Letter to the Editor

A novel frameshift mutation of C19ORF12 causes NBIA4 with cerebellar atrophy and manifests with severe peripheral motor axonal neuropathy

To the Editor:

Neurodegeneration with brain iron accumulation (NBIA) is a group of heterogeneous genetic disorders with extrapyramidal movement disturbance, spasticity and intellectual decline (1). Clinical symptoms overlap between several subtypes, hence their definite classification can only be achieved through molecular testing. Mutations have been found in PANK2 (panothenate kinase–associated neurodegeneration; NBIA1, MIM#234200), PLA2G6 (infantile neuroaxonal dystrophy; NBIA2, MIM#256600), ATP13A2 (Kufor–Rakeb syndrome; NBIA3, MIM#606693), FA2H (fatty-acid-hydroxylase–related neurodegeneration, MIM 612319), and in WDR45 for X-linked NBIA with distinct cranial magnetic resonance imaging (cMRI) features (1).

Mitochondrial membrane protein associated neurodegeneration (NBIA4, MIM#614297) is caused by mutations in C19ORF12 that encodes a highly conserved mitochondrial membrane protein, presumably involved in lipid homeostasis. Clinical features comprise Parkinsonism, dystonia, dysarthria, optic atrophy, spasticity, cognitive decline and psychiatric symptoms. Neuroimaging shows iron accumulation in the globus pallidus and substantia nigra. Half of the patients have a motor axonal neuropathy, however, with no or only mild clinical symptoms (2–4).

We report on a Turkish patient, first son of consanguineous parents, with a novel frameshift mutation, who presented at 11 years with severe peripheral axonal neuropathy antedating NBIA-related symptoms.
by 3 years. Creatine phosphokinase (CPK) elevation (750 U/l) led to a muscle biopsy, which confirmed a neurogenic process (Fig. 1). At 15 years, school performance declined (IQ = 70) and cMRI showed T2-signal attenuation in the globus pallidus and substantia nigra as well as cerebellar atrophy (Fig. 1). One year later he became spastic and developed an extrapyramidal movement disorder with hypomimia and bradykinesia, but without dystonia or tremor. Presently, at 21 years he mainly suffers from neuropathy and severe bradykinesia. Oral treatment with deferiprone (1500 mg/day, starting 9 years after disease manifestation) and L-3,4-Dihydroxyphenylalanine (DOPA)/Carbidopa was without clinical benefit and thus discontinued after 1 year.

After initial exclusion of mutations in PMP22 and PANK2 we combined whole exome sequencing with candidate gene analysis for inherited neuropathies: BSCL2, CTDP1, EGR2, FGD4, FIG4, GDAP1, LITAF, LMNA, MED25, MPZ, MTMR2, NDRG1, NEFL, PMP22, PRX, SBF2, SBF2, SH3TC2, SLC12A6, SOX10, SUMO1; and NBIA:s: PANK2, PLA2G6, FA2H, FTL, MCOLN1, ATP13A2, DCAF17, C19orf12, WDR45. In the 31 candidate genes, we detected 17 homozygous variants; 16 were found homozygous in the 1000 Genomes Project (frequency >0.05) and thus removed. A cytosine insertion at Chr19:30,193,874 (hg19) in C19orf12 remained as single pathogenic variant. Its genotype–phenotype segregation in the family was verified by Sanger sequencing (Fig. 2). The c.177-178insG mutation (NM_031448.3) truncates the protein by 51% (p.Leu60Alafs10X). On Western blot the specific C19orf12-band was absent, probably due to degradation of the mutant protein. As the primary rabbit polyclonal antibody was generated against full-length human C19orf12 protein (2) even a truncated variant should have been detected.

In contrast to previously described patients, in whom the neuropathy was mainly an electrophysiological finding (2, 4), our patient presented with the full clinical picture of a motor axonal neuropathy. Initially those findings let us suspect a primary neuropathy until central nervous system-related symptoms started later. Axonal neuropathies have been reported in NBIA2 and NBIA3, albeit at more advanced disease stages and not as presenting complaints. His extrapyramidal movement disorder is characterized by severe bradykinesia, but no dystonia, tremor, or rigor as seen in other NBIA subtypes. Repeated ophthalmologic investigations did neither reveal optic atrophy, a frequent NBIA4 symptom (2), nor iris paralysis, pigmented retinopathy, or abnormalities of eye movement as seen in other NBIA subtypes. Although no obvious cerebellar symptoms could be elicited, his cMRI revealed a marked cerebellar atrophy. This has not been described in NBIA4 patients, but rather in other NBIA:s, such as infantile neuroaxonal dystrophy, again showing considerable phenotypic overlap between NBIA subtypes. Disease severity of NBIA4 seemingly follows a genotype–phenotype relation, with homozygous frameshift or nonsense mutations leading to an earlier manifestation and more rapid progression than those with missense mutations (2) thus placing our patient into the severe disease category.

Fig. 2. (a) Pedigree and genotypes of the consanguineous family. (b) Sanger sequencing of the mutant exon in the index patient, his mother and a wild-type control. (c) Complete absence of the C19orf12-protein on Western blot from cultured patient fibroblasts. C19orf12 (isoform 1) has a calculated molecular weight of 15.5 kDa. As the band runs at 12 kDa a mitochondrial targeting sequence is possibly cleaved upon mitochondrial import; β-actin as loading control.
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As C19ORF12 is highly expressed in differentiating adipocytes and co-regulated with genes involved in fatty acid biogenesis and degeneration of valine, leucine and isoleucine (2), we measured serum amino acids, free fatty acids, ketone bodies, and the acyl-carnitine profile but did not find any abnormalities. Unspecific markers for disturbances of lipid metabolism (e.g. acanthocytes) were absent.

Here we show the power, swiftness and cost-efficiency of whole exome sequencing combined with candidate gene analysis to find disease mutations, even in a single patient with atypical presentation.

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