AChR deficiency due to ε-subunit mutations: two common mutations in the Netherlands

Catharina G. Faber · Peter C. Molenaar · Johannes S. H. Vles · Domenic M. Bonifati · Jan J. G. M. Verschuuren · Pieter A. van Doorn · Jan B. M. Kuks · John H. J. Wokke · David Beeson · Marc De Baets

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Abstract Congenital myasthenic syndromes are a clinically and genetically heterogeneous group of hereditary disorders affecting neuromuscular transmission. We have identified mutations within the acetylcholine receptor (AChR) ε-subunit gene underlying congenital myasthenic syndromes in nine patients (seven kinships) of Dutch origin. Previously reported mutations ε1369delG and εR311Q were found to be common; ε1369delG was present on at least one allele in seven of the nine patients, and εR311Q in six. Phenotypes ranged from relatively mild ptosis and external ophthalmploegia to generalized myasthenia. The common occurrence of εR311Q and ε1369delG suggests a possible founder for each of these mutations originating in North Western Europe, possibly in Holland. Knowledge of the ethnic or geographic origin within Europe of AChR deficiency patients can help in targeting genetic screening and it may be possible to provide a rapid genetic diagnosis for patients of Dutch origin by screening first for εR311Q and ε1369delG.

Keywords Congenital myasthenic syndrome · AChR ε-subunit gene · AChR mutations

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Abbreviations

- BuTx $^{125}$I-
- Bungarotoxin

AChR Acetylcholine receptor
CHRNE AChR $\varepsilon$-subunit gene
CMS Congenital myasthenic syndrome
PCR Polymerase chain reaction

Introduction

The congenital myasthenic syndromes (CMSs) are a group of hereditary disorders affecting neuromuscular transmission [1–3]. The disorders are clinically and genetically heterogeneous [4]. Patients frequently present at birth or within the first two years of life. Most present with ocular, bulbar or respiratory muscle symptoms, worsened by crying or activity. The majority of CMSs primarily affect postsynaptic function, and are caused by mutations in subunits of the acetylcholine receptor (AChR), in the AChR-clustering protein rapsyn, or in DOK7. The AChR mutations may alter kinetic properties (slow- and fast-channel syndromes), decrease expression of the AChR pentamer (AChR deficiency) [5] or alter kinetic properties and decrease expression. Many mutations that decrease AChR expression reside in the AChR $\varepsilon$-subunit gene [6], with the resulting AChR deficiency phenotype inherited as an autosomal–recessive trait [3, 5, 7].

CMSs are relatively rare disorders that may be difficult to diagnose in infants and early childhood. Many of the AChR $\varepsilon$-subunit gene mutations are restricted to a single or only a few kinships. Consequently, genetic screening for AChR deficiency frequently requires thorough analysis of the whole of the $\varepsilon$-subunit gene. Here we present the cases of nine patients (seven kinships) for whom the diagnosis of congenital myasthenia has recently been confirmed by genetic analysis. We find a common occurrence of mutations $\varepsilon$R311Q and $\varepsilon$1369delG in AChR deficiency patients of Dutch origin.

Patients and methods

We identified nine CMS patients in the Netherlands (Table 1). All patients were of Dutch origin and Caucasian. Patients 3–8 have been reported before by the late Dr. Oosterhuis before mutation analysis was performed [8]. Antibodies to the AChR were absent in all cases. Patients generally presented with ptosis and ophthalmoparesis from infancy or early childhood. Some of them had swallowing difficulties or proximal muscle weakness later on. Neostigmine had a moderate effect in most patients. Some patients remained stable throughout life, whereas others slowly declined in muscle strength.

Patient 1, a 14-year-old boy with non-consanguine parents, presented at the age of two and a half years with ptosis and ophthalmoparesis. The ptosis had been present from birth. He remained stable until the age of 10, when he presented with a rapid progressive weakness involving his arms, legs and bulbar muscles, following a flu-like disease. These symptoms resolved spontaneously after a few days. He improved after administration of neostigmine. Repetitive nerve stimulation showed no decrement of compound muscle action potentials (CMAPs), nor repetitive CMAPs.

Patient 2 is the younger brother of patient 1, who according to the parents had symptoms of ophthalmoparesis and ptosis at the age of two years. No muscle weakness of arms or legs was noted. His parents did not request further investigations to be made. His current age is 9 years, and he has apparently been stable so far.

Patient 3 had drooping eyelids from early infancy, and was never able to run. At the age of 23 she had ptosis at both eyes, a nearly complete ophthalmoplegia, mild facial weakness and mild weakness of the limbs. She improved after treatment with neostigmine. During the following years her symptoms remained unchanged.

Patient 4 is a 24-year-old man who has had swallowing difficulties from birth. At the time of the biopsy (when he was 5 years old) he had drooping eyelids and difficulties

| Patient number | Age at onset (years) | Sex | Age at diagnosis (years) | First symptoms | Actual age (years) | Actual symptoms | Mutations found in AChR $\varepsilon$-subunit gene |
|----------------|----------------------|-----|--------------------------|----------------|-------------------|----------------|-----------------------------------------------|
| 1              | Birth                | Male| 2                        | P              | 14                | P, O, B, L      | $\varepsilon$1369delG $\varepsilon$R311Q       |
| 2              | 2                    | Male| 2                        | P, O           | 9                 | P, O           | $\varepsilon$1369delG $\varepsilon$R311Q       |
| 3              | Birth                | Female| 7                        | P              | 48                | P, O, L        | $\varepsilon$1369delG $\varepsilon$1369delG    |
| 4              | Birth                | Male| 4                        | B              | 24                | P, O, L        | $\varepsilon$1369delG $\varepsilon$509insA     |
| 5              | 3                    | Female| 54                       | P              | 74                | P, O, L        | $\varepsilon$1369delG $\varepsilon$R311Q       |
| 6              | Birth                | Male| 38                       | P              | 80                | P, O, L        | $\varepsilon$1369delG $\varepsilon$R311Q       |
| 7              | Infancy              | Male| 18                       | B              | 32                | P, O, L        | $\varepsilon$R311Q                              |
| 8              | Infancy              | Female| 50                       | P              | 53                | P, O, L        | $\varepsilon$R64X $\varepsilon$R311Q            |

P ptosis, O external ophthalmoplegia, B bulbar symptoms, L limb weakness

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with swallowing. Running with other boys was difficult. Neostigmine, and later mytelase was given from 4 years onwards which provided some improvement. On his last neurologic examination in 2004 he still used neostigmine, with a moderate effect, and his symptoms remained unchanged. Repetitive nerve stimulation showed a decrement of CMAPs.

Patient 5, a 74-year-old woman with unrelated parents, had drooping eyelids from the age of 1 year. Mild generalised weakness especially in arms and hands, impeded her with children’s games. At 15 years myasthenia gravis was diagnosed and pyridostigmine was found to have a beneficial effect. Her condition gradually worsened and when she was 51 years old she had diplopia, chewing difficulties and outdoors she became wheelchair dependent. At the time of the biopsy (when she was 58 years old) the muscle bulk was very small, with weakness of the deltoid, arm and leg muscles. The 5th CMAP (3 Hz) of the abductor pollicis brevis showed a 10% decrement. In the following 17 years the generalised weakness progressed further.

Patient 6, an 80-year-old man (brother of patient 5) had drooping eyelids from early childhood. He had dyspnea after climbing the stairs while he was at primary school. There were no diurnal fluctuations of symptoms, but there were bad days, especially when it was cold. He responded well to treatment with pyridostigmine, which was initiated only when he was 60 years old. At the time of the biopsy (when he was 64 years old) the muscle bulk was small but without obvious atrophy. He could not rise from his chair without the help from his arms. The 5th CMAP (3 Hz) of the abductor digiti minimi muscle showed a 10% decrement. In the following 20 years the generalised weakness progressed further.

Patient 7 is a 38-year-old man who had drooping eyelids and weak facial muscles since birth. At the time of the biopsy (when he was 19 years old) he had double vision in the morning, ptosis, and diffuse weakness of the limbs.

Patient 8 is a 32-year-old man who experienced feeding difficulties due to a weak suck after birth. At the end of his first year he developed drooping eyelids. At the age of 4 years he developed limb weakness. He was never able to stand up without aid of his hand, and could not run. At the age of 18 years he had generalized proximal weakness of his limbs, ptosis, and intermittent diplopia.

Patient 9 is a 53-year-old woman with symmetrical ptosis without double vision since infancy and walking difficulties in childhood. She had to drink with her meals to make swallowing easier. At the age of 50 years she had severe ptosis with very restricted eye movements and sometimes double vision. She is quickly tired in her limbs and is unable to rise from a chair without support. Her fatigue disappeared completely with pyridostigmine.

Genetic analysis

Genomic DNA from patient and control samples was isolated from peripheral blood using the Nucleon™ II DNA extraction kit (Nucleon Biosciences). Coding exons and regions of the promoter of the AChR ε-subunit gene were amplified using the polymerase chain reaction (PCR) and amplicons screened for mutations by direct DNA sequencing. DNA changes were confirmed by restriction endonuclease digestion and size fractionation by agarose gel electrophoresis. The detected mutations were not observed in restriction digests of 100 control samples. Functional effects of the missense mutations were confirmed by studies of the mutant AChR expressed in HEK 293 cells [9].

Oligonucleotides 5’-CAACAGGATATCCAAGGCC ACTAC-3’ and 5’-CTTCCCCAAAATCCAGGAAATG GAG-3’ were used in PCR reactions to amplify a section of the CHRNE 3’ untranslated region that harbours a cluster of SNPs (http://www.ncbi.nlm.nih.gov/SNP/).

Haplotypes were determined by DNA sequence analysis of CMS patients and relatives. Gene frequency for the G or C nucleotide at SNP rs9914357 was determined by DNA sequencing of 100 control chromosomes. SNPs with minor allele frequency >25% (CEPH) were selected.

Data from screening over 100 suspected CMS cases of Northern European origin that did not have CHRNE mutations gave similar allele frequencies to the CEPH population.

Results

Mutation analysis

Screening of DNA from patients 1–9 identified mutations in the AChR ε-subunit gene (CHRNE). Five different mutations were identified: εY15H, εR64X, εS091nsA, εR311Q, ε1369delG. Previous functional studies of these mutations have shown that εR311Q results in severely reduced expression of adult-type AChR with near normal ion-channel properties, whereas ε1369delG is a null mutation [9]. Four patients (two were siblings) are compound heterozygous for εR311Q/ε1369delG, one is homozygous for εR311Q, one homozygous for ε1369delG, two harbour a null allele in combination with ε1369delG, and one a null allele in combination with εR311Q (Table 1; Fig. 1). Strikingly, all patients harboured at least one copy of either εR311Q or ε1369delG.

Haplotype analysis

To investigate the possibility that εR311Q and ε1369delG may be derived from original founder mutations we analysed a cluster of four single nucleotide polymorphisms (SNPs), rs1053754, rs8834, rs7774, rs9914357, that are

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located within the 3' untranslated region of CHRNE.
Haplotypes for patients and family members for the four SNPs were determined (Table 2; Fig. 1b). In all cases mutation \( \varepsilon 1369 \text{del} G \) associated with the haplotype T A T G at the four SNPs \( (P = 0.001) \) and mutation R311Q with SNP haplotype G A G C \( (P = 0.00006) \).

### Intercostal muscle biopsy

Histological and electrophysiological characteristics of endplates from patients 4–7 are given in supplementary material. In patients 5 and 6 there was a reduction in the sensitivity of the endplates towards ACh and a decrease of the number of secondary synaptic clefts. However, in patients 4 and 7 the sensitivity of the endplates towards ACh was almost normal despite the clinical weakness, suggesting that in these patients the level of expression of the AChR \( \gamma \) subunit is sufficient to effectively compensate for loss of the \( \varepsilon \).

### Discussion

To date, the Oxford Myasthenia Service has identified mutations that cause congenital myasthenic syndromes in 17 patients of Dutch origin. Four have mutations that cause slow channel syndrome, four have mutations in the RAPSN gene, and nine have mutations that underlie an AChR deficiency syndrome due to mutations within CHRNE. Mutation \( \varepsilon 1369 \text{del} G \) was present on at least one allele in seven of the nine \( \varepsilon \)-subunit gene AChR deficiency patients, and \( \varepsilon R311Q \) in six. At Oxford the \( \varepsilon 1369 \text{del} G \) and \( \varepsilon R311Q \)
mutations have not been identified in patients with CMS from other ethnic origins, but e1369delG has been reported in a patient from Northern Germany [10]. The common occurrence of e1369delG and eR311Q, and their strict association with the respective CHRNE SNP haplotypes (rs1053754-rs8834-rs7774-rs9914357: T A T G and G A G C) strongly suggests a founder for each of these mutations originating in North Western Europe, possibly in Holland.

Most CMS with severe endplate AChR deficiency result from recessive mutations in AChR ε-subunit gene, or the gene encoding rapsyn. The mutations in the ε-subunit are found along the length of the gene, and result in frameshifts or nonsense codons that truncate the subunit polypeptide chain, or loss of residues essential for AChR assembly or function. More than 90 different AChR ε-subunit gene mutations have been described thus far [11, 12] (and D. Beeson, unpublished data).

Previous functional studies of the e1369delG mutation, located close to the subunit C-terminus, have shown that it is not incorporated into cell surface AChR and thus is a null allele [9, 10]. Studies of eR311Q show that it is incorporated into surface AChR pentamers although at severely reduced levels, and in contrast to another mutation identified in the same site, eR311W [13], does not affect AChR kinetics [9]. It is thought that patients harbouring homozygous or heteroallelic ε-subunit null mutations show partial phenotypic rescue through residual expression of the AChR γ-subunit [7, 13]. Interestingly, symptoms in patient 3, homozygous for e1369delG, were not substantially more severe than in patients 1, 2, 5 and 6 who are heteroallelic for e1369delG and eR311Q.

In many cases of AChR deficiency syndrome the kinship is found to have ‘private’ mutations and it may be necessary to screen the entire ε-subunit gene coding sequence. However, it is becoming clear that some ε-subunit gene mutations underlying AChR deficiency syndrome have an increased incidence in particular geographic regions, presumably arising from original founder mutations. The best examples of this are the 1293insG mutation arising in the Magreb population in North Africa, and in the Gypsy population of South Eastern Europe where e1267delIG is common [14, 15]. Here we highlight two mutations, eR311Q and e1369delIG, that are common in AChR deficiency syndrome patients of Dutch origin. Knowledge of the ethnic or geographic origin within Europe of AChR deficiency patients can help in targeting genetic screening, and our results suggest that for many patients of Dutch origin, screening first for eR311Q and e1369delIG may provide a rapid genetic diagnosis.

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