First Characterization of Congenital Myasthenic Syndrome Type 5 in North Africa

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Short Report

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Abstract

Congenital myasthenic syndromes (CMS) are associated with defects in the structure and the function of neuromuscular junctions. These rare disorders can result from mutations in the collagenic tail of endplate acetylcholinesterase (*COLQ*) essentially associated with autosomal recessive inheritance.

With the lowered cost of genetic testing and increased access to next-generation sequencing, many mutations have been reported to date. In this study we identified the first *COLQ* homozygous mutation c.1193T>A in the North African population.

This study outlines the genetic and phenotypic features of a CMS patient in a Moroccan family. It also describes a novel *COLQ* missense mutation associated with CMS-5. *COLQ* mutations are probably underdiagnosed in these North African populations, this is an issue as CMS-5 may be treated with ephedrine, and albuterol. Indeed, patients can seriously benefit and even recover after the treatment that should be planned according to genetic tests and clinical findings.

Introduction

The congenital myasthenic syndromes (CMS) are rare disorders described by abnormal neuromuscular transmission. Most cases of CMS are associated with autosomal recessive inheritance. The onset of symptoms is typically at birth, however certain patients are diagnosed later in childhood or early adult life[1]. Patients present different manifestations depending on the type of the congenital myasthenic syndrome. Symptoms may appear at birth or until infancy, other cases may present signs until adolescence or adulthood due to the under diagnosis[2]. Respiratory involvement is common. Treatment uses ephedrine and albuterol/salbutamol[3]. Treatment with albuterol brought about the reformist increment of muscle quality, practice resistance, and ophthalmoplegia[4].

In the last decade, next-generation sequencing (NGS) has helped to identify new genes responsible for CMS. Those genes are ubiquitously expressed, proving that not only neuromuscular junction (NMJ) defects can cause myasthenia. So far, at least 30 genes have been associated with CMS[5]. Both autosomal dominant and recessive genetic inheritance patterns have been described. Mutations in *DOK7*, *AGRN*, *CHAT*, *COLQ*, and *RAPSN* genes (among others) are inherited in an autosomal recessive pattern, while genes encoding the acetylcholine receptor (AChR) subunits (*CHRNA1*, *CHRNB1*, *CHRND*, and *CHRNE*) are inherited in an both mode of inheritance autosomal dominant and autosomal recessive[6,5].

These disorders have been classified according to the location of the defect: presynaptic, synaptic, and postsynaptic. Endplate acetyl-cholinesterase deficiency is characterized by a deficiency inside the synapse at the NMJ[1,7]. Pathogenic variants in the collagen-like tail subunit of asymmetric acetylcholinesterase, *COLQ*, lead to an endplate acetyl-cholinesterase (AChE) deficiency. The prolonged time of acetylcholine at the NMJ leads to the desensitization of AChRs and secondary endplatemyasthenia.

To this date, *COLQ* variants associated with CMS have been reported in the literature (56 listed as Pathogenic or Likely Pathogenic in LOVD and 31 in ClinVar). Both missense and loss of function variants have been shown to be associated with CMS. In our study we report a novel missense *COLQ* variant in a patient with a typical CMS-5.

Material And Methods

Molecular genetic analysis

Following informed consent, peripheral blood was collected from the affected patient and his parents. Genomic DNA was extracted from peripheral leukocytes with Qiagen Kit, QIAamp DNA Blood Mini Kits.

Our high-throughput sequencing panel consisted of 306 neuromuscular genes[8], containing most of the genes proposed by FILNEMUS network guidelines[9]. Library preparation for Next-Generation Sequencing (NGS) was performed using the HaloPlex Target Enrichment System (Agilent, CA, USA) for Illumina sequencing. The enriched libraries were sequenced using a NextSeq 550 sequencer (Illumina, CA, USA).

Data analysis and pathogenicity assessment of candidate variants

Annotated VCF files containing all variants were analyzed using VarAFT software that provides a full graphical interface and includes unique features to improve mutation annotation and interpretation[10].

VarAFT interactive filtration features allow the progressive reduction of the candidate mutations list by combining various annotations data. The identification of the disease-causing mutation from the index patient's VCF was performed in many steps using data from the variant annotation and the phenotype layers. We prioritized rare functional variants by excluding all variant types except splicing and exonic, as well as excluding variants with a Minor Allele Frequency (MAF) > 0.01 in the GnomAD database[11].

Finally, assumed polymorphism, and probable polymorphism using pathogenicity predictions software, especially UMD-predictor[12], were also discarded. The obtained variants were classified according to the ACMG classification system[13].

Sanger verification

COLQ exon 15 targeted Sanger sequencing confirmation was performed on DNA samples of affected and unaffected family members using ABI Big-Dye terminator v3.1 sequencing reaction Kit (Applied Biosystems) and analyzed on an ABI 3130 Genetic analyzer (Applied Biosystems). Sequences were analyzed with the ChromasPro software.

Results

Here we identified a novel mutation in the collagenic tail of endplate acetylcholinesterase (COLQ) gene, responsible for CMS-5, in addition to the previously reported mutations in the literature (Table 1). Previous gene by gene analysis for the *SGCG* (SarcoGlyCan Gamma; ***** 608896) gene (mutation c.del525T[14]), the test bore witness to a negative yield. The novel test based on NGS methods has been started and After bionformatic filtration and genetic interpretation, The patient with a diagnosis of CMS-5 harbors a homozygous *COLQ* variant one remaining homozygous COLQ variant NM_005677.4:c.1193T>A p.(Ile398Asn) (hg19) chr3:15497408 was retained for the molecular diagnosis of this patient. This probably pathogenic (class 4 ACMG)[13] *COLQ* variant has never been described in any locus databases, or scientific literature reports.

Using different pathogenicity prediction softwares (SIFT[15], REVEL[16], MetaLR[17], UMD-predictor[12] and Human Splice Finder (HSF)[18]) combined, this novel missense *COLQ* variant was found to have a severe predicted pathogenic effect (Table 2).

Moreover, the VarAFT filters led us to conclude that the c.1193T>A *COLQ* mutation (NM_005677.4, exon 15) is probably associated with the myasthenic congenital phenotype observed for the patient (Table 3). Indeed, the resemblance of clinical features of the index case and the phenotype associated with CMS-5 reported in OMIM (# 603034) and literature are in favor of this molecular diagnosis for our patient.

Moreover, the absence of this variant in control datasets (1000G and GnomAD), as well as predicted pathogenicity effects, suggest that it's indeed the likely cause of the observed phenotype for the patient analyzed in this study.

Confirmation of the presence of this *COLQ* variant identified by NGS as well as variant familial segregation analyses were undertaken by targeted Sanger sequencing (Figure 1) and is consistent with this molecular diagnosis.

Discussion

CMS are rare disorders caused by a defect in the structure and the function of neuromuscular junctions. Many genes were found to be reported associated with CMS using next-generation DNA sequencing for which the cost has been considerably decreasing these last few years. Most of the mutations found are in the *CHRNE* gene resulting in a loss of function and defects of the AChR at the endplate. Other genes commonly leading to CMS are *DOK7*, *COLQ*, and *RAPSN*[19].

A previous study identified a single truncating mutation (c.1293insG) in the acetylcholine receptor epsilon subunit gene (*CHRNE*) that was the most often identified in CMS families originating from North Africa with a possibly founder effect in this population[20]. The homozygous mutation p.Gly240* in *COLQ* encoding the collagen tail of acetylcholinesterase, has been reported in several Palestinian Arab patients and an Iraqi Jewish patient, also suggesting a founder effect in the Middle East population for this *COLQ* mutation[21].

In this study, we report a patient, presenting with clinical features and a neuromuscular phenotype suggestive of CMS who was initially analyzed with a gene-by-gene strategy. The lack of categorical molecular diagnosis with this initial analysis led us to explore a wider spectrum of genes associated with NMD using a high-throughput sequencing gene panel approach.

Next-generation sequencing has transformed the paradigm of clinical genetic testing. Now there are various molecular tests available, including gene panels that provide a comprehensive and feasible approach for heterogeneous genetic disorders[22]. Thus, the gene panel approach is appropriate for efficient molecular diagnosis. This NGS approach is perfectly suited for disorders associated with significant phenotypic and genetic heterogeneity such as neuromuscular disorders (NMD)[23]. Using a panel of 306 neuromuscular genes, we were able to identify a novel *COLQ* homozygous variant in the index case, thus, describing the first patient presenting with Congenital Myasthenic Syndrome-5 in Morocco and North Africa. Indeed, the c.1193T>A (p.(Ile398Asn) mutation was suggested to impact the function of the protein *COLQ* (Acetylcholinesterase - Associated Collagen) associated with Myasthenic Syndrome, Congenital, 5 in the first IC.

VarAFT software interactive filtration features allow us the progressive reduction of the list of candidate mutations by combining the various annotations, as well as OMIM who provided detailed clinical features about such disorders to compare with the phenotype information available for our patient. The patient had been analyzed with a gene-by-gene strategy for the *SGCG* (SarcoGlyCan Gamma; ***** 608896) gene (mutation c.del525T[14]), and the test attested a negative yield. This 12 years old boy had walking difficulties and the electromyogram showed myogenic impairment.

By referring to the VarAFT prioritization of causative variants, applying several filters provided by this software, using data from the variant annotation and the phenotype layers, we excluded all variant types except splicing and exonic, and also removed variants with frequency >1% in the GnomAD database. In addition, supposedly polymorphisms and probable polymorphisms were also excluded based on multiple pathogenicity prediction softwares. After bioinformatic filtration, the remaining *COLQ* homozygous variant, consistent with the reported consanguinity in the family, correlating with the CMS phenotype of the patient, was retained as a probable molecular diagnostic.

Finally, targeted *COLQ* exon 15 Sanger sequencing confirmed the unaffected parents heterozygous status for the variant. Further, this variant has never been reported neither in the gnomAD database, or scientific literature.

The missense variant identified in this study is located in the conserved C-terminal domain. Other missense variants in this region have been shown to alter bond of collagen-tailed AChE to the NMJ[24]. Further Functional studies are necessary to confirm the pathogenicity of c.1193T>A p.(Ile398Asn) variant.

In conclusion, CMS are rare but important neuromuscular disorders, and most of them are responding to medication[25]. Many proteins are crucial to maintain the function or the structure of the NMJ, the multifunctional protein collagenic tail of endplate acetylcholinesterase protein is encoded by the *COLQ* gene, its function is essential for securing AChE to the basal lamina and gathering AChE at the NMJ[5], *COLQ* variants may alter the essential function of this protein. Recent studies have highlighted the potential of NGS in mutation detection and are being used increasingly as the initial molecular analysis for heterogeneous genetic disorders. Our study demonstrated the efficacy of the NGS targeted approach by implementing a high-throughput gene panel for the identification of variants involved in heterogeneous genetic disorders. The main panel's limitation is requiring a targeted Sanger sequencing for test fulfillment. However, the NGS panel approach has higher clinical yield compared to a sequential Sanger sequencing approach. Clinical NMD NGS panels allow cost-effective and more accelerated turn-around molecular diagnostic testing than the conventional sequential Sanger sequencing of associated genes[26].

To date, mutations in the *COLQ* gene leading to the CMS-5 have never been reported in Morocco or in North Africa before, revealing a probable underdiagnosed condition. The high rate of consanguinity in this region is responsible for the diversity of genetic diseases, hence the need to implement a targeted and efficient diagnosis. Thus, NGS approach systematization in the near future, suited for genetic diagnosis of heterogeneous disorders, could be an efficient solution for this problem, especially in the context of NMD that may benefit from an appropriate therapeutic option and treatment.

Declarations

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Conflicts of interest: The authors declare that they have no conflict of interest

Availability of data and material: The data are available on request from the corresponding author

Code availability: None

Ethics approval: the study protocol was approved by committee on ethics of Mohamed V university of Rabat, faculty of medicine and pharmacy (ID 09/19).

Consent to participate: All patients gave written informed consent for genetic testing and study related procedures.

Consent for publication: Obtained.

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Tables

Table 1. *COLQ* mutations in patients with Congenital Myasthenic Syndrome-5 identified in this study and earlier reports

Nucleotide change NM_005677.4	yyyyggggrrr	Effect on coding sequence	Number of reported patients	Ethnicity	Reference
c.1292A>C	p.Tyr431Ser		7	Spain	24
	p.Glu214X			NR	27
	p.Ser169X			NR	27
	p.Arg282X			NR	27
c.788insC	p.X262fs*36			NR	27
c.1082delC	p.P361Lfs*64			NR	27
c.176C>A	p.Pro59Gln		1	NR	28
c.1026C>G	p.Asp342Glu		1	NR	28
c.375delT IVS15 1+ IG>A	- Frameshift after codon 125 in collagen domain - Skipped exon 15; frameshift after codon 358 in C-terminal region; stop codon after 26 missense codons		1	NR	28
c.444G>A	p.Trp148X		1	NR	28
c.806insC	Frameshift after codon 269 in collagen domain; stop codon after 31 missense codons		1	NR	28
IVS15 1 + 1G>A	Skipped exon 15; frameshift after codon 358 in C-terminal region; stop codon after 26 missense codons		1	NR	28
c.1111C>T c.1219G>A c.1331G>A	p.Gln371X p.Arg410Gln p.Cys444Tyr		1	NR	28
	p.Cys417Tyr/p.Cyr417Tyr			Saudi Arabian	29
IVS15 + 1G > T	p.Gln211X			Hungarian	29
c.158insC/c.158insC				Spanish	29
c.109delC/c.1324delCAG				Serbian	29
	p.Arg227X/p.Arg227X			Pakistanian	29
	p.Trp148X/p.Cys386Ser			German	29
c.797insC	p.Gly237Asp			Czech	29
c.738delA/c.738delA				Saudi Arabian	29
IVS2-1G > A			1	Philippine-Chinese	4
IVS2+1G >C/IVS2+1G > C				Brazilian	29
c.275insC ; c.631C>T	p.Gln211X		1	Palestinian arab and Iraki jew	21
c.718G>T	p.Gly240X		6	Palestinian arab and Iraki jew	21
c.950delC	Frame-shift in exon 13 and truncates the collagen encoding region.		1	German	30
c.443G>A	p.Trp148Ter		1	Turkish	19
c.1169A>G	p.Asn390Ser		1	Turkish	19
c.1281C>T	p.Cys427Cys		1	Turkish	31

c.107-1G>A	**p.Arg236X**		1	French	31
c.157dup	**p.Leu53ProfsX81**		1	Portuguese	31
c.219+1G>C	**p.Arg340His**		1	French	31
c.788insC	**Splice mutation frame shift mutation**		1	French	31
c.1082delC	**p.Pro361LeufsX65**		1	Italian	31
c.175C>T	**p.Pro59Ser**		1	Filipino	31
c.1321A>G	**p.Thr441Ala**		1	French	31
c.1337T>C	**p.Ile446Thr**		1	Italian	31
c.679C>T	**p.Arg227X**		1	Kurdish	31
c.1169A>G	**p.Asn390Ser**		1	Turkish	25
c.444G>A/c.706C>T	**p.W148*/p.R236***		1	Turkish	25
c.1010T>C	**; p.Ile337Thr**	**p.Ile337Thr**	2	Syrian	32
c.1193T>A	**p.Ile398Asn**		1	Moroccan	This study

Table2. Mutational data

RefSeq Transcript	HGVS coding	HGVS Protein	Variant type[33]	Location (exon)	Coding impact[33]	ACMG classification[13]	SIFT[15]	REVEL[16]	UMD[12]	HSF[18]	G
NM_005677.4	c.1193T>A	p.(Ile398Asn)	SNV	exon 15	Missense	Likely Pathogenic	Damaging	Pathogenic	Pathogenic	no impact	4

The table provides information about the Index Case (ICs) and variant pathogenicity predictions by several tools.

SNV: Single nucleotide Variant

NA: Not available (SIFT, REVEL, DANN do not provide prediction for variants creating a stop or a frameshift)

UMD[12]: UMD-Predictor ACMG[13]: American College of Medical Genetics SIFT[15]: Sort Intolerant From Tolerant; REVEL[16]: Rare Exome Variant Ensemble Learner; DANN[35]: Deleterious Annotation of genetic variants using Neural Networks, HSF[18]: Human Splicing Finder; GERP[34]: Genomic Evolutionary Rate Profiling

Table 3. *COLQ* Genetic disorder associated data

Disorder[36]	Protein name	Locus/ Gene	Cellular functions[37]	Disease clinical features[36]	Inheritance	Prevalence[38]	Treatment[38]
Myasthenic Syndrome, Congenital, 5 **CMS5** # **603034**	COLQ Acetylcholinesterase-Associated Collagen OMIM: 603033 UniProtKB: Q9Y215 Ensembl: ENSG00000206561	3p25.1	This gene encodes the subunit of a collagen-like molecule associated with acetylcholinesterase (AChE) in skeletal muscle. Each molecule is composed of three identical subunits. Each subunit contains a proline-rich attachment domain (PRAD) that binds an acetylcholinesterase tetramer to anchor the catalytic subunit of the enzyme to the basal lamina.	Clinical features are easy fatigability and muscle weakness affecting the axial and limb muscles (with hypotonia in early-onset forms), the ocular muscles (leading to ptosis and ophthalmoplegia), and the facial and bulbar musculature (affecting sucking and swallowing, and leading to dysphonia). The symptoms fluctuate and worsen with physical effort.	Autosomal Recessive	1-9 / 1 000 000 (worldwide)	Treatment with ephedrine/ Albuterol may be beneficial.

The table provide several information about the disorder, Protein affected, phenotype and prevalence.

Figures

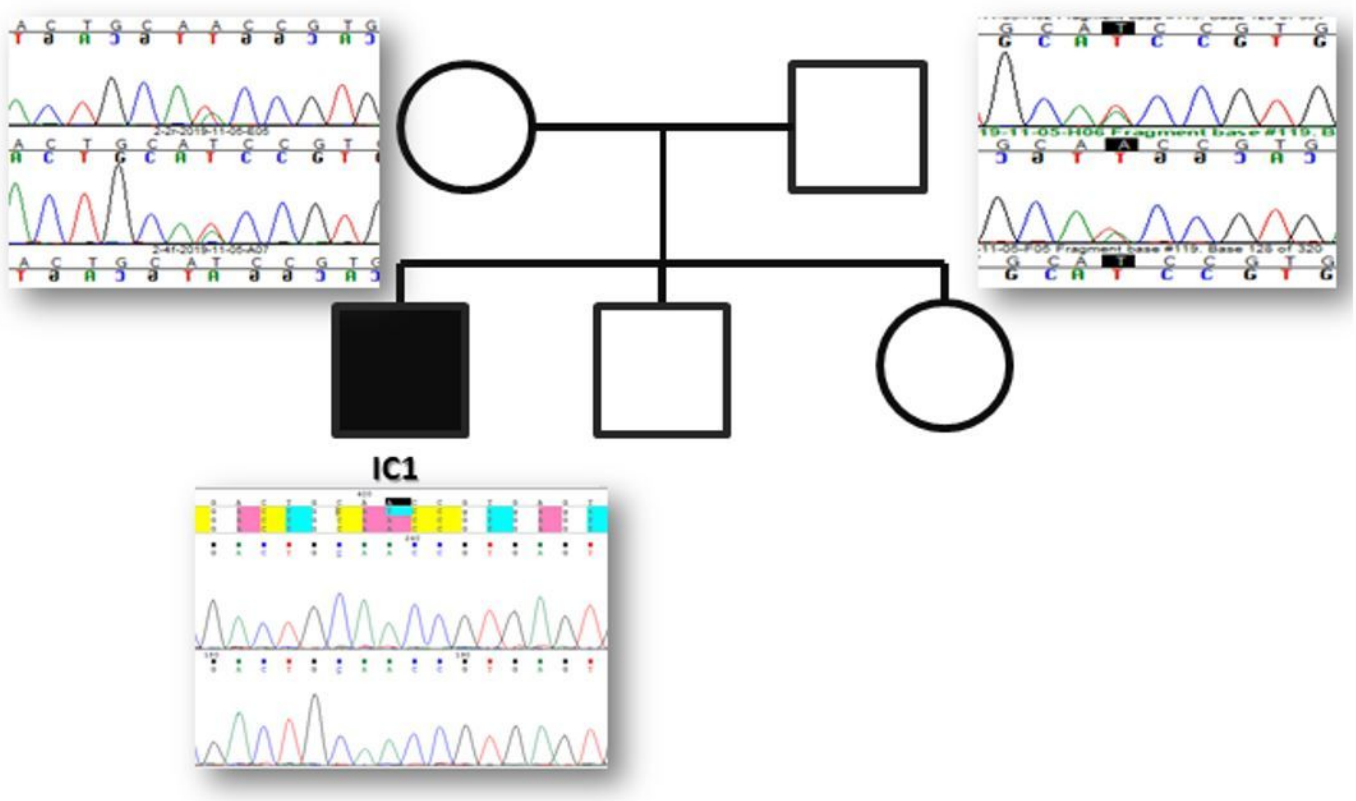

Figure 1

Pedigree and electrophoregram corresponding to the Sanger sequencing confirmation performed for the Index Case (IC) and his parents.