

SUPPLEMENTARY MATERIAL

Supplementary Methods

As part of a larger cohort whole genome sequencing was performed at the National Institutes of Health (NIH) Intramural Sequencing Center (NISC) using high molecular weight DNA extracted from the patient's cultured fibroblasts. Illumina sequencing libraries are generated from 1 microgram genomic DNA using the TruSeq[®] DNA PCR-Free HT Sample Preparation Kit (Illumina, San Diego, CA, USA) with median insert sizes of approximately 400 bp. Libraries are tagged with unique dual index DNA barcodes to allow pooling of libraries and minimize the impact of barcode hopping. Libraries are pooled for sequencing on the NovaSeq 6000 (Illumina, San Diego, CA, USA) to obtain at least 300 million 151-base read pairs per individual library.

The bioinformatics pipeline follows the best practices guidelines for the Genome Analysis Toolkit (GATK).¹ Reads were aligned to the human b37_decoy reference sequence (UCSC assembly hg19, NCBI build 37) using BWA² and variants were called using the GATK Haplotype Caller and filtered based on the best practices recommendations. Using ANNOVAR,³ rare variants were filtered using a minor allele frequency (MAF) of < 0.05 annotated in gnomAD v2.11 (<https://gnomad.broadinstitute.org/>) and their functional effect was evaluated using SIFT, PolyPhen-2 HVAR, PolyPhen-2 HDIV, LRT, Mutationtaster, MutationAssessor, FATHMM, MetaSVM, MetaLR and PROVEAN.

Genes included in the NIH Genetic testing registry's panel for dystonia were evaluated and only one variant, rs74315457 in Arylsulfatase A (ARSA), was detected as deleterious by more than one program. The variant is also reported as pathogenic in ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>). When evaluating the entire genome > 70 variants were called by > 6/10 of the programs used for functional effect assessment. Apart from the known pathogenic mutations in GBA1, two other identified variants were classified as pathogenic in ClinVar, rs121908646 in Argininosuccinate Synthase 1 (ASS1) and rs150343959 in ClpB Family Mitochondrial Disaggregase (CLPB).

REFERENCES

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3. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010; 38:e164.