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CONGENITAL MYASTHENIC SYNDROMES IN TURKEY: CLINICAL CLUES AND PROGNOSIS WITH LONG TERM FOLLOW-UP

Hacer Durmus¹, Xin-Ming Shen², Piraye Serdaroglu-Oflazer¹, Bulent Kara¹, Yesim Gulsen-Parman¹, Coskun Ozdemir¹, Joan Brengman², Feza Deymeer¹, and Andrew G. Engel²

¹Department of Neurology, Istanbul Medical Faculty, Istanbul University, Capa 34390, Istanbul, Turkey

²Department of Neurology, Mayo Clinic, Rochester, MN, USA

Abstract

Congenital myasthenic syndromes (CMS) are a group of hereditary disorders affecting the neuromuscular junction. Here, we present clinical, electrophysiological and genetic findings of 69 patients from 51 unrelated kinships from Turkey. Genetic tests of 60 patients were performed at Mayo Clinic. Median follow-up time was 9.8 years (range 1-22 years). The most common CMS was primary acetylcholine receptor (AChR) deficiency (31/51) and the most common mutations in AChR were c.1219+2T>G (12/51) and c.1327delG (6/51) in *CHRNE*. Four of our 5 kinships with AChE deficiency carried p.W148X that truncates the collagen domain of COLQ, and was previously reported only in patients from Turkey. These were followed by mutations in *GFPT1* (4/51), *DOK7* (3/51), slow channel CMS (3/51), fast channel CMS (3/51), choline acetyltransferase deficiency (1/51) and a CMS associated with desmin deficiency (1/51). Distribution of muscle weakness was sometimes useful in giving a clue to the CMS subtype. Presence of repetitive compound muscle action potentials pointed to AChE deficiency or slow channel CMS. Our experience confirmed that one needs to be cautious using pyridostigmine, since it can worsen some types of CMS. Ephedrine/salbutamol were very effective in AChE and *DOK7* deficiencies and were useful as adjuncts in other types of CMS. Long follow-up gave us a chance to assess progression of the disease, and to witness 12 mainly uneventful pregnancies in 8 patients. In this study, we describe some new phenotypes and indicate the clinical features of the well-known CMS.

Keywords

Congenital myasthenic syndromes; Turkey; genetic; myasthenia

Corresponding author: Feza Deymeer, MD, Department of Neurology, Istanbul Faculty of Medicine, Istanbul University, Millet Caddesi, Capa 34390, Istanbul, Turkey. fezadeymeer@gmail.com, **Fax number:** 90-212-5338575, **Phone number:** 90-532-6049534.

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INTRODUCTION

Congenital myasthenic syndromes (CMS) are heterogeneous disorders in which one or more specific mechanisms impair the safety margin of neuromuscular transmission. They usually present in the first year of life with fluctuating weakness affecting ocular, bulbar and extremity muscles, but late-onset phenotypes with proximal limb muscle weakness and a progressive course also exist [1-5]. Defective proteins causing CMS reside in the presynaptic region, the synaptic space, the postsynaptic region, or are ubiquitous. This differentiation is difficult clinically but can be established by electrophysiologic and molecular genetic tests. Defects in no fewer than 30 genes are currently known to cause CMS with 90% being in one of the following genes: *CHRNE*, *RAPSN*, *COLQ*, *DOK7*, *GFPT1*, and *CHAT* [1-2, 6-7]. To date, a limited number of CMS patients have been identified in Turkey [8-10], but the genotype/phenotype features of the CMS in this population remain unclear. Recently, a series of CMS patients was reported from Israel, another country in the region [11]. Here, we describe our findings in CMS patients from Turkey identified in the past three decades.

PATIENTS AND METHODS

We retrospectively evaluated the clinical, electrophysiologic, and genetic findings of 69 patients from 51 unrelated kinships diagnosed with CMS at the Department of Neurology, Istanbul Faculty of Medicine, Istanbul University. Clinical information was extracted from the detailed medical records. Repetitive nerve stimulation was performed at 3 Hz and rarely at 2 Hz. A decrement $\geq 10\%$ of the fourth compared with the first evoked muscle action potential was accepted as positive for a defect of neuromuscular transmission.

Genetic tests of 60 patients were performed between 1996 and 2016 by the laboratory of A. G. Engel at Mayo Clinic, USA. Whole-exome sequencing was done in 4 other patients. All genetic tests were performed with written informed consent from the participants according to the Declaration of Helsinki. The study protocol was approved by the Istanbul University Institutional Review Board for Research with Human Participants and by the Institutional Review Board of Mayo Clinic.

RESULTS

Thirty-six of the 69 patients were women. The peak age at onset of symptoms was the neonatal period. Fifty percent of the patients were symptomatic at birth and fewer than 20% presented after the age of 24 months. The most commonly reported initial finding was poor suck and cry (26/69), followed by eyelid ptosis (24/69). Mean time to accurate diagnosis was 12.9 ± 10.35 years. Only 52% of the patients were referred with the accurate diagnosis of CMS. The most common misdiagnoses were autoimmune myasthenia gravis (MG) (12/69) and congenital myopathy (12/69). Median follow-up time was 9.8 years (range 1-22 years). Mean age of patients at last examination was 32.6 ± 10.35 years (range 10-52 years).

The most common CMS among our families was primary acetylcholine receptor (AChR) deficiency (31/51). This was followed by endplate acetylcholinesterase (AChE) deficiency caused by mutations in *COLQ* which encodes the structural subunit of AChE (5/51), GFPT1-myasthenia (4/51), DOK7 deficiency (3/51), slow channel CMS (SCCMS) (3/51),

fast channel CMS (FCCMS) (3/51), choline acetyltransferase (ChAT) deficiency (1/51) and desmin deficiency CMS (1/51) (Table 1). All causative mutations including those previously reported are in Table 1.[12-25]

1. Primary acetylcholine receptor deficiency

Forty-six (23F/23M) patients from 31 unrelated kinships were diagnosed with primary AChR deficiency. Seven of these patients from unrelated kinships were previously reported [12]. In our cohort, mutations in the *CHRNE* gene that encodes the AChR ϵ subunit (30/31) were the commonest cause of AChR deficiency. Only one patient from a consanguineous family carried a homozygous c.727C>T (p.R243C) mutation in *CHRNA1*. Only 3 patients were compound heterozygotes for the mutations; all others harboured homozygous mutations. The commonest mutations in *CHRNE* were c.1219+2T>G (12/30) and c.1327delG mutation (6/30), present in 58% of our families. Mutations in the remaining patients were very heterogeneous with 14 different mutations found in 12 families. Two of these mutations were novel c.501-2A>G, and c.1220_1227 (p.C410Pfs*51).

The peak age at onset of symptoms was the neonatal period. About half of the patients (52%) were symptomatic in the neonatal period and 75% were symptomatic during infancy. Six affected siblings had died in the neonatal period. Only 6 patients presented with myasthenic symptoms after the age of 5 years. The most frequent initial symptoms were poor suck and cry (22/46) and eyelid ptosis (16/46). Varying degrees of eyelid ptosis and ophthalmoparesis were present in all patients. Bilateral symmetric eyelid ptosis was usually noted after 3 months of age; it was asymmetric in 5 patients. Despite the marked limitation of eye movements, double vision was not a pronounced symptom. Mild, short lasting and non-disturbing diplopia, usually upon awakening, during a febrile illness or at the peak effective hour of pyridostigmine was reported in about one-half of the patients. In many patients, the diplopia disappeared after puberty, but in 2 patients it was disabling and persisted into adulthood. Bulbar symptoms were not pronounced in any patient. Most patients (34/46) had proximal lower extremity predominant weakness. In all patients who had weakness, triceps and iliopsoas muscles were the weakest. Quadriceps femoris weakness was found in about half of the patients, but was always milder than the iliopsoas weakness. Tibialis anterior muscle was usually strong; mild weakness was present in only 6 patients who also had severe iliopsoas weakness. Mild fluctuations, usually related to exertion, were reported by most of the patients. All except three patients had a decremental response on repetitive nerve stimulation (RNS). These three patients only had abnormal single fiber EMG studies. All were improved by pyridostigmine treatment (90-360 mg/day).

Median follow-up time was 12 years (range 2-26 years). In most patients, the course of the disease was benign with only mild exacerbations, but 2 adult patients needed intubation following respiratory infections. The commonest complaint in patients over age 20 years was easy fatigability on exertion. Thirteen patients had no limb muscle weakness at last follow-up and 5 of them never had any weakness (median follow-up time: 8 years [ranges 5-21 years]) Eleven patients had been examined after age 40 years, all were ambulatory except one who required a device supporting ambulation. Five women gave birth to 8 healthy children with no complications. Only one patient reported moderate worsening of

her symptoms during pregnancy and never regained her strength prior to pregnancy. Three deliveries in 2 patients were vaginal; others were by caesarean section with epidural anaesthesia.

2. Endplate acetylcholinesterase deficiency due to mutations in *COLQ*

Five patients (4F/1M) from 5 unrelated families carried mutations in *COLQ* gene and 4 were homozygous for the c.444G>A (p.W148X) mutations (Table 1). All had severe myasthenic symptoms since birth. One patient's sister - followed elsewhere - died at the age of 18 months of severe respiratory insufficiency, apparently after receiving several doses of pyridostigmine. Three patients required intermittent mechanical ventilation. All patients were over the age of 20 years (range 20-29 years) at the last examination. Median follow-up time was 8.5 years (range 2-12 years). Ptosis and ophthalmoparesis were present in 4, but one patient had only minimal ptosis. Two had a slow pupillary light reflex. All patients had bulbar involvement at the last examination. Swallowing difficulties improved as the patients grew older but all still needed bilevel positive airway pressure (BIPAP) machine assistance, especially at night. In all patients, the weakest muscles were neck flexors and wrist and finger extensors in upper limbs. Proximal muscles were also affected, more in the legs than in the arms. Quadriceps femoris was weak in all 4 patients, although less weak than iliopsoas.

All patients had a decremental response on RNS as well as repetitive compound muscle action potentials (CMAPs) with the second component of each CMAP decrementing faster than the first component. All patients were worsened by exposure to pyridostigmine treatment and improved by ephedrine or salbutamol (8-12 mg/day). In each patient, ephedrine or salbutamol improved the quality of life apparently by strengthening the proximal limb muscles although ocular, cervical and distal limb muscles did not appear to respond to therapy. For example, one patient, unable to attend classes at the University before therapy, was able to graduate and work as a psychologist after treatment initially with ephedrine, then with salbutamol when ephedrine was no longer available. His exam showed improvement in proximal muscles only. Three patients had scoliosis; 2 were treated surgically; this improved their respiratory status and reduced the need for BIPAP.

3. CMS caused by kinetic defects in AChR

3.1. Slow-channel syndrome (SCCMS)—Three sporadic patients from 3 unrelated families had dominant mutations causing SCCMS. One previously published patient carried a heterozygous c.1314C>G (p.C438W) in *CHRNA* [21]. Another patient had a heterozygous c.711C>A (p.N237K) mutation, also in *CHRNA* and the third carried a heterozygous c.865C>A (p.L289F) mutation in *CHRNE*. The patients with p.N237K and p.L289F mutations presented with poor suck and cry at birth. They were last examined at 20 and 34 years of age. At that time, they had dyspnoea on exertion, mild ptosis and ophthalmoparesis, no diplopia or dysphagia, and cervical and predominantly distal limb muscle weakness. Both of the patients had severe weakness and atrophy in the hands (Figure 1), the patient with heterozygous p.N237K mutation had additional weakness/atrophy in the leg with pes cavus in the feet. The patient harboring the p.C418W mutation had mild fatigable ptosis since birth that was ignored. At age 22, he presented with fatiguing legs. At age 34, he had persistent

mild ptosis, mild neck weakness, severe upper distal and mild upper and lower proximal extremity weakness. He had difficulty walking, required help for activities of daily living, and needed BIPAP ventilatory support at night.

All 3 patients had decremental responses to RNS and repetitive CMAPs, with the second component of each CMAPs decrementing faster than the first component. All 3 initially responded to pyridostigmine, but then became weaker. All 3 benefited from fluoxetine (40-60 mg/day) and improved further with the additional use of salbutamol (12 mg/day).

One patient delivered healthy twins by caesarean section and epidural anaesthesia. She fatigued more easily during pregnancy and for 3 months postpartum, but later returned to her clinical state before pregnancy.

3.2. Fast-channel syndromes (FCCMS)—Three patients were diagnosed with FCCMS, one with homozygous c.610G>A (p.E204K) mutation and the other with homozygous c.712C>T (p.R238W) mutation, and one with compound heterozygous c.778C>A and c.965G>A mutation in *CHRNAE*. Except for easy fatigability, all patients were independent in most of activities of daily living. All were greatly improved by pyridostigmine (180-240 mg/day). One of our patients gave birth to a healthy child with no complications. She fatigued more easily for 2 months postpartum, but later returned to her clinical state before pregnancy.

4. GFPT1 deficiency

Six patients from 4 unrelated families were diagnosed with GFPT1-myasthenia. Clinical data in 2 families were previously published [17]. Four patients presented with a classical limb girdle phenotype having proximal limb but no facial muscle weakness. One of these patients carried a novel homozygous c.496G>A (p.R111C) mutation (Table 1). They began having difficulty walking and climbing stairs in the second decade of life. The other 2 patients from a consanguineous family carried homozygous c.684A>2G mutation that disrupts the function of the GFPTL1 muscle isoform. Both patients were mildly hypotonic at birth, had delayed motor milestones, and had progressive weakness with prominent involvement of distal limb muscles, and autophagic vacuoles in their muscle biopsy.

Serum creatine kinase was mildly elevated in 5 of the 6 patients. Electrophysiologic studies revealed decremental responses to RNS and myopathic motor unit potentials. All patients benefited from pyridostigmine (120-300 mg/day) and derived additional benefit from 3,4-diaminopyridine (20-40 mg/day) and salbutamol (4-12 mg/day).

5. DOK 7 deficiency

Three patients from 3 unrelated families were diagnosed with DOK 7 deficiency. Three different homozygous mutations were detected in these families (Table 1). The mutation c.922_922delG (p.E309Kfs*147) in *DOK7* was reported for the first time in this study. All patients had delayed motor milestones. All presented with difficulty walking, climbing stairs and easy fatigability in early childhood. All patients had limb girdle weakness with no facial involvement. All patients reported periods (from 5 days to several weeks) when they were much improved. One patient gave birth to 2 healthy children with normal vaginal delivery

and without any problems. She had mild worsening during pregnancy but returned to her pre-pregnancy clinical status within 6 months.

Serum creatine kinase levels were mildly elevated in all patients. Electrophysiologic studies revealed decremental responses to RNS except in one patient without RNS studies who had an abnormal single fiber EMG. All patients had myopathic motor unit potentials. The patients were not improved by pyridostigmine, but were improved after salbutamol therapy (8-12 mg/day) was initiated.

6. Choline acetyltransferase (ChAT) deficiency

Only one patient in our series harboured homozygous c.1007T>C (p.I336T) mutation in *CHAT*; clinical aspects of her case were previously published [24]. She presented with eyelid ptosis and a waddling gait around one year of age. At age 2 and 6 years, she had severe hypoventilation episodes requiring intubation. The mild ptosis persisted and generalized mild to moderately severe fatigable weakness became apparent after age 5. A marked decremental response to RNS occurring only after exercise followed by a slow recovery over about 15 minutes was evident. She was treated with pyridostigmine (240-360 mg/day) and salbutamol (4 mg/day) was added at age 10 years. When last examined at age 14 years, she still fatigued easily, became dyspneic even on mild exertion, was wheelchair-dependent outside her home, and required BIPAP at night. A decremental response was elicited only after prolonged exercise (climbing stairs) of the tested muscle.

7. Desmin deficiency related CMS

Two cousins, carrying homozygous c.345dupC (p.N116Qfs*2) mutation in *DES*, presented with fatigable weakness in infancy [25]. Neurological examination revealed a myopathic face, symmetric eyelid ptosis with ophthalmoparesis, and generalized fatigable muscle weakness and atrophy. Both patients had cardiomyopathy. One died at the age of 17 years due to cardio-respiratory failure. The other patient was improved with salbutamol therapy (8 mg/day). At age 15 years, she fatigued easily but was independent in her daily activities and was able to attend high school. RNS revealed an abnormal decremental response. Immunostains of a muscle specimen revealed absence of desmin.

DISCUSSION

The most common mutations in our cohort of 51 unrelated CMS kinships were by far mutations causing AChR deficiency. Heterozygous kinetic mutations in AChR causing SCCMS, homozygous mutations causing FCCMS, homozygous mutations in the *ColQ* subunit of AChE, *GFPT1*, *DOK7*, *CHAT*, *DES* were less frequent. We describe their detailed clinical, electrophysiological and genetic features. To our knowledge, this is the most comprehensive study on CMS from Turkey.

Similar to previously published two large case series, which comprised patients from all over the world [1-2], the most common clinical syndrome among our families was AChR deficiency [12]. The most common mutation was c.1219+2T>G mutation in *CHRNE* which was previously identified only in patients who originated from Turkey [12]. The second most common mutation was c.1327delG mutation in *CHRNE* which is known to be a

common mutation in patients of Gypsy ethnic origin [26]. Our study also identified four previously unreported mutations in *CHRNE*.

The most prominent, though not pathognomonic, clinical feature of AChR deficiency was bilateral ptosis with marked limitation of the ocular ductions. Bilateral symmetrical or rarely asymmetrical ptosis was usually reported after 3 months of age. Mild diplopia was a rare complaint and was usually associated with pyridostigmine treatment (at its peak effective time) or was present briefly upon awakening, both situations when the eyes were apparently at a different position from where they were accustomed to be. Diplopia was a persistent symptom in rare patients. In adulthood, bulbar symptoms faded in most of the patients. Fatigability was the most important symptom that hampered the quality of life in adulthood. Some previous studies stated that the disease could be progressive and patients could lose ambulation in the course of the disease [27-28]. Only one of our patients needed a supporting device for ambulation at age 37.

The patients with AChR mutations shared some similarities with MG regarding the distribution of muscle weakness [29]. As in MG, the weakest muscles were triceps in the upper and iliopsoas in the lower extremities while tibialis anterior was spared unless the iliopsoas was very weak [29]. However, quadriceps femoris was weak in about half of the CMS patients with AChR deficiency, in contrast to autoimmune MG where this muscle is seldom weak.

The second most common clinical syndrome in our cohort was AChE deficiency due to mutations in *COLQ* [16]. Four of our 5 kinships carried c.444G>A (p.W148X) that truncates the collagen domain of *COLQ*, and was previously reported only in patients from Turkey [10, 16]. Our patients with *COLQ* mutations had severe myasthenic symptoms since birth and continued to experience swallowing difficulties, recurrent episodes of respiratory insufficiency, and extremity weakness as previously described [30-32]. In all patients, weakness in neck flexors, wrist and finger extensors in upper extremities were most severe and proximal muscles had moderate weakness. Scoliosis was common among our patients and could point to AChE deficiency, as previously proposed [33].

Slow-channel congenital myasthenic syndrome, a rare subtype of CMS, was observed in 3 kinships in our cohort. The clinical phenotypes were variable. As noted previously, the ocular findings were mild, but there was selective weakness and atrophy of cervical and distal arm muscles as well as impaired respiratory muscle strength [21, 34]. Interestingly, one patient had, in addition to severe hand weakness/atrophy (figure 1A), pronounced distal leg weakness and atrophy that was not previously emphasized in this syndrome (figure 1B). Another patient required BIPAP at night despite the late onset of symptoms and milder phenotype. We observed three patients with fast-channel CMS, both with homozygous mutations and one with compound heterozygous mutations in *CHRNE*. There were no clinical or electrophysiological clues to the diagnosis.

Limb-girdle type weakness without facial involvement was associated with mutations in *DOK7* and *GFPT1* in our cohort, as previously described [3]. Some common mutations in Europe such as c.1124_1127dupTGCC in *DOK7* were also identified in our families and a

novel mutation c.922_922delG (p.E309Kfs*147) was described for the first time [35]. GFPT1-myasthenia was heterogeneous [17]. We also noted a mild distal-prominent phenotype with autophagic vacuoles in our cohort. Myasthenia associated with congenital myopathies was described in patients with centronuclear myopathies [36-37]. We had one patient with truncating mutation in *DES* whose findings included eyelid ptosis, ophthalmoparesis, facial and fatigable extremity weakness, a decremental response on RNS, and response to salbutamol therapy [25]. Desmin deficiency should be considered in patients with fatigable early onset myopathy with generalized weakness including facial and extraocular muscles, cardiomyopathy and respiratory involvement.

Electrophysiological tests were very helpful in the differential diagnosis of CMS. RNS revealed 10% decrement in most patients. Single fiber EMG was abnormal in the few patients in whom RNS was not performed. Repetitive CMAPs were observed in AChE deficiency and in SCCMS. The importance of searching for repetitive CMAPs cannot be overemphasized in patients worsened by pyridostigmine.

The most common misdiagnosis of CMS was MG in our cohort. Later onset disease (age onset was >5 years in 11% of our patients), presence of asymmetrical ptosis, persistent diplopia and worsening with infections could cause diagnostic challenges. Juvenile onset seronegative MG patients unresponsive to immunosuppressive treatment should always be re-evaluated for CMS. Another common misdiagnosis was myopathy in our cohort, especially in the subgroup of late-onset patients with limb girdle type weakness who did not have common and well-known findings such as ptosis and marked eye movement limitation, since they also had elevated serum CK levels and myopathic changes in EMG. Presence of fluctuations, daily or over longer time periods, should raise suspicion of CMS even in patients diagnosed with myopathy previously and careful electrophysiological studies are needed including a search for repetitive CMAP and single fiber EMG if RNS studies are negative.

The differentiation of CMS subtypes, which is usually very difficult clinically, can be done by molecular genetic tests. Recent studies emphasize that ethnical background should be taken into consideration with regard to genetic testing algorithms [11, 38]. AChR deficiency (primarily epsilon subunit) should be the first choice in patients with early ptosis/marked limitation of eye movements and proximal weakness in patients from Turkey. Patients with limb girdle type weakness should be tested for glycosylation defects and *DOK7* deficiency. Marked neck and distal weakness with repetitive CMAP's should lead to search for AChE deficiency and SCCMS. Slow pupillary light response, scoliosis and persistent and marked bulbar weakness in adulthood could be additional clues for *COLQ* mutations. Clinical clues for different CMS subtypes are summarised in Table 2. CMS sequencing panel, which includes *CHRNE*, *RAPSN*, *COLQ*, *DOK7*, *GFPT1*, and *CHAT* would cover 95% of our families. Mutations in other genes are rare and whole exome sequencing should be considered in those patients.

Consistent with previous reports, all patients with AChR, GFPT1, and ChAT deficiency and patients with FCCMS benefited from pyridostigmine; patients with FCCMS responded particularly well [1-4, 7, 39]. Patients with AChE deficiency worsened with pyridostigmine

and those with DOK-7 derived no benefit, or worsened with pyridostigmine. All patients with SCCMS derived some benefit from pyridostigmine at first, but this response diminished with repeated doses and caused worsening. SCCMS patients derived marked benefit from fluoxetine. Patients with GFPT1 deficiency derived additional benefit from 3,4-diaminopyridine.

Ephedrine/salbutamol were very effective therapeutic agents in CMS patients, and especially those with COLQ and DOK 7 deficiency, and provided additional benefit in other types of CMS (SCCMS, GFPT1, FCCMS, ChAT and desmin deficiencies). The benefit became more evident with use over several months. Of note, the benefit of ephedrine/salbutamol was most evident in proximal extremity muscles and not in ocular, neck and distal muscles, particularly in AChE deficiency. All patients should be closely monitored for respiratory problems and the use of BIPAP should be considered. Surgery may be necessary for severe scoliosis which can aggravate respiratory problems, especially in patients with AChE deficiency.

As treatments are optimized in time, patients survive to reach older ages and live healthier lives, so that new issues such as pregnancy arise. We report 12 pregnancies in 8 patients. Pregnancies were in general uneventful except for increased fatigue in some. There was no difference in clinical outcome between vaginal delivery and caesarean section with epidural anaesthesia. Outcome after pregnancy was good in all of our patients except in one patient with AChR deficiency who experienced sustained worsening after pregnancy [40]. These patients should be closely monitored by a multidisciplinary team including neurologists, Ob/Gyn specialists, and pulmonologists during the course of pregnancy and in the postpartum period.

A limitation of our study is that in our neuromuscular unit we do not see the CMS patients when they are infants and in early childhood. For this reason, it is difficult to claim that our population represents CMS in Turkey, but it rather reflects the experience of a large referral center. As such, our population does not include some types of CMS, such as rapsyn deficiency and we very rarely see patients with ChAT deficiency. However, many patients with *ChAT* deficiency were reported from other pediatric neurology centers in Turkey [8]. *Rapsyn* deficiency may be mild in older children and adults and are often misdiagnosed as seronegative MG. Our CMS cohort would likely increase if all undiagnosed myopathy patients were evaluated for a defect of neuromuscular transmission by RNS and/or single fiber EMG, and if all seronegative myasthenic patients were re-evaluated for CMS.

Most of our patients were followed from a single center for more than 10 years, which allowed us to closely observe the course of different CMS in different age groups. We described some new phenotypes, and detailed the clinical characteristics of the well-known CMS. We were also able to witness the course of several pregnancies and births.

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Highlights

- We present clinical, electrophysiological and genetic findings of 69 patients from 51 families.
- The most common CMS was primary acetylcholine receptor deficiency (31/51 families).
- Fifteen families had COLQ, *GFPT1*, *DOK7* deficiencies or slow channel CMS.
- Distribution of muscle weakness and EMG were useful in giving a clue to the CMS subtype.
- Due to the long follow-up, we were able to assess progression and to witness several pregnancies.

Figure 1.

A-B. Eighteen years old female patient with slow channel congenital myasthenic syndrome caused by c.865C>A (p.L289F) mutation in *CHRNE*. Note the marked hand and finger extensor weakness (A) and pronounced distal leg weakness and atrophy (B).

Mutations in a CMS cohort from Turkey

Table 1

Clinical Syndrome	Gene	Mutations	Number of Families (%)	Reference
Primary acetylcholine receptor deficiency	<i>CHRNAE</i> NM_000080 NP_000071	c.1219+2T>G (c.1159+2T>G)	12/51 (24%)	12
		c.1327delG (c.1267delG)	6/51 (12%)	12
		c.452_454delAGG (c.392_394delAGG)	3/51 (6%) [#]	13 (No functional analysis)
		c.442T>A (c.382T>A)	2/51 (4%)	
		Others* :	7/51 (16%)	14
		c.1367_1369delACA (c.1307_1309delACA)	p.N456del (p.N436del)	
		c.778C>A (c.718C>A)	p.L260I (p.L240I)	
		c.1192C>T (c.1132C>T)	p.Q398X (p.Q378X)	13
		c.601+1G>T (c.541+1G>T)		
		c.130msG (c.70msG)	p.E44Gfs*3 (p.E24Gfs*3)	
		c.803-2A>G (c.743-2A>G)		
		c.813_820dup (c.753_760dup)	p.T274Rfs*29 (p.T254Rfs*29)	12
		c.971delT (c.911delT)	p.I324Tfs*61 (p.I304Tfs*61)	
		c.501-2A>G (c.441-2A>G)		
		c.1220_1227dup (c.1160_1167dup)	p.C410Pfs*51 (p.C390Pfs*51)	
	<i>CHRNA1</i> NM_000747 NP_000738	c.727C>T (c.668C>T)	1/51 (2%)	15 (No functional analysis)
Endplate acetylcholinesterase deficiency	<i>ColQ</i> NM_005677 NP_005668	c.444G>A	4/51 (8%)	16
		c.1082delC	1/51 (2%)	
Limb Girdle Myasthenia	<i>GFP11</i>	c.2002G>A*	1/51 (2%)	17

Clinical Syndrome	Gene	Mutations	Number of Families (%)	Reference
	NM_002056 NP_002047	c.821T>C* c.686-2A>G c.496G>A	p.I274T p.R111C	17 17 18
	<i>DOK7</i> NM_173600 NP_775931	c.922delG c.1124_1127dup c.496G>A	p.E309Kfs*147 p.A378Sfs*30 p.G166R	19, 20
			1/51 (2%) 1/51 (2%) 1/51 (2%)	
Slow channel CMS	<i>CHRNA1</i> NM_000079 NP_000070	c.1314C>G (c.1254C>G) c.711C>A (c.651C>A)	p.C438W (p.C418W) p.N237K (p.N217K)	21 22
	<i>CHRNE</i> NM_000080 NP_000071	c.865C>A (c.805C>A)	p.L289F (p.L269F)	22, 23
			1/51 (2%)	
Fast channel CMS	<i>CHRNE</i> NM_000080 NP_000071	c.610G>A (c.550G>A) c.712C>T (c.652C>T) c.965G>A** (c.905G>A)	p.E204K (p.E184K) p.R238W (p.R218W) p.C322Y (p.C302Y)	1/51 (2%) 1/51 (2%) 1/51 (2%)
Choline acetyltransferase deficiency	<i>CHAT</i> NM_020549 NP_065574	c.1007T>C	p.I336T	24
Desmin deficiency CMS	<i>DES</i> NM_001927	c.345dupC	p.N116Qfs*2	25

Clinical Syndrome	Gene	Mutations	Number of Families (%)	Reference
	NP_001918			

The Table shows all AChR subunit mutations using the human genome variation society (HGVS) nomenclature followed by the legacy nomenclature in parenthesis. The cDNA numbers start with the first nucleotide of the start codon in the HGVS nomenclature and with the first nucleotide of mature peptide in legacy nomenclature. The amino acid numbers start with the first residue (Met) in the HGVS nomenclature and with the first residue of mature peptide in legacy nomenclature. Mutations in all other genes are shown using the HGVS nomenclature.

* Three patients were compound heterozygous for the mutations and each mutation was found in one family.

One patient was compound heterozygous for c.452_454delAGG and c.130insG.

** This patient was compound heterozygous for c.778C>A and c.965G>A.

Table 2

Clinical clues for congenital myasthenic syndromes (CMS) subtypes in our cohort

CMS subtype	Clinical Clues	Rare symptoms/signs	EMG	Response to treatment
Acetylcholine receptor deficiency	<ul style="list-style-type: none"> • Neonatal period • Onset with poor suck and cry • Marked ptosis and ophthalmoplegia • Mild bulbar symptoms • Weakest muscles triceps and iliopsoas • Frequent quadriceps femoris weakness (in contrast to MG) • Strong tibialis anterior (as in MG) • Improvement with age 	<ul style="list-style-type: none"> • Asymmetric ptosis • Double vision • Severe exacerbations with infections 	•	• Improvement with pyridostigmine
	Endplate acetylcholinesterase deficiency	<ul style="list-style-type: none"> • Severe bulbar symptoms since birth • Mild ptosis • Slow pupillary light reflex may be present • Frequent scoliosis • Distal>Proximal muscle weakness • Persistent respiratory problems in adulthood 	<ul style="list-style-type: none"> • • 	<ul style="list-style-type: none"> • Deterioration with pyridostigmine • Improvement with ephedrine/salbutamol
Slow-channel syndrome	<ul style="list-style-type: none"> • Onset in early childhood-adulthood • Variable clinical phenotypes • Distal>Proximal muscle weakness • May have respiratory problems 	<ul style="list-style-type: none"> • Severe hand weakness/atrophy • Pes cavus 	<ul style="list-style-type: none"> • • 	<ul style="list-style-type: none"> • Deterioration with pyridostigmine • Improvement with fluoxetine and ephedrine/salbutamol

CMS subtype	Clinical Clues	Rare symptoms/signs	EMG	Response to treatment
CMS subtype	Clinical Clues	Rare symptoms/signs	EMG	Response to treatment
Fast-channel syndromes	<ul style="list-style-type: none"> Variable clinical phenotypes 		<ul style="list-style-type: none"> Decremental response to RNS 	<ul style="list-style-type: none"> Marked improvement with pyridostigmine Additional benefit from salbutamol
GFPT1 deficiency	<ul style="list-style-type: none"> Limb girdle phenotype No facial muscle weakness 	<ul style="list-style-type: none"> Hypotonia at birth Prominent involvement of distal limb muscles 	<ul style="list-style-type: none"> Decremental responses to RNS Myopathic MUPs 	<ul style="list-style-type: none"> Improvement with pyridostigmine Additional benefit from 3,4-DAP and salbutamol
DOK 7 deficiency	<ul style="list-style-type: none"> Limb girdle phenotype No facial muscle weakness 		<ul style="list-style-type: none"> Decremental responses to RNS Myopathic MUPs 	<ul style="list-style-type: none"> No improvement with pyridostigmine Marked improvement with salbutamol
Choline acetyltransferase (ChAT) deficiency	<ul style="list-style-type: none"> Hypoventilation episodes requiring intubation Mild ptosis and generalized weakness 		<ul style="list-style-type: none"> Decremental response, occurring only after exercise followed by slow recovery in over 15 minutes 	<ul style="list-style-type: none"> Improvement with pyridostigmine and salbutamol
Desmin deficiency related CMS	<ul style="list-style-type: none"> Myopathic face, symmetric ptosis, ophthalmoparesis, generalized weakness and atrophy Cardiomyopathy 		<ul style="list-style-type: none"> Decremental responses to RNS Myopathic MUPs 	<ul style="list-style-type: none"> Improvement with salbutamol

RNS= repetitive nerve stimulation, MUP= motor unit potential, CMAPs= compound muscle action potentials