Silent but Not Harmless: A Synonymous SLC5A5 Gene Variant Leading to Dyshormonogenic Congenital Hypothyroidism

Romina Celeste Geysels1,2, Carlos Eduardo Bernal Barquero1,2, Mariano Martín1,2, Victoria Peyret1,2†, Martina Nocent1,2, Gabriela Sobrero3, Liliana Muñoz3, Malvina Signorino3, Graciela Testa3, Ricardo Belisario Castro4†, Ana María Masini-Repiso1,2, Mirta Beatriz Miras3 and Juan Pablo Nicola1,2*

1 Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina, 2 Centro de Investigaciones en Bioquímica Clínica e Inmunología - Consejo Nacional de Investigaciones Científicas y Técnicas (CIBICI-CONICET), Córdoba, Argentina, 3 Programa Provincial de Pesquisa Neonatal, Servicio de Endocrinología, Hospital de Niños de la Santísima Trinidad de Córdoba, Córdoba, Argentina, 4 Servicio de Medicina Nuclear, Hospital Nacional de Clínicas, Córdoba, Argentina

Background: Congenital iodide transport defect (ITD) is an uncommon cause of dyshormonogenic congenital hypothyroidism characterized by the absence of active iodide accumulation in the thyroid gland. ITD is an autosomal recessive disorder caused by loss-of-function variants in the sodium/iodide symporter (NIS)-coding SLC5A5 gene.

Objective: We aimed to identify, and if so to functionally characterize, novel ITD-causing SLC5A5 gene variants in a cohort of five unrelated pediatric patients diagnosed with dyshormonogenic congenital hypothyroidism with minimal to absent 99mTc-pertechnetate accumulation in the thyroid gland.

Methods: The coding region of the SLC5A5 gene was sequenced using Sanger sequencing. In silico analysis and functional in vitro characterization of a novel synonymous variant were performed.

Results: Sanger sequencing revealed a novel homozygous synonymous SLC5A5 gene variant (c.1326A>C in exon 11). In silico analysis revealed that the c.1326A>C variant is potentially deleterious for NIS pre-mRNA splicing. The c.1326A>C variant was predicted to lie within a putative exonic splicing enhancer reducing the binding of splicing regulatory trans-acting protein SRSF5. Splicing minigene reporter assay revealed that c.1326A>C causes exon 11 or exon 11 and 12 skipping during NIS pre-mRNA splicing leading to the NIS pathogenic variants p.G415_P443del and p.G415Lfs*32, respectively. Significantly, the frameshift variant p.G415Lfs*32 is predicted to be subjected to degradation by nonsense-mediated decay.
Conclusions: We identified the first exonic synonymous SLC5A5 gene variant causing aberrant NIS pre-mRNA splicing, thus expanding the mutational landscape of the SLC5A5 gene leading to dyshormonogenic congenital hypothyroidism.

Keywords: congenital hypothyroidism, iodide transport defect, sodium/iodide symporter, pathogenic synonymous variant, pre-mRNA splicing defect

INTRODUCTION

Congenital iodide transport defect (Online Mendelian Inheritance in Man #274400) is a rare autosomal recessive disorder whose hallmark is the inability of the thyroid follicular cell to actively accumulate the iodide required for thyroid hormonogenesis, thus leading to dyshormonogenic congenital hypothyroidism (1). The general clinical presentation of the disease includes a variable degree of hypothyroidism, reduced to absent radioiodide accumulation in an eutopic thyroid gland, and low saliva-to-plasma iodide ratio (2–4).

The sodium/iodide symporter (NIS) is an integral basolateral plasma membrane glycoprotein that mediates active iodide accumulation into the thyroid follicular cell, which is the first step in the biosynthesis of the iodine-containing thyroid hormones (5). The intracellularly-located carboxy-terminus of the protein is determinant for NIS expression at the basolateral plasma membrane (6, 7). Moreover, NIS-mediated iodide transport is electrogenic (2 sodium:1 iodide stoichiometry) and remarkably efficient considering the submicromolar extracellular iodide concentration (8, 9).

To date, over thirty pathogenic variants in the NIS-coding SLC5A5 gene have been identified in patients with congenital iodide transport defect. The molecular characterization of several disease-causing NIS variants has revealed critical amino acids for substrate binding, specificity, and stoichiometry, as well as folding and plasma membrane targeting (5). A detailed analysis of the p.G561E NIS variant revealed the importance of the kinesin-1 subunit kinesin light chain 2 (KLC2) in thyroid hormonogenesis (10). Moreover, the functional characterization of the p.D396V NIS variant uncovered a critical intramolecular ionic interaction— involving the β carboxyl group of D369 and the guanidinium group of R130—for the correct folding required for NIS maturation and transport to the plasma membrane (11). Recently, based on structure-function analysis of pathogenic NIS variants, we developed a machine learning-based NIS-specific variant classifier aiming to improve the prediction of pathogenicity of missense NIS variants in clinical practice (12).

Here, we explored the presence of pathogenic SLC5A5 gene variants in a cohort of five unrelated pediatric patients with dyshormonogenic congenital hypothyroidism suspected of having a congenital iodide transport defect based on reduced 99mTc-pertechnetate accumulation in an eutopic thyroid gland. We identified the homozygous synonymous SLC5A5 variant c.1326A>C. In silico analysis predicted that c.1326A>C disrupts the binding of the splicing regulatory protein SRSF5 to an exonic splicing enhancer located in exon 11. Functional in vitro characterization using a splicing minigene reporter assay revealed that the c.1326A>C variant causes exon 11 or exons 11 and 12 skipping during NIS pre-mRNA splicing leading to the NIS pathogenic variants p.G415_P443del and p.G415Lfs*32, respectively. Significantly, the frameshift variant p.G415Lfs*32 is predicted to be subjected to degradation by nonsense-mediated decay.

MATERIAL AND METHODS

Patients

Five patients with dyshormonogenic congenital hypothyroidism with a suspected defect in iodide accumulation were included in the study. All patients were full-term infants born from non-consanguineous Caucasian (of European descent) parents showing an abnormally high TSH level during the neonatal screening program (Table 1). The patients did not have clinical signs of hypothyroidism at birth. The cut-off value adopted for basal TSH level in blood spot determined by UMELISA TSH Neonatal (TecnoSuma International SA, La Habana, Cuba) for neonatal screening was 10 µIU/ml. The diagnosis of congenital hypothyroidism was confirmed based on elevated TSH serum levels, with or without total or free T4 levels below the normal range (Table 1). Inclusion criteria were congenital hypothyroidism with eutopic thyroid gland explored by ultrasonography and reduced to absent 99mTc-pertechnetate accumulation in the thyroid gland assessed by radionuclide scintigraphy. The reference range for 99mTc-pertechnetate uptake adopted in the Division of Nuclear Medicine is 0.5–4% (13); therefore, we considered values lower than 0.4% to be reduced, and suggestive of defective iodide accumulation. The accumulation of 99mTc-pertechnetate in salivary glands was not tested. Determination of saliva-to-plasma iodide ratio was not technically available in the Division of Nuclear Medicine. Levothyroxine therapy was introduced immediately after diagnosis at initial dose of 5–15 µg/kg per day according to the degree of hypothyroidism. Patient 2 was re-evaluated at the age of three years as recommended by consensus guidelines (14); discontinuation of levothyroxine treatment revealed transient hypothyroidism.

Thyroid Function Tests

Thyroid function analyses were performed by electrochemiluminescence immunoassay by Cobas e412 analyzer (Roche Diagnostics, Indianapolis, IN). Age-specific thyroglobulin reference intervals were established in-house (15).

SLC5A5 Gene Sequencing

Genomic DNA was extracted from whole blood using the standard cetyltrimethylammonium bromide-based method. All 15 coding exons and exon-intron boundaries of the SLC5A5 gene
algorithm NMDEscPredictor (19) was used to predict whether frameshift variants are sensitive to nonsense-mediated mRNA decay.

Ultrasonography

| Patient | 1 | 2 | 3 | 4 | 5 |
|---------|---|---|---|---|---|
| Neonatal screening | | | | | |
| Age (days) | 36 | 7 | 4 | 7 | 4 |
| TSH (<10 mU/l) | >20 | 18 | 20 | 29 | 67 |
| Biochemical analysis | | | | | |
| Age (days) | 49 | 25 | 15 | 50 | 15 |
| TSH (0.8-7.8 µg/dl) | 300 | 16 | 16 | 46 | 62 |
| T₄ (6-16.5 µg/dl) | 2 | 5 | 8 | 9 | 4 |
| Free T₄ (1-2.1 ng/dl) | 0.1 | 1.3 | 1.2 | 1.0 | 1.2 |
| T₃ (100-310 ng/dl) | 45 | 156 | 155 | 218 | 164 |
| Tg (6-83 ng/ml) | 5.2 | 59 | 221 | 67 | 157 |

In Silico Analysis

Splice Site Prediction by Neural Network (NNsplice), Max Entropy Scan (MES), GeneSplicer, Human Splicing Finder (HSF), SpliceSiteFinder-like (SSF-like), ESEFinder and RESCUE-ESE integrated into the splicing module of Alamut Visual version 2.9.0 (Interactive Biosoftware, Rouen, France) were used to investigate the effect of synonymous variants on consensus acceptor and donor splice-site sequences at the intron-exon boundaries and exonic splicing regulatory elements. Additional data analysis to assess the effect of synonymous variants on exonic splicing regulatory elements was accomplished using the algorithm HExoSplice (18). The algorithm NMDEscPredictor (19) was used to predict whether frameshift variants are sensitive to nonsense-mediated mRNA decay.

Cloning and Site-Directed Mutagenesis

Human genomic sequence containing exons 11 (87 nucleotides) and 12 (197 nucleotides) of the SLC5A5 gene along with the last 311 nucleotides of intron 10, the 82 nucleotides of intron 11, and the first 367 nucleotides of intron 12 were amplified using the primers and PCR conditions previously reported (16, 17). The nucleotide sequence of all PCR products was determined in both orientations by Sanger sequencing by capillary electrophoresis (Macrogen, Seoul, South Korea). The nucleotide position in SLC5A5 mRNA was named according to the National Center for Biotechnology Information reference sequence NM_000453.3, considering the adenine of the ATG translation start codon as nucleotide +1.

In Silico Analysis

Splice Site Prediction by Neural Network (NNsplice), Max Entropy Scan (MES), GeneSplicer, Human Splicing Finder (HSF), SpliceSiteFinder-like (SSF-like), ESEFinder and RESCUE-ESE integrated into the splicing module of Alamut Visual version 2.9.0 (Interactive Biosoftware, Rouen, France) were used to investigate the effect of synonymous variants on consensus acceptor and donor splice-site sequences at the intron-exon boundaries and exonic splicing regulatory elements. Additional data analysis to assess the effect of synonymous variants on exonic splicing regulatory elements was accomplished using the algorithm HExoSplice (18). The algorithm NMDEscPredictor (19) was used to predict whether frameshift variants are sensitive to nonsense-mediated mRNA decay.

Cell Culture and Transfections

HeLa cells (CCL-2, American Type Culture Collection, Rockville, MD) were obtained from our institutional cell line repository. Cells were cultured in Dulbecco Modified Eagle’s Medium (Thermo-Fisher Scientific - Waltham, MA) supplemented with 10% fetal bovine serum (Natocor, Córdoba, Argentina). Cells were transiently transfected at the ratio of 4 µg plasmid/10 cm dish using Lipofectamine 2000 (Thermo-Fisher Scientific) (22).

Splicing Minigene Reporter Assays

Total RNA was extracted from HeLa cells 24 hours after transient transfection with pSPL3-based minigene reporters using the Direct-zol RNA MiniPrep Kit (Zymo Research - Irvine, CA). Complementary DNA was synthesized from 1 µg total RNA. PCR reactions were performed as described (23). The pSPL3-specific primer sets were as follows: SD6 5'- TCTGAGTCACCTGACAACCC and SA2 5'- ATCTCAGTGGTATTTGTGAGC. RT-PCR products were resolved by electrophoresis on 2.5% agarose gels containing ethidium bromide and gel purified using Wizard SV Gel and PCR Clean-Up System (Promega) according to the manufacturer’s protocol. All PCR products were sequenced to verify the identity of spliced exons (Macrogen).

RESULTS

Identification of Synonymous SLC5A5 Variants in Patients With Dishormonogenic Congenital Hypothyroidism

Five patients with congenital hypothyroidism showing diffuse and reduced to non-detectable ⁹⁹mTc-pertechnetate uptake by the thyroid gland, which suggested a congenital iodide transport...
defect, were included in the study (Table 1). The presence of an eutopic normal-shaped thyroid gland was ascertained by ultrasonography. All patients were detected through neonatal screening assessing TSH levels (Table 1). Confirmatory thyroid function test showed increased TSH and normal to reduced total and free thyroxine (T4) serum levels. Thyroglobulin serum levels were variable and all patients were tested negative for thyroid autoantibodies (Table 1).

Patients’ SLC5A5 gene sequencing revealed the homozygous synonymous variants c.1326A>C located in exon 11 (patient 4) (Figure 1A) and c.1626C>T located in exon 13 (patient 2) (Figure 1B), whereas no variants were identified in the remaining patients. The variants c.1326A>C and c.1626C>T have been reported in the Single Nucleotide Polymorphism database (rs73520743 and rs45602038, respectively). The variant c.1326A>C showed an allele frequency of 0.001103 in the European (non-Finnish) population, while c.1626C>T was 0.02997 in the same population, according to The Genome Aggregation Database (accessed on August 2021). We did not observe the variants in the genome of 50 age-matched healthy pediatric patients. Moreover, the variants c.1326A>C and c.1626C>T were observed in homozygosis in 1 and 56, respectively, out of 61659 European (non-Finnish) individuals, according to The Genome Aggregation Database (accessed on August 2021). Hence, according to the standards and guidelines provided by the American College of Medical Genetics and Genomics (24), the c.1626C>T variant was classified as benign (BS1).

**In Silico Analysis Predicts the Synonymous c.1326A>C Variant as Deleterious for Normal NIS Pre-mRNA Splicing**

Synonymous variants are frequently considered benign as these do not alter the corresponding amino acid residue and have no direct effect on the protein sequence. However, synonymous variants may impact on transcription, mRNA processing and translation (25).

In silico analysis using splicing prediction algorithms revealed that the c.1326A>C variant, located at position +4 of the exon/intron 11 junction, has no impact on the splice consensus sequence nor the creation of a new splice site. However, complementary in silico analysis assessing the effect of the c.1326A>C variant on splicing regulatory elements revealed that the variant is potentially deleterious for normal NIS premRNA splicing. The variant c.1326A>C was predicted to lie at a potential exonic splicing enhancer (ESE) motif (ACAC[A/C]CC) using ESEfinder software. The motif carrying the c.1326A>C variant exhibited a decrease in the splicing regulatory protein SRSF5 binding score from 4.12 to below the threshold level set at 2.67. In addition, the analysis using HExoSplice software revealed that the variant c.1326A>C causes a significant negative effect on an exonic splicing regulatory (ESR) sequence (ΔtESRseq = -0.5932) whose identity overlaps with the ESEfinder-predicted SRSF5 binding site. Together, in silico analysis suggests that the synonymous variant c.1326A>C might reduce the affinity of SRSF5 binding to the mutant ESE motif. In agreement, previous reports suggested that variants in ESE could cause exon skipping leading to aberrant pre-mRNA splicing (26–28).

**The Pathogenic Variant c.1326A>C Impairs Normal NIS Pre-Messenger RNA Splicing**

To functionally assess the impact of the synonymous c.1326A>C variant, pSPL3-based minigene reporter constructs were generated and functionally tested in transiently transfected HeLa cells (Figure 2A). Minigene reporter assay revealed that the variant c.1326A>C generates a transcript of 263 bp compatible in size with the skipping of exons 11 and 12 (α splicing product), as a similar pattern was observed when cells were transfected with the empty reporter vector (Figure 2B). By contrast, the WT minigene generates a transcript of 547 bp compatible with the canonical spliced transcript including exons 11 and 12 (β splicing product) and a transcript of 263 bp (α splicing product) (Figure 2B). Moreover, the variant c.1326A>C also generated a transcript of 457 bp compatible with the spliced transcripts including exon 12 alone (γ splicing product) (Figure 2B), and an additional artificial splicing transcript of 573 bp including a pseudoexon from the intron of the minigene reporter vector (Supplementary Figure 1). Sequence analysis uncovered the identity of all PCR products (Figure 2C and Supplementary Figure 1).

If the exon-excluded transcripts of the SLC5A5 gene were successfully translated, as the c.1326A>C variant-caused exons 11 and 12 skipping induces a change in the open reading frame of the transcript, it would lead to the NIS variant p.G415Lfs*32, whereas exon 11 skipping would generate the in-frame NIS deletion variant p.G415_P443del. Significantly, NMDEscPredictor analysis predicted that the transcript encoding the frameshift variant p.G415Lfs*32 undergoes nonsense-mediated decay.
Together, combining *in silico* and functional *in vitro* assays, we determined that the synonymous c.1326A>C variant disturbed normal NIS pre-mRNA splicing, thus leading to dyshormonogenic congenital hypothyroidism due to a defect in iodide accumulation.

**DISCUSSION**

Here, we explored the presence of pathogenic SLC5A5 gene variants in a cohort of five unrelated pediatric patients with dyshormonogenic congenital hypothyroidism suspected of...
having a congenital iodide transport defect. Significantly, we identified the homozygous synonymous c.1326A>G variant causing aberrant NIS pre-mRNA splicing in one of the patient. Consistent with the recessive nature of the disease, the mother of the patient showed conserved thyroid function although her genetic material was not available for segregation analysis. Our analysis did not reveal pathogenic variants in the coding region of the SLC5A5 gene in three out of four patients with permanent congenital hypothyroidism. Therefore, although we focused on the coding region and exon-intron boundaries, other potential mechanisms, such as large genomic rearrangements and variants likely affecting regulatory elements, including the promoter, enhancers, and deep intronic regions should also be considered. Interestingly, pathogenic variants in TSHR and PAX8 genes have been reported in patients with permanent congenital hypothyroidism showing reduced to normal-sized eutopic thyroid glands with low to absent radiiodide uptake on thyroid scintigraphy (29, 30). Regarding the patient with transient congenital hypothyroidism, Castellnou et al. (31) recently reported a case of transient congenital hypothyroidism with eutopic gland and undetectable radiiodide uptake on thyroid scintigraphy due to maternal thyrotropin receptor-blocking antibodies (TRAbs). This report highlights the importance to analyze TRAbs in hypothyroid mothers during pregnancy, which is particularly useful when congenital hypothyroidism is diagnosed in their newborns. Unfortunately, maternal TRAbs data were not available for our patients.

Most disease-causing exonic single nucleotide variants are frequently assumed to exert their effects by altering the amino acid sequence of the protein. However, many human genetic diseases are caused by exonic variants that disrupt canonical splice sites at exon-intron boundaries or splicing regulatory elements that enhance the recognition of the splice sites, which are relevant to pre-mRNA splicing, leading to abnormal splicing outcomes (32). Indeed, one-third of disease-causing mutations were predicted to result in aberrant splicing (33). Interestingly, exonic synonymous variants—which do not alter protein sequences—or even non-synonymous single nucleotide variants have been demonstrated to contribute to human diseases by affecting transcription and splicing regulatory factors within protein-coding regions (33). Particularly, synonymous variants were reported as a disease-causing mechanism in several endocrine disorders including familial pheochromocytoma (34), combined pituitary hormone deficiency (35, 36), pseudohypoparathyroidism type 1 (37) and maturity-onset diabetes of the young (38). Therefore, synonymous variants should not be neglected in gene variant prioritization pipelines as they may produce abnormal mRNAs and dysfunctional proteins.

Patients with congenital hypothyroidism due to deficient iodide accumulation show a substantial clinical and biochemical heterogeneity (39). Under conditions of iodine sufficiency, thyroid function at diagnosis reflects differences in residual mutant NIS protein activity (39, 40). Thus, thyroid function in the patient carrying the pathogenic variant c.1326A>G was sufficient to preserve normal peripheral thyroid hormone levels. A likely explanation for this situation is the presence of residual properly-spliced NIS expression, which correlates with the clinical observation of reduced 99mTc-pertechnetate when the patient was examined by thyroid scintigraphy. Considering that NIS-mediated iodide uptake is a crucial step in the synthesis of thyroid hormones, our results indicate that the variant c.1326A>G impairs iodide uptake by interfering with normal NIS pre-mRNA splicing. Therefore, defective NIS pre-mRNA splicing leading to lack of sufficient NIS molecules at the basolateral plasma membrane in the thyroid follicular cells may explain the mechanism underlying the deficient accumulation of iodide causing dyshormonogenic hypothyroidism. In this context, the increase in TSH levels following a decrease in thyroid hormone production may partially overcome the splicing defect by upregulating SLC5A5 gene transcription, as has been observed in patients with different loss-of-function NIS variants (41, 42).

Disruption of pre-mRNA splicing has been implicated in the etiology of numerous congenital human disorders (43). Several reports support splicing defects as a disease-causing mechanism in congenital hypothyroidism (44–48). Particularly, NIS splicing defect-caused dyshormonogenic congenital hypothyroidism has been described in the literature. Pohlenz et al. (49) reported the nonsense variant c.1593C>G (originally named c.1940C>G) that generates a cryptic 3’ splice acceptor site in exon 13 leading to the mis-splicing variant p.S509Rfs*6 NIS. Moreover, two recent reports provided functional evidence that the variants c.970-3C>A and c.970-48G>C disrupts canonical and non-canonical splice sites, respectively, located in the intron 7 causing exon 8 skipping during NIS mRNA splicing, thus leading to the nonsense variant p.Y324Hfs*148 NIS (11, 50). Here, we report the exonic synonymous SLC5A5 gene variant c.1326A>G causing aberrant NIS pre-mRNA splicing. The variant c.1326A>G is predicted to disrupt a potential ESE motif located in exon 11, thus promoting exon 11 or exons 11 and 12 skipping during the splicing process. The skipping of exon 11 during NIS pre-mRNA splicing leads to the in-frame deletion variant p.G415_P443del, which is likely inactive, as the ITD-causing in-frame deletion variant p.A439_P443del NIS identified in a patient showing not-detectable 131I-iodide uptake by the thyroid gland is intracellularly retained and does not accumulate iodide (51, 52). Moreover, the skipping of exons 11 and 12 change the open reading frame of the transcript leading to the NIS pathogenic variant p.G415Lfs*32, whose transcript is predicted to undergo nonsense-mediated mRNA decay.

The implementation of next-generation sequencing has been instrumental in expanding the mutational landscape of monogenic forms of congenital hypothyroidism (53). Recently, whole-exome sequencing revealed pathogenic GBP1 variants in patients with defective developmental thyroid morphogenesis (54). The future implementation of next-generation sequencing-based approaches might reveal novel disease-causing gene variants in patients with permanent dyshormonogenic congenital hypothyroidism showing a phenotype of defective iodide accumulation. A precedent for this hypothesis is provided by the observation that the KCNQ1/KCNE2 potassium channel is crucial in facilitating NIS-mediated iodide transport (55).
DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the medical ethics committee of the Hospital de Niños de la Santísima Trinidad (Córdoba, Argentina). Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

RG performed research and analyzed the data. CBB, MM, VP, MN, and RC provided critical technical support and analyzed the data. GS, LM, MS, GT, and MM diagnosed and followed-up the patients enrolled in the study. AM-R, MM, and JN conceived the project. RG and JN designed research. JN drafted the manuscript. All authors edited and approved the final version of the manuscript.

REFERENCES

1. Targovnik HM, Citterio CE, Rivolta CM. Iodide Handling Disorders (NIS, TPO, TG, ITD). Best Pract Res Clin Endocrinol Metab (2017) 31:195–212. doi: 10.1016/j.beem.2017.03.006
2. Zhang CX, Zhang JX, Yang L, Zhang CR, Cheng F, Zhang RJ, et al. Novel Compound Heterozygous Pathogenic Mutations of SLC5A5 in a Chinese Patient With Congenital Hypothyroidism. Front Endocrinol (Lausanne) (2021) 12:620117. doi: 10.3389/fendo.2021.620117
3. Durgia H, Nicholas AK, Schoenmakers E, Dickens JA, Halanaik D, Sahoo J, et al. Brief Report: A Novel Sodium/Iodide Symporter Mutation, S356F, Causing Congenital Hypothyroidism. Thyroid (2022) 32:215–8. doi: 10.1089/thy.2021.0478
4. Reyna-Nearya A, Jung L, Chakrabarti M, Suarez MX, Amzel LM, Carrasco N. The Iodide Transport Defect-Causing Y348D Mutation in the Na(+)/I(-) Symporter Renders the Protein Intrinsically Inactive and Impairs Its Targeting to the Plasma Membrane. Thyroid (2021) 31:1277–81. doi: 10.1089/thy.2020.0931
5. Ravera S, Reyna-Nearya A, Ferrandino G, Amzel LM, Carrasco N. The Sodium/Iodide Symporter (NIS): Molecular Physiology and Preclinical and Clinical Applications. Anna Rev Physiol (2017) 79:261–89. doi: 10.1146/annurev-physiol-022516-034125
6. Martin M, Modenutti CP, Peyret V, GeyseLS RC, Darrouzet E, Pourcher T, et al. A Carboxy-Terminal Monoleucine-Based Motif Participates in the Basolateral Targeting of the Na(+)/I(-) Symporter. Endocrinology (2019) 160:156–68. doi: 10.1210/en.2018-00603
7. Martin M, Salleron L, Peyret V, GeyseLS RC, Darrouzet E, Lindenhalh S, et al. The PDZ Protein SCRAPIB Regulates Sodium/Iodide Symporter (NIS) Expression at the Basolateral Plasma Membrane. FASEB J (2021) 35: e21681. doi: 10.1096/fj.202100303R
8. Ravera S, Quick M, Nicola JP, Carrasco N, Amzel LM. Beyond non-Integer Hill Coefficients: A Novel Approach to Analyzing Binding Data, Applied to Na+ Driven Transporters. J Gen Physiol (2015) 145:555–63. doi: 10.1085/jgp.201511365
9. Nicola JP, Carrasco N, Amzel LM. Physiological Sodium Concentrations Enhance the Iodide Affinity of the Na(+)/I(-) Symporter. Nat Commun (2014) 5:3948. doi: 10.1038/ncomms4948
10. Martin M, Modenutti CP, Gil Rosas ML, Peyret V, GeyseLS RC, Bernal Barquero CE, et al. A Novel SLC5A5 Variant Reveals the Crucial Role of Kinesin Light Chain 2 in Thyroid Hormonogenesis. J Clin Endocrinol Metab (2021) 106:1867–81. doi: 10.1210/cen/dgb283
11. Bernal Barquero CE, Martin M, GeyseLS RC, Peyret V, Papendieck P, Masini-Repiso AM, et al. An Intramolecular Ionic Interaction Linking Defective Sodium/Iodide Symporter Transport to the Plasma Membrane and Dysmorphonogenic Congenital Hypothyroidism. Thyroid (2022) 32:19–27. doi: 10.1089/thy.2021.0344
12. Martin M, Nicola JP. Impact of the Mutational Landscape of the Sodium/Iodide Symporter in Congenital Hypothyroidism. Thyroid (2021) 31:1776–85. doi: 10.1089/thy.2021.0381
13. Atkins HL. Technetium-99m Pertechnetate Uptake and Scanning in the Evaluation of Thyroid Function. Semin Nucl Med (1971) 1:345–55. doi: 10.1016/S0001-2998(71)80007-7
14. van Trotsenburg P, Stoupaa A, Leger J, Rohtrer T, Peters C, Fugazzolla L, et al. Congenital Hypothyroidism: A 2020-2021 Consensus Guidelines Update. An ENDO-European Reference Network Initiative Endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. Thyroid (2021) 31:387–419. doi: 10.1089/thy.2020.0333
15. Sobrero G, Munoz L, Bazzara L, Martin S, Silvano L, Iorkansky S, et al. Thyroglobulin Reference Values in a Pediatric Infant Population. Thyroid (2020) 30:1006–15. doi: 10.1089/thy.2019.0046
16. Martin M, Bernal Barquero CE, GeyseLS RC, Papendieck P, Peyret V, Masini-Repiso AM, et al. Novel Sodium/Iodide Symporter Compound Heterozygous Pathogenic Variants Causing Dyshormonogenic Congenital Hypothyroidism. Thyroid (2019) 29:1023–6. doi: 10.1089/thy.2019.0046
17. Nicola JP, Nazar M, Serrano-Nascimiento C, Goulart-Silva F, Sobrero G, Testa G, et al. Iodide Transport Defect: Functional Characterization of a Novel Mutation in the Na(+)/I(-) Symporter S5’-Untranslated Region in a Patient With Congenital Hypothyroidism. J Clin Endocrinol Metab (2011) 96:E1100–1107. doi: 10.1210/jc.2011-0349
18. Toubon C, Charbonnier C, Sokarjih B, Claverie B, Lebvre B, Dauchel H, et al. Large-Scale Comparative Evaluation of User-Friendly Tools for Predicting Variant-Induced Alterations of Splicing Regulatory Elements. Hum Mutat (2020) 41:1811–29. doi: 10.1002/humu.24091
19. Coban-Akdemir Z, White JJ, Song X, Ijangiani SN, Fatih JM, Gambin T, et al. Identifying Genes Whose Mutant Transcripts Cause Dominant Disease Traits by Potential Gain-Of-Function Alleles. Am J Hum Genet (2018) 103:171–87. doi: 10.1016/j.ajhg.2018.06.009

FUNDING

This work was supported by Fondo para la Investigación Científica y Tecnológica - Agencia Nacional de Promoción Científica y Tecnológica (grants number PICT-2018-1596 and PICT-2019-1772), Secretaría de Políticas Universitarias - Ministerio de Educación (grant number VT12-UNCOR4153), and Secretaría de Ciencia y Tecnología - Universidad Nacional de Córdoba (grant number 33620180100772CB).

ACKNOWLEDGMENTS

We thank Dr. Thomas v. O. Hansen (Copenhagen University Hospital, Denmark) for kindly providing the reporter vector pSPL3.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.868891/full#supplementary-material
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Geysels, Bernal Barquero, Martín, Peyret, Nocent, Sobrero, Muñoz, Signorino, Testa, Castro, Masini-Repiso, Miras and Nicola. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.