Sanfilippo Syndrome Type D

Natural History and Identification of 3 Novel Mutations in the GNS Gene

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Background: Mucopolysaccharidosis type IIID (MPS-IIID), or Sanfilippo syndrome type D, is a rare autosomal recessive lysosomal storage disorder caused by mutations in the N-acetylglucosamine-6-sulfatase (GNS) gene, leading to impaired degradation of heparan sulfate.

Objectives: To report the natural history of MPS-IIID in 2 siblings described by Kaplan and Wolfe in 1987 and to study the phenotype in 2 other unrelated families with MPS-IIID.

Design, Setting, and Patients: Case series of 4 patients with MPS-IIID: 2 siblings followed up at the Montreal Neurological Hospital and Institute, 1 patient followed up at the UZ Brussel, and 1 patient recruited through the prenatal counseling program at the UZ Brussel.

Main Outcome Measures: Clinical and molecular data collected from 3 families with enzyme-based diagnosis of MPS-IIID.

Results: The course of the disease was characteristic of MPS-IIID in all patients, although survival may be longer than was previously reported. In family 1, both siblings were homozygous for a novel nonsense mutation in the GNS gene (c.1168C>T). In family 2, the proband carried a heterozygous mutation occurring in a splice recognition site in the intron 7 boundary (c.876-2A>G). The second mutation in this patient remains to be identified. In family 3, the proband was homozygous for a novel frameshift mutation in GNS due to the insertion of 5 nucleotides (c.1138_1139insGTCCT).

Conclusions: Major issues in the care of patients with MPS-IIID include behavioral problems, sleep problems, recurrent infections, dysphagia, and pain from orthopedic complications. To date, all mutations in GNS predict protein truncation, and there is no obvious genotype-phenotype correlation.

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drome type D. We report 3 novel mutations in the GNS gene and clinical follow-up of the 2 siblings described by Kaplan and Wolfe in 1987 as well as the phenotypes of 2 other unrelated families with MPS-IIID.

### METHODS

Detailed medical and family histories were obtained for 2 of the families. Patients were examined, and medical records were reviewed. The third family was identified through a prenatal counseling program, and clinical information was limited. Blood samples were collected for genetic studies. Genomic DNA was isolated using a DNA isolation kit (Puregene; Gentra Systems Inc, Minneapolis, Minnesota), according to the manufacturer’s instructions. Amplification of coding regions and intron-exon boundaries of GNS from genomic DNA was performed using the primers and conditions described previously. Polymerase chain reaction products were purified and directly sequenced in both directions (ABI Prism 3730; PE Applied Biosystems, Mississauga, Ontario, Canada). Each mutation was confirmed using an independent sequencing reaction on another day. Control subjects were genotyped for each mutation using dedicated allele-specific detection methods (details of reagents and conditions are available on request). The DNA analysis protocol was approved by the ethics review panel of the University of Western Ontario.

### RESULTS

#### FAMILY 1 (PATIENTS 1 AND 2)

In this family, both parents originated from a small community in Abruzzo, Italy, but were unaware of any consanguinity despite the fact that the maternal grandmother had the same surname as the father. The proband, a 31-year-old man, presented with developmental delay at age 2 years (Figure 1 A). He started saying single words at age 3 years and phrases at age 4 years. He started school in a regular kindergarten, had to repeat grade 2, and later required special education. The diagnosis of Sanfilippo syndrome type D was suspected at age 8 years because of excess amounts of urinary heparan sulfate, and it was confirmed at age 9 years based on the complete absence of N-acetylglucosamine-6-sulfatase activity in fibroblasts. He had surgery for left pes cavus deformity at ages 12 and 19 years and for a pialodinal sinus tract at age 17 years. During adolescence he had severe oppositional behavior, and school performance regressed. He had increasing difficulties walking beginning at age 25 years and gradually became wheelchair bound. Sleep problems have occurred from time to time but have never been a major burden. He sometimes had periods of restless sleep with frequent awakenings, and he recently had an inverted sleep-wake cycle for 3 weeks. At age 28 years, he was investigated for brief episodes characterized by holding his head, followed by facial redness, drooling, and crying. No changes were recorded on continuous video electroencephalography during these events. There was no hydrocephalus.

At age 31 years he understands simple commands; he moans or screams to draw attention, but he does not talk. He has a quiet nature and, despite limited communication, a good relationship with his brother and parents. He is fully dependent for dressing, eating, and personal hygiene. He has dysphagia for liquids and needs to be spoon-fed. He has low-set ears, coarse facial features, a short prominent forehead, deep-set eyes, hypertrichosis of the eyebrows, synophrys, a low nasal bridge, anteverted nares, and thick lips with a wide alveolar ridge. He has a

### Table. Patients With MPS-IIID Described in the Literature

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of Patients/Sex</th>
<th>Ethnic Origin</th>
<th>Age Last Examined, y</th>
<th>Mutation</th>
<th>GNS Intron/Exon</th>
<th>Type of Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kresse et al</td>
<td>1/M</td>
<td>East Indian</td>
<td>7</td>
<td>See Mok et al</td>
<td>See Mok et al</td>
<td>See Mok et al</td>
</tr>
<tr>
<td>Gatti et al</td>
<td>1/F</td>
<td>Italian</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Coppa et al</td>
<td>1/M</td>
<td>Italian</td>
<td>9.7</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Kaplan and Wolfe</td>
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<td>Italian-Canadian</td>
<td>11/3.5</td>
<td>See present report</td>
<td>See present report</td>
<td>See present report</td>
</tr>
<tr>
<td>Siciliano et al</td>
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<td>Italian</td>
<td>19/14</td>
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<td>NA</td>
</tr>
<tr>
<td>del Canho et al</td>
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<td>Dutch</td>
<td>8</td>
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<td>NA</td>
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<tr>
<td>Ozand et al</td>
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<td>Saudi Arabian</td>
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<td>NA</td>
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<tr>
<td>Jones et al</td>
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<td>White NS</td>
<td>14†</td>
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<td>NA</td>
<td>NA</td>
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<td>NA</td>
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<tr>
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<td>7</td>
<td>c.1063C&gt;T</td>
<td>Exon 9</td>
<td>Nonsense</td>
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<tr>
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<td>3.8</td>
<td>c.1169delA</td>
<td>Exon 10</td>
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<td>10/7</td>
<td>c.192 + 2488_296del8723</td>
<td>Exons 2 and 3</td>
<td>Intragenic deletion</td>
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<tr>
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<td>6</td>
<td>c.814C&gt;T</td>
<td>Exon 7</td>
<td>Nonsense</td>
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<tr>
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<td>31/23</td>
<td>c.1168C&gt;T</td>
<td>Exon 10</td>
<td>Nonsense</td>
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<tr>
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<td>15</td>
<td>c.876-2A-&gt;G (HTZ)</td>
<td>Intron 7/exon 8</td>
<td>Splice site</td>
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<tr>
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<td>15</td>
<td>c.1138_1139insGCTCT</td>
<td>Exon 10</td>
<td>Frameshift</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: bp, base pair; HTZ, heterozygous; MPS-IIID, mucopolysaccharidosis type IIID; NA, not available; NS, not specified; sib, siblings; †, deceased. 

^a^ Same as second patient under Kresse et al.

^b^ Same as first patient under Kresse et al.

^c^ Same as patient under Kaplan et al.
short neck, broad hands and feet with short blunt fingers and toes, fixed contractures of the elbows and heel cords, and bilateral pes cavus (Figure 1B). His vision is reduced and he has night blindness. Deep tendon reflexes are brisk and equal bilaterally. The plantar responses are equivocal. Sensory deficits cannot be assessed because of poor cooperation. He can walk with difficulty with bilateral support.

His 23-year-old brother was diagnosed in infancy based on family history, clinical features, and elevated levels of MPS in the urine (Figure 1C). Elbow contractures were noted in the first year of life. Development slowed after age 1 year, and his facial features coarsened. He started kindergarten in a regular school, but he was transferred to special education at age 7 years. He had recurrent otitis media treated with tympanostomy tubes and tonsillectomy.

In his late teens, the predominant problems were behavioral, with agitation, hyperactivity, and occasional fugue, for which he was treated with risperidone and procyclidine hydrochloride for 6 months without any improvement. Between ages 18 and 22 years, he had severe sleep problems characterized by frequent awakenings. He would get up at night and wander around the house, and he experienced much difficulty in getting to sleep again. Treatment with benzodiazepines resulted in increased daytime somnolence. Since age 20 years, he has had increasing drooling, with abundant mucus secretions, and dysphagia, mainly for liquids, which was a major cause of morbidity. Drooling and dysphagia improved markedly with botulinum toxin injections in the salivary glands.

At age 23 years, he has progressive hearing loss. He can dress with some supervision and needs help with personal hygiene. He is good-humored and has an outgoing nature. He is dysarthric, uses simple words, and understands simple commands. He has a prominent forehead and occiput, coarse facial features, thick eyebrows with hypertrichosis, a flat nasal bridge, a wide mouth, a short neck, moderate thoracic scoliosis, and fixed contractures of the elbows and heel cords (Figure 1D). He experiences increasing difficulty getting up from a chair but can still run quickly. Deep tendon reflexes are present and brisk bilaterally. There are no cerebellar signs. Neurosensory tests are noncontributory. He has a cardiac systolic ejection murmur.

When the brothers were 29 and 21 years of age, respectively, formal neuropsychological testing could not be performed, but full IQ scores were estimated to be below 50. On the basis of previous evidence from school records and early drawings, it was evident that their IQ scores had been significantly higher.
Genomic DNA analysis showed that both brothers were homozygous for a single nucleotide change in exon 10, designated c.1168C>T, which predicted a nonsense mutation at the amino acid level in residue 390, namely, glutamine (CAG) to a stop codon (TAG) or p.Gln390Ter (Figure 2A). The DNA sequence analysis subsequently showed that each parent was heterozygous for the c.1168C>T mutation. The mutation was absent from 200 chromosomes of healthy individuals.

**FAMILY 2 (PATIENT 3)**

The proband, a 15-year-old boy, is the third child of healthy, nonconsanguineous parents of Belgian origin (Figure 1E). Early developmental milestones were normal, but at age 5 years delayed psychomotor development with severe speech delay was diagnosed, and speech therapy and physiotherapy were initiated. Surgery for bilateral inguinal hernia was performed at age 21/2 years. He had recurrent upper respiratory tract infections, for which he received bilateral tympanostomy tubes at ages 2 and 31/2 years and tonsillectomy at age 5 years. He was treated successfully with melatonin from age 8 years until age 14 years. He then became very agitated at night and would sleep for only 4 to 5 hours. Melatonin was continued to age 14 years and was characterized by difficulty falling asleep, frequent awakenings, and restless sleep and were treated successfully with melatonin from age 8 years until age 14 years. He then became very agitated at night and would sleep for only 4 to 5 hours. Melatonin was replaced by lormetazepam, 1 mg/d, with good results. Behavioral problems included restlessness, hyperactivity, and aggressive outbursts and were a significant burden for the family. Trials with lorazepam, prazepam, pipamperone, risperidone, melitracen hydrochloride, and flupentixol had little effect. Valgus position and epiphyseal dysplasia of the hips caused severe pain and required bilateral osteotomy at age 11 years. The postoperative course was complicated by wound infection, increased speech problems, swallowing problems that required tube feeding, and severe behavioral problems, for which he received sedation during several weeks. He gradually recovered his preoperative level of functioning, but he continued to have pain in both hips that required continuous treatment with nonsteroidal anti-inflammatory drugs and local infiltrations with analgesics. At age 15 years, the osteosynthesis material (plate and screws) had to be removed from the femur because of pain and scarring of the overlying skin. The surgery was complicated by unilateral osteomyelitis. Six months later, he had a luxation of the left hip that required removal of the femoral head. He had multiple infections and needed a percutaneous endoscopic gastrostomy tube for feeding.

At age 151/2 years he is dysarthric, uses simple words, and understands simple commands. He does not like to be touched, but he holds on to every person who comes close. In the past 2 years, he has lost any form of occupation or play but remains good-humored when comforted. He has coarse features closely resembling those of the siblings described in family 1, short stature, and bilateral pes cavus (Figure 1F). Stretch responses are present and equal bilaterally. Sensory functions are impossible to assess. He is wheelchair bound and is fully dependent for all activities of daily living.

Magnetic resonance imaging of the brain at age 15 years showed severe corticosubcortical atrophy and more discrete cerebellar atrophy. Electroencephalograms showed slow background activity of low voltage. Continuous 24-hour video electroencephalographic monitoring was per-
formed at age 14 years because of episodes of holding the head and crying for 15 minutes, similar to what was described in the first patient. No epileptiform changes were recorded. Evoked potentials were normal. He has fusion of the vertebral bodies of C2 and C3, hypoplasia of the vertebral bodies of C4 to C7, a discrete insufficiency of the mitral valve, and hepatosplenomegaly. Results of the ophthalmologic examination at age 10 years were normal.

Mutation analysis of the GNS gene revealed a heterozygous mutation in a splice recognition site in the intron 7/exon 8 boundary, namely, c.876-2A>G (Figure 2B). The mutation was absent from 200 chromosomes of control subjects. The patient was also heterozygous for a previously described single nucleotide polymorphism (c.198A/G) in exon 2. A second potential disease-causing mutation could not be found. The unaffected mother was heterozygous for the same 2 DNA changes.

FAMILY 3 (PATIENT 4)

The proband is a 15-year-old girl of Turkish-English descent who was diagnosed as having Sanfilippo syndrome type D at age 13 years based on the phenotype, positive MPS screen in the urine, and enzyme assessment. She is severely retarded and is fully dependent for all activities of daily living.

She was homozygous for a novel 5–base pair insertion in exon 10, c.1138_1139insGTCCT, resulting in a frameshift starting from codon 380 and in premature termination of the protein at amino acid position 389 (p.Aspartate388GlyfsX9) (Figure 2C). The mutation was absent from 200 chromosomes of control subjects.

Mutations in the GNS gene result in the rare lysosomal storage disorder Sanfilippo syndrome type D. There is no effective treatment for the disorder: bone marrow transplantation in a presymptomatic patient with MPS-IIIA and enzyme replacement in the caprine model for MPS-IIID did not result in any change in the neurologic features of the disease. Reports on the natural history of MPS-IIID are scarce and, unlike for Fabry or Hunter disease, a systematic follow-up registry does not exist. The disease evolution in all 4 patients has followed the classic pattern of Sanfilippo syndrome.

Sleep problems are common in patients with MPS-IIID and are characterized by sleep maintenance insomnia with frequent nocturnal awakenings and by an extremely irregular sleep pattern on polygraphic recordings. Sleep problems were present in all 3 patients described herein on whom information was available. Treatment with benzodiazepines often results in increased daytime somnolence, as was reported in patient 2. Melatonin is the treatment of choice and was applied successfully in patient 3. Evidence of the implication of melatonin in the pathogenesis of sleep problems in Sanfilippo syndrome has been provided by Guerrero et al, who demonstrated an alteration in the circadian rhythm of melatonin in patients with Sanfilippo syndrome.

Behavioral problems proved to be extremely difficult to treat and were a major cause of distress for the family. Recurrent infections and pain often led to episodic regression in general functioning, affecting mobility, speech, swallowing, and behavior. Life expectancy in patients with Sanfilippo type D was found to be longer compared with that of patients with type A, illustrating intratype variability.

The brothers in family 1 are the oldest patients with MPS-IIID described to date, with survival into the fourth decade for patient 1. The rate of disease progression in this family seems more benign compared with that in families 2 and 3 and other patients with MPS-IIID described in the literature (life expectancy of 14 and 17 years reported by Jones et al). This is consistent with previous reports on clinical heterogeneity in MPS-IIID. This intratype variability may be related to multiple factors, including the nature of the mutation or differences in the care that the patients have received. Survival into adulthood requires a well-orchestrated transition from pediatric to adult care. In everyday practice, parents of children with special needs often experience a gap between pediatric and adult care, and in family 1 this has added significant stress to the care of the siblings.

Comparing the phenotypic features of all reported patients with MPS-IIID reveals considerable interfamilial phenotypic variability. This could be the result of different functional consequences of the various mutations in the GNS gene or to the potential modulating effects of other genes or to differences in genetic backgrounds and possibly even environmental effects, such as intrauterine environment. However, all reported GNS mutations in MPS-IIID to date are protein truncating mutations, including a large intragenic deletion, premature stop codons, frameshift mutations, and a probable RNA splicing mutation. So far, no GNS missense mutation has been found in MPS-IIID, suggesting that a more severe genomic change predicting more severe biochemical deficiency is the underlying cause.

The siblings in family 1 were each homozygous for a single nucleotide change in exon 10, predicting a nonsense mutation at the amino acid level (p.Gln390Ter). Family 1 is the third family with MPS-IIID of Italian origin in whom the molecular defect has been defined. So far, all the families have a different molecular defect underlying the disease. However, since 65% of patients reported to have MPS-IIID are of Italian ancestry, it would be of interest to investigate whether the other Italian patients with MPS-IIID share one of the known mutations, suggesting a common founder.

In family 3, the proband was homozygous for an insertion that is predicted to lead to a frameshift, is likely to cause premature termination at residue 389, and would have 8 abnormal C-terminal residues.

The absence of these mutations from chromosomes of control subjects together with the fact that the truncated products of the mutant alleles would likely be subject to nonsense-mediated messenger RNA degradation argues strongly in favor of disease causality in the case of both mutations.

In family 2, the proband and his healthy mother were each heterozygous for a common single nucleotide polymorphism in exon 2 of GNS (c.198A/G) (data not shown) and a novel mutation occurring directly in the acceptor splice site in intron 7 (c.876-2A>G). The nature of this
likely splicing mutation and its absence from control subjects make it highly probable that it is a disease-related mutation. These findings suggest the existence of a possible second GNS mutation causing the disease in the probe band, but so far we have not identified any additional mutations. Such a mutation might be located in the promoter region and might affect transcription, or might be situated deep in an intron and affect splicing, or there might be a large deletion affecting 1 or more exons that may be detectable using multiplex ligation-dependent probe amplification. Further analysis to distinguish between these possibilities is not possible or warranted at present and lies outside the scope of this article.

To date, 7 different mutations have been identified in all 7 families studied (Table). Further mutational analysis in these patients will help clarify genotype-phenotype correlations.

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REFERENCES