A new heterozygous mutation (L338N) in the human Gsα (GNAS1) gene as a cause for congenital hypothyroidism in Albright’s hereditary osteodystrophy

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Abstract

Objective: To identify the molecular defect by which psychomotor retardation is caused in two brothers with congenital hypothyroidism who received adequate treatment with L-thyroxine.

Case report: A six-year-old boy presented with psychomotor retardation and congenital primary hypothyroidism (CH). The patient had a normal blood thyrotrophin (TSH) level on neonatal screening, but low total serum thyroxine and triiodothyronine concentrations prompting thyroid hormone substitution shortly after birth. Nevertheless, psychomotor development was retarded and the patient underwent further investigation. Typical features of Albright’s hereditary osteodystrophy (AHO) such as round face, obesity, and shortened 1st, 4th and 5th metacarpals were found.

Methods and results: Further investigation confirmed AHO with pseudohypoparathyroidism (PHP) type Ia. The boy had a mild resistance to parathyroid hormone and a reduced adenylyl cyclase stimulating protein (Gsα) activity in erythrocytes. DNA analysis detected a new heterozygous mutation (L338N) in the Gsα protein (GNAS1) gene. This mutation was also present in the patient’s brother who had similar features and was also treated with thyroid hormone because of CH, and in the phenotypically normal-looking mother who had a normal calcium metabolism but a reduced Gsα protein activity in erythrocytes suggestive of pseudopseudohypoparathyroidism.

Conclusion: In patients with CH, in whom the neurological outcome is poor even under adequate thyroid hormone substitution, PHP Ia may be suspected, especially when symptoms of AHO are present.

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Introduction

Congenital hypothyroidism (CH) occurs in 1 of 4000 births and is caused, in decreasing order of frequency, by thyroid dysgenesis, thyroid dyshormonogenesis, defects of thyrotrophin (TSH) or TSH-receptor, or hypothalamic–pituitary deficiency (1). Due to screening programs and early diagnosis, treatment of affected individuals is usually started within the first two weeks of life. With adequate therapy, growth and mental development proceed normally in infants with CH (1). However, in some patients with CH the neurological outcome is poor even under adequate hormone substitution. In such patients the retarded mental development may be related to other defects.

Pseudohypoparathyroidism (PHP) is biochemically characterized by hypocalcemia and hyperphosphatemia in association with an increased secretion of parathyroid hormone (PTH) due to target tissue unresponsiveness to PTH (2). PHP type Ia represents one variant of this condition in which patients are not only resistant to PTH but also to other hormones that bind to receptors coupled to stimulatory G protein (Gs) to activate adenylyl cyclase (3). In addition, these patients have characteristic features of Albright’s hereditary osteodystrophy (AHO) which are a round face, brachymetacarpy, short stature, obesity, mental retardation and subcutaneous calcifications (2). PHP Ia and AHO are caused by a reduced activity of the adenylyl cyclase stimulating protein (Gsα). The gene encoding this protein (GNAS1) is located on chromosome 20q13.2-13.3. Heterozygous inactivating GNAS1 mutations have been identified in patients with AHO and PHP (4), following an autosomal-dominant trait.

We studied a six-year-old boy and his eight-year-old brother with CH, who developed mental retardation...
TSH associated with high levels of total and free T4, sound. Laboratory tests revealed a suppressed serum were normal in size, a finding confirmed by ultra-broad necks and brachydactyly. Their thyroid glands retarded. Both were obese with round faces and had developed. The children appeared physically and mentally year-old brother presented to our outpatient clinic. Subsequently for his age and weight. Thyroid hormone substitution was started and adjusted concentrations. Therefore (normal range 3.0 – 18 mU/l) but low blood thyroxine levels were below the lower limits of normal so that serum TSH level was slightly elevated and his total T3 and prompted further evaluation at the age of 2 months. His CH with a TSH level below 15 mU/l. Prolonged jaundice was expressed as per cent of the mean of healthy individuals suggesting a defect in the Gsα protein. To characterize the underlying molecular defect, the GNAS1 gene was sequenced. All family members gave informed consent to participate in the study.

Clinical tests
Serum TSH, total T4, total T3, fT4 and fT3 concentrations were measured by chemiluminescent immunoassays (Chiron Diagnostics, Fernwald, Germany). For the measurement of PTH an Immulite-Assay (DPC-Biermann, Bad Nauheim, Germany) was used.

Gsα protein activity
The activity of the Gsα protein in erythrocyte membranes was analysed in heparanized blood samples as described previously (5). Briefly, after solubilization of the Gs protein from the patients’ erythrocyte membranes and activation with GTPγS, cAMP generation was measured by radioimmunoassay (Immuno Biological Laboratories, Hamburg, Germany) using adenyl cyclase from turkey red cell membranes in the presence of ATP. Results obtained in triplicate were expressed as per cent of the mean of healthy controls (normal range 85–115%).

Molecular studies
Genomic deoxyribonucleic acid (DNA) was extracted from circulating white blood cells of all family members and was used for amplification of exons 2–13 of the GNAS1 gene. The sequences of oligonucleotide primers and conditions for the polymerase chain reactions (PCR) have been described previously (6). Total ribonucleic acid (RNA) was extracted from cultured skin fibroblasts of the index patient using the phenol/guanidine isothiocyanate method (Trizol; Gibco BRL, Carlsruhe, Germany). The first complementary DNA (cDNA) strand that was used for PCR was synthesized
with avian myeloblastosis virus reverse transcriptase (Promega, Mannheim, Germany) and an oligo-DT primer.

The PCR amplified products were purified and sequenced directly using an automated fluorescence-based sequencer (ABI 377, Perkin-Elmer, Weiterstadt, Germany).

The mutation L338N which is located in exon 12 of the GNAS1 gene removes a restriction site for Rsal. Genomic DNA was used to amplify exon 12 of all family members. The PCR products were then digested with Rsal, electrophoresed on a 10% polyacrylamide gel and, after exposure to ethidium bromide, visualized under UV-light. In normal individuals the amplified PCR product of 252 bp is cut into a 130 bp and a 122 bp fragment. In the mutant allele which carries L338N the amplified band remains uncut.

**Results**

Direct sequencing of exons 2–13 of the GNAS1 gene of the propositus using genomic DNA revealed a heterozygous transition of the normal guanine at nucleotide 1082 (according to the published sequence in Genbank Acc. No. NM000516) to a mutant cytosine (AAG → AAC). The same result was obtained by sequencing fibroblast cDNA. This mutation results in the replacement of the normal lysine with an asparagine in codon 338 (L338N) (Fig. 2).

The pedigree and genotyping analysis of all family members are shown in Fig. 1. The mutation removes a restriction site for Rsal allowing verification and rapid screening for the mutation by digestion of the amplification product. The result shows that the propositus is indeed heterozygous for L338N (Fig. 1).
association with an elevated serum TSH concentration had low peripheral thyroid hormone levels in hormone substitution was initiated in the older boy, he individuals with PHP Ia at birth (7–10). When thyroid made by others who found elevated TSH levels in concentrations which is in contrast to the observation with thyroid hormone.

Discussion

This study demonstrates that patients with CH and poor mental development despite sufficient thyroid hormone substitution therapy should be considered to have a deficiency of Gs protein activity, especially when they display symptoms of AHO or PHP. In our patients the diagnosis of PHP Ia was delayed because neither boy developed hypocalcemia which could have led to the correct diagnosis earlier. It is known that patients with PHP Ia have resistance to other hormones that stimulate cAMP production in target cells via the Gs pathway. This explains the elevated serum TSH levels in such patients (4). However, it is unclear whether the resistance to TSH results in true hypothyroidism and whether these patients benefit from treatment with thyroid hormone.

On neonatal screening both boys had normal TSH concentrations which is in contrast to the observation made by others who found elevated TSH levels in individuals with PHP Ia at birth (7–10). When thyroid hormone substitution was initiated in the older boy, he had low peripheral thyroid hormone levels in association with an elevated serum TSH concentration indicating true primary hypothyroidism. The younger boy was treated with thyroid hormone because it was known that his older brother had hypothyroidism. He had allegedly a normal serum TSH but low peripheral thyroid hormone levels when thyroid hormone substitution was started. However, when thyroid hormone was stopped in both boys for a period of two weeks, serum thyroid hormone levels were still within the normal range whereas the TSH levels were elevated (see Results). The ability to further respond to TRH and for the thyroid gland to respond to TSH by an increase in serum T3, indicates that both brothers had partial resistance to TSH rather than primary thyroid gland failure.

This finding prompted us to compare our patients with others with PHP Ia and mutation in the GNAS1 gene and resistance to TSH or who were considered to be hypothyroid (5, 6, 10–22). Of the 13 patients who were reported to have elevated serum TSH concentrations (10, 11, 13–15, 17–19, 22, 23) eight were treated with thyroid hormone (10, 13, 15, 17, 19). Four had low thyroid hormone levels (10, 13, 14, 17), whereas in four other individuals the levels were normal (11, 15, 22, 23). In the remaining five cases thyroid hormone levels were not mentioned (13, 17, 19). Furthermore, in the vast majority of the published cases no detailed information regarding thyroid function tests and thyroid hormone treatment is given. Thus, it is uncertain how many patients with PHP Ia and known GNAS1 mutation had hyperthyrotrophinemia. Consequently, a conclusion with regard to a beneficial effect from thyroid hormone substitution cannot be drawn.

It is not known why patients with PHP Ia are mentally retarded. The decreased Gs protein activity which leads to a reduced cAMP concentration may explain why mental deficiency is associated with Gs protein defects (24). Because serum T3 and T4 concentrations were within the normal ranges in both boys and thyroid hormone substitution was initiated immediately, hypothyroidism is unlikely to be the cause of the delayed mental development. This observation would be supported if individuals with pseudopseudohypoparathyroidism (PPHP) who have AHO and Gs protein deficiency but no hormonal resistance frequently developed psychomotor retardation. Unfortunately, the diagnosis of PHP versus PPHP is not clearly established in many hitherto reported cases and the description of the mental development of individuals with PPHP is limited.

Defects in the Gs protein may result in either loss or gain of endocrine function. So far, 52 different loss-of-function mutations have been reported (4–6, 10–23, 25–35) in more than 70 affected individuals. A complete updated list of all known mutations is available online (http://mammary.nih.gov/aho/). In the present study a missense mutation in codon 338 of exon 12 of the GNAS1 gene was identified which to our
knowledge has not previously been described. Since the Gsα protein activity was severely reduced in erythrocyte membranes of both boys we demonstrate that the mutant protein is defective. It is conceivable that substitution of a leucine which has a nonpolar side chain, by an asparagine with an uncharged polar side chain could result in a conformational change leading to a defective protein. However, based on our studies we cannot predict the precise mechanism by which loss-of-function is caused by L338N. Further studies are needed to exclude a targeting defect. Furthermore, we have no explanation for the absence of mental retardation in the mother despite a similar degree of impaired Gsα function.

In conclusion, Gsα protein defects can cause CH. This diagnosis should be considered in children with hypothyroidism that have a poor mental development despite adequate treatment with thyroid hormone and who display features of AHO. Whether these patients benefit from thyroid hormone substitution remains unclear although our patients did not. Elevated TSH levels simply reflect a defect in TSH signaling and do not necessarily indicate true hypothyroidism. Thyroid hormone levels should be monitored in these patients.

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