

Supplement 4: Burden analysis of missense variants in the total *SYNE1* gene and in the actin-binding domain of *SYNE1*

We analyzed whether rare, predicted damaging **missense** variants in *SYNE1* were more frequent in whole exome (WES) datasets of ataxia cases versus controls. Cases comprised a consecutive series of n=96 index patients with early-onset degenerative ataxia (age of onset <40 years) compatible with autosomal recessive inheritance (no ataxia in the parental generation) and negative for trinucleotide repeat expansions causing Friedreich's ataxia (FRDA). Controls comprised a consecutive series of n=250 index subjects with early-onset Alzheimer's Disease (EOAD). This disease was selected for control as this condition is not part of the *SYNE1* disease spectrum, not even of the extended disease spectrum described in this manuscript.

Exome analysis of both cases and controls was performed at the Hussman Institute for Human Genomics in Miami, Florida. WES was performed using a SureSelect Human All Exon 50Mb kit (Agilent, Santa Clara, CA, USA) for in-solution enrichment and a HiSeq2000 and HiSeq2500 instruments for exome sequencing (Illumina, San Diego, CA, USA). Paired-end reads of 100 bp length were produced. BWA and FreeBayes software packages (Li and Durbin, 2009, McKenna *et al.*, 2010, DePristo *et al.*, 2011, Garrison and Marth, 2012) were used to align sequence reads to the reference and call variant positions, respectively. All data were then annotated and imported into GENESIS, a web-based tool for next generation sequencing data analysis (Gonzalez *et al.*, 2013, Gonzalez *et al.*, 2015). Exome datasets were filtered for non-synonymous missense variants in *SYNE1*, with low frequency in public databases (minor allele frequency in EVS, 1000 Genomes and ExAc each <0.5%, AND variant alleles in GENESIS <40), at good genotype quality (GQ > 50 OR QUAL > 35) AND depth > 10), and predicted to be damaging by at least two out of three in silico software predictions (MutationTaster, SIFT, PolyPhen2).

Cases (ataxia)

| Sample ID | chrom | position | reference | minor allele | MutationTaster | SIFT | PolyPhen2 |
|--------------|-------|-----------|-----------|--------------|-----------------|-----------|-------------------|
| 201510203385 | 6 | 152461162 | C | T | disease causing | NULL | possibly-damaging |
| 201246854 | 6 | 152563538 | C | T | disease causing | NULL | probably-damaging |
| 400003331 | 6 | 152804308 | G | A | disease causing | NULL | probably-damaging |
| 201504300122 | 6 | 152776635 | C | T | disease causing | damaging | probably-damaging |
| 400002588 | 6 | 152651971 | T | G | disease causing | NULL | probably-damaging |
| 201246895 | 6 | 152553376 | G | A | disease causing | NULL | probably-damaging |
| 201246835 | 6 | 152464786 | G | A | disease causing | NULL | probably-damaging |
| 400002014 | 6 | 152614793 | G | C | disease causing | NULL | probably-damaging |
| 400002572 | 6 | 152768726 | T | G | disease causing | damaging | probably-damaging |
| 400001969 | 6 | 152647194 | C | T | disease causing | tolerated | probably-damaging |
| 400002565 | 6 | 152651557 | G | A | disease causing | NULL | probably-damaging |
| 201246926 | 6 | 152806014 | C | T | disease causing | NULL | probably-damaging |
| 400002015 | 6 | 152466622 | C | T | disease causing | damaging | possibly-damaging |
| 400004048 | 6 | 152466622 | C | T | disease causing | damaging | possibly-damaging |

Controls (EOAD)

| Sample ID | chrom | position | reference | minor allele | MutationTaster | SIFT | PolyPhen2 |
|--------------|-------|-----------|-----------|--------------|-----------------|-----------|-------------------|
| 201508252619 | 6 | 152461162 | C | T | disease causing | NULL | possibly-damaging |
| 201508252623 | 6 | 152454556 | A | G | disease causing | damaging | probably-damaging |
| 201508110031 | 6 | 152762307 | A | T | disease causing | NULL | possibly-damaging |
| 201508252620 | 6 | 152717943 | T | A | disease causing | NULL | probably-damaging |
| 201508252653 | 6 | 152651911 | C | T | disease causing | NULL | probably-damaging |
| 201508252653 | 6 | 152501416 | C | T | disease causing | NULL | probably-damaging |
| 201508180988 | 6 | 152501416 | C | T | disease causing | NULL | probably-damaging |
| 201508282749 | 6 | 152697692 | G | C | disease causing | NULL | probably-damaging |
| 201508252701 | 6 | 152762307 | A | T | disease causing | NULL | possibly-damaging |
| 201508252668 | 6 | 152615118 | A | G | disease causing | NULL | probably-damaging |
| 201508110029 | 6 | 152472789 | G | A | disease causing | NULL | probably-damaging |
| 201508282760 | 6 | 152501416 | C | T | disease causing | NULL | probably-damaging |
| 201508180970 | 6 | 152456276 | T | G | disease causing | NULL | probably-damaging |
| 201508252659 | 6 | 152501416 | C | T | disease causing | NULL | probably-damaging |
| 201508252612 | 6 | 152697692 | G | C | disease causing | NULL | probably-damaging |
| 201508110008 | 6 | 152680581 | C | T | disease causing | damaging | probably-damaging |
| 201508252636 | 6 | 152806014 | C | T | disease causing | NULL | probably-damaging |
| 201508252649 | 6 | 152472789 | G | A | disease causing | NULL | probably-damaging |
| 201508252628 | 6 | 152776571 | C | T | polymorphism | damaging | possibly-damaging |
| 201508252613 | 6 | 152485384 | C | T | disease causing | NULL | probably-damaging |
| 201508110056 | 6 | 152652439 | C | T | disease causing | tolerated | probably-damaging |
| 201508110034 | 6 | 152720912 | G | T | disease causing | NULL | probably-damaging |
| 201508252628 | 6 | 152501416 | C | T | disease causing | NULL | probably-damaging |

| | | | | | | | |
|--------------|---|-----------|---|---|-----------------|------|-------------------|
| 201508252706 | 6 | 152697692 | G | C | disease causing | NULL | probably-damaging |
| 201508180967 | 6 | 152831401 | G | A | disease causing | NULL | probably-damaging |
| 201508252705 | 6 | 152673440 | G | A | disease causing | NULL | probably-damaging |
| 201508180975 | 6 | 152757129 | C | A | disease causing | NULL | probably-damaging |
| 201508180946 | 6 | 152469377 | G | A | disease causing | NULL | probably-damaging |

Table: Rare missense variants in SYNE1 in ataxia cases (top) and controls (EOAD patients, bottom) predicted to be damaging by at least two software predictions. Chrom= chromosome.

This analysis yielded 14/192 (=7.3%) rare missense *SYNE1* variant alleles in cases and 28/500 (=5.6%) rare missense *SYNE1* variant alleles in controls in the whole *SYNE1* gene (GRCh37/hg19: chr6:152442822-152957986), without statistical difference between cases and controls ($p=0.48$; Fisher's exact test, two-tailed).

We next tested the possibility that rare missense variants might be more frequent in ataxia cases than in controls only in the N-terminal actin-binding domain of *SYNE1* (codon 1-289 [transcript NM_0033071, NP_149062]; chromosomal position according to GRCh37/hg19: chr6:152823810-152949466). This analysis yielded 0/192 (=0%) rare missense *SYNE1* variants in cases and 1/500 (=0.2%) rare missense *SYNE1* variants in controls, without statistical difference between cases and controls ($p=1$; Fisher's exact test, two-tailed). Please note that our case cohort for the missense burden analysis comprised only of 96 early-onset ataxia patients who received WES. The index case with the missense (plus truncating) *SYNE1* mutation reported in the main text (family #20) was not part of this cohort as this case received only panel sequencing and was thus not part of the cohort entered into the burden analysis.

Taken together, these findings do not support the hypothesis that rare missense variants in *SYNE1* are more frequent in ataxia patients than in controls- neither in the total *SYNE1* gene nor in the actin binding domain. This argues against a significant contribution of rare missense alleles in *SYNE1* to autosomal recessive ataxia.

References:

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