D6S1573	6p21.1	21	14	56	0.00	0.28	2.20e-16	1.78e-13	2.95e-16
	D6S1630	6p21.1	20	11	54	0.00	0.63	2.20e-16	1.78e-13	2.20e-16
	D6S1549	6q16	15	7	41	0.01	0.33	2.20e-16	1.78e-13	1.05e-09
	D6S1698	6q16	23	6	69	0.00	0.37	2.20e-16	1.78e-13	1.51e-15
	D6S1719	6q21	8	2	54	0.38	0.28	2.20e-16	1.78e-13	4.16e-03
	D7S2560	7q34	16	5	43	0.00	0.19	2.02e-13	1.63e-10	7.71e-09
	D9S269	9p23	13	0	-	-	-	4.39e-11	3.56e-08	-
	D10S1649	10p14	17	8	56	0.04	0.23	2.20e-16	1.78e-13	6.07e-09
	D10S1686	10q23	12	1	60	0.08	0.00	3.30e-05	2.68e-02	2.05e-03
	D10S1709	10q24	23	12	52	0.05	0.49	2.20e-16	1.78e-13	6.52e-11
	D12S1675	12q24.3	15	8	50	0.00	0.60	2.20e-16	1.78e-13	2.20e-16
	D14S1037	14q31	14	2	30	0.23	0.00	3.48e-05	2.82e-02	5.56e-05
	D15S988	15q22	8	2	56	0.12	0.09	8.93e-06	7.24e-03	1.36e-03
	D17S1816	17q24	17	1	58	0.00	0.05	3.40e-13	2.76e-10	1.06e-02
	D18S465	18q22	16	12	52	0.00	0.37	2.20e-16	1.78e-13	2.20e-16
	D21S263	21q22	18	6	67	0.02	0.35	2.20e-16	1.78e-13	1.24e-09
	DXS987	Xp22.2	22	12	60	0.00	0.30	2.20e-16	1.78e-13	2.97e-12
	DXS7108	Xp22.2	10	3	66	0.04	0.02	1.93e-05	1.57e-02	1.65e-03
	DXS1196	Xq21	20	5	50	0.00	0.49	2.20e-16	1.78e-13	2.20e-16
	DXS984	Xq27	9	2	39	0.00	0.02	3.29e-05	2.67e-02	7.20e-04
ADEFS+	D1S423	1q44	5	3	51	0.00	0.24	1.22e-13	9.89e-11	1.67e-09
	D2S347	2q19.4	14	3	30	0.00	0.76	2.20e-16	1.78e-13	2.20e-16
	D2S2241	2q23	17	5	46	0.05	0.79	2.20e-16	1.78e-13	2.20e-16
	D3S3697	3p14	22	3	57	0.00	0.12	1.27e-08	1.03e-05	2.85e-04
	D3S3567	3p21	9	0	-	-	-	1.05e-09	8.48e-07	-
	D4S2986	4q24	6	2	54	0.00	0.24	7.03e-13	5.70e-10	8.18e-04
	D5S630	5p15.2	29	7	74	0.02	0.29	1.09e-13	8.80e-11	5.07e-07
	D5S422	5q34	10	1	48	0.01	0.00	2.20e-16	1.78e-13	1.74e-04
	D6S1575	6p12	22	12	50	0.00	0.47	2.20e-16	1.78e-13	1.10e-10
	D6S1671	6p12	31	16	50	0.00	0.26	2.20e-16	1.78e-13	1.70e-07
	D6S300	6p15.1	14	8	56	0.00	0.97	2.20e-16	1.78e-13	2.20e-16
	D6S1573	6p21.1	21	9	50	0.00	0.56	2.20e-16	1.78e-13	2.19e-14
	D6S1630	6p21.1	20	9	54	0.00	0.79	2.20e-16	1.78e-13	2.20e-16
	D6S1574	6p25	8	2	60	0.00	0.06	2.20e-16	1.78e-13	1.74e-03
	D6S1698	6q16	19	5	69	0.00	0.65	2.20e-16	1.78e-13	2.68e-11
	D7S515	7q27	17	7	50	0.00	0.56	2.20e-16	1.78e-13	2.19e-15
	D7S2560	7q34	15	4	43	0.00	0.26	4.62e-13	3.75e-10	6.09e-09
	D9S269	9p23	14	4	48	0.45	1.21	2.10e-07	1.70e-04	9.98e-06
	D10S1649	10p14	17	7	54	0.08	0.59	3.24e-13	2.62e-10	1.45e-07
	D10S1709	10q24	23	8	52	0.05	0.65	2.20e-16	1.78e-13	1.32e-13
	D12S1675	12q24.3	16	6	50	0.00	1.00	2.20e-16	1.78e-13	2.20e-16
	D18S465	18q22	17	9	54	0.00	0.68	2.20e-16	1.78e-13	1.66e-15
	D21S263	21q22	19	6	67	0.02	0.62	2.20e-16	1.78e-13	5.69e-07
	DXS1196	Xq21	21	6	50	0.00	0.74	2.20e-16	1.78e-13	1.17e-08
	DXS987	Xp22.2	19	9	60	0.00	0.35	2.20e-16	1.78e-13	2.20e-16
SMEI	D1S423	1q44	4	3	47	0.01	0.57	2.20e-16	1.78e-13	2.20e-16
	D2S2569	2p16	8	3	47	0.00	0.40	1.01e-08	8.20e-06	2.62e-08
	D2S347	2q19.4	16	5	24	0.00	0.81	2.20e-16	1.78e-13	2.20e-16
	D2S2241	2q23	24	9	49	0.40	0.04	9.44e-08	7.66e-05	1.42e-08
	D5S428	5q14	15	3	39	0.04	0.25	6.78e-06	5.49e-03	2.71e-05
	D6S1573	6p12	23	14	36	0.00	0.60	2.20e-16	1.78e-13	2.20e-16
	D6S1630	6p12	22	11	34	0.00	0.69	2.20e-16	1.78e-13	2.20e-16
	D6S300	6p15.1	15	10	36	0.00	0.82	2.20e-16	1.78e-13	2.20e-16
	D6S1549	6p21.1	17	6	30	0.01	0.87	2.20e-16	1.78e-13	2.20e-16
	D6S1575	6p21.1	27	14	55	0.38	0.00	2.20e-16	1.78e-13	2.17e-13
	D6S1671	6q16	28	20	41	0.30	0.00	2.20e-16	1.78e-13	1.25e-10
	D9S1853	9p21	14	8	55	0.00	0.42	2.20e-16	1.78e-13	5.51e-12
	D9S269	9p23	15	5	44	0.00	0.48	2.20e-16	1.78e-13	2.20e-16
	D10S1649	10p14	17	9	47	0.51	0.08	2.20e-16	1.78e-13	2.05e-10
	D10S1709	10q24	25	17	15	0.00	0.45	2.20e-16	1.78e-13	1.17e-12
	D12S1675	12q24.3	16	8	209	0.00	0.67	2.20e-16	1.78e-13	2.20e-16
	D14S1037	14q31	14	3	23	0.00	0.23	1.51e-08	1.22e-05	1.37e-07
	D15S988	15q22	8	2	33	0.00	0.40	1.37e-07	1.11e-04	4.05e-12
	D18S465	18q22	17	13	237	0.00	0.52	2.20e-16	1.78e-13	2.20e-16
	D21S263	21q22	20	6	59	0.00	0.59	2.20e-16	1.78e-13	5.43e-12
	DXS1196	Xq21	24	9	210	0.00	0.48	2.20e-16	1.78e-13	2.20e-16

*: Pc-value = P-value × total number of contingency tables (811), corrected by Bonferroni correction.

**: Mean allele frequency with the lowest P-value in each locus.

multi-factorial disorder because they are provoked by high fever or, in cases of SMEI, the first seizures are mostly associated with fever [10]. These data suggest that FS, ADEFS+ and SMEI represent a continuum of epileptic disorders; FS is the mild type, SMEI is the most severe type, and ADEFS+ is an intermediate phenotype between FS and SMEI spectrum.

The existence of responsible loci common to all the three phenotypes of FS, ADEFS+ and SMEI suggests that these loci may be related to epileptogenesis. Loci specific to each phenotype have relevance to seizure-specific candidate genes, and the low allele frequencies (13~15%) of these loci may relate to the diversity of seizure in each phenotype. Thus, the epilepsy phenotypes may be determined by the interaction between common epileptogenesis-related genes and phenotype-specific candidate genes, although the current definitions of epilepsy phenotypes in clinical situations classified these phenotypes into two epilepsy syndromes; FS and SMEI. ADEFS+ is a newly introduced group based upon genetic studies. ADEFS+ encompasses uncomplicated FS, FS persisting beyond 6 years of age (FS+), as well as afebrile generalized tonic-clonic, absence, myoclonic, atonic, and temporal lobe seizures. Both ADEFS+ and FS are triggered by fever and patients are heterozygous for the mutant alleles, but mutations in the former are dominantly inherited and mutations in the latter are polygenic with some exceptions. The prognosis and symptoms are different from each other, and they are considered to be different disorders. Although SMEI arises de novo, febrile seizures

tend to be long and resistant to anti-epilepsy drug (AED) treatment. Psychomotor development is retarded from the second of year age, and the first seizures are mostly associated with fever [10].

Mutations of sodium ion channel gene (*SCN1A*) have been identified in these epilepsy phenotypes. The symptoms in patients with *SCN1A* mutations encompass a continuum of phenotypes with mild and severe forms of generalized or partial epilepsy. The mild type is FS and the most severe type is SMEI, and ADEFS+ may be in between. However, it remains to be clarified why missense mutations of *SCN1A* are associated with clinically distinct phenotypes such as ADEFS+ and FS, or even with SMEI [40]. Carriers of *SCN1A* mutations associated with ADEFS+ do not always exhibit seizures, and only a few FS cases with *SCN1A* mutation have been reported to date. The *SCN1A* mutations may result in greater phenotypic diversity perhaps by being more sensitive to the effect of modifier gene, location of mutations and/or kinds of amino acid exchanged. Precise understanding of phenotype-genotype relationship is necessary for the development of DNA diagnosis and individualized medical treatment based upon genetic polymorphisms/mutations of related genes [2, 28, 40, 41].

4.2 Strategy to uncover FS/ADEFS+/SMEI related genes

FS represent a heterogeneous group of disorders, and the mode of inheritance in most of the cases is unknown. Thus, the model-free or nonparametric method of linkage analysis

such as sib-pair analysis or genome-wide case-controlled study (association analysis) using micro-satellite markers is useful to identify the responsible genes. With this method, analysis of the difference in allele frequency between patients and control groups can potentially identify the region where the candidate genes may exist. In this region, mutations of known candidate genes, susceptible genes and SNPs are detected, and their allele frequencies are then calculated between patient and control groups. If there is a difference between two groups, the candidate gene is considered to be responsible for the disease.

Another whole genome screening approach is a genome-wide copy number analysis using high-density oligonucleotide genotyping arrays [11]. This is a new methodology to investigate genes in common complex disorders. By combining the results of this study (genome-wide case-control study using micro-satellite markers) and those of whole genome screening approach using high-density oligonucleotide genotyping arrays, false-positive candidate genes will be excluded in our subsequent study design.

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