

A de novo *C19orf12* heterozygous mutation in a patient with MPAN

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Main Text

Mitochondrial Membrane Protein-Associated Neurodegeneration (MPAN or NBIA4) is caused by biallelic mutations in the *C19orf12* gene. MPAN accounts for the largest proportion of NBIA after PKAN (*PANK2* mutations) and PLAN (*PLA2G6* mutations). It is characterized by juvenile-onset spastic paraparesis, levodopa-unresponsive parkinsonism, dystonia and neuropsychiatric symptoms. Additional features are optic atrophy, dysphagia, dysarthria, and motor axonal neuropathy. Brain MRI displays T2-weighted symmetrical hypointensities in globus pallidus (GP) and substantia nigra (SN) [1]. Missense, frameshift and nonsense mutations were found in exons 2 and 3 of *C19orf12* (NM_001031726 transcript). One splice-site mutation (c.194-2A>G) was identified in intron 2 (Figure 1a). Specific variants were found to be frequent in MPAN cases of selected populations (c.204_214del - p.G69Rfs*10 in Eastern Europeans and p.T11M in Turkish) [2, 3]. Here we report a case of NBIA with a novel *C19orf12* mutation with molecular evidence of de novo occurrence

The relevant ethical authorities approved the study and written informed consent was obtained from all involved subjects. The proband is the only child of non-consanguineous Italian parents. Familial history was negative for neurological disorders (Figure 1b). She was born at term by spontaneous delivery after an uneventful pregnancy. Motor development was at first normal, while a delay in language development was reported at the age of 3. Height-weight growth has been always at the lower normal limits (3-10 centiles). At the age of 5 she developed a progressive imbalanced gait associated with lower limbs rigidity and later onset of right hand dystonia forced her to use the left hand to write. She developed mild hirsutism at 9 years (pubis and limbs) and precocious puberty. Ocular involvement was present and included low vision, hypermetropia and astigmatism. She attends high school with a support teacher for learning disability (IQ 74). She came to the attention of our outpatient clinic at the age of 16 years. The neurological examination showed moderate dysarthria, cervical dystonia, dysdiadochokinesia, mild intentional and postural upper limbs tremor, lower limbs spastic hypertonia associated with movement-exacerbated dystonic postures of feet,

patellar hyperreflexia and bilateral Babinski sign. Typical radiological findings of SN and GP hypointensity in SWI, T2* and T2-weighted MRI with a T2-hyperintense medial medullary lamina (MML) were present, with the additional finding of subthalamic nucleus involvement. No cortical or cerebellar atrophy was evident (Figure 1d). Electroencephalogram and electroretinogram were normal, while visual evoked potential displayed an increased latency of P100 wave bilaterally with reduced amplitude and EMG showed diffuse axonal motor neuropathy. Fundus examination revealed optic atrophy.

Genetic analysis revealed a novel heterozygous *C19orf12* c.265_266delAT (NM_001031726) - p.M89Gfs*12 (NP_001026896) mutation in the proband, but not in her parents (Figure 1b-c). False paternity was excluded using eight polymorphic short tandem repeats (STR) on chromosome X. This suggests the p.M89Gfs*12 being a de novo mutation. Sanger sequencing of all exons and intron-exons boundaries of *PANK2*, *PLA2G6*, *C19orf12*, *FA2H*, *WDR45*, *COASY*, *CP*, and *FTL*, and full-length amplification followed by sequencing of *PANK2* and *WDR45* transcript did not detect other pathogenic mutations or rearrangements.

The amount of *C19orf12* transcripts was measured by quantitative reverse-transcription PCR in lymphocytes of patient, parents and controls. This assay failed to detect a reduced mRNA quantity in the proband cDNA, suggesting the absence of degradation through nonsense-mRNA decay. Sequence analysis of *C19orf12* transcripts revealed the presence of the heterozygous c.265_266delAT mutation in the proband and ruled-out splicing aberrations on both mutated and non-mutated alleles.

In order to explore a possible mosaicism, the mutation was studied in DNA from several tissues (hair, saliva and urine). The presence of the p.M89Gfs*12 in all tissues suggests a germ-line mosaicism in a parent.

MPAN is considered an autosomal recessive disorder; however, five monoallelic *C19orf12* mutations have been already described [1, 3, 4]. A second occult mutation was suspected to be

present in these cases. Deletion screening and promoter regions sequencing in some of these cases did not identify a second mutation [1, 3, 4]. Interestingly, all these mutations localize on exon 3 (G69R, p.Q86*, p.A94Cfs*8, p.A120Gfs*32 and p.L99fs*102), including deleterious frameshift/stop mutations on the *C19orf12* C-terminal domain, as in the case described in this study. Moreover, in a family with a heterozygous p.A120Gfs*32 mutation family history suggested a possible autosomal-dominant inheritance; indeed, the father of the patient, who died at age 47 following a long course of progressive dementia with parkinsonism, showed a typical MPAN neuropathology at postmortem examination [3].

Several lines of evidence support the pathogenic role of the identified variant reported here: the association with clinical-radiological features of MPAN, the de novo occurrence of the variant, the lack of additional mutations or aberrant splice sites on *C19orf12* transcripts and the absence of genetic mutations in other NBIA genes. The lack of a second mutation may indicate a dominant negative effect of this variant. Alternatively, the combination of this p.M89Gfs*12 with mutations in other still unknown causative genes may be hypothesized. In this view, studies on additional MPAN cases with monoallelic mutations are required in order to explore their role in NBIA.

Figure Legend

A) Representation of the *C19orf12* gene mutations reported in MPAN phenotypes so far. B) Pedigree of the family under study. Black symbol denotes affected individual. C) Electropherograms of the mutation c.265_266delAT of *C19orf12* gene in the proband (II.1) and wild type sequence in her parents (I.1 and I.2). D) Patient Brain MRI (T2, FLAIR and SWI sequence), performed at the age of 16, showing hypointensity of substantia nigra, globus pallidus and interestingly, subthalamic nucleus. (Abbreviations: wt = Wild type; mut = Mutated; GP = Globus Pallidus; GPe = External Globus Pallidus; GPi = Internal Globus Pallidus; SN = Substantia Nigra; STN = Subthalamic nucleus; MML = Medial Medullary Lamina).

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