Microarray based molecular diagnostic testing in HSP

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Background:

Hereditary spastic paraplegias (HSP) are a genetically highly heterogeneous group of disorders. To date 30 genetic loci and 13 genes have been described (SPG1-33), two of which only this year. The most common form of HSP is SPG4, caused by spastin mutations, that accounts for about 50% of autosomal dominant disease. Other forms of HSP like SPG13 (HSP60 mutations) have been identified in single families only. Therefore, especially for rarer genetic subtypes of HSP, only rudimentary data on genotype-phenotype correlations exist. This renders cost-effective use of molecular genetic testing in HSP almost impossible. Furthermore routine molecular genetic testing is currently available for 4 out of 11 HSP genes only.

Objective:

To provide fast, reliable and cost-effective molecular genetic testing for most forms of HSP, where the disease causing genes have been identified (SPG1/2/3/4/6/7/10/13/17/20/21).

Methods:

DNA is amplified using specific primer sequences for all coding exons of eleven known HSP genes.

The amplified DNA is then fragmentated, labelled and hybridized to the CustomSeq Resequencing array (Affymetrix). The data is read out by the GeneChip Scanner 3000. One array can analyze 50kb of double stranded DNA (100kb total); accuracy is 99.998%, specificity is 99.9999%.

REEP1 (SPG31) and ZFYVE27 (SPG33) were identified after chip design was finished so they were not included on the chip.

Results:

PCR amplification was established for 145 coding exons of eleven known HSP genes. DNA samples containing known mutations in HSP genes were collected. Six known genes causing autosomal dominant HSP were sequenced conventionally in 20 index patients of autosomal dominant HSP families. The five known autosomal recessive HSP genes and two genes known to cause X linked disease were sequenced in ten index patients of autosomal recessive / X-linked HSP families respectively. These DNA samples served as controls to validate the Resequencing Array.

We present microarray data of >20 autosomal dominant HSP patients.

Conclusions:

Microarray based molecular diagnostic testing will increase the proportion of HSP patient where genetic diagnosis is available. Based on this genetic data genotype-phenotype correlations will be studied. Identification of novel HSP mutations might provide valuable insights in disease pathogenesis.