4H Syndrome With Late-Onset Growth Hormone Deficiency Caused by POLR3A Mutations

Ana Potic, MD; Bernard Brais, MD, MPhil, PhD; Karine Choquet; Raphael Schiffmann, MD, MHS; Geneviève Bernard, MD, MSc, FRCPC

Objective: To report a novel clinical and genetic presentation of a patient with 4H syndrome, which is a recently described leukodystrophy syndrome characterized by ataxia, hypomyelination, hypodontia, and hypogonadotropic hypogonadism.

Design: Case report.

Setting: University teaching hospital.

Patient: A 20-year-old male patient with 4H syndrome.

Results: The patient was found to have delayed tooth eruption and a late-onset growth hormone deficiency without overt growth failure. He was a compound heterozygote for the novel missense mutations R1005H and A1331T of POLR3A, which codes for the largest subunit of RNA polymerase III.

Conclusion: This is the first report of this type of leukodystrophy from southeastern Europe, which suggests that POLR3A mutations should be suspected in patients with hypomyelination and various central nervous system–based endocrine abnormalities.

METHODS

IDENTIFICATION OF MUTATIONS

Genomic DNA was extracted from peripheral blood lymphocytes using a standard method. Polymerase chain reaction primers were designed using ExonPrimer (http://genome.ucsc.edu) or Primer3 (http://frodo.wi.mit.edu/primer3/) and synthesized by Invitrogen.11 Polymerase chain reactions for the screening of polymerase (RNA) III (DNA directed) polypeptide A, 155-kDa (POLR3A [OMIM 614258]) exons, 5′/H11032 and 3′/H11032 untranslated regions, and intron-exon boundaries were performed using 40-ng genomic DNA in 10-µL polymerase chain reactions containing 1× polymerase chain reaction buffer, 3mM of magnesium chloride, 10mM of primer mix, and 0.4 U of Taq DNA polymerase (Qiagen). For an amplification reaction, a touchdown program was used. Sequencing analyses were performed at the Genome Quebec Innovation Center, McGill University (Montreal, Quebec, Canada) using an ABI3730 Genetic Analyser (Applied Biosystems). Sequences were aligned using SeqMan 4.03 (DNAnstar) with POLR3A GRCh27/hg19 (http://genome.ucsc.edu) as a reference sequence.

REPORT OF A CASE

A male patient aged 20 years was the second born to nonconsanguineous, healthy parents with no family history of neurological dis-
cases. After an uneventful gestation, birth, and early psychomotor development, the patient first presented at 4 years of age with progressive cerebellar ataxia, bilateral pyramidal deficit, and cognitive decline. Three years later, vertical gaze palsy and loss of pursuit without nystagmus became evident. Focal motor seizures that appeared at 10 years of age were easily controlled with valproate sodium, but replacement with levetiracetam led to noticeable motor function improvement. Dentition was perturbed and delayed: the first teeth were deciduous mandibular molars erupting at 20 months of age; deciduous incisors were never replaced by permanent teeth.

The results of a neurological examination at 20 years of age showed a normal head circumference, a severe cerebellar syndrome, mild spastic quadriparesis with mild pseudobulbar signs, and pre-existing oculomotor abnormalities with normal fundoscopic findings. He was anarthric and confined to a wheelchair, with titubation and poor head control. His full-scale IQ was less than 20, which indicated severe mental retardation according to the Wechsler Intelligence Scale for Children, 3rd edition, confirming a decline from the previous evaluation at 11 years of age (with a full-scale IQ of 42, which indicated moderate mental retardation).

The results of magnetic resonance imaging of the brain performed at 12, 14, and 20 years of age (Figure) showed supratentorial and infratentorial hypomyelination: diffuse white matter hypointensity on T2-weighted images and an isointense T1-weighted signal (Figure). A mild vermian cerebellar atrophy, moderate cortical atrophy, and thinned corpus callosum were evident at all examinations (data not shown). A small pituitary structure of 5 mm was recorded at 20 years of age (data not shown; with 13 mm × 9 mm × 13 mm being the normal mean structure for the age and sex).11 No other hypophysial structural anomaly existed. Proton magnetic resonance spectroscopy performed at 12 and 20 years of age revealed a decreased choline to creatine ratio as the only metabolic anomaly within the affected white matter (data not shown).

Table 1. Hormonal Status of Patient With 4H Syndrome at Different Ages

<table>
<thead>
<tr>
<th>Hormone</th>
<th>At 17 y and 9 m of Age</th>
<th>At 20 y and 3 m of Age</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH, mIU/mL</td>
<td>0.4</td>
<td>0.2</td>
<td>1.0-12.0</td>
</tr>
<tr>
<td>Maximal FSH peak value, mIU/mL</td>
<td>0.5</td>
<td>0.3</td>
<td>&gt;5</td>
</tr>
<tr>
<td>LH, mIU/mL</td>
<td>0.1</td>
<td>0.1</td>
<td>0.8-7.6</td>
</tr>
<tr>
<td>Maximal LH peak value, mIU/mL</td>
<td>0.2</td>
<td>0.1</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Prolactin, µg/L</td>
<td>0.3</td>
<td>0.2</td>
<td>2.1-7.8</td>
</tr>
<tr>
<td>Testosterone, ng/dL</td>
<td>60.5</td>
<td>46.1</td>
<td>160.0-810.0</td>
</tr>
<tr>
<td>Estradiol, pg/mL</td>
<td>46.6</td>
<td>436</td>
<td>0.0-56.1</td>
</tr>
<tr>
<td>Thyrotopin, mIU/L</td>
<td>2.11</td>
<td>2.08</td>
<td>0.27-4.2</td>
</tr>
<tr>
<td>Free thyroxine, ng/dL</td>
<td>1.2</td>
<td>1.2</td>
<td>0.7-1.5</td>
</tr>
<tr>
<td>Free triiodothyronine, pg/dL</td>
<td>290.9</td>
<td>302.0</td>
<td>170.8-370.1</td>
</tr>
<tr>
<td>ACTH, pg/mL</td>
<td>21.8</td>
<td>22.3</td>
<td>7.3-63.2</td>
</tr>
<tr>
<td>Cortisol, pg/dL</td>
<td>17.5</td>
<td>17.4</td>
<td>6.2-19.4</td>
</tr>
<tr>
<td>GH basal peak values, ng/mL</td>
<td>0.7 to &gt;0.5</td>
<td>Undetectable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>to &gt;0.35</td>
<td>0-1</td>
<td></td>
</tr>
<tr>
<td>IGF-1, ng/mL</td>
<td>230</td>
<td>56d</td>
<td>116-358</td>
</tr>
</tbody>
</table>

Abbreviations: ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; GH, growth hormone; IGF-1, insulin-like growth factor 1; LH, luteinizing hormone; LHRH, luteinizing hormone-releasing hormone.

SI conversion factors: To convert FSH and LH to international units per liter, multiply by 1.0; to convert prolactin to picomoles per liter, multiply by 43.478; to convert testosterone to nanomoles per liter, multiply by 0.0347; to convert estradiol to picomoles per liter, multiply by 3.671; to convert free thyroxine to picomoles per liter, multiply by 12.871; to convert free thyroxine to picomoles per liter, multiply by 0.154; to convert ACTH to picomoles per liter, multiply by 0.2; to convert cortisol to nanomoles per liter, multiply by 27.588; to convert GH to micrograms per liter, multiply by 0.1 and to convert IGF-1 to nanomoles per liter, multiply by 0.131.

a After LHRH test.
b Peak value.
c Measured at 3-hour intervals.
d Significant decrement compared with the previous value.

ENDOCRINE EVALUATION

The patient's linear growth has always been unremarkable. At the age of 17 years 9 months, his height was 178 cm, and his weight was 72 kg with a body mass index (calculated as weight in kilograms divided by height in meters squared) of 22.7. However, pubertal development was absent (with a Tanner puberty stage of 0). Hypogonadotropic hypogonadism was confirmed by the low levels of follicle-stimulating hormone, luteinizing hormone, prolactin, and testosterone, along with the absent response to the luteinizing hormone-releasing hormone stimulation test (Table 1). No other hormonal anomaly was revealed at the time. At the age of 20 years, the patient's body mass index and clinical findings were unaltered; genitalia were of prepubertal size and shape, with small-volume testes (1.5 mm³) within the scrotum. His hormonal status remained unchanged except for GH deficiency characterized by undetectable GH during peak basal conditions and a low level of insulin-like growth factor 1 (Table 1). Osteodensitometry revealed advanced osteoporosis resulting from untreated androgen deficiency and loss of ambulation.14

RESULTS

The patient was found to be compound heterozygote for c.3014G>A (p.R1005H) in exon 23 and c.3991G>A (p.A1331T) in exon 30. The mutations were found to be present in highly conserved regions and to segregate within the family; both parents were carriers of 1 mutation. Both mutations were absent in more than 350 control chromosomes.
involved in innate immune responses.15 merase III is also thought to be a cytosolic DNA sensor.

cellular growth, differentiation, and apoptosis. RNA polymerase III is responsible for the noncoding RNA transcription, including all transfer RNA (tRNA). The hypoth-
polymerase III is the largest subunit of Pol III, 1 of the 3 polymerases. RNA

Table 2. Known Hypomyelinating Leukodystrophies

<table>
<thead>
<tr>
<th>Disease(s)</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelizaeus-Merzbacher disease (PNS may be affected)</td>
<td>PLP1</td>
</tr>
<tr>
<td>Pelizaeus-Merzbacher–like disease or hypomyelinating leukodystrophy type 2 (PNS may be affected)</td>
<td>GJC2</td>
</tr>
<tr>
<td>Hypomyelinating leukodystrophy type 3</td>
<td>AIMP1</td>
</tr>
<tr>
<td>Trichothiodystrophy with hypersensitivity to sunlight</td>
<td>ERCC3, GTF2H5, ERCC2</td>
</tr>
<tr>
<td>Free sialic acid storage disease</td>
<td>SLC17A5</td>
</tr>
<tr>
<td>Hypomyelination with atrophy of basal ganglia and cerebellum</td>
<td>Unknown</td>
</tr>
<tr>
<td>Fucosidosis</td>
<td>FUCAI</td>
</tr>
<tr>
<td>Galactosemia</td>
<td>GALT</td>
</tr>
<tr>
<td>Serine synthesis defects</td>
<td>PHGDH, P5AT1, PSPI</td>
</tr>
<tr>
<td>Oculodentodigital dysplasia</td>
<td>GJA1</td>
</tr>
<tr>
<td>18q1 syndrome</td>
<td>MBP (18q22.3-q23)</td>
</tr>
<tr>
<td>Early-onset neuronal degenerative disorders (eg, GM1 and GM2 gangliosidosis, and infantile ceroid lipofuscinosis)</td>
<td>GLB1, HEXA, PPT1</td>
</tr>
</tbody>
</table>

Leukodystrophy Group With Typical Peripheral Nerve Involvement

Cockayne syndrome | ERCC6, ERCC8, POLR3A, POLR3B |
4H syndrome, tremor-ataxia with central hypomyelination, leukodystrophy with oligodontia | HCC |
Hypomyelination with congenital cataracts or hypomyelinating leukodystrophy type 5 | POLR3A, POLR3B |
Peripheral neuropathy, central hypomyelination and Waardenburg syndrome and Hirschsprung disease | SOX10 |

Abbreviation: PNS, peripheral nervous system.

To our knowledge, this is the first report of a patient with 4H syndrome and late-onset GH deficiency. The patient was found to be compound heterozygote for 2 previously un-published POLR3A mutations. The mutation in exon 23 is located in the same codon as another known 4H mutation (c.3013C>T or p.R1005C).11 Mutations in POLR3A were recently found to cause 3 related leukodystrophies: 4H syndrome, leukodystrophy with oligodontia, and tremor-ataxia with central hypomyelination.11 POLR3A encodes for the largest subunit of Pol III, 1 of the 3 polymerases. RNA polymerase III is responsible for the noncoding RNA transcription, including all transfer RNA (tRNA). The hypo-
thesis is that mutations in this gene cause abnormal tRNA transcription leading to cytoplasmic protein synthesis alter-
tations. This mechanism has been suggested for an-
other leukodystrophy: hypomyelinating leukodystrophy type 3, which is caused by mutations in the aminoacyl tRNA synthetase complex-interacting multifunctional protein 1 (AIMP1) gene. The 4H phenotypic spectrum may be explained by the fact that Pol III transcribes genes that are expressed in a cell-type and growth state–dependent man-
ner. These genes play a role in various stresses, such as cell-
growth, differentiation, and apoptosis. RNA poly-
merase III is also thought to be a cytosolic DNA sensor involved in innate immune responses.15

To our knowledge, this is also the first report of such a disorder from southeastern Europe. Only 9 other pa-
tients with 4H syndrome have been identified so far.1-4,8 The overlap between 4H and other leukodystrophies has been observed,1,3,10,16,17 with the 4H/ADDH overlap being the most prominent one.1,2,3,8,10,16,17

Our patient had late-onset GH deficiency in addition to hypogonadotropic hypogonadism and dental anomalies. Among the 8 patients with 4H/ADDH who had short stature,2,5,7 a partial GH deficiency was found in 1 patient at the age of 14 years, and this finding was associated with small sella turcica.7 Pituitary structure was otherwise re-
ported in only 2 patients with 4H syndrome.5,6 However, all these patients presented with short stature during child-
hood, necessitating a prompt evaluation. In contrast, the GH deficiency in our patient did not cause the short stature because this deficiency appeared only during the repeated routine hormonal screenings at 20 years of age. Growth hormone stimulation tests (ie, the insulin, glucagon, and arginine tests) are unnecessary for the eval-
uation of GH deficiency when markedly low levels of insulin-like growth factor 1 and GH are accompanied by other pituitary dysfunctions.18,19 The short stature previously re-
ported in other patients with 4H syndrome indicates that the GH deficiency is likely to be a common feature that should be looked for in this syndrome and in other POLR3A-related disorders. Furthermore, the normal growth rate dur-
ing childhood and adolescence exhibited by the patient sug-
gests that the insufficiency of sex hormones did not nega-
tively influence GH secretion. The delayed and per-
turbed dentition without true hypodontia represents an-
other variation of the wide spectrum of dentition anomali-
ies among patients with 4H/ADDH.1,3,8,16

Unlike the neurological symptoms in some patients with 4H syndrome,5,6,8 our patient’s neurological symp-
toms did progress, and the progression was not associ-
ated with the symptoms of fever and infections de-
scribed in 3 reports on 4H syndrome.2,5,6 Moreover, we ob-
served a clinical progression, even in the context of a lack of change in magnetic resonance imaging findings over time. Although it is unlikely that hormonal status has had an effect on the overall disease course, the untreated androgen deficiency and the new-onset GH defi-
ciency may have contributed to the clinical decline. The clinical improvement of muscle tone and truncal stabil-
ity after valproate therapy was stopped is noteworthy.

The clinical, electrophysiological, and radiological find-
ings and the extensive laboratory screenings ruled out the diagnosis of other hypomyelinating entities (Table 2).20
Although the hypogonadotropic hypogonadism was key to making the diagnosis, the hypomyelination detected by use of magnetic resonance imaging can be the only abnormality associated with POLR3A mutations. The various combinations of neurologic and nonneurologic features should help us to recognize and diagnose Pol III-related leukodystrophy in a timely manner.

Accepted for Publication: September 8, 2011.

Correspondence: Raphael Schiffmann, MD, MHSc, Institute of Metabolic Disease, Baylor Research Institute, 3812 Elm St, Dallas, TX 75226 (raphael.schiffmann@baylorhealth.edu).

Author Contributions: Study concept and design: Potic, Schiffmann, and Bernard. Acquisition of data: Potic, Choquet, and Bernard. Analysis and interpretation of data: Potic, Brais, Choquet, Schiffmann, and Bernard. Drafting of the manuscript: Potic, Schiffmann, and Bernard. Critical revision of the manuscript for important intellectual content: Potic, Brais, Choquet, Schiffmann, and Bernard. Obtained funding: Potic and Bernard. Administrative, technical, and material support: Potic, Choquet, and Bernard. Study supervision: Potic, Brais, Schiffmann, and Bernard.

Financial Disclosure: Dr Bernard has received fellowship scholarships from Fonds de Recherche en Santé du Québec and Réseau de Médecine Génétique Appliquée.

Funding/Support: This project was financed by La Fondation sur les Leucodystrophies and the European Leukodystrophy Association.

Additional Contributions: We thank the patient and his family for participating in the study.

REFERENCES