Supplemental Materials 2: NGS Pipeline and Bioinformatics

Lab 1: Ataxia Exome; Testing on V1 and remaining family members

Exome sequencing was performed using the Agilent SureSelect Clinical Research Exome kit (Agilent Technologies Inc. Santa Clara, USA) that targets the whole exome with improved capture of exons of medically important genes. Sequencing was performed using Illumina NextSeq technology with 150bp paired-end reads (Illumina Inc. San Diego, USA). Variants were analyzed in a pre-defined set of 441 genes.  The 441 genes were generated by a systematic review of databases such as PubMed, OMIM, HGMD and HPO and curated to include genes that had sufficient evidence associating them with ataxia-related conditions.  The 441 genes selected are implicated in pure forms of cerebellar ataxia, genes associated with syndromes that have ataxia as part of the clinical presentation, and genes associated with spasticity and other movement abnormalities that may be misinterpreted as ataxia.  Variants within exons and canonical splice sites in the 441 genes were identified and evaluated using a validated, custom bioinformatic pipeline.  Variants with a global population frequency of ≥1% in ExAC were excluded. Variants were interpreted by a team of board-certified PhD geneticists, MD geneticists, genetic counselors and neurologists. The ACMG guidelines for sequence variant interpretation were utilized to categorize variants. Data was assessed for quality, and to confirm it had a minimum coverage of 30x for at least 90% of targeted regions. The mean depth of coverage per sample was over 150x, and on average more than 96% of the targeted regions were covered at a minimum of 30x. Variants considered likely related to the patient's phenotype were confirmed by Sanger sequencing. For further details see methods section of the following article:

Sun, Miao, Amy Knight Johnson, Viswateja Nelakuditi et al. “Targeted Exome Analysis Identifies the Genetic Basis of Disease in over 50% of Patients with a Wide Range of Ataxia-Related Phenotypes.” *Genetics in Medicine* 21, no. 1 (January 2019): 195. https://doi.org/10.1038/s41436-018-0007-7.

Lab 2: Ataxia Exome; Testing on VI12

The Agilent SureSelect Clinical Research Exome XT capture kit (Agilent Technologies, Santa Clara, CA) was used to prepare genomic DNA for sequencing which was then performed on an Illumina HiSeq2500 machine (Illumina, San Diego, CA). Alignment to the human reference genome (hs37d5) was done using Burrows-Wheeler Aligner. GATK3 was used to generate variant calls which were filtered using the ExAC database for variants with an MAF of 2% or less. Variants of interest were then prioritized for further assessment and interpretation using ACMG guidelines. For further details see methods section of the following article:

Fogel, Brent L., Hane Lee, Joshua L. Deignan et al. “Exome Sequencing in the Clinical Diagnosis of Sporadic or Familial Cerebellar Ataxia.” *JAMA Neurology* 71, no. 10 (October 2014): 1237–1246. https://doi.org/10.1001/jamaneurol.2014.1944.

Lab 3: Retinal Dystrophy Testing; Testing on IV1

The test performed here was an allele-specific pre-screen followed by a retinal disease-gene targeted analysis of whole exome sequencing data. For further details see methods section of the following article:

Stone, Edwin M., Jeaneen L. Andorf, S. Scott Whitmore et al. “Clinically Focused Molecular Investigation of 1000 Consecutive Families with Inherited Retinal Disease.” *Ophthalmology* 124, no. 9 (September 1, 2017): 1314–31. https://doi.org/10.1016/j.ophtha.2017.04.008.