

Genetic basis of autosomal dominant nocturnal frontal lobe epilepsy

Agata RÓŻYCKA, Wiesław H. TRZECIAK

Department of Biochemistry and Molecular Biology, Karol Marcinkowski University of Medical Sciences, Poznań, Poland.

Abstract. In this review the current literature regarding autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is presented and discussed. This disease is caused by mutations of genes coding for subunits of neuronal acetylcholine receptor comprising the sodium/potassium ion channel. To date, three types of mutations of the gene encoding $\alpha 4$ subunit of acetylcholine receptor were described in multi-generation families in Australia, Spain, Norway and Japan. Two other types of mutations of the $\beta 2$ subunit were also reported in two families, one from Italy and the other from Scotland. Mutations were caused by substitutions of a single nucleotide or by several-nucleotide insertions and result in a decrease or an increase in the activity of the receptor, or its changes in the affinity to the ligand. Recent advances in molecular genetics have provided the means for a better understanding of human epileptogenesis at a molecular level, which facilitates clinical diagnosis and provides a more rational basis of therapy of this form of epilepsy.

Key words: sodium-potassium channel, neuronal nicotinic acetylcholine receptor, nocturnal frontal lobe epilepsy.

Introduction

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) was first described in 1994 and has been classified as one of the forms of partial epilepsy (SCHEFFER et al. 1994). This syndrome is characterized by clusters of brief sei-

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Correspondence: W.H. TRZECIAK, Department of Biochemistry and Molecular Biology, K. Marcinkowski University of Medical Sciences, ul. Świącickiego 6, 60-781 Poznań, Poland, e-mail: trzeciak@am.poznan.pl

Table 1. Mutations of *CHRNA4* and *CHRN2* genes in patients with ADNFLE

Chromosome	Gene (OMIM)	Mutation designation		Influence on receptor function	Country ^c	References
		nucleotide variation	amino acid variation			
20q13.2	<i>CHRNA4</i> (#600513)	C743T ^a	S248F ^a	acceleration of desensitisation; reduced Ca ²⁺ permeability	Australia	STEINLEIN et al. 1995
		C755T ^b	S252F ^b	loss of function	Norway	STEINLEIN et al. 2000
		C755T ^a	S252L ^a	acceleration of desensitisation; loss of function	Japan	HIROSE et al. 1999
		C767T ^b	S256L ^b		Lebanon	PHILLIPS et al. 2000
		776insGCT ^a	259insL ^a	increased affinity to acetylcholine; reduced Ca ²⁺ permeability; loss of function	Norway	STEINLEIN et al. 1997
		788insGCT ^b	263insL ^b			
1q21	<i>CHRN2</i> (#605375)	G1025C ^b	V287L ^b	retardation of desensitisation; gain of function	Italy	FUSCO et al. 2000
		G1025A ^b	V287M ^b	increased affinity to acetylcholine; gain of function	Scotland	PHILLIPS et al. 2001

^a According to *Torpedo* $\alpha 4$ -subunit gene numbering (BEAUDET, TSUI 1993);

^b Guidelines for human gene mutation nomenclature (DUNNEN, ANTONARAKIS 2000);

^c In all reports, the relevant mutation was always identified in a single family;

All genetic abnormalities identified so far are heterozygous mutations located in the TM2 domain of each subunit.

zures occurring during the non-rapid eye motion (non-REM) phase of sleep. These symptoms begin predominantly in childhood and persist into adulthood. In most patients, they are mild (often misdiagnosed as nightmares and parasomnias) and respond well to antiepileptic drugs, such as carbamazepine (CBZ) (SCHEFFER et al. 1995). As in most focal epilepsies, interictal EEG abnormalities are rare, and nocturnal video-polysomnography and gene analysis are most helpful for making an accurate diagnosis of ADNFLE (OLDANI et al. 1998). This form of epilepsy is the first idiopathic epilepsy for which specific mutations have been found. It follows autosomal dominant type of inheritance with about 70% penetrance and considerable intrafamilial variation in severity. Linkage studies performed in a single large Australian family assigned the gene to chromosome 20q13.2 (STEINLEIN et al. 1994). This region of chromosome 20q contains

a candidate gene, *CHRNA4*, which encodes the $\alpha 4$ subunit of the nicotinic acetylcholine receptor (nAChR).

However, in most families with ADNFLE the linkage of the disease to the *CHRNA4* gene has been excluded (OLDANI et al. 1998, TENCHINI et al. 1999). The lack of involvement of $\alpha 4$ subunit in a subset of families with ADNFLE makes the other neuronal nAChR subunit genes principal candidates, especially the gene encoding the $\beta 2$ subunit (*CHRNA2*), which combines with $\alpha 4$ to form the major species of brain nAChR in humans. Only five different mutations (four missense mutations and one small insertion) in the two genes (*CHRNA4* and *CHRNA2*) have been associated with ADNFLE (Table 1). Interestingly, both gene mutations reside within the region coding for TM2, which forms the ion channel pore, thus disrupting the electrophysiological properties of the receptor. All genetic abnormalities identified so far comprise heterozygous mutations, responsible for only a minority of cases of ADNFLE. In most ADNFLE families, the genetic background of the disease remains unknown.

The nicotinic acetylcholine receptor: structure and function

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels present in the central and peripheral nervous systems (UNWIN 1995). To date, 9 distinct genes encoding neuronal nAChR subunits ($\alpha 2$ to $\alpha 10$ and $\beta 2$ to $\beta 4$) have been found in various species (MC GEHEE 1999). The subunits co-assemble to form hetero- or homo-pentameric nAChR channels (Figure 1). Subunit composition determines the electrophysiological and pharmacological properties of the nAChR channels. Functional receptors, as homomers, can be obtained by assembling $\alpha 7$, $\alpha 8$ and $\alpha 9$ subunits, whereas other α subunits may co-assemble with at least one type of β subunit to form heteromers. Receptors composed of $\alpha 4/\beta 2$ (with or without $\alpha 5$) seem to be the most abundant nAChRs in the brain. In mammalian brains, the $(\alpha 4)_2(\beta 2)_3$ and $\alpha 4$ variants comprise the major AChR subtype, with high affinity to the ligand (GOTTI et al. 1997).

All nAChR channels are permeable to sodium, potassium and calcium ions. They display strong inward rectification resulting in activity at only hyperpolarised or resting membrane potentials (ALBUQUERQUE et al. 1995), and their conductance depends on the concentration of extracellular calcium ions, which are considered to be particularly important for their presynaptic function. The release of numerous neurotransmitters can be regulated via the activation of presynaptic nAChRs, including the secretion of both excitatory and inhibitory transmitters, such as acetylcholine (ACh), dopamine, γ -aminobutyrate (GABA), glutamate, norepinephrine and serotonin (SUMMERS, GIACOBINI 1995). Neurotransmitter release may be affected by Ca^{2+} influx through presynaptic nAChRs,

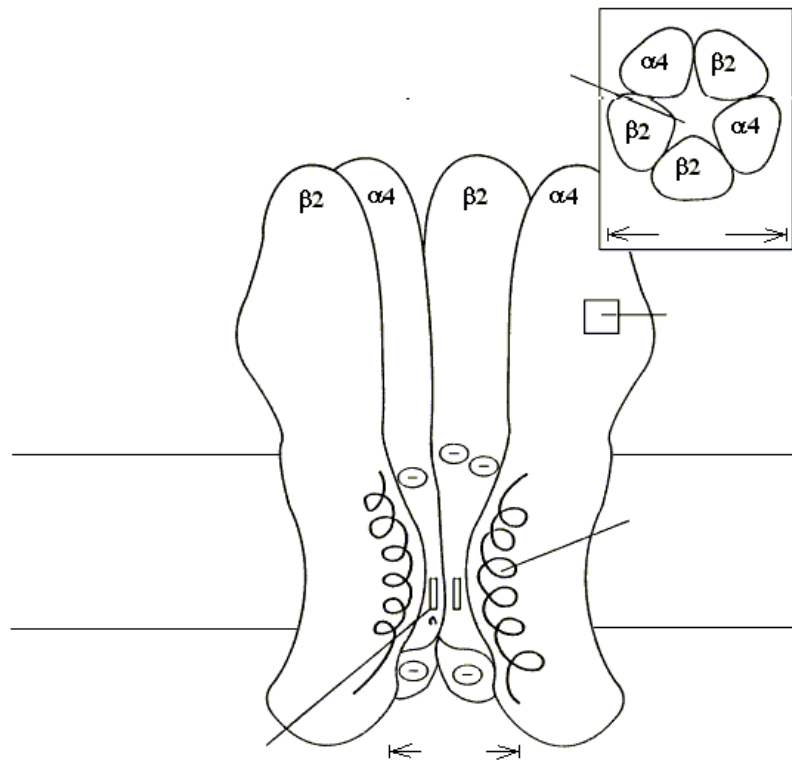


Figure 1. The structure of neuronal acetylcholine receptor in an open state (from UNWIN 1995, modified).

enhancing the fusion of vesicles with presynaptic membrane. On the basis of the notion that nAChR plays an excitatory role in neuronal transmission, it had been hypothesised that dysfunctional AChR might impair GABA-ergic synaptic transmission between neocortical neurons, since defects of this system are often associated with the occurrence of paroxysmal activity and consequently might induce seizures (WEILAND et al. 1996).

Each nAChR subunit contains four segments of transmembrane domain (TM1-TM4) and a large extracellular N-terminal domain that contains the ligand-binding site (Figure 2). The presence or absence of specific amino acids involved in binding of acetylcholine, makes it possible to distinguish between α -subunits and other subunits. Characteristically, α -subunits contain adjacent cysteine residues in the N-terminal region (amino acid positions: 197 and 198) that are thought to form a disulphide bond (KAO, KARLIN 1986). All TM2 segments of each nAChR subunit form the walls of the channel pore and therefore they are crucial both for the ion selectivity of the receptor and the energy level for allosteric transition. In the absence of acetylcholine, they remain close to the central axis, thus closing the gate of the ion channel. After binding of two molecules

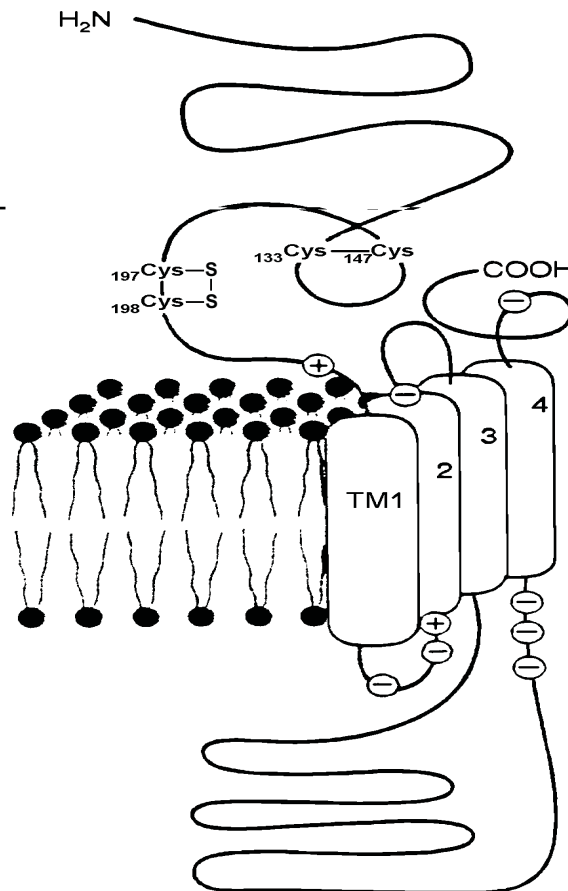


Figure 2. The structure of 4 sub-unit of neuronal acetylcholine receptor (from KAO, KARLIN 1986, modified).

of agonist to the ligand-binding sites, the protein undergoes a major change in the three-dimensional structure (Figure 3). This allosteric transition to the open state allows the flow of ions through the pore. When confronted with ACh for a long time, the receptor enters a number of desensitised states, in which the ligand is tightly bound with high affinity, but the channel is non-conducting (AUERBACH, AKK 1998).

The *CHRNA4* gene: structure and mutations

Although the ADNFLE phenotype seems clinically homogeneous, there are various of molecular defects responsible for this syndrome. So far, a causative role of a defect in neuronal nAChR in human disease has been found only for a partial epilepsy syndrome. This is interesting with respect to the finding that nAChRs are

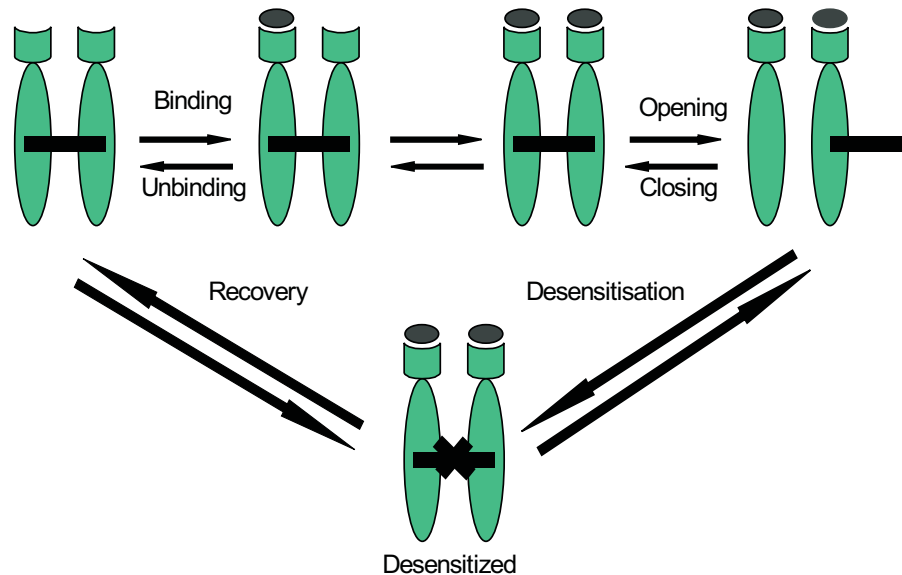


Figure 3. Allosteric states of the nicotinic acetylcholine receptor (from AUERBACH, AKK 1998, modified). When the receptor binds two molecules of agonist, the protein may enter a conducting conformation. The open channel may adopt a desensitized state in which the channel is nonconducting and the agonist is bound with high affinity.

most likely located presynaptically and the predominant role of this receptor type is to modify neuronal excitability. The role of mutations in the *CHRNA4* gene in ADNFLE has been supported by the finding of three site-specific mutations within exon 5 of the *CHRNA4* gene, encoding the TM2 segment of the $\alpha 4$ subunit (Table 1). The *CHRNA4* gene is located on chromosome 20q13 and contains six exons (Figure 4). The exact size of the first and the last exon is not known (STEINLEIN et al. 1996). Exon 1 encodes the ATG start codon and the 5'-untranslated region. Exons 2 to 4 are rather small (44 to 152 bp) and introns vary in size from 1.0 kbp (intron 1) to 5.5 kbp (intron 4). The open reading frame encodes 627 amino acid residues. The hydrophobic transmembrane domains I to III are located in the large exon 5 (harbouring the main part of the coding region). Exon 6 codes for transmembrane domain IV as well as the translation stop codon and the 3'-untranslated region.

Two mutations in the *CHRNA4* gene: S252F and S256L substitutions (corresponding to S248F and S252L, basing on the sequence of *Torpedo* α -subunit) (BEAUDET, TSUI 1993), have been found (STEINLEIN et al. 1995, SAENZ et al. 1999, HIROSE et al. 1999, STEINLEIN et al. 2000, PHILLIPS et al. 2000). An insertional mutation in the *CHRNA4* gene, 263insL (259insL corresponding to the sequence of *Torpedo*), was detected in a Norwegian family with ADNFLE

Studies of other genes

Linkage studies have presented evidence for yet other genes involved in the pathogenesis of ADNFLE. They are located on chromosome 15q24, within a region containing the *CHRNA3/CHRNA5/CHRNA4* clusters of nAChR subunits (PHILLIPS et al. 1998). Indeed, three types of nocturnal frontal lobe epilepsies have been linked to the loci encoding subunits of nAChR (ENFL1 for *CHRNA4*, ENFL2 for *CHRNA3/CHRNA5/CHRNA4* and ENFL3 for *CHRNA2*) [OMIM: 600513, 603204 and 605375 respectively]. Recently, it has been shown that ADNFLE can also be caused by mutations in the corresponding *CHRNA2* gene (Table 1). So far, two unrelated families with ADNFLE, carrying two different substitutions of the same amino acid residue in the β 2-subunit (V287L and V287M) have been described (FUSCO et al. 2000, PHILLIPS et al. 2001). Intriguingly, the two mutations exhibit gain of function and confer hyperactivity of the receptor in different ways. The V287L mutation leads to retardation of desensitization of the mutant nAChRs, while receptors harbouring V287M exhibit increased sensitivity to acetylcholine. Although ADNFLE, resulting from *CHRNA2* abnormalities, is referred to as ENFL2, its phenotype is discernible from that of ENFL1, which is caused by *CHRNA4* mutations. Electrophysiological properties of *CHRNA2* and *CHRNA4* mutants are apparently different from each other *in vitro*. The accelerated desensitization and the reduced Ca^{2+} permeability of nAChRs containing the α 4 subunit could result in an overall reduced presynaptic cation influx and lower secretion of inhibitory transmitters (e.g. GABA), thereby facilitating the genesis of an inappropriate excitation of postsynaptic neurons. Alternatively, at the postsynaptic membrane, the altered electrophysiological features of the receptor may cause an impaired depolarisation, which leads to the reduced inhibition and overexcitation of communicating neurons.

Concluding remarks

Uncovering the exact pathogenetic mechanisms of ADNFLE *in vivo* may offer a clue to comprehensively understand common epilepsies, beyond issues of neuronal excitability as a focal event (HIROSE et al. 2000). A comprehensive search for mutations in candidate genes is needed to evaluate the possibility that these genes contribute to the lowered seizure threshold in idiopathic epilepsies. Moreover, recent advances in molecular genetics have provided the means for better understanding of human epileptogenesis at a molecular level, which facilitates clinical diagnosis and provides a more rational basis of therapy for this form of epilepsy.

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