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Association of SCN1A gene polymorphisms with infantile spasms and adrenocorticotropic hormone responsiveness

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Abstract. – OBJECTIVES: Infantile spasms (IS) are severe epileptic encephalopathy during infancy. The SCN1A encodes the α1 subunit of the neuronal voltage-gated sodium channels, and mutations in SCN1A have been frequently detected in idiopathic epilepsy and encephalopathy, which had similar symptoms as IS. Therefore, we investigated the association of SCN1A polymorphism with the IS and the responsiveness to adrenocorticotropic hormone (ACTH) treatment in the present study.

PATIENTS AND METHODS: We totally collected 113 IS patients and and 122 age-matched healthy controls. All of the subjects were Han Chinese descent, and the 113 cases were further divided into subgroups of cryptogenic and symptomatic patients. Nine tag SNPs within the SCN1A gene were selected and genotyped by the direct sequencing of PCR-amplified products. The ACTH was then applied to all of the cases.

RESULTS: Two SNPs in high linkage disequilibrium, rs13397210 and rs760543, were significantly associated with IS under genotype model (p = 0.015). In addition, we also found that a 4-SNP haplotype (CAGC) which contains the aforementioned 2 SNPs, was associated with increased responsiveness to ACTH therapy in IS (p = 0.018, OR = 4.8) under recessive model. Of the 2 subgroups of cases, more cryptogenic patients responded to the ACTH treatment than the symptomatic patients.

CONCLUSIONS: The results suggested that genetic variants of the SCN1A gene were associated with IS and ACTH responsiveness.

Key words:
Infantile spasms, SCN1A, SNP, Association study.

Introduction

Infantile spasms (IS), a catastrophic childhood epileptic syndrome, is characterized by clusters of epileptic spasms and mental retardation[1]. The incidence of IS has been reported to be around 0.025% in the new-born[2]. Approximately half of IS patients progressed into other types of epilepsy[3], which was intractable and always accompanied by severe developmental delay in intelligence[4]. Identifying the causative mutations and clarifying the molecular mechanisms of IS could help develop early prevention and intervention measures.

A few hypotheses and models have been proposed regarding the pathogenesis of IS, including the corticotropin-releasing hormone (CRH) hypothesis, the N-methyl-D-aspartate (NMDA) hypothesis, the serotonin hypothesis, prenatal stress exposure hypothesis, and aristaless-related homeobox (ARX) mutation model[5-11]. However, the mechanism of IS pathophysiology has not been fully clarified yet. It was reported that some chromosomal aberrations were implicated in the pathogenesis of IS, including 1p36 deletion, 5q14.3 deletion, 15q11-13 duplication, 16p11.2 deletion, 16p13.11 deletion, 17q21.31 deletion, 19p13 deletion, 21q21 duplication[12-19]. Additionally, several genes, such as ATXN2, NR3C1, SCN1A, SCN2A, KPNA7, STXBP1, ABCB1, GRIN1, ARX, TSC2[10,20-29], which were located within or outside the aforementioned aberration region, were found to be associated with the pathogenesis of IS. For instance, the nonsynonymous mutations in the ARX gene was reported to be associated with X-chromosome linked IS[11]. Moreover, according to the function cluster and annotation analyses, a large number of these genes were involved in the biological pathways of ventral forebrain development and forebrain synaptic transmission[12,30].

Adrenocorticotropic hormone (ACTH) was widely applied as the first-line treatment option for the IS[31-35]. However, a portion of IS patients are clinically resistant to ACTH[36,37]. The respon-

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siveness to ACTH is likely to be affected by genetic variants of the subject. For example, polymorphisms in NR3C1, GRIN1, and MC2R were found to be associated with the ACTH responsiveness[10,21,38].

The SCN1A gene encodes the α1 subunit of the neuronal voltage-gated sodium channels, which is involved in mediating the generation and propagation of action potentials in electrically excitable cells[39]. SCN1A mutations have been detected in severe idiopathic genetic epilepsy and encephalopathy of infancy[40-44]. In SCN1A knockout mice, the sodium currents was significantly declined in the hippocampal interneurons that were critical for GABA-mediated neuronal inhibition[45]. Previous studies have also shown that a missense mutation in SCN1A led to a dysfunction of inhibitory GABAergic neurons, which consequently resulted in the hyperexcitability of the brain and seizures of infants[46,47]. All of these findings highlighted the necessity to investigate the association between SCN1A polymorphisms and the pathogenesis of IS, which has not been evaluated before.

In attempt to investigate the association of genetic polymorphisms of the SCN1A gene with IS, we selected the tag SNPs in SCN1A gene, and conducted a case-control analysis among the Han Chinese patients. Additionally, we also investigated the association between SCN1A polymorphisms and the responsiveness to ACTH treatment.

Patients and Methods

Participants

As shown in Table I, a total of 113 unrelated patients with IS collected between January 2006 and May 2010 by Beijing Children's Hospital and Chinese PLA General Hospital were involved in this study. The control was consisted of 122 age-matched healthy children. None of the controls had diseases of the nerve system or family history of epilepsy. No neurological or medical condition suspected to be associated with IS was observed for the controls. All individuals involved in the study were Han Chinese descent and the written informed consent was provided by their parents or guardians. The study was approved by the Committee on Human Study of Chinese PLA General Hospital and the Ethics Review Committee of Beijing Children's Hospital.

The diagnosis of IS was based on the criteria proposed by the International League Against Epilepsy[48]. Either classic or modified hypsarrhythmia was observed for the electroencephalogram (EEG) of the affected children. The affected children were further divided into subgroups of cryptogenic and symptomatic patients. The cryptogenic patients was defined as the children who had no identified underlying etiology of IS with a normal developmental process and a normal brain imaging by computer tomography (CT) or magnetic resonance image (MRI). Symptomatic IS patients referred to the children who had the seizures resulted from identifiable causes, unequivocal developmental delay before the onset of spasms, and abnormal brain-imaging. We excluded the patients if (1) they received ACTH treatment within 28 days before the study; (2) they were contraindicated for hormonal treatments, including a lethal or potentially lethal disease other than IS; (3) the parents or guardians of the patients refused to provide informed consent. Every patient received thorough clinical history evaluation, developmental and neurological assessments, brain-imaging using CT or MRI, and metabolic examination of the uric acid.

Tag SNP selection and genotyping

Genomic DNA of the participants was extracted from peripheral blood leukocytes using standard methods. Nine tag SNPs in the SCN1A gene were selected based on the genotype of the Han Chinese population in the HapMap database (http://www.hapmap.org). The tag SNPs were selected using the HaploView software according

Table I. IS patients collected in this study and the responsiveness to ACTH therapy.

	Total	Cryptogenic	Symptomatic
Patients (percentage)	133	28 (25%)	85 (75%)
Sex (male/female)	70/43	17/11	52/33
Age at onset (months)	6.4±2.7	6.7±1.5	6.0±2.1
ACTH response	61	20	41
ACTH non-response	52	8	44

to a minor allele frequency (MAF) of 0.1, a pairwise correlation coefficient (r^2) of at least 0.80, and SNPs that met the criteria for aggressive pairwise tagging[49]. The genotypes of the tag SNPs were determined by direct sequencing of PCR products by 3730xl DNA analyzer (Applied Biosystems Inc., Foster City, CA, USA). All of the sequencing in this study was performed using both forward and reverse primers.

Assessment of ACTH responsiveness

All 113 patients involved in this study received ACTH treatment for a total of 14 days. Other drugs they received before the ACTH treatment were still applied during this period. ACTH responsiveness was assessed by parents/caregivers according to the change of patient seizure frequency at 2 weeks and 1 month after the ACTH treatment. At each time point, changes in seizure frequency were classified as seizure free, reduced (reduction from baseline of 50%-100%), no change (reduction from baseline of < 50%), or increased. The rate of response, defined as a reduction from baseline in seizure frequency ≥ 50%, was calculated for both the cryptogenic and symptomatic IS patients group. EEG of the patients were categorized as complete recovery (normalized EEG), partial improvement (multifocal spike wave), or no improvement (hypsarrhythmia or modified hypsarrhythmia) as previously described[10].

Statistical analysis

The SHEsis software was used to assess deviations from the Hardy-Weinberg equilibrium (HWE), and detect the genotypic and allelic association with IS of the tag SNPs[50]. HaploView software was used to estimate the haplotype blocks and their frequencies. Two-sided nominal p value less than 0.05 was considered as statistical significant.

Results

No ARX gene mutations detected in the IS patients

To perform association analysis of a candidate gene using a case-control design, we collected 113 IS-affected children and 122 age-matched healthy controls (Table I). In order to preclude the possibility that the IS of these patients was caused by mutations in ARX gene, we initially conducted a genetic pre-screening for ARX mutations when the samples were collected. No ARX mutation was found in the screening (data not shown).

Table II. Nine tag SNPs in the SCN1A gene.

SNP	Chromosome	Position (hg18)
rs10182473	2	166581969
rs11691603	2	166600592
rs3812718	2	166617790
rs10188577	2	166624143
rs10497278	2	166625837
rs1381108	2	166626648
rs7607543	2	166629033
rs13397210	2	166630274
rs1824549	2	166633217

Association of SCN1A with IS

In attempt to study the association of SCN1A polymorphism with IS, nine tag SNPs located in the introns of SCN1A gene were selected for genotyping (Materials and Methods, and Table II). Although located in the introns of the SCN1A gene, the tag SNPs covered the whole block ranging from 1 Kb upstream of SCN1A gene 5' end to 1 Kb downstream the gene 3' end. Frequencies of genotypes and alleles of these SNPs showed no significant difference against HWE.

We then conducted genotypic and allelic association analyses on all of the single SNPs using SHEsis software[50]. Two SNPs, rs13397210 and rs7607543, showed a nominal association with IS under genotypic model (p = 0.015). Since these two SNPs were in high linkage disequilibrium (LD, pairwise r^2 = 0.99), only the result of rs13397210 was presented (Table III). Furthermore, under the dominant model in the allelic association analysis, these two SNPs were also associated with IS (Table IV, only rs13397210 was presented as a proxy). As shown in Table IV, the G-allele of rs13397210 was enriched in the IS patients group (p = 0.02, OR = 3.26), suggesting that A allele of this SNP might be associated with lower risk of IS.

We then analyzed the association of SCN1A haplotypes with IS. The haplotype blocks were estimat-

Table III. Genotypic association of rs13397210 with IS.

	Genotype			p
	AA	AG	GG	
Cases	5 (0.044)	65 (0.575)	43 (0.381)	0.015
Controls	16 (0.131)	52 (0.426)	54 (0.443)	

The digits in the parentheses showed the genotype frequency in different groups.

Table IV. Significant association of rs13397210 with IS under dominant model.

	G+	G-	p	OR [95% CI]
Cases	108	5	0.02	3.26 [1.2-9.2]
Controls	106	16		

ed using the HaploView program[49]. Only two haplotype blocks were estimated for the 9 SNPs. The rs13397210 and rs7607543, together with another 2 SNPs, rs1381108 and rs1824549, were located within a haplotype block at the 6-kb region of the first intron in SCN1A. Three common haplotypes (frequency >5%) were detected (Table V). Although two haplotypes, CAGC and TAGA, contained the G allele of rs13397210, no association with IS was detected for these haplotypes (Table V).

Most of the cryptogenic IS patients responded to ACTH treatment

In attempt to study the different responsiveness of the IS patients to ACTH, we classified the subjects into cryptogenic and symptomatic groups (Materials and Methods), and applied ACTH to all of the IS-affected children.

After the ACTH therapy, totally 61 patients (53.9%) were responsive to the ACTH therapy, and 52 patients (46.1%) were resistant. We also examined efficacy of ACTH in cryptogenic and symptomatic patients. Forty-one of eighty-five (48.2%) symptomatic patients were responsive to ACTH therapy, while twenty of twenty-eight (71.4%) cryptogenic patients were responsive. This result indicated that cryptogenic patients were more sensitive to ACTH treatment.

SCN1A haplotype associated with ACTH responsiveness

We further tested the association of the haplotypes harboring G-allele of rs13397210 with the responsiveness to ACTH treatment. As shown in Table VI, the most common haplotype, CAGC, was associated with ACTH responsiveness under recessive model ($p = 0.018$, OR = 4.8), which indicated that homozygous carriers of this haplotype were more sensitive to the ACTH than heterozygous and non-CAGC carriers.

Discussion

A total of 113 IS patients and 122 age-matched healthy children were analyzed in this study. Of

Table V. Haplotypes at the first intron of SCN1A.

Haplotype	Frequency	Cases (n = 226)	Controls (n = 236)
CAGC	0.368	82 (0.363)	88 (0.373)
TGAC	0.338	75 (0.332)	81 (0.343)
TAGA	0.281	65 (0.288)	65 (0.275)

The digits in the parentheses showed the haplotype frequency in different groups.

the affected children, eighty-five (75%) were classified as symptomatic patients and twenty-eight (25%) were classified as cryptogenic patients. We selected 9 tag SNPs spanning the candidate gene SCN1A, and conducted a case-control association analysis. Among the 9 tag SNPs, rs13397210 and rs7607543 were in high LD SNPs and were associated with IS. We also detected that the efficacy of ACTH was higher in cryptogenic patients than that of symptomatic patients. Homozygote carriers of a 4-SNP haplotype, which contained the aforementioned two SNPs, were more sensitive to ACTH than the carriers of other haplotypes. The results supported the hypothesis that ACTH responsiveness could be genetically different for distinct patients.

It has been hypothesized that brain dysfunction such as brain stem dysfunction, cortex-subcortex brain tissue function asynchrony, and hypothalamus-hypophysis-adrenal gland axial dysfunction[10,51], might be the main mechanism of IS. Among the identified causative mutations of some kind of epilepsy, the majority of these mutation were located in genes encoding ion channels or receptors such as voltage-gated sodium, potassium, calcium, and chloride channels and receptors for acetylcholine and c-aminobutyric acid (GABA). Nonsynonymous mutations in these coding genes could result in the dysfunction of nerve system. The SCN1A encoded the central pore-forming glycosylated alpha subunit of voltage-gated ion channel, which is essential for the generation and propagation of action potentials in neuron and muscle. In this study, we found that the SCN1A polymorphisms were associated with IS and homozygous carriers of a common haplotype in SCN1A were more sensitive to ACTH therapy, suggesting that the voltage-gated sodium channels, an important group of candidate genes for epilepsy[52], might play a role in IS onset and ACTH treatment.

However, it should be mentioned that, because this study was focused on the association of

SCN1A to IS, we cannot exclude the possibility that the SCN1A gene might also be associated with other types of epilepsy. In addition, although the missense mutations in SCN1A have been detected in patients of Lennox-Gastaut syndrome and in patients with IS[42,44], all of the SNPs associated with IS and the haplotype associated with higher ACTH responsiveness in this study were located in the first intron of SCN1A. Whether these SNPs were located in gene expression regulatory regions or the polymorphisms at these SNPs could induce the alternative splicing of SCN1A should be further explored by experiments in vitro as well as *in vivo*.

Conclusions

Although the molecular mechanism and function of variant identified in this study deserved further investigations in the future, the genetic polymorphisms in SCN1A gene was associated with IS as well as the ACTH responsiveness.

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Conflict of interest

The Authors have no conflict of interests of declare.

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