Supp. Figure S1. Heterozygous variants detected in the \textit{AARS} gene. Chromatograms from control and affected (‘Patient’) individuals are shown for R329H (left) and E778A (right). The nucleotides and resulting amino-acid sequences are shown, with ‘R’ indicating heterozygosity for a ‘G’ and ‘A’ nucleotide, and ‘M’ indicating heterozygosity for an ‘A’ and ‘C’ nucleotide.
Supp. Figure S2. Effect of AARS variants on editing activity. Deacylation of the incorrectly charged Ser-tRNA\textsuperscript{Ala} by the wild-type (red), E778A (black), and the previously-described A734E (yellow; see discussion) AARS enzymes is plotted over time. The uncatalyzed deacylation (the no-enzyme reaction, indicated in orange) was run in parallel as a control for background hydrolysis. Values represent the average of two independent experiments, and error bars indicate the standard deviation.
Supp. Figure S3. Evaluation of axonal AARS localization in differentiated MN-1 cells. A, B: Differentiated MN-1 cells were stained with an anti-AARS antibody and visualized via confocal microscopy. Endogenous AARS protein was localized diffusely throughout the cell body, nucleus, and neurite projections. C: Wild-type AARS tagged with DsRed on the C-termus was transiently expressed in MN-1 cells. After differentiation, localization was analyzed via confocal microscopy. Wild-type AARS was localized diffusely throughout the cell body and neurite projections (arrow). D: Similar analyses as described in (C) for E778A AARS. Similar to wild-type AARS, E778A AARS was localized diffusely throughout the cell body and neurite projections (arrow).
Supp. Figure S4. Bisulfite sequencing analysis of \textit{AARS} exon 7, \textit{AARS} exon 9, and \textit{SOX3}. A: Evaluation of the methylation status of 5 CpGs in \textit{AARS} exon 7. A representation of bisulfite
sequencing products of \textit{AARS} exon 7 is shown for two control individuals (Control 1 and Control 2). Seventeen and eighteen clones were analyzed for Control 1 and Control 2, respectively. Filled circles indicate methylated CpGs. \textbf{B}: Similar analyses as described in (\textbf{A}) for two CpG dinucleotides within \textit{AARS} Exon 9. Eighteen and nineteen clones were evaluated for Control 1 and Control 2, respectively. \textbf{C}: Evaluation of the methylation status of 19 CpGs in a \textit{SOX3} CpG island. The female control (19 clones evaluated) is denoted ♀, while the male control (18 clones evaluated) is denoted ♂.