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Exome sequencing identifies a significant variant in methionyl-tRNA synthetase (*MARS*) in a family with late-onset CMT2

Michael Gonzalez[1], **Heather McLaughlin**[2], **Henry Houlden**[3], **Min Guo**[4], **Liu Yo-Tsen**[3], **Marios Hadjivassilious**[5], **Fiorella Speziani**[1], **Xiang-Lei Yang**[6], **Anthony Antonellis**[2,7], **Mary M Reilly**[3], **Stephan Züchner**[1], and **Inherited Neuropathy Consortium (INC)**

[1]Dr John T McDonald Foundation Department of Human Genetics, John P Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, Florida, USA

[2]Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan, USA

[3]Department of Molecular Neurosciences, MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, London, UK

[4]Department of Cancer Biology, The Scripps Research Institute, Jupiter, Florida, USA

[5]Department of Neurosciences, Royal Hallamshire Hospital, Sheffield, UK

[6]Departments of Chemical Physiology and Molecular Biology, The Scripps Research Institute, La Jolla, California, USA

[7]Department of Neurology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Abstract

Charcot–Marie–Tooth (CMT) disease is a genetically heterogeneous condition with >50 genes now being identified. Thanks to new technological developments, namely, exome sequencing, the ability to identify additional rare genes in CMT has been drastically improved. Here we present data suggesting that *MARS* is a very rare novel cause of late-onset CMT2. This is supported by strong functional and evolutionary evidence, yet the absence of additional unrelated cases warrant future studies to substantiate this conclusion.

Corresponding Author: Dr. Stephan Züchner Dr. JT Macdonald Foundation Department for Human Genetics, Hussman Institute for Human Genomics University of Miami Miller School of Medicine Biomedical Research Building (BRB) Room 523, LC: M-860 1501 NW 10th Avenue, Miami, FL 33136, USA szuchner@med.miami.edu.

Contributors

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Report

Charcot–Marie–Tooth (CMT) disease is a genetically heterogeneous disorder of the peripheral nerve, which is clinically divided into primarily demyelinating CMT1 and axonal CMT2. Despite astounding progress in gene identification in CMT, a large part of up to an estimated 70% of CMT2 patients do not have a mutation in any of the known genes.[1,2] The identification of the remaining CMT2 genes is expected to yield important insights into the pathways and pathophysiology associated with axonal degeneration. This will ultimately lead to focused studies aimed at drug development. In addition, it is becoming evident that the phenotypic and genotypic intersection of CMT2 with related motor neurone, axon degeneration and other diseases is more extended than previously thought. The recent introduction of exome sequencing and ultimately whole genome sequencing will be essential to the mapping of exact genotype/phenotype relationships in the coming decade. Currently, exome sequencing offers an economic opportunity to increase the gene discovery pace and, importantly, investigators are able to take advantage of relatively small families or even single cases. However, the presented case study offers insight into the challenges that may come with describing new rare causes of CMT2.

We have studied a family with late-onset CMT2 and incomplete phenotypic penetrance. Examination of the index case at age 50 (figure 1A; III.1) revealed bilateral foot drop, distal wasting in the upper and lower limbs, mild distal weakness in the upper limbs to Medical Research Council scale grade 4, but equal proximal and distal weakness in the lower limbs with both hip flexion and ankle dorsiflexion and plantar flexion being grade 4. The 81-year-old uncle (figure 1A; II.3) of the index case has an axonal neuropathy presenting at age 67. Other causes of a peripheral neuropathy including diabetes mellitus were excluded. The mother (figure 1A; II.2) of the index case was clinically unaffected and showed normal neurophysiology at age 85. Unusual features in the family include equal proximal and distal motor involvement in the lower limbs and the presence of neuropathic pain (see online supplementary data). These clinical results and the pedigree suggested a hereditary late-onset X linked or autosomal-dominant CMT2 with incomplete penetrance.

To identify the underlying genetic cause we applied next-generation sequencing of whole exomes (figure 1A). Analysis focused on nonsynonymous, splice-site and coding indel variants that segregated under an autosomal-dominant and X linked model. First, we analysed the known X linked CMT genes GJB1, AIFM1, PRPS1 and PDK3, but did not identify any changes despite excellent coverage in two male exomes. A total of 3044 variants that met the initial filtering criteria were further filtered for conservation (GERP>3 OR PhastCons>0.7), predicted consequence on protein function (PolyPhen2>0.5 OR unknown) and a minor allele frequency of less than 0.005% in the Exome Variant Server (http://evs.gs.washington.edu/EVS/) and dbSNP137. We also compared the results with 1236 exomes from different phenotypes available in our own database. In this family, only four missense variants passed these filters (see online supplementary table S1). The variant with the highest conservation score (GERP=5.04, PhastCons=1) resided in the methionyl-tRNA synthetase (*MARS* or *MetRS*) gene. Sanger sequencing validated segregation of the c.1852C<T (p.Arg618Cys, chr12 : 57906632 (hg19)) variant in *MARS* in the two affected male family members. As expected, the clinically unaffected mother of the index case, II.2, was an obligate gene carrier. *MARS* is an excellent CMT candidate gene because four aminoacyl-tRNA synthetase (ARS) genes have been shown to cause axonal forms of CMT: glycyl-tRNA synthetase (GARS), tyrosyl-tRNA synthetase (YARS), alanyl-tRNA synthetase (AARS) and lysyl-tRNA synthetase (KARS).[3–6]

We then Sanger sequenced all coding exons of *MARS* in 400 unrelated CMT2 patients, but did not identify any additional pathogenic changes. A search of the entire database of 1236

exomes, including 466 families with CMT, hereditary spastic paraplegia, amyotrophic lateral sclerosis (ALS) and other related phenotypes, only revealed one additional non-synonymous variant in *MARS* (c.1448G->A, p.Arg483His). The affected family, however, was previously diagnosed with CMT1A due to PMP22 gene duplication.

The change identified in the described family is located in the catalytic domain of *MARS* (*MetRS*) and the residue Arg618 is unusually strictly conserved from bacteria to human (figure 1B). In absence of a crystal structure, we performed in silico modeling of the core domains of human *MetRS* (figure 1C). Arg618 is located at the interface of the catalytic domain and the anticodon-binding domain. Interactions with both domains suggest that Arg618 plays an important role in stabilising the domain interface. The Arg618Cys substitution could potentially cause a neomorphic structural opening in the protein. The hypothetic structural opening between the catalytic domain and the anticodon-binding domain may also affect the tRNA aminoacylation function of *MARS*, which is essential for the translation process, and for viability of all organisms. The result may be a gain of function or a loss of function as observed in other CMT-associated ARS mutants.[7]

To assess the functional consequences of Arg618Cys *MARS* in vivo, we modelled the variant in the yeast ortholog *MES1*, and determined the ability of Arg618Cys *MES1* to rescue deletion of endogenous *MES1* compared with wild-type *MES1*, and a common, non-synonymous, non pathogenic missense change (Arg727Gln *MARS*, dbSNP rs113808165; see online supplementary table S2). An insert-free pRS315 construct was unable to rescue the *mes1*Δ allele, whereas both wild-type and Arg727Gln *MES1* were able to fully complement *mes1*Δ (figure 1D). These data are consistent with *MES1* being an essential gene, and with the wild-type and R727Q experimental *MES1* vectors expressing functional proteins, respectively. In contrast, Arg618Cys *MES1* was unable to rescue the *mes1*Δ allele (figure 1D). Combined, these data indicate that Arg618Cys *MARS* represents a loss-of-function allele in vivo.

Our results represent a typical conundrum in the new arena of exome sequencing. While a gene has been identified in a single small CMT2 family with strong functional and evolutionary support, sequencing of 1218 exomes from a diverse set of related and unrelated phenotypes and Sanger sequencing of 400 unrelated CMT2 cases revealed only a single additional relevant change in *MARS*, albeit in a CMT1 family already carrying a PMP22 duplication (CMT1A). This is somewhat further complicated by the late-onset of CMT2 in this family, which we believe contributes to the incomplete penetrance. A wide range of age-of-onset is common in CMT2 and can complicate segregation studies.[8] With over 50 genes known in CMTonly ~30% of CMT2 cases are explained as of today.[1,2] It is to be expected that future genes are exceedingly rare causes of the disease and it will thus become difficult to fully verify their relevance. We believe however that it will be valuable to carefully report such gene identifications as to provide the research field the opportunity to further support or disprove such claims in the future. Although our finding required a number of assumptions on variant filtering typical for exome sequencing studies, we are suggesting *MARS* as a novel rare CMT2 gene for the following reasons: (1) near complete evaluation of functionally strong variants in the studied family; (2) unusually strong conservation of the mutation across species; (3) four pre-existing ARS genes causing CMT phenotypes; and (4) a demonstrated loss-of-function effect of Arg618Cys *MARS* similar to many other CMT-associated ARS alleles.[4,6,9]

In summary, we are presenting data for a late-onset CMT2 family suggesting *MARS* as a novel, yet very rare cause of the disease. These results warrant further evaluation of the *MARS* locus for pathogenic mutations in patients with axonal CMT disease, and further

underscore the importance of relevant functional evidence toward implicating rare variants in disease-onset in pedigrees too small for significant linkage analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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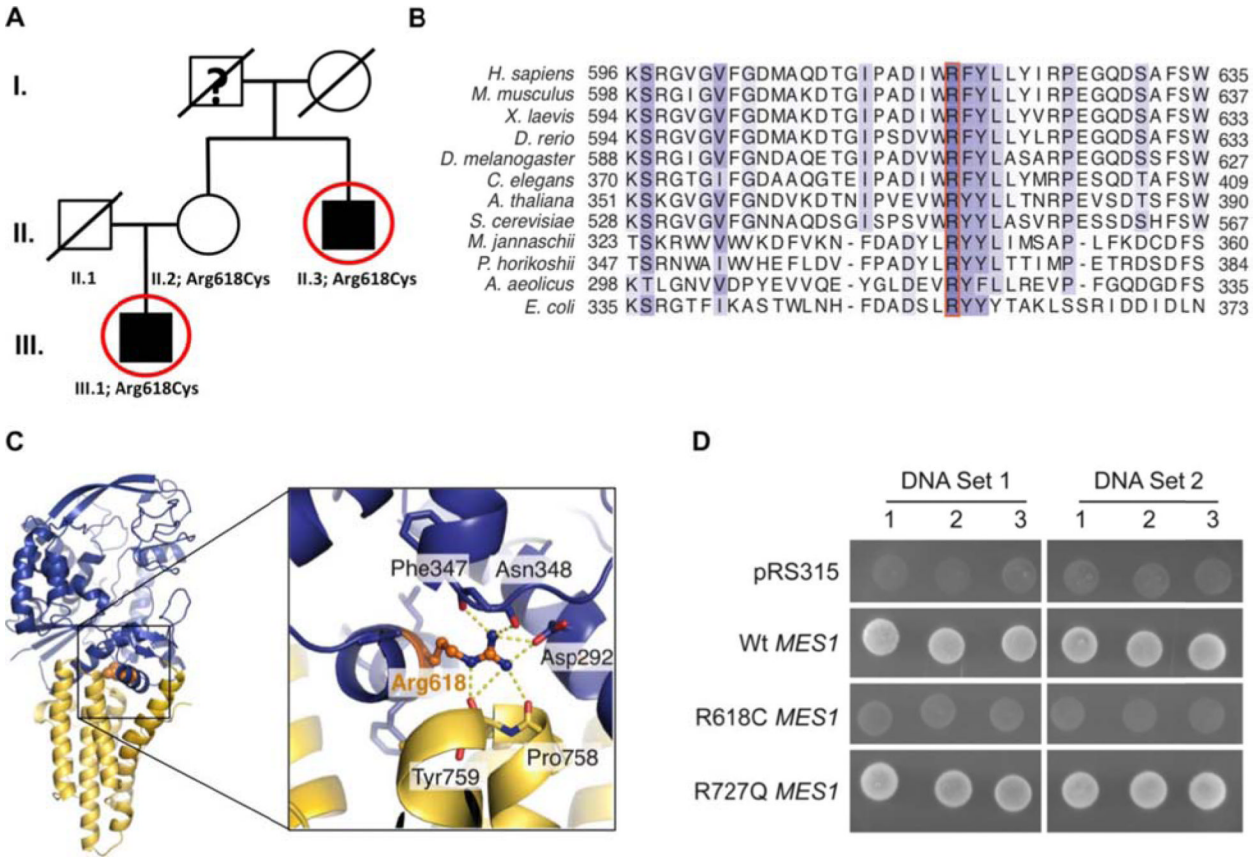

Figure 1.

(A) Pedigree of dominant CMT2 family displaying segregation of Arg618Cys. Two affected male individuals were analysed by exome sequencing (circles). (B) Sequence alignment of the MetRS proteins from becteria to human showing that Arg618 is a strictly conserved residue during evolution. (C) Structural model of human MetRS showing that Arg618 is located at the interface of the catalytic domain (blue) and the anticodon-binding domain (yellow), with the guanidinium side chain forming a strong salt-bridge with the side chain of Asp292 from the catalytic domain, and extensive hydrogen-bonding interactions with the backbone carbonly oxygens of Phe347 and Asn348 from the catalytic domain and of Pro758 and Tyr759 from the anticodon-binding domain. (D) Three representative cultures of each yeast strain (indicated along the top of each panel) were inoculated and grown on solid growth medium containing 5-FOA (see Methods for details). Each strain was previously transfected with a vector containing no insert (pRS315), wild-type *MES1* (wt *MES1*) or the indicated variant form of *MES1*. Two independently generated mutant-bearing constructs were analysed (DNA Set 1 and DNA Set 2). Before inoculating on 5-FOA-containing medium, each strain was resuspended in 100 μl water, then diluted 1:10.