

SUPPLEMENTARY FILE 1 – SUPPLEMENTARY METHODS

Generation of WGS data

A total of 850 DNA samples from 638 SALS patients, 103 FALS patients and 109 FTD patients underwent library preparation using the TruSeq PCR free library preparation kit (Illumina, v2.5). Prepared libraries underwent multiplex 150bp paired-end sequencing on an Illumina HiSeq X Ten instrument (Kinghorn Centre for Clinical Genomics, Sydney, Australia). Sequencing data was quality controlled based on per lane metrics. Percentage of bases with phred base quality score ≥ 30 exceeded 75, Mean coverage for aligned reads was set at a minimum of 30X. Sequencing yield (GB) and Duplicate read rate was also estimated. Samples were demultiplexed using bcl2fastq [version 2.16.0], aligned to hs37d5x using bwa mem [version 0.7.15-r1140] and bamsortdup [version 2.0.65] was used for sorting, duplicate marking and indexing BAM files. GATK [version 3.7-0-gcfedb67] IndelRealigner and BaseRecalibrator were then used, followed by sambamba merge [version 0.6.5] to produce the final per-sample BAM file. GATK HaplotypeCaller was run to produce the final per-sample Genomic VCF (gVCF). gVCF files were merged using GATK CombineGVCFs, and then input into GATK GenotypeGVCFs to genotype the complete cohort of samples. Variant Recalibration was performed on all variants in gVCF files using GATK VariantRecalibrator and ApplyRecalibration to yield a single joint-called VCF for all samples. The final joint-called VCF variants were annotated using ANNOVAR (version 2017Jul16).

Filtering of WGS data

Samples from joint VCF file were excluded if they did not pass a number of quality control criteria. Human-Human contamination rate was estimated using VerifyBamID [version 1.1.3] and samples with a contamination rate > 0.03 were excluded. Principle Component Analysis was used to identify samples from similar population background. Samples which were outliers to the main population group cluster were excluded. Samples which had a high level ($>5\%$) of missing genotypes were also filtered out. One sample whose autopsy result came back negative for ALS was also removed. A total of 616 SALS cases remained.