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Rapid communication

The *C9ORF72* expansion does not affect the phenotype in Nasu-Hakola disease with the *DAP12* mutation

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ABSTRACT

Nasu-Hakola disease (NHD) is a rare autosomal recessive disease that is characterized by cyst-like bone lesions and pathologic fractures combined with an early-onset frontal type of dementia. Mutations in DNAX-activation protein 12 (*DAP12*) and triggering receptor expressed on myeloid cells 2 (*TREM2*) are the known genetic causes of NHD. However, the role of both these genes in the neurodegenerative process is still partly unclear, and the input of other modifying factors has been postulated. Frontotemporal lobar degeneration (FTLD) is a neuropathologically and genetically heterogeneous neurodegenerative disease. A hexanucleotide repeat expansion in the chromosome 9–associated open reading frame 72 (*C9ORF72*) gene is the most common cause of familial FTLD in Finland. Here, we describe a family with 3 siblings with a clinical diagnosis of NHD. All patients had an equivalent age of onset of the behavioral/cognitive symptoms, and brain imaging revealed a similar pattern of brain atrophy and calcification in putamen and caudate nucleus. Case II-3 had the most severe phenotype with epilepsy and a rapid cognitive decline. Genetic analyses were performed in 2 patients (cases II-2 and II-3), and both had a homozygous *DAP12* deletion. Because the role of *DAP12* and *TREM2* in neurodegeneration in NHD is partly unclear, our aim was to evaluate the role of other genetic variations as modifiers. The *C9ORF72* expansion was found in case II-2. Exome sequencing did not reveal any other mutations that could be involved in FTLD. Case II-3 had a novel predictably deleterious mutation in the progressive myoclonic epilepsy type 2 (*EPM2*), which may have influenced his epilepsy as the *EPM2* has been implicated in Lafora progressive myoclonic epilepsy. We conclude that the *C9ORF72* expansion is probably an incidental finding because it did not have any apparent influence on the phenotype. Exome sequencing identified several rare missense variants and indels. Additional analyses in other NHD patients will be needed to elucidate their clinical relevance.

1. Introduction

Nasu-Hakola disease (NHD, polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy, OMIM 221770) is a rare autosomal recessive disease that is characterized by painful cyst-like bone lesions and pathologic fractures mostly encountered in the ankles and feet in the early stages of the disease at a mean age of 27 years. Personality changes and cognitive decline become symptomatic at the mean age of 33 years leading to frontal type of dementia and death at a mean age of 43 years (Hakola, 1990; Hakola and Iivanainen, 1973; Paloneva et al., 2001). Mutations in the DNAX-activation protein 12 (*DAP12*), which is also called the TYRO protein tyrosine kinase–binding protein (*TYROBP*), and the triggering receptor expressed on myeloid cells 2 (*TREM2*) genes are the known genetic causes of NHD (Paloneva et al., 2000, 2002).

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Table 1
Clinical findings of the patients with NHD

Clinical findings				Mean[a]	Range[a]
Case	II-1	II-2	II-3		
Gender	M	M	M		
Age at the first fracture (y)	—	32	29	27	18–33
Bone cysts in X-ray	+	+	+		
Age at onset of personality changes and cognitive decline (y)	32	32	31	33	25–40
Age at the first epileptic seizure (y)	—	40	28		
Myoclonia	+	—	+		
Inability to walk	—	?	40	42.5	34–47
Age at death (y)	40[b]	46	42	42.8	37–48
Brain imaging					
Age at examination (y)	37	39	38		
Calcifications					
Putamen	+	+	+		
Nucleus caudatus	+	+	+		
Atrophy					
Nucleus caudatus	+	+	+		
Thalamus	+	+	+		
Frontal neocortic	Mild	Severe	Severe		
Frontal gg. (med)	Mild	Severe	Severe		
Corpus callosum genu	Evident	Evident	Evident		
Parietal	Mild	Mild	Mild		
Genetics					
DAP12 (5.3 kbp deletion)	NA	+	+		
C9ORF72 expansion	NA	+	—		
SOD (D90A)	NA	—	+		
EPM2 (A46P)	NA	—	+		

Key: *C9ORF72*, chromosome 9–associated open reading frame 72; *DAP12*, DNAX-activation protein 12; *EPM2*, progressive myoclonic epilepsy type 2; M, male; NA, not available; NHD, Nasu-Hakola disease.
[a] According to Paloneva et al. (2001).
[b] Accident.

Inactivating mutations in both *DAP12* and *TREM2* are thought in some undetermined way to impair the function of osteoclasts and microglial cells, though the major role of these genes seems to be involved in bone metabolism (Satoh et al., 2011). Other factors, such as disturbances in calcium homeostasis (Satoh et al., 2011) or other genetic factors, have been postulated to play a role in the neurodegenerative processes of NHD.

Frontotemporal lobar degeneration (FTLD) is a clinically, neuropathologically, and genetically heterogeneous syndrome. The most prominent symptoms of FTLD are changes in personality, accompanied by deteriorations in cognition and language. The chromosome 9–associated open reading frame 72 (*C9ORF72*) repeat expansion is the most common cause of the FTLD and amyotrophic lateral sclerosis (DeJesus-Hernandez et al., 2011; Renton et al., 2011). There is a broad phenotype variation between FTLD patients with the *C9ORF72* expansion, and it is possible that there are other still unknown factors that can modify the phenotype.

Here, we describe 3 siblings with clinically diagnosed NHD. DNA was available in 2 of the patients, and both subjects had a homozygous deletion in the *DAP12*. Because the role of *DAP12* and *TREM2* in neurodegeneration in NHD is partly unclear, our aim was to evaluate the role of other genetic variations as modifiers using the analysis of the *C9ORF72* expansion and whole-exome sequencing in these siblings with NHD.

2. Methods and results

All 3 siblings (cases II-1, II-2, and II-3) showed the typical clinical features of NHD (Table 1, Fig. 1). The patients suffered bone cysts or fractures in their early 30s and neuropsychiatric symptoms with impaired social behavior, disinhibition, and cognitive decline appeared gradually after the fractures. The cognitive decline

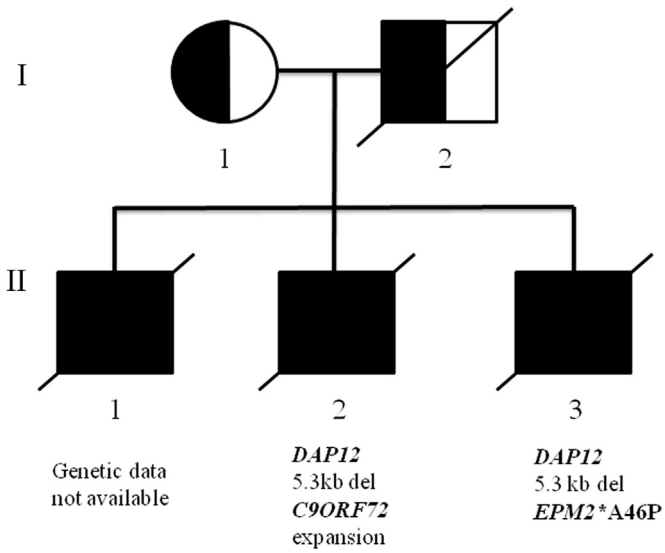

Fig. 1. The pedigree of the siblings with Nasu-Hakola disease.

included prominent executive dysfunction leading to severe frontal dementia during the subsequent 10 years. Brain imaging of the patients revealed calcification in putamen and caudate nucleus in all 3 patients. The atrophy was predominantly frontal, but it was also detected in parietal regions, and central atrophy was evident in all patients (Table 1, Fig. 2). Neuropathologic autopsy was not performed in any of the patients.

Case II-3 suffered from the most severe phenotype with severe epilepsy and rapid cognitive decline. He also became hypokinetic and lost his ability to walk in the last years of his life. However, electroneuromyography revealed no signs of motor neuron disease.

The father of the siblings suffered from the frontal type of dementia when he reached his 80s. He also had tremor, rigidity, and difficulties in walking. He died at the age of 84 years. The mother was cognitively, neurologically, and psychiatrically healthy at the age of 86 years.

DNA was available from cases II-2 and II-3 but not from case II-1. The homozygous 5.3 kbp deletion in *DAP12* (Paloneva et al., 2000) was analyzed using a direct polymerase chain reaction assay with 1 forward primer (FP: 5′-GGCCACATCCGTATGACTG-3′) and 2 reverse primers (RP1: 5′-TAGTATGTCCAG TCTCGAGTTCTCA-3′ and RP2: 5′-CTAGTCTGGGCGTGCATTC-3′). In the *DAP12* deletion allele, the assay produces a 695-bp amplicon (primers FP and RP2) and no product with primers FP and RP1. In the wild-type allele, the assay produces a 454-bp amplicon (primers FP and RP1) and no product with primers FP and RP2 (theoretical product size 5959 bp). The homozygous *DAP12* deletion was detected in both of the siblings (cases II-2 and II-3) providing a genetic confirmation to the diagnosis of NHD. The *C9ORF72* expansion (>40 repeats) was found in case II-2 using the repeat-primed polymerase chain reaction assay (Renton et al., 2011). Exome sequencing was performed in cases II-2 and II-3 using an Agilent HaloPlex Exome kit for target enrichment followed by sequencing on an Illumina MiSeq instrument to the average depth of 80× coverage. The resulting reads were mapped with Burrows-Wheeler Aligner (Li and Durbin, 2009) after which The Genome Analysis Toolkit was used to identify variants (McKenna et al., 2010). The variants were annotated with ANNOVAR (Wang et al., 2010). The number of variants in cases II-2 and II-3 is illustrated in Supplementary Table 1. The homozygous *DAP12* deletion was readily detected in the exome data of cases II-2 and II-3 as evidenced by the lack of any sequence reads in the *DAP12* exons 1–4 (Supplementary Fig. 1). Rare nonsynonymous variants