Genomic copy number of HSP genes in HSP patients

Mutation screening strategies are largely confined to exon-by-exon sequencing of genomic DNA. In genetically heterogeneous disorders, a negative screen is generally considered to indicate a mutation in an alternative disease gene. This notion, however, is challanged when linkage to the gene under analysis has been established.
Several pertinet SPG4 hereditary spastic paraplegia (HSP) pedigrees, i.e. families linking to the SPG4 locus (SPAST gene, spastin protein) but apparently lacking a SPAST mutation, have been published. Explanations put forward include (i) mutation of the promotor region or other regulatory sequences, (ii) deep intronic mutations with a deleterious effect on proper splicing, (iii) existence of another HSP gene within the SPG4 locus, and (iv) presence of large genomic rearrangements undetectable by conventional screening methods. In order to systematically test the latter of these hypotheses, a multiplex ligation-dependent probe amplification (MLPA) assay targeting all SPAST exons was developed and validated on a DNA sample known to carry a multi-exonic SPAST deletion. Subsequently, a world-wide collaborative project established access to eight of the 11 SPG4-linked HSP families screened negative for “small” mutations. Partial SPAST deletions segregating with the phenotype were identified in seven of the eight families, indicating that presence of this kind of mutation is the major, if not the only, cause for an apparent lack of SPAST alterations in SPG4-linked HSP (Beetz et al., 2007, Human Mutation). Subsequent screening of the large cohort of German HSP patients collected by the GeNeMove consortium revealed that approximately 10% of all cases of HSP are due to partial SPAST deletions and that haploinsufficiency is a relevant disease mechanism for SPG4 HSP (Beetz et al., 2006, Neurology). Extension of copy number screening to other HSP genes has, so far, led to the identification of a whole gene deletion of the second major disease gene SPG3A (atlastin protein). This genomic variant, however, does not segragate with nor modify the HSP phenotype in a pedigree completely explained by a SPAST mutation. SPG3A haploinsufficiency is, therefore, not a disease mechanism relevant for HSP (Beetz eta l., 2007, Neurogenetics).
Analysing genomic copy number of the SPAST gene has substantially increased mutation detection frequency in HSP patients. Moreover, the very findings impact on the long-standing controversy regarding the disease mechanism relavant for SPG4 HSP. Preliminary results suggest that equally informative data can be obtained by extending this kind of analysis to other HSP genes; pertinent investigations are underway.