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Neuronal nicotinic receptors in human epilepsy

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Abstract

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is a rare monogenic idiopathic partial epilepsy characterized by clusters of frontal lobe motor seizures during sleep. Recently, it has been shown that mutations of the chromosome-20q-located neuronal nicotinic acetylcholine receptor α4-subunit (CHRNA4) are associated with ADNFLE in some families, but that other families are not linked to this locus. Both CHRNA4 mutations (Ser248Phe and 776ins3) identified so far are found in the pore-forming second transmembrane region of the gene. Electrophysiological studies showed that mutations in this functional important part of the receptor subunit have a profound effect on the permeability for calcium ions. Interestingly, the Ser248Phe mutation was found again in a second ADNFLE family. Haplotype analysis excluded a founder effect and showed that Ser248Phe occurred independently twice. This provides the possibility to study the effect of the same mutation on different genetic backgrounds. Several attempts have been made to identify additional genes responsible for ADNFLE. But despite some positive linkage results including the CHRNA3–CHRNA5–CHRNB2 cluster on chromosome 15q24, no further mutations have been found so far. The mutation screening of functionally important parts of CHRNA5 in 12 ADNFLE patients did not support a causative role of this nicotinic acetylcholine receptor subunit. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Acetylcholine receptor; Epilepsy; Mutation

1. Introduction

Epilepsies can be roughly divided into symptomatic and idiopathic disorders. Idiopathic epilepsies are a heterogenous group of conditions characterized by different types of seizures, ages of onset, and EEG features. By definition, idiopathic epilepsies show no underlying cause other than a possible inherited predisposition (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). For most syndromes, especially the common ones, the mode of inheritance is complex rather than monogenetic. For example, juvenile myoclonic epilepsy or the absence epilepsies of childhood and juvenile age are probably either caused by oligogenic or polygenic inheritance. In oligogenic inheritance, a few genes are involved, their gene products interfering with each other and with the phenotype. In polygenic inheritance, however, a large number of genes might be involved, and a given single gene might have only a very small impact on the disease. But some rare idiopathic epilepsies show an autosomal dominant mode of inheritance, and so far, genetic studies have been most successful in these diseases. To date, gene defects underlying three different idiopathic epilepsies have been discovered: autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (Steinlein et al. 1995), benign familial neonatal convulsions (BFNCs) (Biervert et al. 1998; Charlier et al. 1998; Singh et al. 1998) and generalized epilepsy with febrile seizures plus (GEFS +) (Wallace et al. 1998).

2. Autosomal dominant nocturnal frontal lobe epilepsy ADNFLE

2.1. The phenotype

ADNFLE was the first human idiopathic epilepsy to be linked to a specific gene defect. The disease is characterised by clusters of brief nocturnal motor seizures, which occur mostly during light sleep, either shortly after falling asleep or in the early morning hours. The disease shows considerable intrafamilial variation in age of onset as well as in severity. In more than half of the patients, the seizures start in the first or second decade of life, but onset

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later in life has also been observed. Seizures might be preceded by an aura, and can start with a gasp or grunt, or a vocalisation. The motor features are described as thrashing hyperkinetic activity or tonic stiffening with superimposed clonic jerking. Secondary generalization with loss of consciousness can occur, but the majority of patients remain conscious through most of their seizures. Interictal EEG abnormalities are rare, and for anatomical and technical reasons, ictal recordings often show nonspecific or inconclusive patterns. Therefore, nocturnal videopolysomnography is most helpful for differential diagnosis. Without it, ADNFLE is often misdiagnosed as, e.g., benign nocturnal parasomnia, night terror, hyperactivity, or hysteria (Scheffer et al., 1994; Oldani et al., 1996; Hayman et al., 1997).

2.2. The CHRNA4 locus on chromosome 20

A gene locus for ADNFLE has been assigned to the genomic region 20q13.3 by linkage analysis in a large Australian pedigree of European origin (Phillips et al., 1995). In this family, 27 individuals were affected by nocturnal seizures. Surprisingly, another monogenic idiopathic epilepsy has been previously assigned to the very same genomic region: BFNCs. However, it is now known that this epilepsy is caused by mutations in a different gene which belongs to the class of voltage-gated potassium channels (Biervert et al., 1998; Singh et al., 1998).

After the localisation of an ADNFLE gene on chromosome 20q13.3, further studies showed a point mutation in the DNA from the affected members of the Australian family. The mutation leads to an amino acid exchange (Ser248Phe mutation) in the second transmembrane region of the α4-subunit gene of the neuronal nicotinic acetylcholine receptor (CHRNA4) (Steinlein et al. 1995). It was found in all affected family members, as well as in obligate carriers and some non-manifesting individuals. Contributing to the walls of the ion channel, the second transmembrane region presents one of the most important functional parts of a nicotinic acetylcholine receptor subunit. Electrophysiological experiments in *Xenopus* oocytes were carried out by coexpression of the mutant subunit together with the wild type β2-subunit. These experiments revealed a profound effect of the mutation on the function of the receptor. The mutant receptor exhibited an accelerated desensitization rate as well as a prolonged resensitization time, possibly indicating a destabilization of the ion channel open configuration (Weiland et al., 1996). The apparent affinity to acetylcholine was decreased sevenfold, and agonist concentrations 3000 times lower as in controls were sufficient to cause desensitization of the mutant receptor (Bertrand et al., 1998).

The observation of a second mutation (776ins3) in an Norwegian ADNFLE family supports the etiological role of CHRNA4 in ADNFLE. An insertion of three additional nucleotides into the extracellular end of the second transmembrane region of the CHRNA4 gene was found to be associated with the epilepsy in this family (Steinlein et al. 1997). Electrophysiological studies performed with this mutant showed that although it reconstitutes a functional channel if co-expressed with the β2-subunit, the receptor channel permeability for calcium is significantly reduced. The 776ins3 mutation causes a 10-fold increase in the apparent affinity for acetylcholine. The data suggested that this mutation increases the probability for the receptor of a transition to the active state (Bertrand et al., 1998). Both the Ser248Phe and the 776ins3 mutations lead to a reduced calcium influx and probably cause ADNFLE by hypoactivity of CHRNA4 protein containing neuronal nicotinic receptors.

Recently, another family has been identified in which ADNFLE is associated with the Ser248Phe mutation in the CHRNA4 gene (Steinlein et al, in preparation). This second family was collected in Norway; thus, it was possible that the mutation in both this and the Australian family was due to a common founder effect. However, analysis of two polymorphisms close to the Ser248Phe mutation did not support this hypothesis. Sequencing of parts of exon 5 showed that in the Norwegian Ser248Phe family, the rarer alleles of both the bp555/*Fok*I and the bp594/*Cfo*I polymorphisms (Steinlein, 1995; Guipponi et al., 1997) were coupled in *cis* to the Ser248Phe mutation, while in the Australian families, both the common alleles were found. Thus, the mutations must have occurred independently in both families, suggesting that ADNFLE causing mutations in the CHRNA4 gene might not only be restricted to certain functionally important parts of the gene but that the character of the mutation is important for the etiology of the disease.

We now tested the possibility that other nicotinic acetylcholine receptor subunits might be involved in the etiology of ADNFLE by screening the transmembrane regions 1–3 of the α5-subunit gene of the neuronal nicotinic acetylcholine receptor (CHRNA5).

3. Materials and methods

3.1. Subjects

DNA was collected from 12 independent patients with nocturnal frontal lobe epilepsy. Each patient had at least one family member which also reportedly was affected by nocturnal frontal seizures (mean number of affected family members 2.75, SD 2.09, range from one to seven affected family members). Nine patients came from Germany, one from Spain, one from the Netherlands and one from England. All patients had frequent nocturnal motor attacks, but no seizures during daytime. Neurological and neuroradiological examination showed no abnormalities. Mean dura-

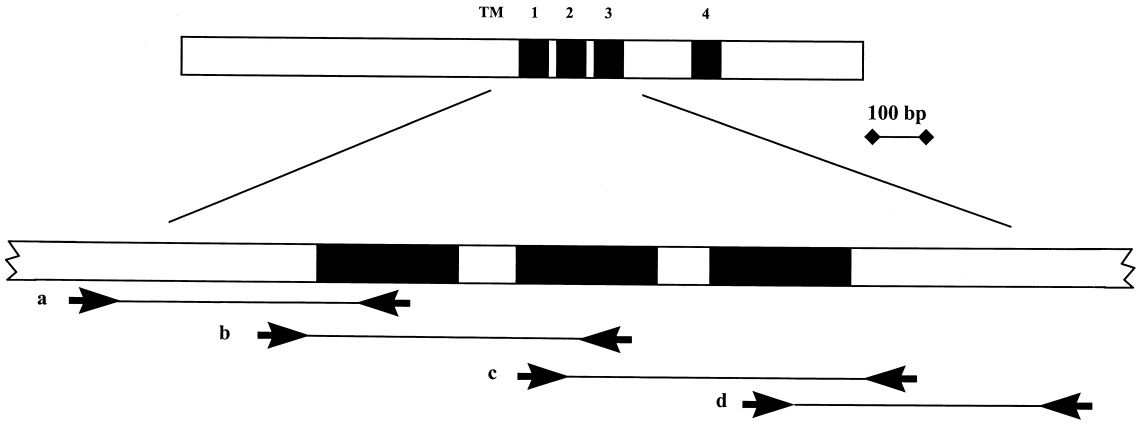

Fig. 1. Schematic presentation of CHRNA5 mutation screening. Upper part: cDNA structure of CHRNA5 including the four transmembrane regions (TM). Lower part: localisation of primer pairs within the TM 1–3 area. To avoid amplification of homologous sequences from other nicotinic acetylcholine subunits, one primer of each pair was placed outside the highly conserved transmembrane regions.

tion and frequency of seizures varied between the patients. None of the families was large enough for linkage analysis. All patients had been previously tested negative for mutations in the CHRNA4 gene (Steinlein et al., unpublished results).

3.2. Single-strand confirmation analysis (SSCA) and heteroduplex analysis

The functionally important transmembrane domain regions 1–3 of the CHRNA5 gene were screened for mutations by SSCA and heteroduplex analysis (Fig. 1). Primer pairs for four overlapping polymerase chain reaction (PCR) fragments were designed and optimised. Primer pair a: 5′ AGAGGACCAAGATGTAGACAAGA and 5′ CAGGGTATTATAAGGAACAAGGT (180 bp). Primer pair b: 5′ ATTTGTAATCAAGCGCCTGCCT and 5′ GGTATGATCTCTTCAATAACCAG (182 bp). Primer pair c: 5′’ TGTCTCTGCACTTCAGTACTTGT and 5′ GCCATGGCATTATGTGTTGAGGA (198 bp). Primer pair d: 5′ CTGTCAATTATGGTAACCGTCTT and 5′ GAGTGAAGTACCTGTCTACATGA (144 bp).

PCR was performed with 50 ng genomic DNA, 10 pmol of each primer, 200 μM dNTP, 1.5 mM $MgCl_2$, 50mM KCl, 10 mM Tris–HCl, 2.5 U Taq Polymerase (Gibco BRL) in a total volume of 25 μl. PCRs were carried out in a Thermocycler 9600 (Applied Biosystems). For SSCA, 10 μl PCR products were mixed with 14 μl formamide-containing loading buffer (95% formamide, 20 mM EDTA, 0.05 bromphenol blue, 0.05% xylene cyanole) and heat-denatured. Five to seven microliters of the buffer/PCR mix was loaded onto 10% polyacrylamide gels. The fragments were separated overnight at 6–7 V/cm at room temperature and at 4°C and then silver-stained (Budowle et al., 1991). For heteroduplex analysis, 5 μl of the PCR product was denatured (95°C, 5 min), re-annealed (50°C, 30 min), and mixed with 1.5 μl loading buffer. Re-annealed products were separated on a 10% gel at 18 V/cm at room temperature for 2 h and then silver-stained.

4. Results

Because the exon–intron structure of the CHRNA5 gene has not been analysed, mutation analysis from genomic DNA can only be performed for certain parts of the gene. One of the functionally most important parts of nicotinic acetylcholine receptor subunits is the second transmembrane region which contributes to the wall of the ion channel. So far, all mutations identified in ADNFLE families are located in this specific structure. Thus, the analysis of the second transmembrane region and its vicinity can be used as a first screening test for a candidate gene. Here, SSCA and heteroduplex analysis of the transmembrane regions 1–3 of the CHRNA5 gene in 12 independent patients with familiar nocturnal frontal lobe epilepsy did not reveal any mutations in this area.

5. Discussion

The neuronal nicotinic acetylcholine receptors have a pentameric structure which can contain either only α-subunits or both α- and β-subunits. So far, 11 distinct subunits have been identified in different species, most of them expressed in human brain (Sargent, 1993) (Table 1). Because ADNFLE was found to be a heterogenous disease with so far only three families linked to CHRNA4, other subunit genes can be considered as candidate genes. Linkage studies indicated a possible second locus for ADNFLE on chromosome 15q24, close to a cluster of three other acetylcholine receptor subunit genes (Phillips et al., 1998). However, molecular studies of the CHRNA3, CHRNA5 and CHRNB4 genes located in this region so far did not show mutations. Here we screened the functionally important first three transmembrane domain regions of CHRNA5

Table 1
Cloned subunits of the human neuronal nicotinic acetylcholine receptor

CHRNA2	α2 subunit	8p21[a]
CHRNA3	α3 subunit	15q24[a]
CHRNA4	α4 subunit	20q13.3[a,b]
CHRNA5	α5 subunit	15q24[a]
CHRNA7	α7 subunit	15q14[c]
CHRNB2	β2 subunit	1q21–q22[a,d]
CHRNB3	β3 subunit	8[a]
CHRNB4	β4 subunit	15q24[a]

[a]Anand and Lindstrom (1992).
[b]Steinlein et al. (1994).
[c]Chini et al. (1994).
[d]Rempel et al. (1998).

in 12 patients with familiar nocturnal frontal lobe epilepsy without finding any mutations. However, considering the heterogeneous etiology of nocturnal frontal lobe epilepsy, ADNFLE probably accounts only for a small number of cases. In the majority of patients, the disease might be due to complex rather than monogenic inheritance. Thus, although all our patients had a positive family history, the negative findings reported here do not necessarily exclude CHRNA5 as a candidate gene for ADNFLE.

Another putative gene locus for ADNFLE would be the CHRNA7 locus on chromosome 15q14 (Chini et al., 1994). This locus has been previously discussed as a candidate gene for myoclonic epilepsy (Elmslie et al., 1997; Sander et al., 1999) and Rolando epilepsy (Neubauer et al., 1998). However, mutation analysis of this gene is complicated by the complex genomic structure including partial gene duplication in this region (Gault et al., 1998) and no mutations have been found so far.

The genomic organisations and exon–intron boundaries of the CHRNA3 and CHRNB2 genes have been recently published (Rempel et al., 1998). CHRNB2 and CHRNA4 together contribute to one of the most abundant receptor subtypes in mammalian brain (Whiting and Lindstrom, 1987). The sequence information provided the possibility to analyse both genes in patients with ADNFLE. However, screening of eight unrelated individuals did not reveal any mutation, excluding both genes as a common cause of this partial idiopathic epilepsy (Rempel et al., 1998).

In summary, familial frontal lobe epilepsy is a heterogeneous disorder for which one minor gene locus has been identified in chromosome 20q, but additional loci are still waiting to be discovered.

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