Next Generation Sequencing diagnostics for HSPs

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Hereditary spastic paraplegias are challenging for diagnostic laboratories because overwhelming heterogeneity precluded comprehensive testing approaches in the past. With the advent of next generation sequencing, virtually all known HSP genes can be tested at once. This increases the expected diagnostic yield significantly, as we tented to analyze only one to a few “good” disease genes in the past. After setting up a disease gene list for all known HSPs gene and several “HSP-related” genes, we develop a HaloPlex exon enrichment assay (Agilent) for 62 genes with > 150kb coding sequences for Illumina MiSeq V2 paired-end sequencing. A pilot study with 14, yet undiagnosed HSP patients revealed that we could sequence > 2/3 of these genes for all coding bases at more than 20 reads coverage. Mean coverage was 500-800 reads. A standard bioinformatic pipeline for mapping (stampy) and annotation (annovar) yielded at total of 82 ± 5 variants in our disease genes. Only 3 ± 2 variants were rare and therefore further evaluated as a potential cause for the spastic phenotype. Interestingly, we identified three known HSP mutations in for patients (in NIPA1, ATL1, and KIF5A). Another three patients carried known heterozygous mutations for recessive diseases (in the GBA, and PANK2 gene, respectively). Whether these genotypes represent risk factors for HSP or rather have been detected by chance without any pathogenic involvement remains to be determined. In conclusion, next-generation sequencing can provide high throughput diagnostics for HSPs by genotyping all known and potentially associated genes in these heterogenic disease.