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Letter to the Editor

A rare *PANK2* deletion in the first north African patient affected with pantothenate kinase associated neurodegeneration

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Dear Editor,

Neurodegeneration with brain iron accumulation (NBIA) disorders are a heterogeneous set of inherited, rare and clinically diverse neurological diseases often characterised by neuropathology of the basal ganglia as a consequence of iron deposition. They are usually childhood-onset genetic conditions and the majority of affected individuals present with developmental delay, abnormal behavior, progressive cognitive impairment and pyramidal/extrapyramidal movement disruption. Post-mortem pathology highlights axonal swellings with ubiquitinated aggregates, tau tangles or Lewy bodies, depending on the NBIA subtype [1].

Variants in at least 10 genes have been established to cause NBIA disorders. Each of these disease genes encode a protein with distinct cellular functions, including regulation of iron metabolism, mitochondrial metabolism, lipid homeostasis and autophagy [2]. The most common NBIA subtype, accounting for 35–50% of NBIA cases [2,3] is pantothenate kinase-associated neurodegeneration (PKAN) caused by biallelic variants in *PANK2* (MIM #606157). *PANK2* was the first causal gene discovered in NBIA, with cases reported from nearly all continents [1,4–8]. *PANK2* encodes a mitochondrial protein implicated in the synthesis of coenzyme A (CoA), an important molecule for an efficient metabolism of the cell.

The clinical entity PKAN can be divided into atypical and typical PKAN. Typical PKAN patients show early childhood-onset, severe presentation and more rapid progression. The mutational spectrum includes homozygous variants causing protein truncation more often than in atypical, later-onset PKAN cases, where variants tend to be compound heterozygous and more often result in amino acid changes [9]. Later disease onset and speech defects as well as psychiatric and cognitive decline are observed more often in atypical cases [10].

Here we report a novel *PANK2* homozygous deletion in a Moroccan girl with a typical PKAN phenotype. To the best of our knowledge this report represents the first PKAN case from North Africa. The proband, a 10 year old girl, was born from first degree consanguineous parents. History of previous neurological or genetic diseases was unremarkable in the family (Fig. 1A). She was born at full-term without birth injury and in good health. At the age of 16 months, she presented with loss of walking and standing, imbalance and frequent forward head falls. She presented with frontal humps and scars during her clinical visit. Examination of the nervous system revealed spasticity and cognitive delay. At 7 years of age, she developed sphincter dysfunction, athetosis of the upper limbs and 4-limb dystonia which later on spread to involve trunk, neck and face with opisthotonus, oromandibular dystonia and severe retrocollis. Her language and speech was normal until the age of 7 after which her speech regressed and became slow with dysarthria, but no stuttering. She also presented with behavioural disturbances including agitation, irritability and significant sleep disorder with frequent awakenings leading to insomnia. The patient received psychomotor rehabilitation and physiotherapy in conjuction with high-dose baclofen administered through oral route. Adjunct pharmacological therapy included trials of haloperidol, l-dopa and benzodiazepines that were largely non-effective. No intrathecal baclofen or DBS was available to her.

Laboratory tests including blood biochemistry, ceruloplasmin, thyroid function, parathormone, calcitonin, serum anti-HIV antibodies, anti-syphilis antibodies and autoimmune antibodies were normal. Brain MRI (1.5 Tesla) performed at the age of 9 revealed mild diffuse cortical atrophy as well as symmetric mineralisations of the bilateral globus pallidus and central hyperintense foci termed as ‘eye of the tiger’ sign. In essence, there is evidence of blooming artefact within the globi pallidi appearing ‘dark’ on the axial image along with the central gliosis appearing hyperintense or ‘bright’. (Fig. 1D i, T2 WI). These foci are consistent with regions of vacuolisation and gliosis, as suggested in previously reported pathology literature on *PANK2* variants (Fig. 1D ii). There was no evidence of cerebellar atrophy, or calaval hypertrophy as described in other variants such as *PLA2G6* associated with brain iron accumulation (Fig. 1D iii). Lumbar MRI at age 9 showed apophyseal joint damage and intervertebral disc degeneration at the L3-L4, L4-L5, L5-L6 spinal segments without any associated clinical phenotype.

Written informed consent was obtained from the patient and her parents, after which DNA was extracted from peripheral lymphocytes from father and index patient according to a standard protocol of phenol-chloroform extraction. DNA of the mother was unfortunately not available. WES was performed as previously described [11] in both the affected female and the father (Fig. 1A: II-1, I-1) as well as a healthy

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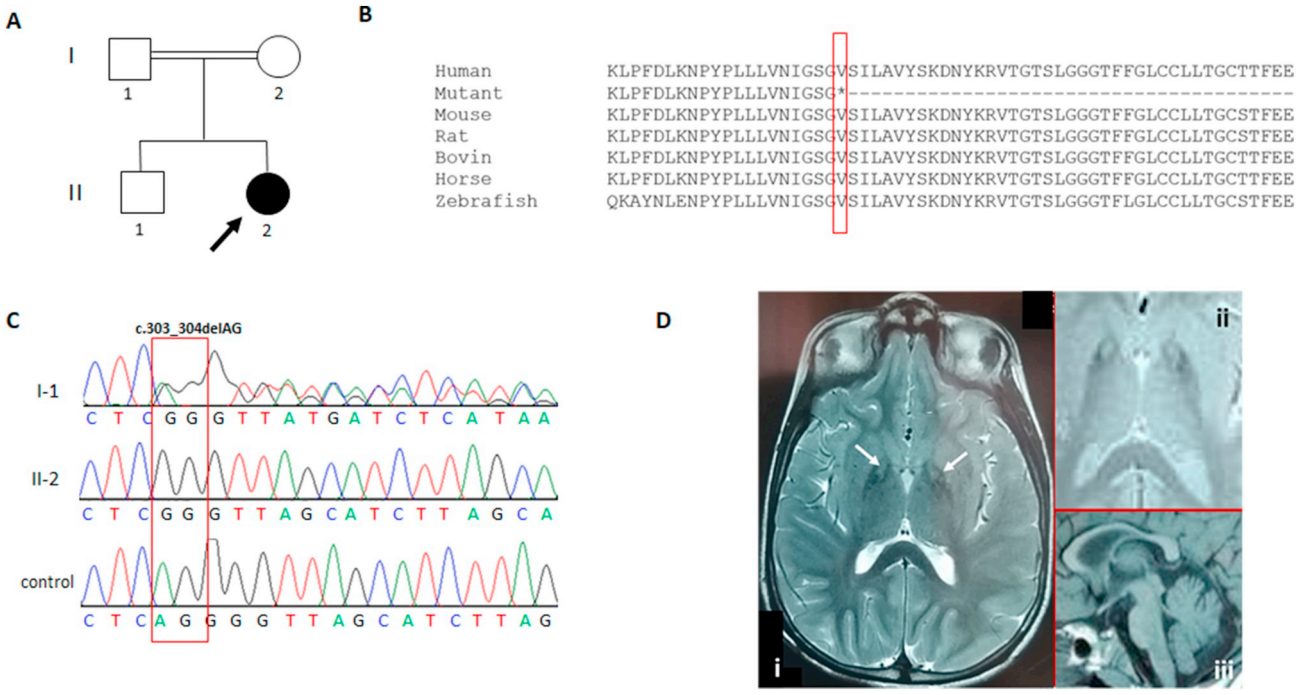

Fig. 1. (A) Family pedigree (B) Interspecies alignment performed with Clustal Omega shows the complete conservation down to invertebrates of the amino acid residues affected by the deletion. (C) Individual results of Sanger sequencing indicating the proband (II-1) to carry the homozygous *PANK2* truncating variant (c.303_304delAG:p.Val103Ter*fs*) while the father (I-1) carries the heterozygous variant. For clarity, only I-1 is shown here, as well as a healthy control homozygous for the reference allele (third lane, wildtype) (D) Axial T2 WI (i), and zoomed-in axial T2* WI (ii) sequences showing hypointense signal return from both globi pallidi, consistent with increased iron deposition. Further, within these areas of hypointensity are defined foci of increased (hyperintense) signal pointed with arrows. These foci are consistent with regions of vacuolisation and gliosis, as suggested in previously reported pathology literature on *PANK2* variants. No other areas of increased brain iron accumulation were noted. Note also that there is no evidence of cerebellar atrophy, or calaval hypertrophy on the sagittal T1 WI (iii) as described in other variants (e.g. *PLA2G6*) associated with brain iron accumulation.

control from our in-house control database. In brief, Nextera Rapid Capture Enrichment kit (Illumina) was used according to the manufacturer instructions. Libraries were sequenced in an Illumina HiSeq3000 platform using a 100-bp paired-end reads protocol. Sequence alignment to the human reference genome (UCSC hg19), and variants call and annotation were performed as described elsewhere [11]. In total, 81,799,534 (II-1) unique reads were generated. All synonymous and in-silico predicted benign changes were discharged. The raw list of single nucleotide variants (SNVs) and indels was then filtered. Only exonic and donor/acceptor splicing variants were considered. In accordance with the pedigree and phenotype, priority was given to rare variants [< 1% in public databases, including 1000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69, and Exome Aggregation Consortium (ExAC v0.2)] that were fitting a recessive model.

The only homozygous variant in a disease-causing gene that we identified was a homozygous frameshift deletion in *PANK2* exon 3 (NM_024960.6:c.303_304delAG; NP_001311120.1: p.Val103Ter*fs*, dbSNP rsID: rs778550409, ClinVar variant ID: 456524) The two base-pair deletion at nucleotide position 303–304 causes a corresponding frameshift at codon 101 and results in a premature stop codon at codon 103. This deletion in *PANK2* emerged as the most likely explanation for the child's phenotype. This deletion has been reported once before according to ClinVar and according to published databases is not frequently implicated in PKAN. It is predicted to be disease-causing on Mutation Taster ($p = 1$) and deleterious on SIFT ($p = 0$) [12,13]. Segregation analysis at the DNA level performed by traditional Sanger sequencing (processed on an ABI 3730 analyser and analysed on Sequencher 4.1.4) confirmed the variant as homozygous in the proband and heterozygous in the father (Fig. 1B). For segregation analysis by Sanger sequencing BigDye terminator v3.1 cycle sequencing chemistry (Applied Biosystems, Weiterstadt, Germany) was used with PCR and sequencing primers as follows: Forward (5′- CGGATTCAATGGACGGT CAC -3′) and Reverse (5′- CCTAACAGGTTCTTGAAGGTGT −3′).

The current study identified a rare homozygous deletion in *PANK2* (c.303_304delAG, p.Val103Ter*fs*) leading to a premature stop codon in a Moroccan patient with a typical NBIA disorder. The deletion of 2 nucleotides at position 303–304 causes a change in the reading frame with premature termination of translation two codons later at codon 103. This is expected to be resulting in an absent or highly disrupted protein suggesting a severe loss of gene function mechanism. Loss-of-function variants in *PANK2* are known to be pathogenic. To date, > 100 pathogenic *PANK2* variants have been described in PKAN patients around the globe but, to the best of our knowledge, the disease has not been described in the North-African population so far. This study further expands the PANK2 ethnic and clinical spectrum and reports the first PKAN case associated with c.303_304del variant in Morocco. This information can help with the genetic screening of north African patients presenting typical PKAN features which could lead to more accurate genetic diagnoses and help in genetic counseling as well as, potentially in the future, prenatal diagnoses in the suspected families.

Declaration of Competing Interest

The authors declare no conflict of interest.

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