

## ORIGINAL ARTICLE

# Autosomal dominant nocturnal frontal lobe epilepsy: a genotypic comparative study of Japanese and Korean families carrying the *CHRNA4* Ser284Leu mutation

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Autosomal dominant nocturnal frontal lobe epilepsy is a familial partial epilepsy syndrome and the first human idiopathic epilepsy known to be related to specific gene defects. Clinically available molecular genetic testing reveals mutations in three genes, *CHRNA4*, *CHRNA2* and *CHRNA2*. Mutations in *CHRNA4* have been found in families from different countries; the Ser280Phe in an Australian, Spanish, Norwegian and Scottish families, and the Ser284Leu in a Japanese, Korean, Polish and Lebanese families. Clear evidence for founder effect was not reported among them, including a haplotype study carried out on the Australian and Norwegian families. Japanese and Koreans, because of their geographical closeness and historical interactions, show greater genetic similarities than do the populations of other countries where the mutation is found. Haplotype analysis in the two previously reported families showed, however, independent occurrence of the Ser284Leu mutation. The affected nucleotide was highly conserved and associated with a CpG hypermutable site, while other *CHRNA4* mutations were not in mutation hot spots. Association with a CpG site accounts for independent occurrence of the Ser284Leu mutation.

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## INTRODUCTION

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE; MIM 118504) is a familial partial epilepsy syndrome characterised by clusters of brief frontal lobe motor seizures during drowsiness or sleep.<sup>1,2</sup> Seizures—often misdiagnosed as other nocturnal motor activities such as parasomnia or night terror<sup>1–4</sup>—vary from simple arousals to hyperkinetic activity with tonic or dystonic features. Onset usually occurs during the first two decades (mean age 10 years), but later onset has also been reported.<sup>5</sup>

ADNFLE is the first human idiopathic epilepsy known to be related to specific gene defects.<sup>6</sup> Clinically available molecular genetic testing reveals mutations in three genes encoding the  $\alpha 4$ ,  $\beta 2$  and  $\alpha 2$  subunits of the neuronal nicotinic acetylcholine receptor (*CHRNA4*, *CHRNA2* and *CHRNA2*, respectively).<sup>7–9</sup> Overall mutations are found in less than 20% of individuals with ADNFLE/NFLE phenotypes, suggesting their genetic heterogeneity.<sup>10</sup> Approximately 10–20% of patients have a positive family history and fewer than 5% a negative one.<sup>11</sup>

Among the four mutations in *CHRNA4* (Ser280Phe, Ser284Leu, Leu291dup and Thr293Ile), Ser280Phe and Ser284Leu have been identified in several unrelated families (mutation names may be different from those of previous papers; we use NP\_000735.1):

the Ser280Phe in an Australian, Spanish, Norwegian and Scottish families,<sup>12–15</sup> and the Ser284Leu in a Japanese, Korean, Polish and Lebanese families.<sup>16–19</sup> It has been assumed that founder effect is not relevant to ADNFLE, because most of the families studied come from different countries,<sup>20</sup> and a previous haplotype study of the Australian and Norwegian family<sup>14</sup> showed no genetic connection.

Japanese and Koreans, because of their geographical closeness and historical interactions, show greater genetic similarities than do the populations of other countries where the mutation is found; indeed, previous studies between Japanese and Koreans have shown a closer genetic similarities than that between other east Asians.<sup>21,22</sup> Founder effects between the two countries have also been reported in several autosomal recessive diseases,<sup>23,24</sup> however, not in autosomal dominant ones. For ADNFLE patients, however, propagating their pathological genes is easier than it is for individuals with other more visible epilepsy syndromes. They have no seizures during the daytime, show normal neurological examinations and have a normal life expectancy and reproductive capacity. In addition, family members or the affected individual himself may believe that his symptoms are not an indication of illness and thus may not seek medical attention (or, knowing himself to be ill, he may deliberately refuse to reveal his condition to others).<sup>25</sup> To determine whether founder effect is present

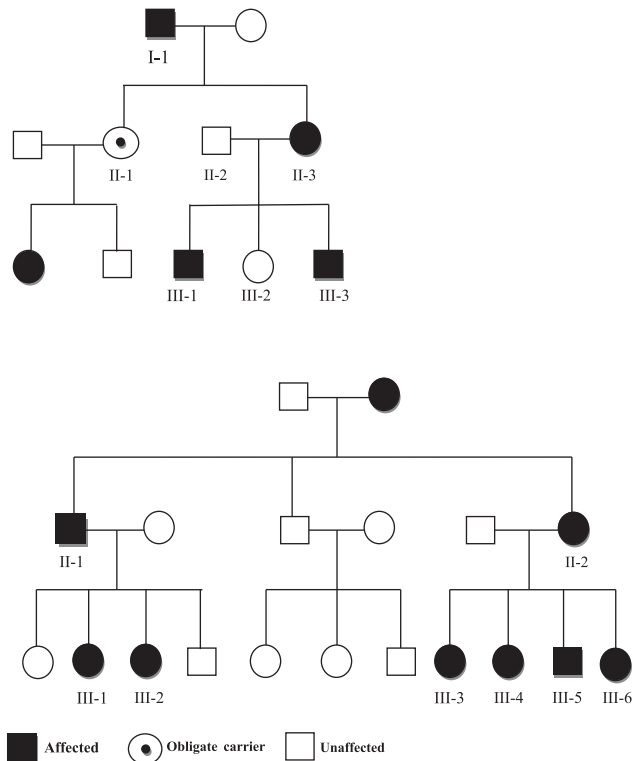
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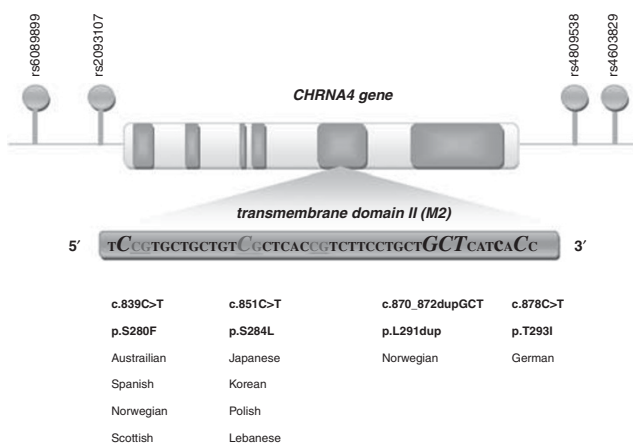
in ADNFLE, we compare haplotype structures in the two previously reported Japanese and Korean families.

## MATERIALS AND METHODS

We reviewed the medical histories and electroencephalogram findings of a three-generational family from Japan and one from Korea (pedigrees in Figure 1).



**Figure 1** Pedigrees of a Japanese (the upper) and Korean (the lower) ADNFLE family. Only members-assigned numbers were evaluated.



**Figure 2** Schematic representation of SNPs around the *CHRNA4* gene and overview of all *CHRNA4* mutations identified in ADNFLE. All the mutations are located in the second transmembrane domain (M2). Affected nucleotides are in larger italic fonts and CpG dinucleotide sites are underlined.

Samples were collected from the two families and DNA was extracted from whole blood using standard protocols. A genetic analysis was carried out for seven members of the Japanese and eight of the Korean family. Among them, four of the Japanese had ADNFLE, as did eight of the Koreans. Four single nucleotide polymorphisms (SNPs) around the *CHRNA4* were selected from a database of Japanese single nucleotide polymorphisms (JSNPs: [http://snp.ims.u-tokyo.ac.jp/index\\_ja.html](http://snp.ims.u-tokyo.ac.jp/index_ja.html)). To identify SNP-based haplotypes, four SNPs including rs 6089899, rs2093107, rs4809538 and rs4603829 (Figure 2) were typed by fluorescent sequencing on an automated DNA sequencer. Sequencing was carried out in both directions using four primer pairs. For comparison, the genotype frequencies of HapMap-JPT are listed in Table 1a.

We next examined the evolutionary conservation and phenotypic effect of Ser280Phe and Ser284Leu, which have shown recurrent occurrence in *CHRNA4*. The evolutionary conservation was estimated using phyloP score, available from UCSC Genome Browser (UCSC Genome Bioinformatics: <http://genome.ucsc.edu/>). The phenotypic effect of mutant protein was predicted using SIFT score (<http://sift.jcvi.org/>). In the phyloP, the conserved nucleotides are assigned positive scores, and the fast-evolving ones negative scores; the SIFT score ranges from 0 to 1. Deleterious amino-acid substitutions are assigned the scores of <0.05; tolerable substitutions are  $\geq 0.05$ .

We also checked hypermutable sites in *CHRNA4*. In the hypermutable sites analysis, we focused on CpG hypermutability. CpG is known as one of the major causes of codon substitution in mammalian genes, and is used to refer to cytosine followed by guanine in the Watson–Crick pair of a cytosine and guanine. The coding sequence of *CHRNA4* was analysed and the adjacent nucleotides were taken into account to distinguish CpG hypermutations from non-CpG transitions.

## RESULTS

As shown in the pedigrees (Figure 1), there were five affected members and an obligate carrier in the Japanese family and nine affected members in the Korean one. Mutation in the two pedigrees showed an autosomal dominant mode with incomplete penetrance. All the affected individuals had a seizure semiology consistent with frontal lobe epilepsy and carried the Ser284Leu mutation in the *CHRNA4*. Both families had similar clinical manifestations, and their electroencephalogram findings were consistent with those of ADNFLE. They had brief motor seizures during sleep and no auras were reported. They shared features such as drug resistance and mental retardation, which are uncommon findings in ADNFLE. Still, SNP-based haplotypes were different in each family (Table 1b).

Ser280Phe and Ser284Leu showed higher phyloP scores (5.88 and 5.80, respectively) than the other nucleotides in *CHRNA4* (mean, 0.08). SIFT scores were lesser than 0.05 (0.00, each). CpG dinucleotide sites are shown in Figure 2. Nine CpG dinucleotide sites were detected in the coding sequence of *CHRNA4*, three of them in the second transmembrane domain (M2). Among the four *CHRNA4* mutations, only Ser284Leu mutation is associated with a CpG hypermutable site.

## DISCUSSION

All *CHRNA4* mutations identified so far in ADNFLE have been located only in the M2 region, which has one of the most important functional roles in the neuronal nicotinic acetylcholine receptor. This receptor has a pentameric structure comprised of various combinations of alpha and beta subunits encoded by *CHRNA4* and *CHRN2*. The overall tertiary structure of nicotinic acetylcholine receptor subunits is similar; each subunit comprises four segments (M1 to M4) of the transmembrane domain and the N and C termini on the extracellular side of the membrane. M2 lines the central pore of the receptor and determines the ion selectivity of the receptor.<sup>26</sup>

**Table 1 Genotype frequency (a) and SNP-based haplotypes in the two families (b)**

RefSNP	HGVS names	Genotype frequency (HapMap-JPT)		
<i>(a)</i>				
rs6089899	NT_011333.6:g.735939G>A	AA 0.13	AG 0.40	GG 0.47
rs2093107	NG_011931.1:g.3754T>C NT_011333.6:g.730573A>G	AA 0.11	AG 0.53	GG 0.36
rs4809538	NT_011333.6:g.706807G>A	AA 0.11	AG 0.47	GG 0.42
rs4603829	NT_011333.6:g.705523C>T	CC 0.13	CT 0.53	TT 0.33
		<i>rs 6089899</i>	<i>rs 2093107</i>	<i>rs 4809538</i>
				<i>rs 4603829</i>
<i>(b)</i>				
<i>Japanese family</i>	<b>G</b>	<b>G</b>	<b>G</b>	<b>T</b>
I-1	G/G	G/G	G/G	T/T
II-1	G/G	G/G	A/G	C/T
II-2	G/G	G/G	G/G	T/T
II-3	ND	G/G	ND	T/T
III-1	A/G	G/G	G/G	T/T
III-2	A/A	G/G	G/G	T/T
III-3	ND	G/G	G/G	T/T
<i>Korean family</i>	<b>G</b>	<b>A</b>	<b>A</b>	<b>C</b>
II-1	A/G	G/A	ND	C/T
II-2	G/G	A/A	A/A	C/C
III-1	G/G	G/A	A/G	C/T
III-2	G/G	G/A	A/G	C/T
III-3	G/G	A/A	A/A	C/C
III-4	G/G	A/A	A/A	C/C
III-5	G/G	A/A	A/A	C/C
III-6	G/G	A/A	A/A	C/C

Abbreviations: HGVS, Human Genome Variation Society (<http://www.hgvs.org/>); SNP, single nucleotide polymorphism. Bold letters represent the most common allele of each SNPs.

The results obtained here do not support the hypothesis that the Ser284Leu mutations originated with a common founder. Association with a CpG site accounts for the independent occurrence of the Ser284Leu mutation. The other three mutations, however, are not associated with hypermutable sites such as homonucleotide runs, direct and inverted repeats and CpG dinucleotide sites.<sup>27</sup> Thus, the repetitive occurrence of Ser280Phe is not easily understood.

To explain it, we need to take into account ADNFLE development. One possible explanation is that ADNFLE is caused by mutations on a few functionally important sites within the M2.<sup>14</sup> Together with S280F, *in vitro* expression studies indicate that all CHRNA4 mutations increase receptor sensitivity to acetylcholine, suggesting gain of function.<sup>28,29</sup> In our results, both S280F and S284L showed high phyloP scores and low SIFT scores, indicating that the affected nucleotides are highly conserved and that their amino-acid substitutions will be deleterious.

Another explanation is that rare mutations have strong phenotypic effects in complex disorders.<sup>30</sup> A recent report uncovered significant excess of rare variants in neurological disorders, providing a 'rare allele-major effect model', and suggesting that the rare variants or *de novo* mutations in neurologically expressed genes are more likely to accumulate.

This is the first report comparing the haplotype structure of Japanese and Korean ADNFLE families. Further functional studies will be required to ascertain the ADNFLE's pathophysiology.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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