A Japanese Case with Nasu-Hakola Disease of DAP12 Gene Mutation Exhibiting Precuneus Hypoperfusion

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Abstract

A 38-year-old Japanese man with Nasu-Hakola disease (NHD) had repeated pathological fractures and frontal lobe symptoms which developed when he was 18 and 26 years old, respectively. Neuropsychological testing showed memory impairment, and in particular, visuo-spatial memory at the age of 35. Furthermore, single-photon emission computed tomography revealed precuneus hypoperfusion. The patient later suffered prolonged convulsive seizures, which left him in a persistent vegetative state. Genetic testing confirmed a heterozygous mutation in the DAP12 gene (a single-base deletion of 141 G in exon 3) specific to NHD. Precuneus dysfunction might contribute to characteristic memory impairment of NHD.

Key words: Nasu-Hakola disease, DAP12, TREM2, SPECT, MRI, precuneus

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Introduction

Nasu-Hakola disease (NHD), an extremely rare autosomal recessive inherited disease also referred to as polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), occurs most commonly among the Japanese and Finnish populations. Our PubMed literature search identified approximately 50 reported cases of NHD. The cardinal symptoms include multiple bone cysts accompanied by pathological fractures and early-onset dementia induced by leukoencephalopathy. The clinical course of NHD follows four stages: latent, osseous, early neurological, and late neurological stages (1-5). The latent stage generally takes place before the patient reaches the age of 20. The osseous stage, which begins after the patient is 20, is characterized by the development of multiple bone cysts, which occur most frequently at the epiphyses of long tubular bones, accompanied by bone pain and repeated pathological fractures. The early neurological stage starts after the patient is 30, with the manifestation of frontal lobe symptoms, including disinhibition, euphoria, and personality disorder, as well as the onset of epileptic seizures. The late neurological stage sets in after the patient is 40, and the patient develops a rapidly progressive, profound dementia, and eventually becomes bedridden. Most patients die in their 30s or 40s.

According to previous reports, the homozygous deletion or point mutation of DAP12 (TYRO protein tyrosine kinase-binding protein, TYROBP) or the triggering receptor expressed on myeloid cells 2 (TREM2) is the typical genetic defect observed in NHD (3, 4, 6, 7). Furthermore, Kuroda et al reported a case in which a compound heterozygote of a defective DAP12 gene was detected (8). Genetic mutations of DAP12 and TREM2 lead to losses of function expressed in microglia in the brain and osteoclasts in the bone, which cause symptoms to appear in the central nervous system and bone.

NHD can be diagnosed with relative ease when it is accompanied by bone lesions, in which case the presence of polycystic lipomembranous osteodysplasia, which is specific to NHD, in the bone biopsy specimen confirms the diagnosis. However, without such bone lesions, diagnosis can be very difficult. According to Chouery et al, some patients in whom the mutation of the TREM2 gene was present developed early-onset dementia without exhibiting bone lesions (9). In such patients, genetic testing may be needed to

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Table 1. Summary of Neuropsychological Tests

<table>
<thead>
<tr>
<th>Neuropsychological Tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mini-Mental State Examination (MMSE)</td>
<td>28/30 (errors on calculation and memory)</td>
</tr>
<tr>
<td>Wechsler Memory Scale-Revised</td>
<td>Verbal Memory Index: 82</td>
</tr>
<tr>
<td>(WMS-R; Index scores less than 85 indicate abnormal decline.)</td>
<td>Visual Memory Index: &lt;50</td>
</tr>
<tr>
<td>Alzheimer’s Disease Assessment</td>
<td>General Memory Index: 65</td>
</tr>
<tr>
<td>Scale-cognitive component-Japanese version</td>
<td>Attention/Concentration: 73</td>
</tr>
<tr>
<td>(ADAS-J cog.)</td>
<td>Delayed Memory: &lt;50</td>
</tr>
<tr>
<td>Clock Drawing Test</td>
<td>Error in standard version. Improvement in copied version.</td>
</tr>
<tr>
<td>Verbal Fluency Test</td>
<td>Difficulty in recalling words with initial Japanese letter, ko.</td>
</tr>
</tbody>
</table>

The testing was performed when the patient was 35 years old, before the onset of convulsive seizures. The profile shows the impairment in memory dysfunction (especially, visuospatial memory) and frontal dysfunction, although overall cognitive function (by MMSE) was shown to be still intact.

arrive at a diagnosis of NHD.

Recent research has revealed an immune abnormality induced by genetic mutations associated with NHD (5, 10). In this paper, we report a case of NHD exhibiting a defective DAP12 gene with a focus on the relationship between clinical symptoms and image findings.

Case Report

The present case is a 38-year-old man. In his family history, his brother, who had multiple bone cysts and was suspected of having NHD, died in a traffic accident at the age of 19. The present patient had previously been healthy. At the age of 18, he experienced pain in the joints of both legs and had repeated pathological fractures. His X-rays showed multiple cystic radiolucent areas at the epiphyses of both legs and thickening of the trabecular bone. A bone biopsy, performed following autologous transplantation of the bones of both ankles, revealed polycystic lipomembranous osteodysplasia. Signs of disinhibition, personality changes, and behavioral abnormalities became evident when the patient was 26. Particularly abnormal behaviors, such as stealing and the reckless borrowing of money, escalated over time. T2-enhanced brain MRI images taken when the patient was 34 years old revealed diffuse high-intensity areas in the white matter as well as hippocampal atrophy. Neuropsychological tests performed when the patient was 35 showed higher-order cognitive dysfunction, including memory impairment (Table 1). Nine months after the testing, the patient had generalized seizures and subsequently received the antiepileptic agent phenytoin. Later, hypobulia became evident, and the patient became withdrawn. However, he was still able to perform daily activities, such as eating and walking. At the age of 36, he was admitted to our hospital immediately after having generalized tonic-clonic seizures, which resulted in prolonged convulsive seizures.

Physical findings on admission: The patient exhibited profoundly impaired consciousness with a score of 4 (E1, V1, M3) on the Glasgow Coma Scale. The size of both pupils was 2 mm and the light reflexes were absent from both eyes. The deep tendon reflexes were exaggerated in all limbs. Foot and knee cloni were positive bilaterally. Both Babinski and Chaddock reflexes were positive bilaterally. The palmomental reflex was also positive. Furthermore, the patient had double incontinence.

Disease progression after admission: The patient was put on a mechanical ventilator. Intravenous phenytoin and continuous intravenous midazolam infusion were initiated to treat prolonged generalized tonic-clonic seizures. Shortly afterwards, the generalized seizures disappeared and never returned. The patient had only transient episodes of partial seizures of the left upper limb. As the patient’s condition improved, he was weaned from the mechanical ventilator on the 11th day of hospitalization. Around that time, the patient showed decerebrate rigidity. The convulsions were controlled by administration of 500 mg/day phenytoin via a gastric tube. Since that time the patient remained in a persistent vegetative state for over one year with no sign of improvement in the level of consciousness.

Neuropsychological testing was administered when the patient was 35 years old, prior to the first episode of convulsive seizures (Table 1). The patient scored 28 on the Mini-Mental State Examination (MMSE), indicating normal overall cognitive function. He made some errors on the items related to calculation and memory. On the Wechsler Memory Scale-Revised (WMS-R), the patient disclosed memory impairment in all indexes (less than 85), among which the scores were extremely low in visual memory and delayed memory (less than 50). On the Clock Drawing Test (CDT), the patient could not image the clock face exactly and hesitated to draw by organizing the parts of the clock; however, he could copy the clock face when he was shown a clock drawn by the tester, indicating that his error in CDT was not due to constructive apraxia but frontal executive dysfunction and memory dysfunction. There was also evidence of frontal lobe dysfunction, i.e., difficulty in verbal fluency task (recalling words with Japanese ka as the initial letter). With these results, the neuropsychological profile of this case resembles those often observed in patients with mild cognitive impairment (MCI), i.e., normal overall cognitive function with an abnormal decline in memory and frontal function; however, there is an additional striking feature.
in this case, namely, that extreme dysfunction which was observed in visuo-spatial memory in the WMS-R visual memory index as well as in the errors on the CDT, which seemed to be inconsequential to the normal MMSE score obtained.

**Imaging findings:**

Plain X-ray images of hands and fingers revealed multiple bone cysts at the epiphyses (Fig. 1). T2-enhanced brain MRI images revealed diffuse high-intensity areas in the white matter as well as low-intensity areas in the basal ganglia (Fig. 2). FLAIR images showed progressive brain atrophy, with particularly significant bilateral atrophy of the hippocampus. The atrophy of the brain progressed much more rapidly after the patient suffered prolonged convulsive seizures compared to before the seizures.

A statistical analysis of the brain tomographic images obtained via N-isopropyl-[123]I iodoamphetamine single-photon emission computed tomography ([123]I-IMP SPECT) was performed (Fig. 3). The spatial distribution of abnormal regional cerebral blood flow (rCBF) was evaluated using the Neurological Statistical Image Analysis Software (3), and the Z score was calculated (11). Data on normal controls aged 30 to 39 years were derived from a normal database (ChibaDB_ver2) developed by Chiba University Hospital for age-group analysis. The result showed a decrease in blood flow to the frontal lobe after the convulsive seizures compared to that before the seizures. Diminished blood flow was also observed in the posterior parietal area, including the precuneus.

Genetic analysis: After informed consent was obtained, high-molecular-weight genomic DNA was extracted from whole peripheral blood using the GenomicPreP blood DNA isolation kit (GE Healthcare UK, Ltd., Buckinghamshire, England). All five exons and 5’ and 3’ flanking regions of the DAP12 gene (TYROBP; GenBank accession No. AF 019563) were amplified by PCR using the primer sets specific for individual exons (3). The purified PCR products were processed for direct sequencing by the dideoxynucleotide chain termination method on the 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). A single-base deletion of 141 G (141delG) was found in exon 3 of the DAP12 gene (Fig. 4). Furthermore, this genetic mutation was found to be a homozygous point mutation. These findings confirmed the diagnosis of NHD on the basis of a dem-
DAP12 gene mutation (141delG) (3, 6), including the pre-

141delG in exon 3 of the DAP12 gene. All reported cases of 

world with a homozygous mutation (single-base deletion) of 

onstrated DAP12 gene mutation.

Figure 3. Cerebral blood flow SPECT images. A, B, C, and D show Z-score maps of SPECT imag-

takes when the patient was 34 and 35 years old, after the onset of convulsive seizures when the 

ntinued after the convulsive seizures, respectively. A positive Z 

score represents a decrease in the regional CBF in the patient relative to the control mean. The red 

arrows indicate diminished blood flow to the posterior parietal area, including the precuneus. The 

yellow and white arrows indicate diminished blood flow to the parietal lobe and frontal lobe, respec-

tively. Diminished blood flow to the precuneus, frontal lobe, and parietal lobe was noted even before 

the onset of convulsive seizures (A, B). After the seizures, however, blood flow to the frontal lobe was 

markedly diminished (C) and became progressively worse (D). LAT.: lateral, SUP.: superior, INF.: 

inferior, ANT.: anterior, POST.: posterior, MED.: medial

Discussion

The present case is the fifth case of NHD reported in the 

world with a homozygous mutation (single-base deletion) of 

141delG in exon 3 of the DAP12 gene. All reported cases of 

DAP12 gene mutation (141delG) (3, 6), including the pre-

sent case, have been Japanese, which suggests that this type 

of genetic mutation may be specific to the Japanese popu-

lation.

The clinical characteristics of typical cases of defects in 

the DAP12 and TREM12 genes reported in the previous 

studies and the characteristics of the present case are sum-

marized in Table 2 (4, 7). Compared to the previous cases, 

the present case exhibited an earlier onset of frontal lobe 

and bone symptoms and a shorter period before the patient 

became bedridden.

The DAP12 gene mutation (141delG) of the present case 

involved a frameshift of the open reading frame and the 

coding of the truncated DAP12 protein with no intracellular 

tyrosine-based activation motif (ITAM) (8), which can po-

tentially lead to the dysfunction of TREM2/DAP12 signaling 

cascades. According to the previous reports, TREM2/DAP12 

signaling cascades play an important role in immune regu-

lation, including the stimulation of mononuclear phagocytes 

in the central nervous system (5, 10). Genetic defects lead to 

the dysfunction of TREM2/DAP12 signaling cascades ex-

presed in the cell membrane of microglia. Under this cir-

cumstance, apoptotic nerve cells can no longer be removed 

by phagocytosis; as a result, the inflammation persists and 

becomes protracted. These processes are believed to make 

up the pathological mechanism that underlies the central 

nervous system damage observed in NHD.

In the present case, rapid progression of brain damage 

was observed after the onset of prolonged convulsive sei-

141Gdel in DAP12 exon3

GCGAAGGATCCTATGAGCTTGG

Figure 4. Sequencing analysis of the DAP12 gene. Exons 

and exon-intron boundaries of the DAP12 gene were amplified 

from genomic DNA by a polymerase chain reaction (PCR). 

PCR products were processed for direct sequencing analysis. 

The figure represents a single-base deletion of 141G (141delG) 

in exon 3. This genetic mutation was found to be a homozy-

gous point mutation.
zures. The promotion of brain damage was possibly caused by a failure to phagocytize and delete the apoptotic neurons resulting from the severe epileptic seizures. The control of epileptic seizures is believed to be effective in preventing the progression of brain damage.

The present SPECT findings showed regional cerebral blood flow (rCBF) abnormalities to the cerebral cortex, including the frontal and parietal lobes. These findings were consistent with previously reported results of SPECT and positron emission tomography (PET) images in NHD (7, 12, 13). Unlike the previous reports in which SPECT images were analyzed qualitatively, the present study was designed to identify a statistical image analysis using 3D stereotactic surface projections (3D-SSP). In this way, we were able to examine diminished blood flow to the parietal lobe in a more detailed manner, and we successfully identified diminished blood flow to the posterior parietal area, including the precuneus. The results of the overall psychological tests disclosed the neuropsychological profiles, that is, intact overall cognitive function with memory decline as primary disturbances that resembled MCI (14).

SPECT and PET studies of MCI have demonstrated diminished blood flow and metabolism in the posterior cingulate gyrus and precuneus, which are known to have dense fiber connections with medial temporal lesions, such as the hippocampus (15-17). These findings are an important determinant in the diagnosis of MCI, which is believed to be a precursor to Alzheimer’s disease. In the present case, the diminished blood flow to the precuneus was also suspected to be the distant effect of hippocampal disorder. On the other hand, the close relationship between the precuneus and spatially guided behaviour is becoming clear (18). The precuneus disorder might be a cause of the memory impairment that is similar to MCI and the characteristic visuo-spatial memory impairment of NHD (19).

NHD is an intractable disease accompanied by symptoms of early-onset dementia. There has been a report of a case of a TREM2 gene mutation in a patient who manifested symptoms of dementia without exhibiting bone lesions (9). Similarly, there have been two reported cases of heterozygous carriers of the mutated allele of TREM2 gene mutation who had profound visuo-spatial memory impairment without exhibiting bone lesions and whose SPECT study revealed the diminished blood flow to the basal ganglia (19). Although NHD is a rare disease, we believe it has important implications in the identification of early-onset dementia accompanied mainly by frontal lobe symptoms.

The authors state that they have no Conflict of Interest (COI).

References

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