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COPY NUMBER ANALYSIS REVEALS A NOVEL MULTIEXON DELETION OF THE COLQ GENE IN CONGENITAL MYASTHENIA

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Congenital myasthenic syndrome (CMS) is genetically and clinically heterogeneous.¹ Despite a considerable number of causal genes discovered, many patients are left without a specific diagnosis after genetic testing. The presumption is that novel genes yet to be discovered will account for the majority of such patients. However, it is also possible that we are neglecting a type of genetic variation: copy number changes (>50 bp) as causal for some of these patients. Next-generation sequencing (NGS) can simultaneously screen all known causal genes² and is increasingly being validated to have a potential to identify copy number changes.³ We present a CMS case who did not receive a genetic diagnosis from previous Sanger sequencing, but through a novel copy number analysis algorithm integrated into our targeted NGS panel, we discovered a novel copy number mutation in the *COLQ* gene and made a genetic diagnosis. This discovery expands the genotype-phenotype correlation of CMS, leads to improved genetic counsel, and allows for specific pharmacologic treatment.¹

Case report. The patient is a 16-year-old girl of Han Chinese descent. She had progressive muscle weakness in a limb-girdle pattern and neck weakness since age 6. The muscle weakness appeared more severe after prolonged activity and would partly resolve after minutes of rest. She was seen at the neurology clinic of China-Japan Friendship Hospital in July 2013 and was noted to have a waddling gait and could not walk more than ~600 feet. No ptosis or double vision was present. She had normal sensory testing and was without pain or other sensory symptoms. She had weakness of neck muscles, proximal upper limbs 4/5 (Medical Research Council), and proximal lower limbs 3/5, with normal ankle, hand strengths, and deep tendon reflexes. Her parents and a younger brother did not have any symptoms.

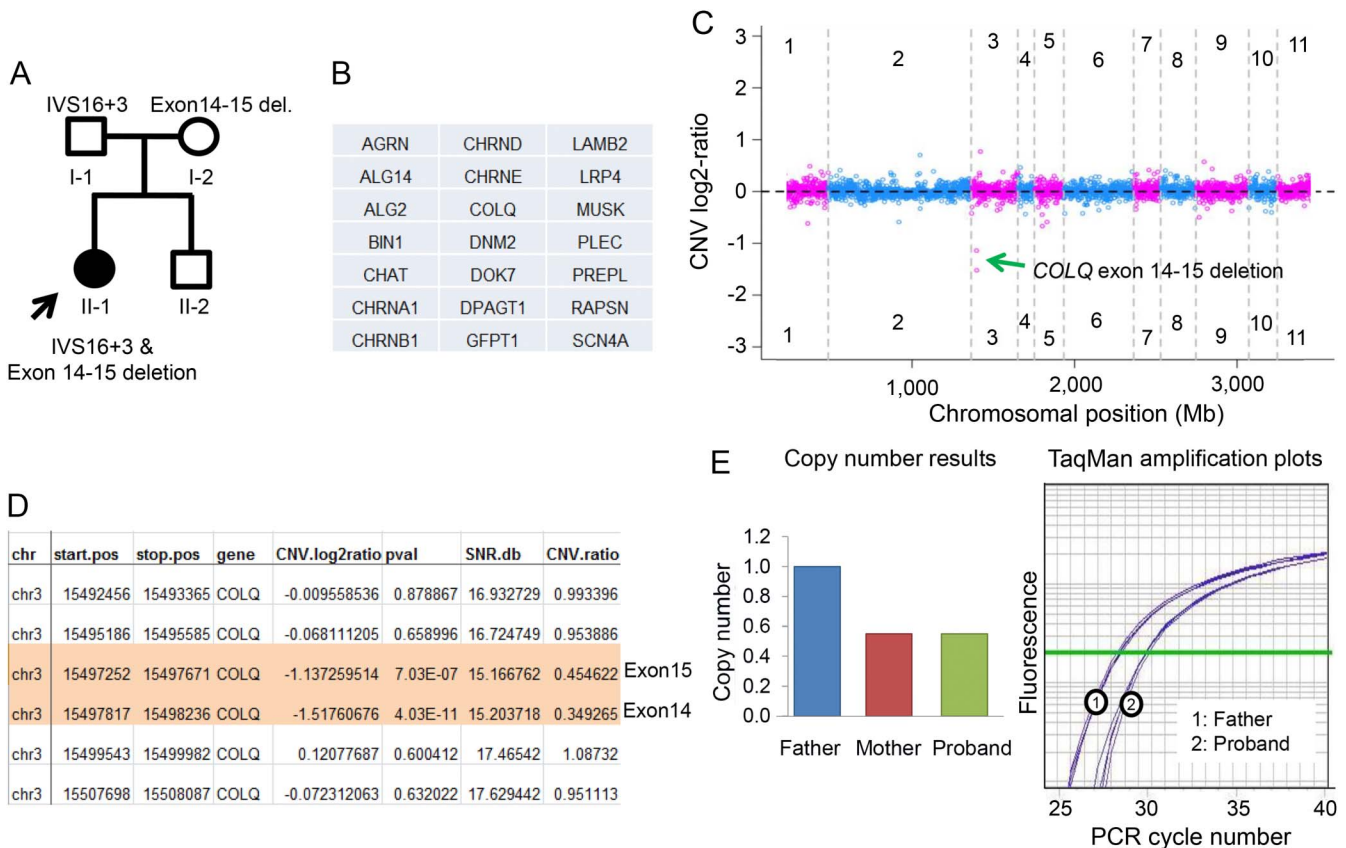
Laboratory testing was unremarkable for creatine kinase, serum lactic acid, anti-acetylcholine receptor, anti-acetylcholinesterase, and anti-Musk antibody. On her EMG, 2 Hz stimulation demonstrated a >10%

decrement without facilitation. Biceps muscle biopsy was without vacuoles, dystrophic changes, or tubular aggregates. Sanger sequencing for *GFPT1*, *DOK-7*, and *COLQ* showed only a heterozygous variant (IVS16+3A→G) in the *COLQ* gene, a previously reported mutation but only deleterious in homozygous state.⁴ This variant was inherited from her father. No genetic diagnosis could be made at that time (figure).

We recently developed a targeted NGS panel including 21 known CMS genes and applied it to this patient. Because a copy number variation (CNV) analysis algorithm (PatternCNV) is incorporated in the bioinformatics evaluation, this panel has a capability of detecting nucleotide changes, small insertion/deletions, and CNVs.³ The result from this panel confirmed the IVS16+3A→G variant and identified a heterozygous copy number deletion encompassing exons 14 and 15 of the *COLQ* gene (~1 kb) (figure, A–C). This copy number change was subsequently confirmed using TaqMan Copy Number Assay (figure, D) and also found in her mother. TaqMan Copy Number Assay was performed using a probe targeting exon 15 of *COLQ* (Hs01393787_cn; Applied Biosystems, Foster City, CA) and housekeeping gene *RNASP* (Applied Biosystems) as copy number control with delta-delta Ct method for copy number calculation. The discovery of this novel copy number mutation of exons 14 and 15 led to the genetic diagnosis of this patient: compound heterozygous *COLQ* mutation, which allowed for more effective genetic counseling in her family. We are initiating drug therapies which have been shown beneficial for patients with *COLQ* mutations.¹

Discussion. Traditional genetic testing often misses large genomic deletions encompassing multiexon (a type of CNV) unless a separate microarray assay is performed. However, comparative genome hybridization arrays would have missed this *COLQ* exons 14 and 15 deletion as the resolution is often inadequate for less than 1 kb deletions. This report underscores the importance of including a validated copy number evaluation in targeted NGS testing in CMS. To date, mutation discovery has been largely focusing on nucleotide changes or small deletion/insertions, mainly due to technology limitations. Current studies estimate that ~10% of the human genome has copy number

Figure Novel compound heterozygous mutation in the *COLQ* gene causes congenital myasthenic syndrome



(A) Pedigree of the proband (II-1). Point mutation (IVS16+3A→G) in the *COLQ* gene was inherited from the father (I-1), and multiexon deletion from the mother (I-2). (B) Gene list of 21 known congenital myasthenic syndrome (CMS) genes included in the targeted CMS panel. (C) Genome view of PatternCNV analysis shows decreased copy number variation (CNV) log₂ ratio for the *COLQ* gene in chromosome 3. (D) Exon-level CNV summary table shows the start and end position of deletion (984 base pairs) in the *COLQ* gene, which indicates 1 copy deletion of exons 14 and 15. SNR.db: signal noise ratio expressed in decibels; CNV.ratio: copy number ratio converted from CNV.log₂ratio. CNV.ratio of 1 indicates no copy number change. (E) TaqMan Copy Number Assay results confirm exon deletions in the *COLQ* gene in the proband, which is also found in her mother (data not shown). Longer PCR cycle number (X-axis) denotes 1 less copy after normalization using *RNASP*.

changes,⁵ a magnitude higher than single nucleotide variants, underlining the importance of understanding copy number changes as we move forward in this molecular diagnostic era. A *COLQ* multiexon deletion (exon 2–3) was first reported once back in 1998.⁶ This is the second multiexon deletion of *COLQ* discovered in almost 20 years. With qPCR method, multiexon deletions of *RAPSN* were recently reported in 15% of previously undiagnosed patients with CMS,⁷ emphasizing the pathogenic role of copy number changes. Validating and providing copy number evaluation in targeted NGS will considerably improve the efficacy of genetic testing and is predicted to reduce the overall cost for CMS and possibly for other genetic neuromuscular disorders.

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