**Supplementary Material**

**Supplemental 2.1 Exome sequencing**

All rare and non-synonymous coding variants were examined. Rare variants were defined as those with a mapping allele frequency less than 1% in the databases Exome Variant Server (EVS; http://evs. gs.washington.edu/EVS/), 1000 Genomes Project (http://www. 1000genomes.org/), ExAC (http://www.exac.broadinstitute.org), and GnomAD (http://www.gnomad.broadinstitute.org). Variants were disregarded if seen in more than 6 samples from our in-house database (~2000 exomes previously sequenced at the McGill University and Genome Quebec Innovation Centre). Various in silico prediction scores were assessed including SIFT, PolyPhen-2, CADD, MutationTaster, and GERP [23,24].

**Supplemental 2.2 Yeast complementation assays**

After mutagenesis using the QuickChange II XL Site-Directed Mutagenesis Kit (Stratagene), resulting expression clones were purified and fully sequenced to confirm successful mutagenesis and to rule out PCR-induced errors. Wild-type or mutant human *MARS* was cloned into pYY1 using Gateway Cloning technology (Invitrogen) [25]. Resulting expression clones were digested with *Bsr*GI (NEB) to confirm successful integration of the open-reading frame. A haploid Δ*Mes1* strain (harboring a maintenance vector to express wild-type *Mes1* and *URA3*) was transformed with an empty vector (‘Empty’ in Fig. 1) or wild-type or p.Ala397Thr *MARS* in a *LEU2*-bearing vector and selected on medium lacking uracil and leucine (Teknova).

**Supplemental Table 1:** Nerve conduction studies

|  |  |  |
| --- | --- | --- |
|  | Normal\* | At 4yr10m old |
|  |  |  |
| **MOTOR:** |  | Right | Left |
| Median nerve  |  |  |  |
|  DML (wrist-APB) | < 3.2 |  | **3.3** |
|  CMAP (mV) | > 3.0 |  | **1.1** |
|  CV (m/sec) | >49 |  | 58 |
| Tibial nerve |  |  |  |
|  DML (msec; ankle-AH) | < 4.7 | **5.5** | 3.1 |
|  CMAP (mV) | > 4.8 | **0.5** | **0.5** |
|  CV (m/sec) | > 40 | **38** | **32** |
| Peroneal nerve |  |  |  |
|  DML (msec; ankle-EDB) | < 4.1 | **NR** | **NR** |
|  CMAP (mV) | > 1.3 |  |  |
|  CV (m/sec) | > 40 |  |  |
| **SENSORY:** |  |  |  |
| Median nerve  |  |  |  |
|  PL (msec; wrist-digitII) | < 3.2 |  | 1.3 |
|  SNAP (V) | > 14 |  | 30.3 |
|  CV (m/sec) | > 48 |  | 69 |
| Sural nerve  |  |  |  |
|  PL (msec; calf-latmall) | < 4.2 | 2.1 | 2.2 |
|  SNAP (V) | > 6 | 17.3 | 10.3 |
|  CV (m/sec) | > 41 | 46 | 48 |
|  |  |  |  |

**Bold values** are abnormal. All sensory responses are antidromic.

**Legend**: DML=distal onset motor latency; CMAP=compound motor action potential; CV=conduction velocity; PL=peak onset latency; NR=no response; APB=abductor pollicus brevis; AH=abductor hallicus; EDB=extensor digitorum brevis.

**\*Normal reference values** from: Kang PB. Normal values tables. In: McMillan HJ, Kang PB, eds. *Pediatric Electromyography: Concepts and clinical applications.* Springer.; 2017:373-378.

N/A: information not available