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LETTER TO THE EDITOR

DNMT1-complex disorder caused by a novel mutation associated with an overlapping phenotype of autosomal-dominant cerebellar ataxia, deafness, and narcolepsy (ADCA-DN) and hereditary sensory neuropathy with dementia and hearing loss (HSN1E)

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Dear Sir,

Autosomal dominant cerebellar ataxias (ADCA) are a group of heterogeneous clinical conditions characterized by frequent association of progressive ataxia with a plethora of different neurological symptoms. They can underlie a broad genetic background with a large group of ADCA causing loci still to be determined.

We have recently identified a novel mutation within the *DNMT1* gene, in an Italian individual presenting with a complicated neurological condition dominated by cerebellar ataxia.

The index patient (Fig. 1a), now 65 years old, presented in his early fifties with slowly progressive hearing impairment, ataxia, and dysarthria. Initial neurologic examination documented the presence of severe gait disturbances and deep sensations impairment. The brain MRI displayed vermian cerebellar atrophy and mild cortical atrophy (Fig. 2). During follow-up visits, the patient manifested a progressive decline of some frontal lobe functions (executive functions, phonemic fluency, and planning) associated with psychosis, urinary urgency, a sensory-motor mixed axonal-demyelinating polyneuropathy with a distal predominance, and lower limbs lymphedema. Patient also reported sleep problems and excessive daytime sleepiness associated with electroencephalographic evidence of bilateral slow activity. Within the last year of follow-up, three undefined syncopal episodes were reported, possibly attributable to cataplexy attacks. Visual disturbance associated with bilateral optic atrophy was also detected.

Family history revealed that his mother, deceased at 85 years, had developed in her adulthood a complex syndrome characterized by a diffuse axonal polyneuropathy, deafness, ataxia, and severe cognitive decline with psychosis. Severe hearing impairment in the maternal grandmother and uncle was also reported. Index patient's father and a younger brother were unaffected (Fig. 1a). We performed clinical exome sequencing (TruSight One Sequencing Panel, Illumina) on our proband. Sequencing data analysis, under the hypothesis of an autosomal dominant disease, led to the identification of a novel heterozygous variant in exon 21 of *DNMT1* (NM_001130823.2:c.1794C > T; NP_001124295.1:p.Arg598Trp; Fig. 1b). The nucleotide change was absent in the healthy brother (aged 61) and not reported in the GnomAD database. It hits a conserved amino acid residue (Fig. 1c) and causes an amino acid substitution predicted deleterious by most commonly used variant predictor bioinformatics tools (Table 1).

DNMT1 is a key DNA methyltransferase with essential roles in methylation pattern maintenance during chromosome replication and repair. This protein is composed of a catalytic C-terminal region and a regulatory N-terminal region. All disease-causing mutations reported to date fall within exons 20 and 21, which encode a small domain of the N-terminal region, called replication foci targeting sequence (Fig. 1d), essential for enzymatic dimerization and binding to DNA hemimethylated CpG sites [1]. Heterozygous mutations in *DNMT1* have been associated with two autosomal dominant pathological phenotypes, namely hereditary sensory neuropathy with dementia and hearing loss (HSN1E; OMIM#614116); and cerebellar ataxia,

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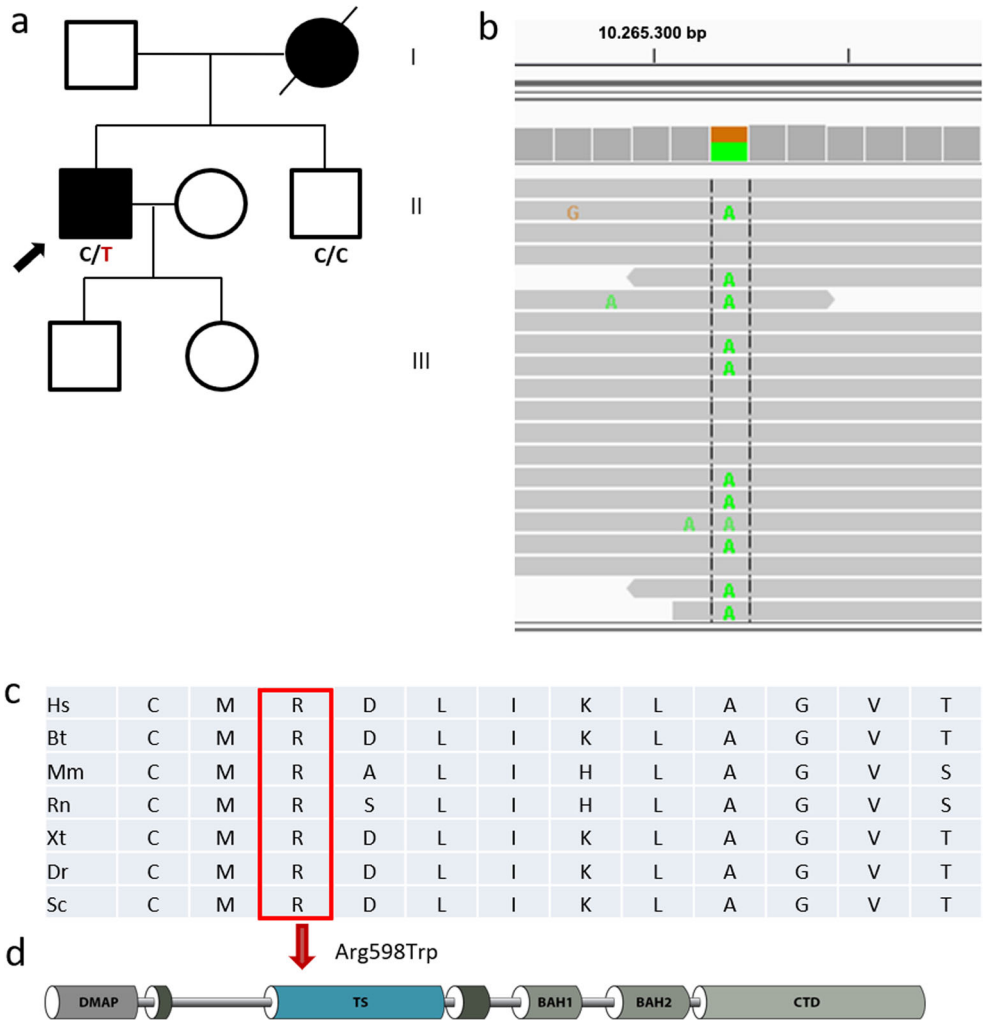

Fig. 1 Pedigree and genetic findings. **a** Family pedigree. Black symbols indicate affected subjects; an arrow indicates the index subject. Genotypes refer to the variant c.1794C > T in *DNMT1*. **b** Snapshot from IGV software of the *DNMT1* variant identified in the proband by clinical exome sequencing. **c** Phylogenetic alignment of the DNMT1 protein region containing the amino acid substitution (p.Arg598Trp) found in the proband. Amino acids corresponding to human Arg598 are boxed in red. Hs, *Homo sapiens*; Bt, *Bos Taurus*; Mm, *Mus musculus*; Rn, *Rattus norvegicus*; Xt, Xenopus tropicalis; Dr:,*Danio rerio*; Sc, *Saccharomyces cerevisiae*. **d** Schematic structure of the DNMT1 protein, the arrow indicates the position of the identified amino acid change. DMAP1, DNMT1-associated protein interaction domain; TS, replication foci targeting sequence; BAH, bromo-adjacent homology; CTD, catalytic domain

deafness, and narcolepsy (ADCA-DN; OMIM#604121). Mutations located in exon 20 typically cause HSN1E, while mutations in exon 21 lead to ADCA-DN. HSN1E is characterized by adult onset of progressive peripheral sensory loss associated with hearing impairment and early-onset dementia. ADCA-DN develops as an adult-onset (30–40 years)

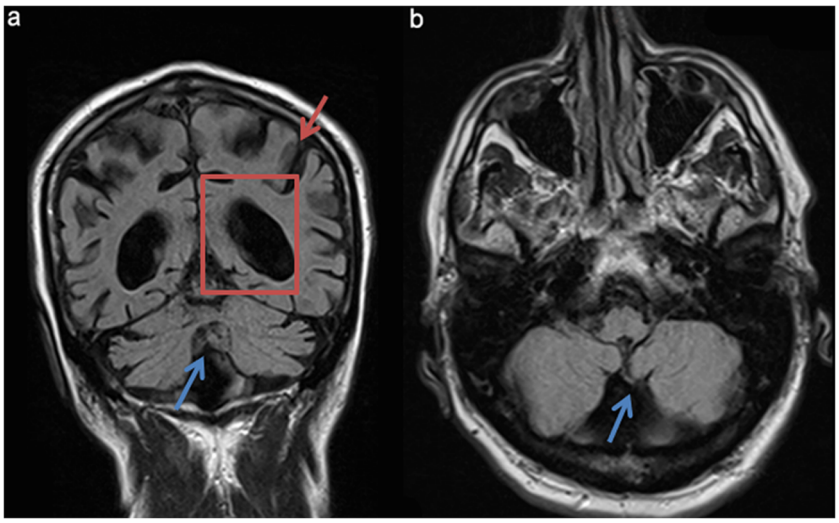

Fig. 2 Brain MRI of the patient. T1 weighted images show enlargement of the lateral ventricles (red square) and cortical sulci (red arrow) (in panel **a**) and prominent vermian cerebellar atrophy (blue arrows in panels **a**–**b**)

Table 1 Information about the identified *DNMT1* mutation

cDNA NM_001130823.2	Protein NP_001124295.1	Exon	GnomAD frequency (%)	Polyphen2, HumVar score	SIFT, score	Mutation taster, prob. value	Provean, score
c.1794C > T	p.Arg598Trp	21	Ø	Probably damaging 1.000	Deleterious 0.01	Disease causing, 0.999	Deleterious, − 7.11

GnomAD database: http://gnomad.broadinstitute.org

Web addresses of software used for predictions are the following:

Polyphen v.2: http://genetics.bwh.harvard.edu/pph2

The PolyPhen-2 score ranges from 0.0 (tolerated) to 1.0 (damaging)

SIFT: http://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html

SIFT scores ≤0.05 correspond to amino acid substitutions predicted to affect protein function

MutationTaster: http://www.mutationtaster.org

The probability value is the probability of the prediction, i.e., a value close to 1 indicates a high "security" of the prediction

Provean: http://provean.jcvi.org

Default threshold is − 2.5, that means variants with a score equal to or below − 2.5 are considered "deleterious" and variants with a score above − 2.5 are considered "neutral"

progressive cerebellar ataxia associated with hearing loss, cognitive decline with possible psychotic symptoms, and narcolepsy/cataplexy. Sensory neuropathy, optic atrophy, and depression are variably reported clinical features [2]. Our patient presents with a phenotype encompassing ADCA-DN and HSN1E, as he shows the most relevant clinical signs and symptoms of both conditions (prominent ataxia, cognitive impairment, sleep disturbances, sensitive neuropathy, and deafness). Indeed, these diseases have been considered as a continuum of a common neurodegenerative spectrum often characterized by overlapping clinical and/or subclinical features, leading to the proposal of the unified disease terminology of "DNMT1-complex disorder" [3]. Moreover, very recently a novel *DNMT1* mutation in exon 20 has been described in a patient with an HSN1E plus ADCA-DN phenotype [4], similar to our index case.

Besides, ADCA-DN shares some common phenotypic traits of mitochondrial encephalomyopathies and several experimental evidences have formerly suggested the involvement of mitochondrial dysfunction in the pathogenesis of the disease [1].

DNMT1 mutations have been associated with alterations of normal genomic methylation pattern [1]. While nuclear DNA (nDNA) methylation is widely established and extensively studied, whether methylation of mitochondrial DNA (mtDNA) occurs or not is still an open question among researchers. The foremost explanations for this controversy lie in the low amount of methylated cytosines found in mtDNA and in bias created by differential sensitivity and reproducibility of experimental techniques employed to assess mtDNA methylation levels. Similarly, DNMT1 involvement in mitochondrial methylation and its possible location inside mitochondria still remains a matter of debate [1]. We investigated mtDNA methylation levels, as previously described [5] using DNAs from the proband and the unaffected brother, as the control. Site-specific methylation analysis of normally hypomethylated mtDNA regions (in three mtDNA genes *MTCO1*, *MTTF*, *MTND6*, and in the D-loop region) did not reveal any statistically significant difference between patient's and control DNAs (Table 2).

Further work will be necessary in order to understand pathophysiological mechanisms underlying the disease and to clarify the potential involvement of altered mitochondrial dysfunction in the pathogenesis. Hence, an enlargement of the case record would be valuable for the collection of multiple biological samples to perform functional studies.

Table 2 Site-specific methylation analysis of mtDNA

Gene	Sample	Percent methylation	Number of CpG analyzed
MTCO1	Patient	2	2 CpG
	Control	2	
MTTF	Patient	2	3 CpG
	Control	3	
mt D-loop	Patient	2	7 CpG
	Control	3	
MTND6	Patient	3	1 CpG
	Control	3	

Cytosine methylation of bisulfite-converted DNAs from the index patient and the healthy brother (control) was assessed by pyrosequencing. Specific amplicons of mtDNA regions were analyzed

mtCO1 mitochondrial cytochrome oxidase subunit 1 gene, *mtTF* mitochondrial tRNA for phenylalanine, *mtND6* mitochondrial NADH ubiquinone oxidoreductase core subunit 6 gene, *mt D-loop* displacement loop, m.6–259

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Maresca A, Zaffagnini M, Caporali L, Carelli V, Zanna C (2015) DNA methyltransferase 1 mutations and mitochondrial pathology: is mtDNA methylated? Front Genet 12(6):90. https://doi.org/10.3389/fgene.2015.00090
2. Winkelmann J, Lin L, Schormair B, Kornum BR, Faraco J, Plazzi G, Melberg A, Cornelio F, Urban AE, Pizza F, Poli F, Grubert F, Wieland T, Graf E, Hallmayer J, Strom TM, Mignot E (2012) Mutations in DNMT1 cause autosomal dominant cerebellar ataxia, deafness and narcolepsy. Hum Mol Genet 15;21(10):2205–2210. https://doi.org/10.1093/hmg/dds035
3. Baets J, Duan X, Wu Y, Smith G, Seeley WW, Mademan I, McGrath NM, Beadell NC, Khoury J, Botuyan MV, Mer G, Worrell GA, Hojo K, DeLeon J, Laura M, Liu YT, Senderek J, Weis J, Van den Bergh P, Merrill SL, Reilly MM, Houlden H, Grossman M, Scherer SS, De Jonghe P, Dyck PJ, Klein CJ (2015 Apr) Defects of mutant DNMT1 are linked to a spectrum of neurological disorders. Brain. 138(Pt 4): 845–861. https://doi.org/10.1093/brain/awv010
4. Zheng W, Yan Z, He R, Huang Y, Lin A, Huang W, Su Y, Li S, Zhang VW, Xie H (2018 Oct 20) Identification of a novel DNMT1 mutation in a Chinese patient with hereditary sensory and autonomic neuropathy type IE. BMC Neurol 18(1):174. https://doi.org/10.1186/s12883-018-1177-2.
5. Novielli C, Mandò C, Tabano S, Anelli GM, Fontana L, Antonazzo P, Miozzo M, Cetin I (2017) Mitochondrial DNA content and methylation in fetal cord blood of pregnancies with placental insufficiency. Placenta 55:63–70. https://doi.org/10.1016/j.placenta.2017.05.008