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CASE REPORT

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A case of Muckle–Wells syndrome caused by a novel H312P mutation in NALP3 (cryopyrin)

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Abstract Here, we report a case of Muckle–Wells syndrome (MWS) caused by a novel mutation in the *CIAS1/NALP3* gene. A 23-year-old woman had recurrent self-limited inflammatory episodes from childhood, with headache, abdominal pain, arthritis, and urticarial rash, associated with profound sensorineural hearing loss. The diagnosis was established on the basis of a typical clinical picture together with a missense mutation, which replaced an amino acid adjacent to one in an earlier reported case of MWS resembling this one.

Key words Autoinflammatory syndromes · Cryopyrin-associated periodic syndromes · Muckle–Wells syndrome · NALP3 · Sensorineural deafness

Introduction

Hereditary autoinflammatory syndromes are systemic disorders characterized by recurrent inflammatory episodes from childhood, caused by mutations within the genes regulating inflammation. Subsequent to the revolution in molecular biology and gene analysis, autoinflammatory syndromes have recently been categorized into several types according to the causal genes: cryopyrin-associated periodic syndromes (CAPS), familial Mediterranean fever, tumor necrosis factor receptor-associated periodic syndrome, hyper IgD syndrome, periodic fever with aphthous stomatitis, and Blau syndrome.[1] CAPS shows autosomal dominant inheritance and includes three distinct but overlapping clinical spectra: (1) neonatal onset multisystemic inflammatory disease (NOMID, also referred to as chronic infantile neurologic cutaneous articular syndrome, CINCA); (2) familial cold autoinflammatory syndrome (FCAS); and, (3) Muckle–Wells syndrome (MWS). Among the CAPS, NOMID has the most severe phenotype, FCAS is the mildest, and MWS shows an intermediate phenotype. The triad of symptoms of MWS consists of (1) intermittent episodes of fever, urticarial rash, and joint pain, (2) progressive sensorineural deafness, and (3) secondary amyloidosis.[2,3] Cases of MWS have been reported mainly from Europe; up to now, no case from Japan has been registered in INFEVERS, an international database of hereditary autoinflammatory syndromes.[4] Here, we describe a case of MWS with a novel mutation within *CIAS1/NALP3*, a gene-encoding a protein called NALP3 (also referred to as cryopyrin) which plays a pivotal role in interleukin (IL)-1β production by monocytes.[5,6]

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Case report

A 23-year-old woman had suffered from recurrent self-limited episodes of fever, conjunctivitis, headache, abdominal pain, arthritis, and cold-induced urticarial rash since childhood. She is a daughter of healthy non-consanguineous Japanese parents and has a healthy brother and sister. When she was 8 years old, she suffered from sensory hearing loss, the cause of which remained undiagnosed despite consultations with several otolaryngologists. One suggested that it might be caused by cytomegalovirus infection because the titer of cytomegalovirus antibody was high, but no more information was available. The hearing loss had worsened thereafter.

At age 20, when she was discovered to have anemia and leukocytosis at a health care check, she consulted a hospital and persistently elevated levels of CRP were found. Anti-SS-A and anti-SS-B antibodies were present, but a lip biopsy revealed no abnormalities, and she was followed at the outpatient department without any diagnosis being established.

In May 2006, at age 23, the patient first visited our hospital seeking evaluation of her condition, and was admitted. She was 144.5 cm tall and weighed 43.9 kg. The mean ± SD of Japanese women's height at age 20–24 was 158.9 ± 5.1 cm in 2005; therefore, this patient was relatively short.[7] She had intermittent fever up to 38°C without chills, and the palatine tonsils were swollen. Bilateral ankle joints were tender and swollen. No neurological symptoms were observed except for bilateral sensorineural hearing loss. She usually communicated with us in writing, and her mental status seemed to be normally developed. Her nasal bridge was smoothly flattened with no inflammatory findings such as tenderness, redness, or edema (Fig. 1). She had conjunctivitis on admission, which diminished spontaneously in a few days. Mild bilateral iriditis was also noticed by an ophthalmologist. She had no symptoms that suggested any complication of amyloidosis.

As shown in Table 1, laboratory studies revealed inflammatory findings such as a markedly elevated erythrocyte sedimentation rate (107 mm/h), C-reactive protein (CRP; 8.8 mg/dl), and serum amyloid A (1070 μg/ml). Leukocytosis (15 100/μl) with microcytic anemia was present. Mild polyclonal hyper γ-globulinemia was also observed. No autoantibodies including anti-SS-A and anti-SS-B antibodies were found. Urinalysis was normal. Audiographic examination showed profound bilateral sensorineural deafness (Fig. 2).

On the evening of admission, an urticarial rash appeared on the trunk and limbs (Fig. 3), but it had disappeared by the next morning without medication. On the third hospital day, she complained of abdominal pain. The most painful point was at the right lower quadrant and associated with slight rebound tenderness. Ultrasonography revealed no

Table 1. Hematological and serological data on admission

White blood cell	15 100 μl^{-1} (Neu 85%, Eo 4%, Ba 0%, Mo 2%, and Lym 9%)
Red blood cell	$437 \times 10^4\,\mu l^{-1}$
Hemoglobin	9.9 g/dl
Hematocrit	31.8%
Platelet	$48.7 \times 10^4\,\mu l^{-1}$
Erythrocyte sedimentation rate	107 mm/h
Albumin	3.4 g/dl
Urea nitrogen	11 mg/dl
Lactate dehydrogenase	104 U/l
Asparate aminotransferase	7 U/l
Alanine aminotransferase	8 U/l
γ-Glutamyltranspeptidase	15 U/l
Creatinine	0.38 mg/dl
Creatine kinase	150 U/l
Iron	8 μg/dl
C-reactive protein	8.8 mg/dl
Amyloid A	1070 μg/ml (normal range < 8 μg/ml)
Immunoglobulin G	2202 mg/dl
Immunoglobulin M	113 mg/dl
Immunoglobulin A	724 mg/dl
Anti-nuclear antibody	Negative

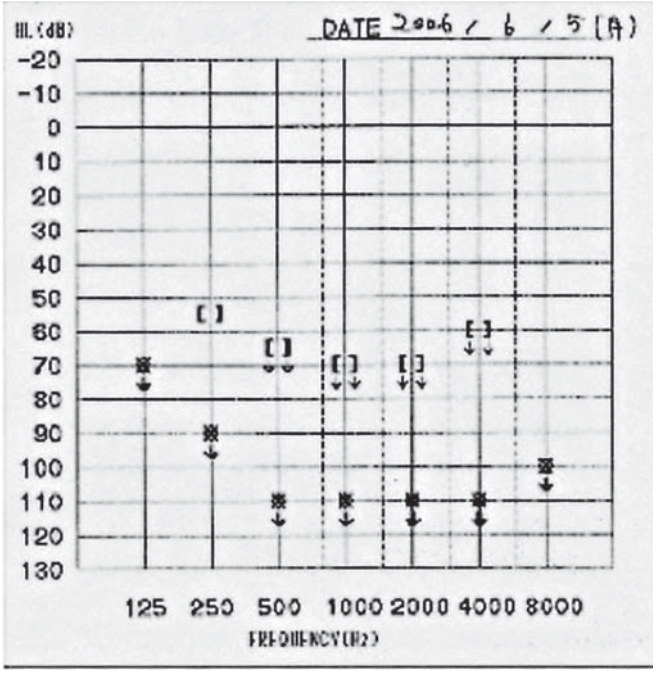

Fig. 2. Audiogram of the patient. Bilateral sensorineural deafness is profound

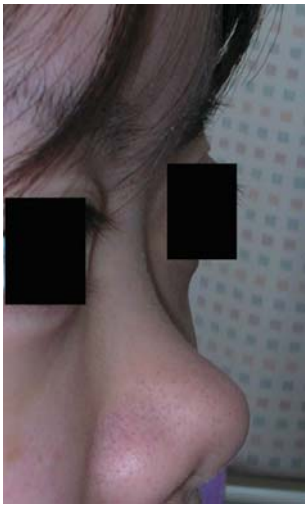

Fig. 1. Saddle nose of the patient. The nasal bridge was smoothly flattened, suggesting that this did not result from destruction

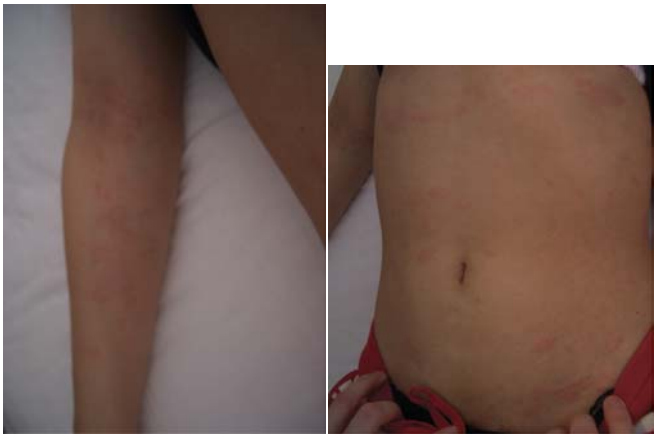

Fig. 3. Urticarial rash of the patient. In the evening on the first hospital day, urticarial rash revealed in her trunk (*left*) and limbs (*right*). This rash disappeared by the next morning

abnormal findings in the internal organs, and this symptom had also decreased by the next morning without any treatment. According to statements by the patient and her mother, she had repeatedly experienced these symptoms since childhood.

On her admission, bacterial culture, viral antibodies, chest and abdominal radiographs, computed tomography, and systemic gallium scintigraphy were all normal. Whole-body fluorodeoxyglucose positron emission tomography revealed only a slight uptake in the axillary and para-aortic lymph nodes that was recognized as reactive. From these results, infectious diseases, malignancies, and autoimmune diseases seemed unlikely, and we suspected autoinflammatory syndromes, especially Muckle–Wells syndrome (MWS). With informed consent, we investigated mutations of the *CIAS1/NALP3* gene by fluorescent sequencing.[8] A heterozygous nucleotide transition A935C (transition from 935th adenine to cytosine) was detected within exon 3 of *CIAS1/NALP3*, which results in a novel H312P (transition from 312nd histidine to proline) amino acid replacement in the NACHT domain of NALP3 (Fig. 4). This transition was not found in 28 alleles of control samples obtained from 14 healthy volunteers. Taking this mutation together with the clinical findings, we established the diagnosis of MWS.

Nonsteroidal antiinflammatory drugs, low-dosage corticosteroids, colchicine, immunosuppressants, or infliximab are known to be ineffective for MWS, and the only agent that can control the systemic inflammation, potentially with an effect on the risk of amyloidosis, is the recombinant human IL-1 receptor antagonist, anakinra.[9–12] Therefore, treatment with anakinra at a dosage of 50 mg daily was commenced after obtaining informed consent from the patient and receiving approval from the institutional review board of Tokyo Medical and Dental University. The patient had an immediate response to anakinra; inflammatory episodes have not recurred thereafter, and CRP and serum amyloid A fell to 0.7 mg/dl and 10 μg/ml, respectively, within a week. However, her hearing has not returned after two months of treatment.

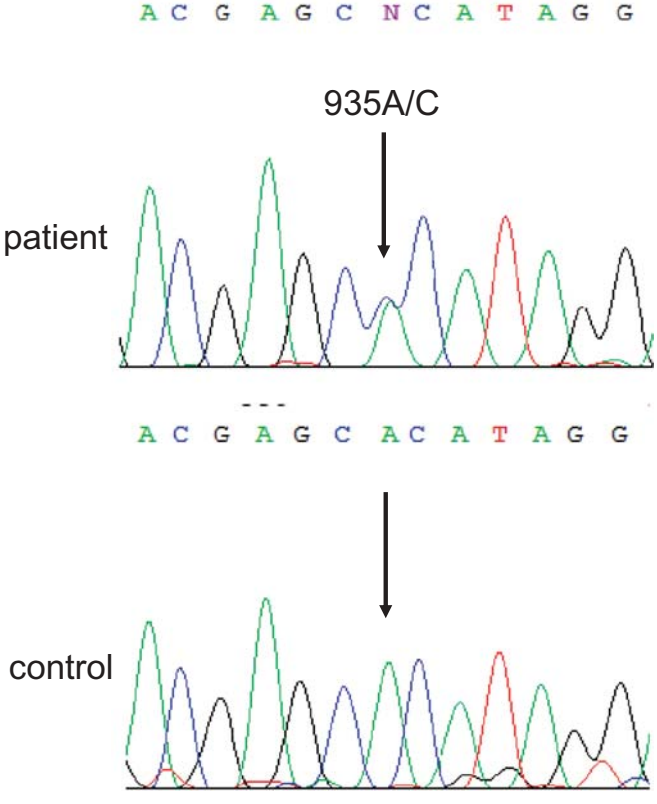

Fig. 4. Sequence analysis of the *CIAS1/NALP3* gene. *Upper panel* shows heterozygous A-to-C transition at position 935 resulting in the new mutation H312P in the NACHT domain of *NALP3*

Discussion

The present case had typical clinical symptoms of MWS, such as recurrent episodes of fever, headache, conjunctivitis, arthritis, abdominal pain, and urticarial rash, as well as sensorineural deafness. In addition, she displayed a relatively short stature and saddle nose, which are typical in NOMID but can be observed in MWS as well.[10] These features may be related to the selective expression of NALP3 in monocytes, neutrophils, and chondrocytes.[13] The fact that accurate diagnosis of this patient had not been made for as long as 20 years needs to be addressed. One reason for this may be that autoinflammatory syndromes are rare and not well recognized by Japanese physicians. Recent genetic studies have succeeded in re-categorizing these syndromes according to the causal genes,[1] and the efficacy of newly developed biologic agents for some of them has been demonstrated. It is therefore important to widely disseminate awareness of the clinical features and pathophysiology of autoinflammatory syndromes to Japanese physicians.[3] They should consider the possibility that patients with persistent or recurrent

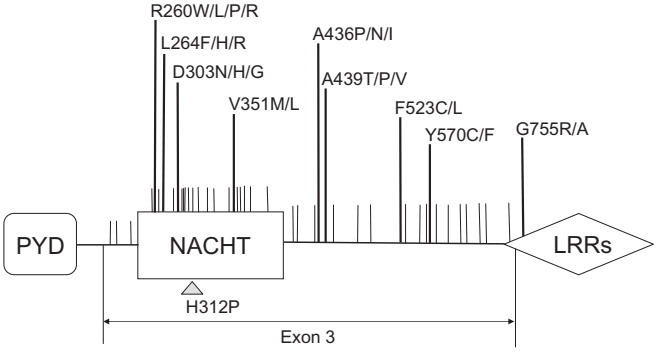

Fig. 5. Scheme and locations of the mutations in *NALP3/CIAS1* encoding protein. All the mutations except for G755R/A (transition from 755th glycine to arginine or alanine) identified to date that cause CAPS are located in exon 3. *Vertical lines* above the protein structure indicate mutations previously reported, *thin line* indicates one case, and *bold line* with amino acid replacement indicates the position from two or more cases. The *triangle* below the protein structure indicates the new mutation identified in this case. *PYD*, pyrin domain; *NACHT*, domain present in neuronal apoptosis inhibitor protein, MHC class II transactivator, HET-E and TP1; *LRRs*, leucin-rich repeats

inflammatory reactions of unknown origin might be suffering from an autoinflammatory disease.

The mutation H312P found in this case is located in the NACHT domain in exon 3 of the *CIAS1/NALP3* gene, the hot spot of mutation in CAPS (Fig. 5).[5] A 22-year-old patient with MWS described by Mirault et al[11] had an E311K mutation which is adjacent to H312P, and interestingly, the clinical picture of that case closely resembled ours. This suggests that amino acids E311 and H312 might be located in a region crucial for NALP3 function. This hypothesis needs confirmation by re-construction experiments using the mutant sequence, which we are now planning. On the other hand, when we sequenced *CIAS1/NALP3*, we found several single nucleotide polymorphisms (SNPs) in healthy controls. Cases of MWS which do not have mutations in *CIAS1/NALP3* have been reported; these are sometimes designated variant MWS.[11] It will be useful to collate information on SNPs in the *CIAS1/NALP3* gene because these may affect the sensitivity of NALP3 to various stimuli, such that a particular combination could possibly lead to a phenotype comparable with MWS. The data on mutations and clinical characteristics of hereditary autoinflammatory syndromes are collected by INFEVERS.[4] As of February 2007, 76 mutations within *CIAS1/NALP3* were registered, of which 10 were associated with MWS. Although three mutations of *CIAS1/NALP3* were reported from Japan, two of them were found in NOMID[14,15] and another was in a healthy individual.[16] Recently, Kagami et al.[17] reported a probable case of MWS, a 26-year-old Japanese woman but without sensorineural hearing loss. The authors state that no *CIAS1/NALP3* mutation was found, but they only established the absence of 5 known mutations. Thus, other mutations in the *CIAS1/NALP3* gene in that patient cannot be excluded.

In summary, to the best of our knowledge, we present here the first definite Japanese case of MWS confirmed by sequencing the *CIAS1/NALP3* gene. This patient had a novel mutation, located adjacent to one reported earlier in a different case of MWS, suggesting an important role for this position in NALP3 function. Because the efficacy of certain biologic agents has now been demonstrated in some autoinflammatory syndromes, it is becoming more important than ever to make an early and accurate diagnosis in order to initiate optimal therapy to prevent irreversible complications.

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