Molecular characterisation of congenital myasthenic syndromes in Southern Brazil

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ABSTRACT

Objective To perform genetic testing of patients with congenital myasthenic syndromes (CMS) from the Southern Brazilian state of Parana.

Patients and methods Twenty-five CMS patients from 18 independent families were included in the study. Known CMS genes were sequenced and restriction digest for the mutation RAPSN p.N88K was performed in all patients.

Results We identified recessive mutations of CHRNA1 in ten families, mutations in DOK7 in three families and mutations in COLQ, CHRNA1 and CHRNB1 in one family each. The mutation CHRNA c.70insG was found in six families. We have repeatedly identified this mutation in patients from Spain and Portugal and haplotype studies indicate that CHRNA c.70insG derives from a common ancestor.

Conclusions Recessive mutations in CHRNA are the major cause of CMS in Southern Brazil with a common mutation introduced by Hispanic settlers. The second most common cause is mutations in DOK7. The minimum prevalence of CMS in Parana is 0.18/100 000.

INTRODUCTION

Congenital myasthenic syndromes (CMS) are a heterogeneous group of inherited disorders characterised by impaired neuromuscular transmission. Genes known to cause CMS if mutated are the presynaptic acetylcholine acetyltransferase gene CHAT, the gene COLQ encoding the triple-stranded collagenic tail (ColQ) of the synaptic acetylcholine-terase, the genes encoding the different subunits of the acetylcholine receptor (ACHr) (CHRNA1, CHRN B1, CHRN D, CHRNAE), the genes for the postsynaptic proteins rapsyn (RAPSN), muscle-specific kinase (MUSK) and MuSK-interacting cytoplasmic protein Dok-7 (DOK7).1 Recently, mutations in the gene encoding the laminin β2 subunit (LAMB2) have been shown to cause severe CMS associated with congenital nephrosis and ocular malformations.2

Here we present the molecular genetic findings of 25 CMS Brazilian patients. We found two novel mutations in CHRNA and one novel mutation in DOK7. The most frequently detected mutation was CHRNA c.70insG which derives from a common ancestor.

RESULTS

We found the molecular defect causing CMS in a total of 22 Brazilian patients. First we screened in all families for the common mutations RAPSN p.N88K and DOK7 c.1124_1127dupTGCC. Subsequently, we sequenced CHRNA in patients with ophthalmoplegia and benefit from esterase inhibitors. Sequencing of the exons encoding the extracellular and transmembrane domains of AChR subunits was performed in patients with slow-channel CMS (SCCMS). Although the clinical phenotype of patients 24 and 25 is compatible with CMS, no mutations were found by sequencing CHRNA1, CHRN B1, CHRN D and RAPSN. In patient 20 the common DOK7 mutation c.1124_1127dupTGCC was found heterozygously. A second DOK7 mutation was not identified on genomic level. However, some DOK7 mutations are identifiable on cDNA only.3 No cDNA of our patient was available for mutation analysis.

All patients presented with myasthenic symptoms at birth or in childhood. The individual clinical data is summarised in table 1. Representative photos of patients are shown in figure 1.

Molecular genetic analysis revealed recessive mutations in CHRNA in a total of 16 patients out


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Electrophysiological studies

| Patient number | Family number | Sex, age | Ethnic origin | Consanguineous family | Age of onset (years) | Delayed motor tones | Respiratory crises | Ptosis/ophthalmoplegia | Myasthenic crisis | Proximal weakness/weakness of scapulomembranous muscles | Facial weakness/waist gait | Generalized weakness of wrist and finger extensors | Axial weakness weakness of neck | Axial weakness | Abnormal tendon reflexes | Progressive course | RNS decrement | Double CMAP | Myopathic potentials | Beneficial response to acetylcholinesterase inhibitors | Gene/Mutations |
|----------------|---------------|----------|---------------|-----------------------|---------------------|---------------------|---------------------|----------------------|-------------------|------------------------|------------------------|--------------------------------|---------------------|----------------|------------------|---------------------|-------------------------|------------------|
| 1              | 1             | F        | Portuguese    | –                     | 5                   | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | nd               | +               |
| 2              | 1             | F        | Portuguese    | –                     | 4                   | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 3              | 2             | F        | Portuguese    | –                     | 8                   | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 4              | 2             | F        | Portuguese    | –                     | 5                   | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 5              | 2             | F        | Portuguese    | –                     | 1                   | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 6              | 3             | M        | Portuguese    | Birth                | 5                   | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 7              | 4             | F        | Portuguese    | Birth                | 5                   | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 8              | 4             | M        | Portuguese    | Childhood            | 5                   | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 9              | 4             | F        | Portuguese    | Birth                | 1                   | <1                  | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 10             | 5             | M        | Portuguese    | Birth                | 7                   | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 11             | 6             | M        | Portuguese    | Birth                | 12                  | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 12             | 7             | F        | Portuguese    | Birth                | 32                  | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 13             | 8             | M        | Portuguese    | Birth                | 19                  | nd                  | +                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 14             | 8             | M        | Portuguese    | Italian Indian       | 15                  | –                   | +                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 15             | 9             | F        | Portuguese    | Italian Indian       | 15                  | –                   | +                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 16             | 10            | F        | Portuguese    | Indian               | 3                   | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 17             | 11            | F        | Portuguese    | Indian               | 12                  | –                   | +                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 18             | 12            | F        | Portuguese    | Indian               | 15                  | –                   | +                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 19             | 12            | M        | Portuguese    | Birth                | 20                  | –                   | +                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |
| 20             | 13            | M        | Portuguese    | Birth                | 20                  | –                   | –                   | +/+/+/-              | –                 | +/+/+/-                 | +/+/+/-                | –                         | –                   | –                 | –                 | –                   | –                       | +               | +               |

Continued
Electrophysiological studies (Table 1 continued)

**Table 1**

<table>
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<th>Ethic origin</th>
<th>Age of family</th>
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<th>Presence of DoubleCMAP</th>
<th>Myopathy</th>
<th>Myasthenia</th>
<th>Delayed motor</th>
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<td>8</td>
<td>F</td>
<td>25</td>
<td>Indian</td>
<td>16 F</td>
<td>CHRNA1</td>
<td>+</td>
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<td>+/+/+</td>
<td>+/+/+</td>
<td>+/+/+</td>
<td>13</td>
<td>12</td>
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<tr>
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<td>13</td>
<td>F</td>
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<td>13</td>
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<td>23</td>
</tr>
</tbody>
</table>

+ indicates yes, - no, no data, nd, no; nt, not tried.

Although the patient reported sustained benefit, there is lack of objective clinical improvement.

Benefit from treatment with fluoxetine.

The newly identified mutations are in bold and underlined.+

We found that CHRNE mutations are the most common cause of CMS in Portuguese or mixed Portuguese, European and African ancestry. The association between CMS and the founder allele of Spanish or Portuguese descent only (9.1%). This may have occurred prior to the immigration of Europeans to South America.

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mutation has been reported causing CMS in patients of North-African, Portuguese and Spanish origin.11

CHRNE mutations are the most common cause of CMS in patients from Portugal analysed in our laboratory (a total of 11 Portuguese patients with identified molecular defect) accounting for 72.7% of the patients, that is, nearly the same frequency that we observed in our Brazilian cohort. The second most common cause is DOK7 mutations. In contrast, approximately half of our German CMS patients (a total of 42 German patients with identified molecular defect) have mutations in RAPSN while mutations in CHRNE are found in 17% of the patients (unpublished results).

Taken together these findings indicate a strong influence of the Portuguese ancestry on the people from Parana which is in line with the data from mtDNA studies showing major European matrilineal genetic contribution to the mtDNA pool in Southern Brazil.12

The second molecular cause of CMS in Parana according to frequency is DOK7 mutations. We found the common mutation DOK7 c.1124_1127dupTGCC in four patients. The mutation DOK7 p.S45L has previously been identified by us in one Portuguese patient and another patient from South America.7

The novel mutation DOK7 p.G64R has not previously been observed in any European CMS patient. The two siblings who carry it derive from a non-consanguineous family of Portuguese, Amerindian and African descent. It can be speculated that DOK7 p.G64R is specific for the indigenous Amerindians or Africans, so testing of CMS patients from Northern and Northeastern Brazil is of particular interest as the Amerindian and African matrilineal genetic contribution to the mtDNA pool in these regions is greater than in the Southern Brazil.12

We did not detect the common European RAPSN p.N88K mutation1 in the Brazilian CMS cohort. The likely reason for this is underdiagnosis of CMS among hospitalised neonates at Intensive Care Units and the benign course of the disease in RAPSN patients with lack of progression with age. Alternatively, this mutation could be very rare in Brazil, similarly to patients from Portugal (RAPSN N88K was detected heterozygously in one patient out of 11 of our CMS Portuguese patients).

The Southern Brazilian state of Parana has a total population of 10 million inhabitants. Eighteen independent CMS families included in the study were referred to a single neuromuscular center in Curitiba. Based on these figures, the estimated minimum prevalence of CMS in Parana is approximately 0.18 in 100 000, which does not differ from figures published for Europe prior to the identification of DOK7 mutations.13 The prevalence

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may be underestimated assuming that there are underdiagnosed patients that have not been referred to Curitiba.

With our findings we show that molecular epidemiology of CMS in Parana - similar to other disorders (eg, spinocerebellar ataxia)—reflects the major Portuguese ancestry of the Brazilian population. Recessive mutations in \textit{CHRNE} are the most common cause of CMS in Southern Brazil with a common founder mutation introduced by Hispanic settlers. In practical terms, we recommend to start genetic testing for CMS in Brazil with screening for mutations in \textit{CHRNE} followed by \textit{DOK7}.

\textbf{Acknowledgements} We wish to thank the patients and their families for participating in this study. We thank Mandy Heiliger and Petra Mitzscherling for excellent technical assistance. AA, AH, MvH, RS and HL are members of the German Muscular Dystrophy Network (MD-NET 01GM0601) funded by the German Ministry of Education and Research (BMBF, Bonn, Germany); http://www.md-net.org. MD-NET is a partner of TREAT-NMD (EC, 6th FP, proposal #036825; http://www.treat-nmd.eu). VM receives a BAYHOST fellowship from the Bavarian state. JSM receives a fellowship from the Deutsche Forschungsgemeinschaft (MU2840/1-1). VG is a research fellow of the Alexander von Humboldt Foundation.

\textbf{Competing interests} None.

\textbf{Patient consent} Obtained.

\textbf{Provenance and peer review} Not commissioned; externally peer reviewed.

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*J Neurol Neurosurg Psychiatry* 2010 81: 973-977 originally published online June 20, 2010
doi: 10.1136/jnnp.2009.177816

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