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

Conclusions Recessive mutations in *CHRNE* 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 choline acetyltransferase gene *CHAT*, the gene *COLQ* encoding the triple-stranded collagenic tail (ColQ) of the synaptic acetylcholinesterase, the genes encoding the different subunits of the acetylcholine receptor (AChR) (*CHRNA1*, *CHRNB1*, *CHRND*, *CHRNE*), 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 *CHRNE* and one novel mutation in *DOK7*. The most frequently detected mutation was *CHRNE* c.70insG which derives from a common ancestor.

PATIENTS AND METHODS

Twenty-five CMS patients from 18 independent families were included in the study. Pedigrees of five CMS families are shown in online figure 1. All patients were referred to a single center in Curitiba and examined by two independent investigators.

Detailed neurological examination and electrophysiological studies including 3-Hz repetitive stimulation of proximal, distal and facial muscles were performed.

Venous blood samples were obtained from the patients as well as from their parents and siblings, if available. In all CMS families screening for the mutation *RAPSN* N88K was performed by restriction digest. 100 healthy controls were screened for each novel mutation.

Known CMS genes (*CHRNE*, *DOK7*, *RAPSN*, *COLQ*, *CHRNA1*, *CHRNB1*, *CHRND*) were sequenced depending on patients' clinical symptoms.

Haplotype studies using six polymorphic microsatellite markers on chromosome 17p13.2 (*D17S1583*, *D17S1828*, *D17S1584*, *D17S1810*, *D17S1832*, *D17S796*) were performed in a total of 38 patients and family members carrying *CHRNE* c.70insG and control individuals of Spanish or Portuguese origin.

All the studies were carried out with informed consent of the patients or parents and comply with the ethical guidelines of the institutions involved.

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 *CHRNE* in patients with ophthalmoparesis 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 *CHRNE*, *CHRNA1*, *CHRNB1*, *CHRND* 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 *CHRNE* in a total of 16 patients out

Table 1 Individual clinical and genetic data of 25 Brazilian CMS patients

Patient number	Family number	Sex, age	Ethnic origin	Consan guineous family	Age of onset (years)	Delayed motor miles tones	Respiratory crises	Ptosis/ ophthal moparesis/ facial weakness/ dysphagia	Proximal weakness/ waddling gait/ scapular winging/ atrophies	Distal muscle weakness/ selective involvement of wrist and finger extensors	Axial/neck muscles weakness/ scoliosis or kyphosis	Abnormal tendon reflexes	Progressive disease course	Electrophysiological studies				Gene/Mutations
														RNS decrement	Double CMAP	Myopathic potentials	Beneficial response to acetylcho-linesterase inhibitors	
1	1	F 26	Portuguese Polish	–	5	–	–	+/+/–/–	+/–/–/+	–/–	–/–/–	–	–	–	–	nd	+	***CHRNE*** 70insG/ 127insCTCAC
2	1	F 23	Portuguese Polish	–	4	–	–	+/+/–/–	+/–/–/–	–/–	–/–/–	–	–	+	–	+	+	***CHRNE*** 70insG/ 127insCTCAC
3	2	F 17	Portuguese	–	8	–	–	+/+/+/+	+/–/–/–	–/–	–/–/–	–	–	+	–	nd	+	***CHRNE*** 70insG/ 70insG
4	2	F 19	Portuguese	–	5	–	–	+/+/–/+	+/–/–/–	–/–	–/–/–	–	–	+	–	nd	+	***CHRNE*** 70insG/ 70insG
5	2	F 23	Portuguese	–	1	–	–	+/+/+/+	+/–/–/–	–/–	–/–/–	–	–	+	–	–	+	***CHRNE*** 70insG/ 70insG
6	3	M 18	Portuguese African	+	Birth	–	–	+/+/–/+	+/–/–/–	–/–	–/–/–	–	–	+	–	–	+	***CHRNE*** **L11P /L11P**
7	4	F	Portuguese	–	5	–	–	+/+/+/+	+/–/–/–	–/–	–/–/–	–	–	+	–	nd	+	***CHRNE*** 1293insG/ 1293insG
8	4	M 33	Portuguese	–	Childhood	–	–	+/+/–/+	+/–/–/–	–/–	–/–/–	–	–	+	–	nd	+	***CHRNE*** 1293insG/ 1293insG
9	4	F <1	Portuguese	–	Birth	–	–	+/+/–/–	–/–/–/–	–/–	–/+/–	–	–	nd	nd	nd	nt	***CHRNE*** 1293insG/ 1293insG
10	5	M 7	Portuguese	–	Birth	–	–	+/+/–/–	+/–/–/+	–/–	–/–/–	+	–	+	–	+	+	***CHRNE*** 1293insG/ 70insG
11	6	M 12	Portuguese	–	Birth	–	–	+/+/–/+	+/–/–/–	–/–	–/–/–	–	–	+	–	+	+	***CHRNE*** 70insG/ **S235L**
12	7	F 32	Portuguese	+	1	–	–	+/+/–/–	+/–/–/–	–/–	–/–/–	–	–	+	nd	+	+	***CHRNE*** 70insG/ 70insG
13	8	F 19	Portuguese Italian Indian	–	Birth	nd	+	+/+/+/+	+/–/+/–	–/–	–/–/–	–	+	+	nd	+	–	***CHRNE*** 70insG/ 70insG
14	8	M 15	Portuguese Italian Indian	–	1	+	+	+/+/+/–	+/–/+/–	–/–	–/–/–	–	+	+	–	nd	–	***CHRNE*** 70insG/ 70insG
15	9	F 33	Portuguese Indian	–	Birth	–	–	+/+/+/+	+/–/–/+	–/–	–/–/–	+	–	+	–	–	+	***CHRNE*** R286M/ R286M
16	10	F 16	Portuguese	–	3	–	–	+/+/–/–	+/–/–/–	–/–	–/–/–	–	–	+	–	–	+	***CHRNE*** R286M/ R286M
17	11	F 12	Portuguese Polish	–	6	–	+	+/–/–/–	+/+/+/+	–/–	+/–/+	+	+	+	–	+	–	***DOK7*** S45L/ 1124_1127dupTGCC
18	12	F 15	Portuguese Indian African	–	1	+	–	+/–/+/–	+/–/+/+	–/–	+/–/+	+	+	+	–	nd	nd	***DOK7*** **G64R**/ 1124_1127dupTGCC
19	12	M 20	Portuguese Indian African	–	Birth	+	–	+/+/+/–	+/+/–/+	–/–	+/–/+	+	–	+	–	+	–	***DOK7*** **G64R**/ 1124_1127dupTGCC
20	13	M 20	Portuguese	–	5	–	–	–/–/–/+	+/–/+/+	–/–	+/–/–	+	+	–	–	+	+	***DOK7*** 1124_1127dupTGCC heterozygously

Continued

Table 1 Continued

Patient number	Family number	Sex, age	Ethnic origin	Consan guineous family	Age of onset (years)	Delayed motor miles tones	Respiratory crises	Ptosis/ ophthal moparesis/ facial weakness/ dysphagia	Proximal weakness/ waddling gait/ scapular winging/ atrophies	Distal muscle weakness/ selective involvement of wrist and finger extensors	Axial/neck muscles weakness/ scoliosis or kyphosis	Abnormal tendon reflexes	Progressive disease course	Electrophysiological studies: RNS decrement	Electrophysiological studies: Double CMAP	Electrophysiological studies: Myopathic potentials	Beneficial response to acetylcholinesterase inhibitors	Gene/Mutations
21	14	M 19	Indian African	+	<1	+	–	+/+/+/–	+/+/+/+	+/–	+/–/+	+	+	+	+	+	+*	***COLQ*** IVS2+1G>C/ IVS2 +1G>C
22	15	M 28	Portuguese Lebanese	–	Birth	–	–	+/+/–/+	+/–/+/+	–/–	+/–/+	–	+	+	+	+	†	***CHRNB1*** V266M
23	16	F 13	Spanish Italian	–	1	+	–	–/–/–/–	+/+/+/+	+/–	–/–/–	+	+	+	+	+	†	***CHRNA1*** G153S
24	17	F 5	nd	–	2	–	–	+/+/–/–	–/–/–/–	–/–	–/–/–	–	–	+	–	nd	–	no identified molecular defect
25	18	F 9	nd	–	3	–	–	+/+/–/–	–/–/–/–	–/–	–/–/–	–	–	+	–	nd	+	no identified molecular defect

The newly identified mutations are in bold and underlined.
+ indicates yes; –, no; nd, no data; nt, not tried.
*Although the patient reported sustained benefit, there is lack of objective clinical improvement.
†Benefit from treatment with fluoxetine.

of ten families, two of them are novel (table 1). The newly identified mutation *CHRNE* p.L11P was detected homozygously in patient 6 and leads to a substitution of a highly conserved leucine. The mutation *CHRNE* p.S235L found in patient 11 has not been previously reported, but was detected homozygously in an Austrian CMS patient by us (unpublished data) and leads to a substitution of a highly conserved serine. Interestingly, the previously published mutation *CHRNE* c.70insG[4] was found on at least one allele in ten patients from six families. All of these patients are of Portuguese or mixed Portuguese, European and Indian origin (table 1).

Other previously published *CHRNE* mutations detected in our patients are: *CHRNE* c.1293insG,[5] found in four patients from two families and *CHRNE* p.R286M[6] detected homozygously in two patients from two kinships.

Thus, mutations in *CHRNE* accounted for approximately 70% of our Brazilian CMS patients with identified molecular defect. The second most common molecular cause in this cohort is *DOK7* mutations, found in four patients (17%) from three kinships (table 1). All of them carry the common mutation *DOK7* c.1124_1127dupTGCC heterozygously.

Two siblings are compound heterozygotes for a newly identified aminoacid exchange *DOK7* p.G64R leading to a substitution of a highly conserved glycine.

Patient 17 carries the previously reported mutation *DOK7* p.S45L[7] together with the mutation *DOK7* c.1124_1127dupTGCC.

Patient 21 (previously reported[8]) has a homozygous splice-site mutation in *COLQ*.

Patients 22 and 23 with SCCMS carry previously reported mutations heterozygously: *CHRNB1* p.V266M[9] and *CHRNA1* p.G153S,[10] respectively.

We and others have repeatedly identified patients from Spain and Portugal harbouring the mutation *CHRNE* c.70insG.[11]

Haplotype studies of patients and family members carrying *CHRNE* c.70insG showed that the distally adjacent polymorphic microsatellite marker *D17S1810* had a fragment length of 260bp in 14 out of 18 70insG alleles (77.8%). In contrast, the same fragment length (260bp) of *D17S1810* was detected in 4 from 44 control alleles of Spanish or Portuguese descent only (9.1%). The difference between controls and c.70insG carriers for marker *D17S1810* is statistically significant ($p<0.01$) providing genetic evidence that the *CHRNE* c.70insG allele derives from a common ancestor. We estimate that a single founder event for the mutation *CHRNE* c.70insG may have occurred prior to the immigration of Europeans to South America.

DISCUSSION

Here we present the molecular genetic findings of 25 Brazilian CMS patients from the Southern Brazilian state of Parana.

Brazilians represent one of the most heterogeneous populations in the world reflecting the admixture of Europeans, Amerindians, Africans and Asians with regional differences of European, Amerindian and African matrilineal genetic contribution to the Brazilian mtDNA pool.[12]

We found that *CHRNE* mutations are the most common cause of CMS in Southern Brazil with *CHRNE* c.70insG mutation being the most frequently detected. Haplotype analysis of *CHRNE* c.70insG families suggests that this is an old founder mutation and the founder allele is shared by patients from Spain and Portugal. Similarly, *CHRNE* p.R286M has been previously identified in four patients from two Portuguese families from our CMS cohort (unpublished observation) and *CHRNE* c.1293insG

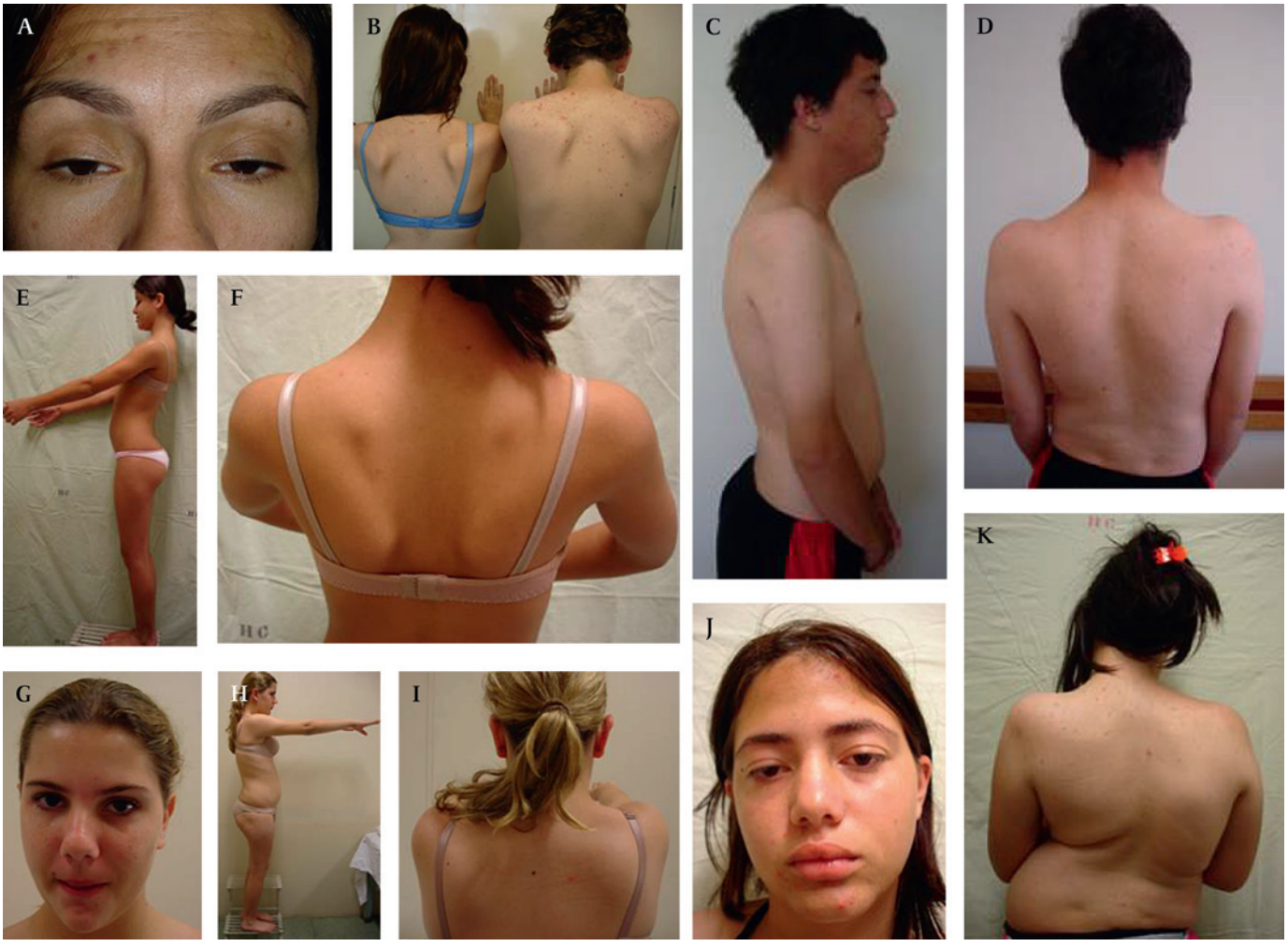

Figure 1 Photos of CMS patients A. Patient 12. The patient has ptosis and ophthalmoparesis. B. Patients 13 and 14 show scapulae alatae. C, D. Patient 19. Note pronounced lordosis and scoliosis. E, F. Patient 17. Note proximal weakness, pronounced lordosis and scapulae alatae. G, H, I. Patient 23. She has no involvement of the ocular muscles. Note pronounced lordosis and scapulae alatae. J, K. Patient 18. She has ptosis and facial weakness. Note severe scoliosis.

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 mixed 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 mutation[1] 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

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 *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 *CHRNE* followed by *DOK7*.

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Competing interests None.

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