Whole genome sequencing analysis of a family with familial dysautonomia and neuropsychiatric symptoms

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Background
We report here our discovery and whole genome sequencing (WGS) analysis of a family with dominantly inherited familial dysautonomia (Figure 1). The mother is affected with dysautonomia, hereditary hemochromatosis, and obsessive compulsive traits. The oldest daughter reports severe dysautonomic syncopal episodes, gastroparesis, glutenoma, visual and auditory hallucinations, urinary retention, and one prior stroke. One son is affected with dysautonomia, Tourette syndrome (TS), attention deficit disorder (ADD), and obsessive-compulsive disorder (OCD). Another son reports dysautonomia, asthma, seizure in response to pertussis, dyslexia, migraine, dysgraphia, ADD, sensory integration disorder, and arthritis.

Methods
WGS was performed on two affected siblings in the Illumina CLIA-certified lab. Three other DNA samples from the family also underwent WGS in the research setting at CSHL. DNA samples from the same five people were genotyped using the Illumina HumanOmni2.5-8 BeadChip. Genome-wide parametric linkage analysis was performed using Merlin (adjusted for linkage disequilibrium). Our group previously reported the low concordance rates across different variant calling pipelines. Thus, to reduce algorithm-induced biases, we used multiple pipelines for quality control, alignment, assembly, variant calling, genotype refinement, variant filtering, and variant annotation (Figure 2). The Empowered Genome Cohort data, implemented in the Ingenuity variant analysis system, was also used to filter out WGS-specific sequencing errors, which might have been identified as rare variants.

Results
Linkage analysis showed not enough power for identifying any disease relevant variant loci in a single family. None of the family members carry any previously reported variants in IKKAP that have been implicated in the autosomal recessive transmission of familial dysautonomia. The WGS data had good sequence coverage for IKKAP in all five people (mean coverage ~40x) but we did not identify any novel rare variants in this gene. ERDS and PennCNV did not reveal any CNVs that of relevance to the phenotype of familial dysautonomia. We have generated a list of rare variants of unknown significance (Table 1 & 2). Sanger sequencing results confirmed an insertion in POU4F1 (Figure 4). We find relatively low concordance across three variant detection pipelines (Figure 5). Pharmacogenomic analyses reveal the recommended dosages of Coumaidin and Simvastatin (Table 3). Five variants in three people with prior clinical evidence are also shown (Table 4).

Conclusions
Here we show one example of individualized medicine in a family with neuropsychiatric symptoms and at least two rare diseases. WGS data provides much more clinically relevant and potentially actionable information when compared to genotyping arrays. Despite limited overall agreement between CLIA certified and non-CLIA certified analysis pipelines, utilizing variants identified by two or more pipelines enables WGS to act as a useful technique for clinical diagnostics. For rare diseases, we argue that family history and other family members’ sequencing data should be incorporated into downstream analyses so as to eliminate false positive findings of disease-associated variants. We highlight the importance of detailed phenotyping and sharing of both genomic and phenotypic data because of extreme heterogeneity across families and insufficient knowledge of rare diseases. Ongoing effort will focus on identifying and proving familial dysautonomia relevant variants, extending the pharmacogenomic analyses to the other people in the pedigree, and including more family members in our study.

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