Iodide transport defect: Identification of a novel mutation in the carboxy-terminus of the sodium/iodide symporter in a pediatric patient with congenital hypothyroidism

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INTRODUCTION

Active iodide accumulation—the first step in the biosynthesis of the iodine-containing thyroid hormones—is mediated by the sodium/iodide symporter (NIS), an plasma membrane protein located on the basolateral surface of thyrocytes (1). The human scl5a5 gene—which encodes NIS, a 643-amino acid protein—is located on chromosome 19p12-13 and consists of 15 exons (1).

Iodide transport defect (ITD) is an autosomal recessive disorder caused by the inability of the thyroid cell to actively accumulate iodide, which leads to dyshormonogenic congenital hypothyroidism (2). The diagnostic criteria for ITD include a variable degree of goiter, low to absent iodide accumulation in the thyroid, and low iodide saliva-to-serum ratio (2). To date, sixteen different loss-of-function NIS mutations have been identified in patients with ITD (Figure 1).

OBJECTIVE

We aimed to analyze the presence of NIS gene mutations in a pediatric patient suspected of ITD on the basis of severely reduced 99mTc-pertechnetate accumulation in a eutopic thyroid gland.

CASE REPORT

The patient was a full-term boy, born from healthy Argentinian, non-consanguineous parents. The patient showed abnormally high TSH level during neonatal screening (64 μU/ml, cut off <20μU/L). Ten days after birth, definitive confirmation of congenital hypothyroidism was achieved by measuring serum TSH 203 μU/ml, free T4 1.6 ng/dl, T3 8.7 μg/dl, and T3 uptake 121 ng/dl. Slightly increased serum thyroglobulin concentration was evidenced (84 ng/ml). Thyroid ultrasonography showed a normal-sized eutopic thyroid gland. Radionuclide scintigraphy revealed a diffuse strongly reduced, although not absent, 99mTc-pertechnetate uptake by the thyroid gland after 30 min, suggesting a diagnosis of ITD. Thyroid hormone supplementation was started immediately after diagnosis, with a daily dose of 14 μg/kg levothyroxine.

RESULTS

We sequenced the gene encoding NIS in order to assess the presence of a genetic abnormality affecting NIS function. Our molecular analysis revealed the presence of a previously unidentified homozygous G to A transition at nucleotide +1682 in exon 14 resulting in a glucamic acid instead of a glycine at position 561 (G561E) (Figure 2).

The hemaglutinin-tagged human NIS cDNA wild-type (WT) or mutated (G561E) was transiently transfected into non-polarized Cos-7 cells which do not express NIS endogenously. Cells transfected with G561E NIS displayed ΔTc uptake levels similar to those of cells expressing WT NIS (Figure 3A). Competitive inhibition by perchlorate confirmed that all observed iodide transport was NIS-mediated. Immunofluorescence studies under non-permeabilized conditions showed that G561E NIS was properly expressed at the plasma membrane (Figure 3B).

To ascertain the role of NIS carboxy terminus in its targeting to the plasma membrane, we generated a NIS deletion mutant lacking the entire 96 amino acid-long, intracellularly facing carboxy terminus (Δ546 NIS). Iodide transport was absent in cells expressing Δ546 NIS as compared to those expressing WT NIS (Figure 4A). Immunofluorescence studies under permeabilized conditions showed that Δ546 NIS was mostly intracellularly retained, whereas WT NIS was clearly expressed at the plasma membrane (Figure 4B).

To determine the roles played by specific region(s) of the intracellular carboxy terminus in NIS trafficking to the plasma membrane, we performed a bioinformatics analysis to identify short linear sequence motifs for polarized sorting. We used the eukaryotic linear motif resource at http://elm.eu.org to identify putative linear motifs in NIS carboxy terminus (Figure 5).

SUMMARY AND DISCUSSION

We report the identification of a novel homozygous missense mutation—G561E—in the gene encoding NIS in a pediatric patient with congenital hypothyroidism. Surprisingly, the mutation G561E is the first NIS mutant to be identified in the intracellular carboxy terminal region of the protein.

The study shows the importance of NIS carboxy terminus in its trafficking to the plasma membrane. Bioinformatics analysis of the carboxy terminus revealed the presence of linear motifs involved in protein targeting to the plasma membrane. We identified a putative di-leucine motif (LLXLLX LL) that could interact with clathrin adapter complex AP-1 involved in the basolateral sorting of proteins (3).

Although the mechanism by which G561E impairs NIS activity remains unknown, we hypothesized that the negative charge of the Glu residue may interfere the recognition of the putative dileucine sorting motif LXXLXLL by adaptor proteins, thus affecting NIS basolateral plasma membrane sorting in polarized cells.

REFERENCES