Supplemental data

Analyzing mutations in known HSP genes

The shared autosomal segments contained 4 known HSP genes: *ATL1*, *GJC2*, *HSPD1*, and *SPG20*. The sequencing coverage was 99% for the coding regions of these genes. We evaluated two patterns of recessive mutation: homozygous and compound heterozygous. For the homozygous mutation, we filtered homozygous variations in patient II.5 that are not homozygous in the parents. Only one variation passed this threshold, chr2: 198070263A->C (*HSPD1*), which is documented in dbSNP and 1000 genomes and causes a synonymous substitution (Supplemental Table 2).

We identified 23 heterozygous variations. We used the following series of exclusion steps: (a) rejecting variations that are in dbSNP (b) rejecting variations that are synonymous (c) rejecting variations in which none of the parents is heterozygous – this excluded calling errors and bystander variations (d) we identified two heterozygous variations: *GJC2* Gly383Arg and *HSPD1* Gly56Glu, but we could not identify additional mutations in those genes as required from compound heterozygous (Supplemental Table 2). The dbSNP variations in those two genes caused synonymous substitutions and therefore cannot account for the second hit. Variations in *HSPD1* have been associated with a dominant form of HSP (Hansen et al. 2002). In our case, both parents were also carriers of the mutation, excluding the possibility of a dominant form. At least half of the sequence reads came from the reference allele in both variations. This removes the possibility of homozygous mutations. We could not exclude a remote possibility of a compound heterozygous that involves a large indel or a change in repeat elements in the two genes.

Analyzing mutations in chromosome X:

We sought to exclude the possibility of an X-linked disorder. We are not aware of any maternal relatives that are affected by the disease and thus treated it as a remote possibility. The genotyping data indicated that the two patients shared the following segments on chromosome X: 4161050-44975296, 72608092-78512036, 116742313-144375939. We covered more than 90% of the coding regions in these segments. We created a list of variations that are homozygous in the exome data of the patient and not homozygous in the mother. This revealed 58 variations. We then excluded: (a) variations that were in dbSNP (b) variations that caused a synonymous change (c) variations also found in the unaffected father. We found four variations that were in very low quality (supported by a single sequence read with no data from the mother). In addition, 3 out of the 4 variations were not in a highly conserved region (GERP<2). MutationTaster found that the only conserved variation (GERP>2) is likely to be a polymorphism (Supplemental Table 3).

Reference for Supplemental data:

Hansen JJ, Durr A, Cournu-Rebeix I, Georgopoulos C, Ang D, Nielsen MN, Davoine CS, Brice A, Fontaine B, Gregersen N et al. 2002. Hereditary spastic paraplegia SPG13 is associated with a mutation in the gene encoding the mitochondrial chaperonin Hsp60. *Am J Hum Genet* **70**(5): 1328-1332.