

Original Article

Genetic screening of solute carrier family 5 member 5 (*SLC5A5*) gene in Chinese Han congenital hypothyroidism patients with goiter

Chang Liu^{1,2}, Guoqing Zhang³, Yucui Zang¹, Fang Wang², Mengmeng Han¹, Wenxiu Han⁴, Qinghua Wang⁵, Shiguo Liu¹, Shengli Yan²

¹Prenatal Diagnosis Center, The Affiliated Hospital of Qingdao University, Qingdao 266003, Shandong, China; Departments of ²Endocrinology and Metabolism, ³Thoracic Surgery, The Affiliated Hospital of Qingdao University, Qingdao 266003, Shandong, China; ⁴Department of Biochemistry and Molecular Biology, Qingdao University, Qingdao 266021, Shandong, China; ⁵Department of Neonatal Screening, Maternal and Child Health-Care Hospital, Heze 274000, Shandong, China

Received March 13, 2016; Accepted June 29, 2016; Epub September 1, 2016; Published September 15, 2016

Abstract: Objective: *SLC5A5* mutations can cause thyroid dysmorphogenesis (an autosomal recessive metabolic disease) and account for approximately 10%-15% of congenital hypothyroidism (CH). In this study, we aimed to investigate the occurrence of *SLC5A5* mutations in CH with goiter patients and to explore the spectrum of phenotypes arising from *SLC5A5* mutations in Shandong province, China. Methods: Blood samples were collected from 110 Chinese CH with goiter patients, and genomic DNA was extracted from peripheral blood leukocytes. All 15 coding exons and exon-intron boundaries of *SLC5A5* were amplified by polymerase chain reaction (PCR) and analyzed by direct sequencing. Results: Although pathogenic mutations in *SLC5A5* were not identified, two rare *SLC5A5* variants (A514S and R569W) were detected in non-consanguineous families. In addition, six polymorphisms (IVS5-51, IVS6+11, IVS8+22, IVS14-8, IVS14+22 and IVS14+28) were found. Conclusion: Our study revealed the rate of *SLC5A5* mutation is low in CH with goiter patients in China. Two rare variants were identified in this study; however, further studies are needed to determine whether these variants may alter function and whether these patients have other reasons for CH.

Keywords: Congenital hypothyroidism, iodide transport defect, *SLC5A5*, mutation

Introduction

Congenital hypothyroidism (CH) is a common congenital endocrine disorder with a prevalence range from 1 in 3000 to 1 in 4000 in live newborns worldwide [1]. If left untreated it is one of the most significant causes of mental and growth retardation. Thyroid dysmorphogenesis (an absence or deficiency of one or more of the enzymes that participate in thyroid hormone biosynthesis or secretion [2]), transmitted by autosomal recessive inheritance, is responsible for approximately 10%-15% of CH cases. Mutations in several genes, including of thyroid hormone receptor (*TR*), thyroperoxidase (*TPO*), thyroglobulin (*TG*), dual oxidase 2 (*DUOX2*) and solute carrier family 5 member 5 (*SLC5A5*) can cause defects in thyroid hormone

biosynthesis [3]. Mutations in *TR* can result in thyroid hormone resistance while mutations in *TPO*, *TG* and *DUOX2* may cause defects in thyroid hormone metabolism. *SLC5A5* mutations can lead to inefficient transport of iodine. The loss of thyroid hormone leads to actin cytoskeleton deficiency and damages cerebellar maturation and neuronal migration [4, 5]. A deficiency in thyroid hormone synthesis also has abnormal consequences on mitochondria and energy expenditure [5], which can damage psychomotor development. Therefore, the manifestations of CH include intellectual impairment and movement disorders.

SLC5A5, located on chromosome 19p12-13.2 [6], has an open reading frame of 1929 nucleotides split up into 15 exons and 14 introns. It

Genetic screening of *SLC5A5* in congenital hypothyroidism patients

encodes a plasma membrane glycoprotein that actively mediates iodide uptake from the bloodstream into thyroid follicular cells by a Na⁺/K⁺-ATPase mechanism that is inverse to sodium concentration gradients. It is expressed in thyroid follicular cells, salivary gland, breast tissue and many other tissues [7, 8]. Because of its essential role in thyroid physiology, *SLC5A5* mutations may change sodium iodide symporter (NIS) expression or function, which may lead to congenital iodide transport defect (ITD) [9]. Patients with ITD have the decreased ability to accumulate I⁻ in thyroid follicular cells. Decreased thyroid hormone secretion feeds back negatively on the adenohypophysis and results in a rise in TSH levels to stimulate the thyroid gland. As a consequent, patients either show an enlarged thyroid gland at birth or develop goiter after birth [10]. The first identified mutation in *SLC5A5* was homozygous missense mutation (T359P) in a Japanese family found by Fujiwara et al in 1997 [11]. The patient with this mutation developed a follicular adenoma at 8 years of age. Another *SLC5A5* mutation (G395R) was located in the 10th transmembrane helix in one ITD family [12]. Interestingly, none of the ITD patients in these families had or developed goiter. To date, 15 mutations in *SLC5A5* that cause ITD have been identified: -54C>T, V59E, G93R, R124H, Q267E, C272X, Δ142-323, Δ287-288, T354P, G395R, M435L, Δ439-443, frameshift 515X, Y531X and G543E [6, 10]. The spectrum of phenotypes arising from *SLC5A5* mutations has not been completely established. Only one mutation (M435L) [13] has been identified in Chinese patients.

The inheritance pattern of thyroid dysmorphogenesis is amenable to analysis by genetic screening. In this study, we collected blood samples from 110 CH with goiter patients to investigate *SLC5A5* mutations in the Chinese Han population. Our study aimed to explore the relationship between clinical features of patients and gene mutations, which may be used for the early diagnosis of infants and for genetic counseling in the Chinese Han population.

Patients and methods

Patients

One hundred and ten unrelated CH patients (62 girls and 48 boys) with thyroid gland enlarge-

ment were recruited for this study. The sex ratio was 1.34:1 and the average age was 5.1 ± 3.8 years. All patients had no other congenital disorders, who had already genetic screening of *TPO*, *DUOX2*, and were originally enrolled from 2008 to 2014 through the neonatal screening program of Shandong Province (consisting of seven Maternal and Child Health care hospitals, including: Qingdao, Jinan, and another five hospitals). Neonatal screening for CH was performed on filter paper blood spots collected between 72 h and 7 days after birth. Blood samples were collected from the heel and TSH levels were measured using an enzyme linked immunosorbent assay (ELISA). When the level of TSH was increased to ≥ 20 μIU/ml (normal range 0.27-4.2 μIU/ml), patients were recalled for further tests. Free thyroxin (FT4, normal range 12-22 pmol/L) and serum TSH were measured using an electro-chemiluminescence immunoassay (ECLI). The patients diagnosed with CH by a high TSH level and a low FT4 level, as well as were examined by thyroid scintiscan and/or ultrasound for goiter. A control group of 300 healthy individuals was recruited from our sample database. This study was approved by the Ethics Committee of all Maternity and Child Health Care Hospitals and the Affiliated Hospital of Qingdao University. After written informed consent was obtained, blood samples were collected from the CH patients.

Methods

Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp blood kit (QIAGEN, Hilden, Germany). PCR was used to amplify all 15 exons and exon-intron boundaries of *SLC5A5* with primers designed by Primer 5. PCR products were analyzed by sequencing. The 25-μL PCR reaction system contained 100 ng of template DNA, 1× reaction buffer with 50 mM KCl, 2.5 mM MgCl₂, 250 nM dNTPs, 1.25 unit of AmpliTaq Gold DNA polymerase, and 0.5 μL of each primer. Samples were denatured at 95°C for 5 minutes, then amplified for 35 cycles. Each cycle included denaturation at 95°C for 30 seconds, a primer specific annealing temperature for 30 seconds, heating-up 72°C for 10 minutes, and primer extension at 72°C for 30 seconds. When the last cycle was completed, the samples were incubated at 4°C for 10 minutes. The amplification products

Genetic screening of *SLC5A5* in congenital hypothyroidism patients

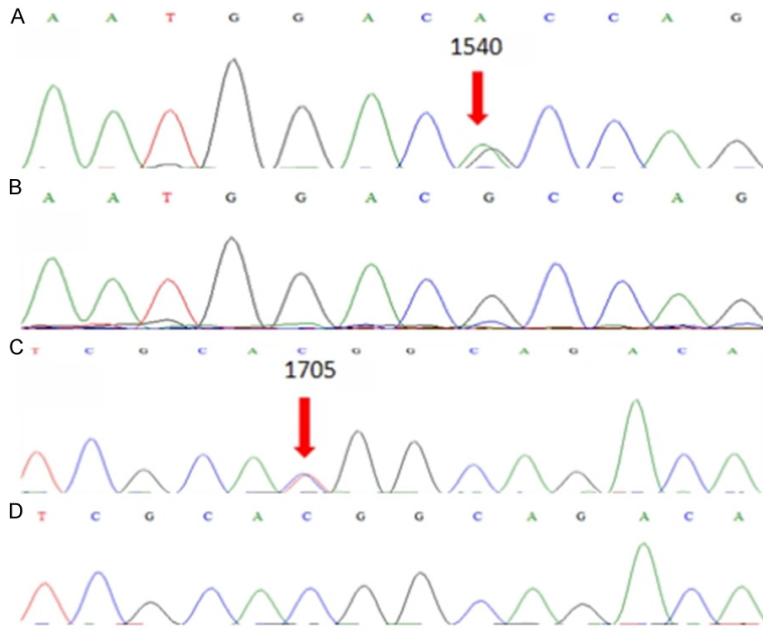


Figure 1. Partial sequence of *SCL5A5* from patient genomic DNA. (A) Sequence from exon 13 shows guanine (G) replaced by adenine (A) at coding position 1540. (B) Control sequence of the same exon 13 position. (C) Sequence of exon 14 shows cytosine (C) replaced by thymine (T) at coding position 1705. (D) Control sequence of the same exon 14 position.

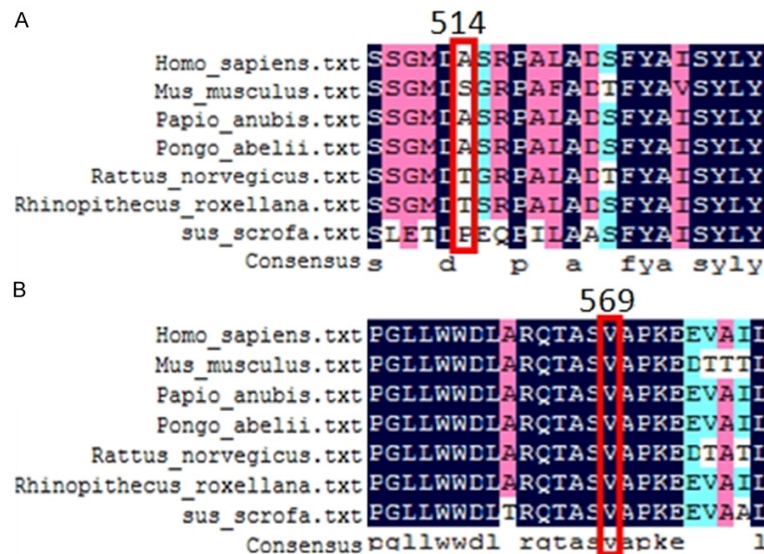


Figure 2. Protein sequence alignment of NIS from various species. A. The A514T variant is located in a non-conserved sequence. B. The R569W variant is located in a highly conserved sequence.

were analyzed on 1.5% agarose gels. PCR products were purified and sequenced using the DNA sequencing kit-BigDye Terminator Ready Reaction Cycle Sequencing Kit (PE Applied Biosystems, Warrington, UK) and an ABI 3730-

XL automated sequencer (Applied Biosystems). The same region was sequenced in 300 control group blood samples.

Results

Eleven *SLC5A5* variations were identified by Sanger sequencing of 110 unrelated Chinese patients, which included two substitution variants (c.1540-C>T, A514T; c.1705G>A, R569W). The rare variant R569W (a C>T nucleotide change at 1705 in exon 14 leading to CGG>TGG, Arg⁵⁶⁹Trp) was located in the last intracellular segment (**Figure 1**). The other variant A514T (a G>A substitution at nucleotide 1540 in exon 13 resulting in GCC>TCC, Ala⁵¹⁴Thr) was located in a chain that connects transmembrane domains XII and XIII (**Figure 1**). In addition, one silent mutation (c.1224C>T, L408L) and six polymorphisms consisting of IVS5-51 (c.-698+52G>A, rs185045651), IVS6+11 (c.839+11C>T, rs182064161), IVS8+22 (c.1058+22T>C, rs764311991), IVS14-8 (c.1652-9G>A, rs4808708), IVS14+22 (c.1767+23A>C, rs148626179), and IVS14+28 (c.1767+29A>G, rs4808709) were identified. Among them, two polymorphisms (c.1767+29A>G and c.1767+23A>C) were detected in one patient.

The NIS protein-sequence from a variety of species, including *Homo sapiens*, *Mus musculus*, *Papio anubis*, *Pongo abelii*, *Rattus norvegicus*, *Rhinopithecus roxellana* and *Sus scrofa* were obtained

from the National Center for Biotechnology Information (NCBI) website. We then used DNAMAN software to perform multiple sequence alignments. The NIS R569W variant was situated in a highly conserved region, while the

Genetic screening of *SLC5A5* in congenital hypothyroidism patients

A514T variant was located in non-conserved sequence (**Figure 2**). These two variants were absent in 300 control individuals. In addition, these variants were not present in the normal Chinese population, as archived in dbSNP and 1000 genomes databases.

Discussion

Patients with CH, when diagnosed through neonatal screening and treated immediately, have normal stature and neurodevelopment [14, 15]. If this condition is not diagnosed, physical and psychomotor development can be irreversibly damaged and patients may have subtle behavioral difficulties, motor delays, and memory impairment [16]. Characterization of genome-wide variation may provide evidence to inform clinical treatment. With medical advances, the precise diagnosis, individualized therapeutic schemes, and following-up examinations for CH have improved. A study suggested that it is necessary for thyroid function to be monitored before treatment because the rate of overtreatment is high [17]. Before diagnosis, patients at least 1 month old, who were monitored the level of TSH were gradually increased in the recent weeks, contrast with TSH the level of FT3, FT4 was decreased, furthermore, patients were diagnosed from minimum dose and blood test weekly in order to prevent overtreatment.

Iodine is crucial to the biosynthesis of thyroid hormone and is, therefore, indispensable for health. Biosynthesis of thyroid hormones consists of two steps: 1) iodide ions (changed from iodine in the intestine) are absorbed into epithelial thyroid cells through NIS [18]. 2) I⁻ is transported into thyroid follicular cells through pendrin or other transporters [19]. TPO catalyses the addition of activated iodine (which was changed from I⁻) to tyrosine residues in TG, to form monoiodotyrosine and diiodotyrosine. One monoiodotyrosine reacts with diiodotyrosine form T3, whereas two diiodotyrosines form T4 [20]. Errors in one of the steps of thyroid hormone synthesis lead to thyroid dysmorphogenesis. Numerous candidate genes for thyroid dysmorphogenesis, such as *DUOX2*, *TPO* and *TG* have been identified. For example, Eastern European CH patients with thyroid dysmorphogenesis and normal or enlarged thyroid glands had *TPO* mutations [21]. Although

early treatment should prevent goitrogenesis, CH patients with *TG* mutations were found to have remarkably large goiters [3]. Hydrogen peroxide is made at the apical membrane of follicular thyroid cells by dual oxidase (DUOX)/dual oxidase maturation factor (DUOXA) NADPH oxidase complexes, which plays a key role in coupling reactions and TPO-catalyzed iodination. The first *DUOXA2* mutation was reported in a Chinese patient with partial iodine organification defect and mild, permanent CH [22]. NIS in the basolateral membrane of thyroid follicular cells mediates uptake of I⁻ and Na⁺ against the electrochemical gradient, with an electrogenic 2 Na⁺:1 I⁻ stoichiometry [23] under normal circumstances. The secondary structure of NIS contains 643 amino acids with 13 transmembrane segments, an extracellular-facing N-terminus, and an intracellular-facing carboxy terminus [24, 25]. NIS is the first and rate-limiting step in the process of thyroid hormone biosynthesis. Consequently, *SLC5A5* mutations are one of the most important causes of CH.

The molecular mechanisms of certain *SLC5A5* mutations are thoroughly understood. A compound heterozygous mutation of T354P/G93P in one patient and a homozygous mutation of G543E in two sibling cases were detected [26] in Japan. These three mutations, through minimal iodide uptake activity, were confirmed to be the direct cause of the disease in the patients. Further investigation confirmed that T354P is a common mutation in Japanese patients that gives rise to NIS over-expression in the basal and lateral plasma membranes of thyrocytes. Because of clinical heterogeneity of T354P mutation, patients may be euthyroid or have severe hypothyroidism in early childhood, with or without goiter [27]. Reed-Tsur et al [28] reported that change from Val at position 59 to the neutral amino acids, including Ala, Asn, Gln, Ile, Leu, Met and Thr, led to a decrease in NIS kinetic turnover and yielded nonfunctional NIS protein, however, I⁻ uptake from blood was partially rescued. As a result, neutral amino acids may rescue the activity of NIS. Another *SLC5A5* mutation, G543E, was discovered as the first ITD-causing mutant that results in only partial maturation, retention of NIS in intracellular organelles and impaired its function. G543 is in a tightly packed region of NIS; when G543 changed to small neutral amino acid (e.g. A, S,

Genetic screening of *SLC5A5* in congenital hypothyroidism patients

T, C), the function of NIS was partially rescued by small neutral amino acid substitutions [29].

We found the frequency of *SLC5A5* mutation to be low, and it may not be an important factor in goiter. This was consistent with previous research by Fu C et al [13] who found the *SLC5A5* mutation rate to be very low in a cohort of 105 patients with CH in Guangxi Zhuang Autonomous Region, China. Two possible reasons may explain the results given above: 1) We did not find all mutations by DNA sequencing, which has many limitations and 2) the mutation is heterozygous (or a SNP). Heterozygous individuals will not present clinically because *SLC5A5* mutations are autosomal-recessive. However, the patient with R569W was diagnosed as CH. This mutation replaces an alkaline amino acid (arginine) by a neutral amino acid (tyrosine) in a highly conserved region of the last side intracellular loop at the amino terminus of NIS. The rate of this variant is extremely rare; its MAF is 0.0006, hence, the variant may not be pathogenic, although this variant had not previously been found in the Chinese Han population. According to population frequency from the 1000 Genome database (NCBI), the allele frequencies at the position c.1705 of *SLC5A5* in the Iberian population were C 99.53% and T 0.47%, in Indian Telugu from the UK were C 99.51% and T 0.049%, and in Peruvians were C 99.41% and T 0.59%, while the remaining population frequencies were all C 100.00% and T 0.00%. According to the evidence discussed above, the R569W variant needs further functional verification studies.

Many unexplained Mendelian diseases can be explained by *de novo* mutation, compound heterozygous mutation or rare heterozygous alleles based on empirical evidence, and disease-associated variants were usually limited to relatives of the affected individuals [30]. Diagnosis and treatment should routinely consider the differences between patients' geographic history [31]. Consequently, the reason of patients with goiter are needed to further study.

Acknowledgements

We thank all their participation in this study. We thank all members of the research group for their contributions to the investigation. This study was supported by the National Nature

Science Foundation of China (81470044, 81500689, and 81170812) and the Science and Technology Project of the Shandong Provincial Population and Family Planning Commission (No.5 in 2013).

Disclosure of conflict of interest

None.

Address correspondence to: Shiguo Liu, Prenatal Diagnosis Center, The Affiliated Hospital of Qingdao University, Qingdao 266003, Shandong, China. Tel: +86 18661807782; E-mail: liushiguo2002@126.com; Shengli Yan, Department of Endocrinology and Metabolism, The Affiliated Hospital of Qingdao University, Qingdao 266003, Shandong, China. Tel: +86 18661807959; E-mail: yansl07@163.com

References

- [1] Agrawal P, Philip R, Saran S, Gutch M, Razi MS, Agroiya P, Gupta K. Congenital hypothyroidism. *Indian J Endocrinol Metab* 2015; 19: 221-7.
- [2] Kumar PG, Anand SS, Sood V, Kotwal N. Thyroid dysmorphogenesis. *Indian J Pediatr* 2005; 42: 1233-5.
- [3] Grasberger H, Refetoff S. Genetic causes of congenital hypothyroidism due to dysmorphogenesis. *Curr Opin Pediatr* 2011; 23: 421-8.
- [4] Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev* 2010; 31: 139-70.
- [5] Vaitkus JA, Farrar JS, Celi FS. Thyroid Hormone Mediated Modulation of Energy Expenditure. *Int J Mol Sci* 2015; 16: 16158-75.
- [6] Smanik PA, Liu Q, Furminger TL, Ryu K, Xing S, Mazzaferri EL, Jhiang SM. Cloning of the human sodium iodide symporter. *Biochem Biophys Res Commun* 1996; 226: 339-45.
- [7] La Perle KM, Kim DC, Hall NC, Bobbey A, Shen DH, Nagy RS, Wakely PE Jr, Lehman A, Jarjoura D, Jhiang SM. Modulation of sodium/iodide symporter expression in the salivary gland. *Thyroid* 2013; 23: 1029-36.
- [8] Cho JY, Leveille R, Kao R, Rousset B, Parlow AF, Burak WE Jr, Mazzaferri EL, Jhiang SM. Hormonal regulation of radioiodide uptake activity and Na⁺/I⁻ symporter expression in mammary glands. *J Clin Endocrinol Metab* 2000; 85: 2936-43.
- [9] Spitzweg C, Morris JC. Genetics and phenomics of hypothyroidism and goiter due to NIS mutations. *Mol Cell Endocrinol* 2010; 322: 56-63.
- [10] De La Vieja A, Dohan O, Levy O, Carrasco N. Molecular analysis of the sodium/iodide symporter: impact on thyroid and extrathyroid

Genetic screening of *SLC5A5* in congenital hypothyroidism patients

- pathophysiology. *Physiol Rev* 2000; 80: 1083-105.
- [11] Fujiwara H. Congenital hypothyroidism caused by a mutation in the Na⁺/I⁻-symporter. *Nat Genet* 1997; 17: 122.
- [12] Kosugi S, Bhayana S, Dean HJ. A novel mutation in the sodium/iodide symporter gene in the largest family with iodide transport defect. *J Clin Endocrinol Metab* 1999; 84: 3248-53.
- [13] Fu C, Chen S, Chen R, Fan X, Luo J, Li C, Qian J. Mutation screening of the sodium iodide symporter gene in a cohort of 105 China patients with congenital hypothyroidism. *Arq Bras Endocrinol Metabol* 2014; 58: 828-32.
- [14] Delvecchio M, Vigone MC, Wasniewska M, Weber G, Lapolla R, Popolo PP, Tronconi GM, Di Mase R, De Luca R, Cavallo L, Salerno M, Faienza MF. Final height in Italian patients with congenital hypothyroidism detected by neonatal screening: a 20-year observational study. *Ital J Pediatr* 2015; 41: 82.
- [15] Soliman AT, Azzam S, Elawwa A, Saleem W, Sabt A. Linear growth and neurodevelopmental outcome of children with congenital hypothyroidism detected by neonatal screening: A controlled study. *Indian J Endocrinol Metab* 2012; 16: 565-8.
- [16] Delahunty C, Falconer S, Hume R, Jackson L, Midgley P, Mirfield M, Ogston S, Perra O, Simpson J, Watson J, Willatts P, Williams F; Scottish Preterm Thyroid Group. Levels of neonatal thyroid hormone in preterm infants and neurodevelopmental outcome at 5 1/2 years: millennium cohort study. *J Clin Endocrinol Metab* 2010; 95: 4898-908.
- [17] Tuhan H, Abaci A, Cicek G, Anik A, Catli G, Demir K, Sober E. Levothyroxine replacement in primary congenital hypothyroidism: the higher the initial dose the higher the rate of overtreatment. *J Pediatr Endocrinol Metab* 2016; 29: 133-8.
- [18] Dai G, Levy O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. *Nature* 1996; 379: 458-60.
- [19] Bizhanova A, Kopp P. Controversies concerning the role of pendrin as an apical iodide transporter in thyroid follicular cells. *Cell Physiol Biochem* 2011; 28: 485-90.
- [20] Yen PM. Physiological and molecular basis of thyroid hormone action. *Physiol Rev* 2001; 81: 1097-142.
- [21] Avbelj M, Tahirovic H, Debeljak M, Kusekova M, Toromanovic A, Krzisnik C, Battelino T. High prevalence of thyroid peroxidase gene mutations in patients with thyroid dysmorphogenesis. *Eur J Endocrinol* 2007; 156: 511-9.
- [22] Zamproni I, Grasberger H, Cortinovis F, Vigone MC, Chiumello G, Mora S, Onigata K, Fugazzola L, Refetodd S, Persani L, Weber G. Biallelic inactivation of the dual oxidase maturation factor 2 (DUOXA2) gene as a novel cause of congenital hypothyroidism. *J Clin Endocrinol Metab* 2008; 93: 605-10.
- [23] Levy O, Dai G, Riedel C, Ginter CS, Paul EM, Lebowitz AN, Carrasco N. Characterization of the thyroid Na⁺/I⁻ symporter with an anti-COOH terminus antibody. *Proc Natl Acad Sci U S A* 1997; 94: 5568-73.
- [24] Levy O, De la Vieja A, Ginter CS, Riedel C, Dai G, Carrasco N. N-linked glycosylation of the thyroid Na⁺/I⁻ symporter (NIS). Implications for its secondary structure model. *J Biol Chem* 1998; 273: 22657-63.
- [25] Paroder V, Nicola JP, Ginter CS, Carrasco N. The iodide-transport-defect-causing mutation R124H: a delta-amino group at position 124 is critical for maturation and trafficking of the Na⁺/I⁻ symporter. *J Cell Sci* 2013; 126: 3305-13.
- [26] Kosugi S, Inoue S, Matsuda A, Jhiang SM. Novel, missense and loss-of-function mutations in the sodium/iodide symporter gene causing iodide transport defect in three Japanese patients. *J Clin Endocrinol Metab* 1998; 83: 3373-6.
- [27] Tatsumi KI, Fujiwara H, Tanaka S, Amino N. Characterization of Thr-354 in the human sodium/iodide symporter (NIS) by site-directed mutagenesis. *Endocr J* 2010; 57: 997-9.
- [28] Reed-Tsur MD, De la Vieja A, Ginter CS, Carrasco N. Molecular characterization of V59-E NIS, a Na⁺/I⁻ symporter mutant that causes congenital I⁻ transport defect. *Endocrinology* 2008; 149: 3077-84.
- [29] De la Vieja A, Ginter CS, Carrasco N. Molecular analysis of a congenital iodide transport defect: G543E impairs maturation and trafficking of the Na⁺/I⁻ symporter. *Mol Endocrinol* 2005; 19: 2847-58.
- [30] Cook MC. Medical case reports in the age of genomic medicine. *Clin Transl Immunology* 2015; 4: e45.
- [31] Kamel Boulos MN, Le Blond J. On the road to personalised and precision geomedicine: medical geology and a renewed call for interdisciplinarity. *Int J Health Geogr* 2016; 15: 5.