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 *slc5a5* 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).

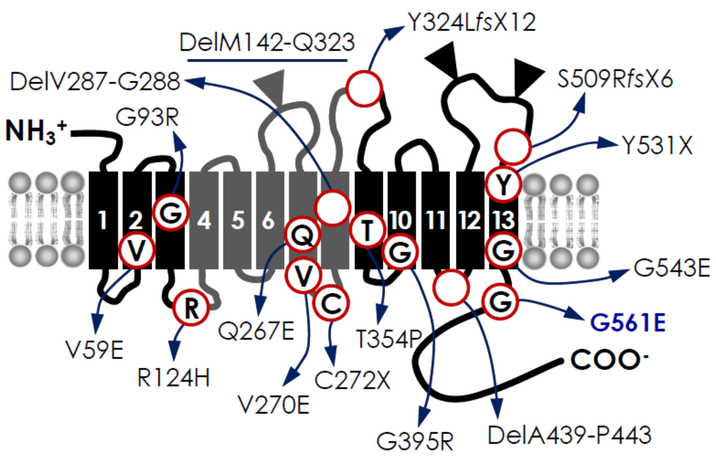

Figure 1. NIS secondary structure model. Experimentally tested NIS secondary structure model predicts a protein with 13 transmembrane segments (TMSs), an extracellularly facing amino terminus, and an intracellularly facing carboxy terminus. Previously reported NIS mutations identified in patients with ITD are indicated in black. The mutant G561E—reported in this poster—is indicated in blue.

OBJECTIVE

We aimed to analyze the presence of NIS gene mutations in a pediatric patient suspected of ITD on the basis of severely reduced ^{99m}Tc-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 µIU/ml, cut off <20mIU/L). Ten days after birth, diagnostic confirmation of congenital hypothyroidism was achieved by measuring serum TSH 203 µIU/ml, free T_4 1.6 ng/dl, T_4 8.7 µg/dl, and T_3 121 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, ^{99m}Tc-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 glutamic acid instead of a glycine at position 561 (G561E) (Figure 2).

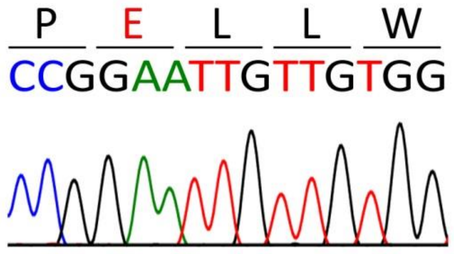

Figure 2. Identification of the homozygous NIS mutation G561E. Chromatogram showing a 15-bp fragment of *slc5a5* exon 14 (nucleotides 1.678 to 1.692). Amino acids (P560 to W564) are indicated using the one-letter code.

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 $^{125}I^-$ 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).

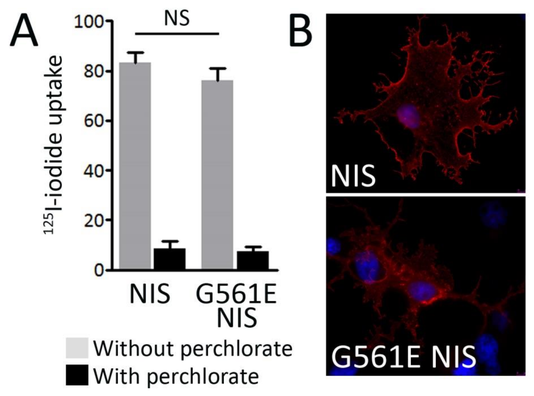

Figure 3. G561E does not affect NIS function in non-polarized Cos-7 cells. A. Steady state iodide uptake in Cos-7 cells transiently expressing WT or G561E NIS. Cells were incubated with 10 µM iodide in the absence (light bars) or presence (dark bars) of 40 µM perchlorate. **B.** Immunofluorescence analysis assessing WT and G561E NIS expression and localization under permeabilized conditions in Cos-7 cells. NIS was stained with Alexa 488 (green) and nuclei with DAPI (blue).

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).

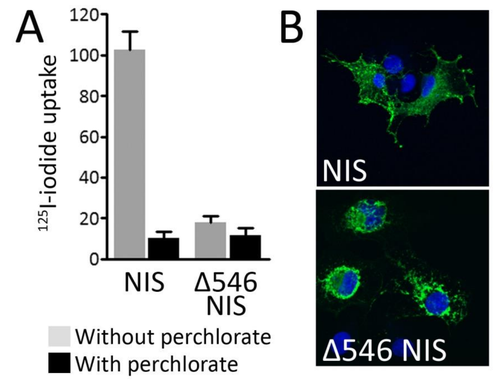

Figure 4. A. Steady state iodide uptake in Cos-7 cells transiently expressing WT or Δ546 NIS. Cells were incubated with 10 µM iodide in the absence (light bars) or presence (dark bars) of 40 µM ClO_4^-. **B.** Immunofluorescence analysis assessing WT and Δ546 NIS expression and localization under permeabilized conditions in Cos-7 cells. NIS was stained with Alexa 488 (green) and nuclei with DAPI (blue).

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).

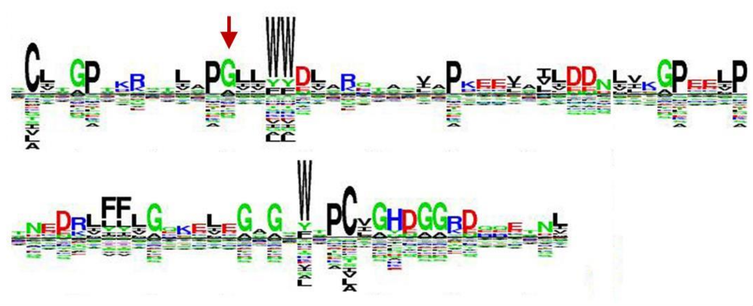

Figure 5. Evaluation of NIS carboxy terminus-located short linear motifs involved in protein trafficking. Bioinformatics analysis was performed using the eukaryotic linear motif resource at http://elm.eu.org. Motif logo reflects relative frequency of residues in each position of different linear motifs. The residue G561 is indicated with a red arrow.

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 carboxyl 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 ($L^{562}L^{563}$) 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 $L^{562}L^{563}$ by adaptor proteins, thus affecting NIS basolateral plasma membrane sorting in polarized cells.

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