Clinical Endocrinology (2003) **59**, 500–506

Congenital hypothyroidism due to a new deletion in the sodium/iodide symporter protein

Massimo Tonacchera*, Patrizia Agretti*, Giuseppina De Marco*, Rossella Elisei*, Anna Perri*, Elena Ambrogini*, Melissa De Servi*, Claudia Ceccarelli*, Paolo Viacava†, Samuel Refetoff¶, Claudio Panunzi‡, Maria Luisa Manca Bitti§, Paolo Vitti*, Luca Chiovato and Aldo Pinchera***

**Dipartimento di Endocrinologia e Metabolismo, and †Dipartimento di Oncologia, Sezione Anatomia Patologica; Università di Pisa, Pisa, ‡UO Endocrinologia, Ospedale Regina Apostolorum, Albano Laziale and §Clinica Pediatrica, Università Tor Vergata, Roma, Italy, ¶Departments of Medicine, Pediatrics and the Committee on Genetics, The University of Chicago, Chicago, Illinois, USA and **Cattedra di Endocrinologia, Fondazione S Maugeri IRCCS, Pavia, Italy*

(Received 26 February 2003; returned for revision 11 March 2003; finally revised 1 May 2003; accepted 5 June 2003)

Summary

OBJECTIVE **Iodide transport defect (ITD) is a rare disorder characterised by an inability of the thyroid to maintain an iodide gradient across the basolateral membrane of thyroid follicular cells, that often results in congenital hypothyroidism. When present the defect is also found in the salivary glands and gastric mucosa and it has been shown to arise from abnormalities of the sodium/iodide symporter (NIS).**

PATIENT **We describe a woman with hypothyroidism identified at the 3rd month of life. The diagnosis of ITD was suspected because of nodular goitre, and little if any iodide uptake by the thyroid and salivary glands. Treatment with iodide partially corrected the hypothyroidism; however, long-term substitution therapy with L-thyroxine was started.**

MEASUREMENTS **Thyroid radioiodide uptake was only 1·4% and 0·3% at 1 and 24 h after the administration of recombinant human TSH. The saliva to plasma I^- ratio was 1·1 indicating that the inability of the thyroid gland to concentrate I^- was also present in the salivary glands.**

RESULTS **Analysis of the patient's NIS gene revealed a 15 nucleotide (nt) deletion of the coding sequence (nt 1314 through nt 1328) and the insertion of 15 nt duplicating the first 15 nt of the adjacent intron. The patient was homozygous for this insertion/deletion, while both consanguineous parents were heterozygous. This deletion predicts the production of a protein lacking the five terminal amino acids of exon XI (439–443) which are located in the 6th intracellular loop.**

COS-7 cells transfected with a vector expressing the mutant del-(439–443) NIS failed to concentrate iodide, suggesting that the mutation was the direct cause of the ITD in this patient.

CONCLUSION **In conclusion we describe the first Italian case of congenital hypothyroidism due to a new deletion in the NIS gene.**

Correspondence: Massimo Tonacchera, Dipartimento di Endocrinologia, Università degli Studi di Pisa, Via Paradisa 2, 56124, Cisanello, Pisa, Italy. Tel: +50/995048; Fax: +50/578772; E-mail: mtonacchera@hotmail.com

Primary congenital hypothyroidism (CH) is a sporadic disorder which in the majority of patients is caused by thyroid dysgenesis (thyroid agenesis, ectopia or hypoplasia; Stanbury & Chapman, 1960; Refetoff *et al*., 2001; Vulsma *et al*., 2001). In about 15% of cases, CH results from defects of thyroid hormonogenesis mostly inherited in an autosomal recessive manner (Stanbury *et al*., 1960; Refetoff *et al*., 2001). Such defects may be due to abnormalities in several steps involved in thyroid hormone synthesis, including TSH, TSH-receptor, iodide (I^-) trapping, I^- organification, thyroglobulin (Tg) and iodothyrosine deiodination. Homozygous or compound heterozygous mutations have been described in the genes encoding TSH, TSH receptor, sodium/iodide symporter (NIS), thyroid peroxidase (TPO), pendrin (PDS), thyroid oxidase (THOX2) and thyroglobulin (Tg) (Stanbury *et al*., 1960; Refetoff *et al*., 2001; Vulsma *et al*., 2001; Moreno *et al*., 2002). Heterozygous mutations of THOX2 may also cause CH (Moreno *et al*., 2002).

Iodide transport defect (ITD) is a rare disorder characterised by an inability of the thyroid gland to maintain a concentration gradient of iodide between the plasma and the thyroid follicular cell which often results in CH (Stanbury *et al*., 1960; Wolff, 1983). When present, the defect is also found in the salivary glands and gastric mucosa and it has been shown to arise from abnormalities of NIS (Stanbury *et al*., 1960; Wolff *et al*., 1983). To date, several loss-of-function mutations of the NIS gene have been identified in patients with ITD (Fujiwara *et al*., 1997, 2000;

© 2003 Blackwell Publishing Ltd

Matsuda & Kosugi, 1997; Pohlenz *et al*., 1997, 1998; Kosugi *et al*., 1998a, 1998b, 1999, 2002).

NIS is a membrane-bound glycoprotein located at the basolateral portion of the thyroid follicular cell (Levy *et al*., 1998). NIS concentrates iodide in thyroid follicular cells by an active transport process, counteracting an electrochemical gradient, to maintain iodide concentration inside the cells at about 20- to 100-fold higher than in serum (Levy *et al*., 1998). NIS co-transports two sodium ions together with an iodide ion. The NIS is predicted to have a serpentine structure of probably 13 transmembrane segments with extracellular N and intracellular C termini (Levy *et al*., 1998). The human NIS (hNIS) gene is located on chromosome 19. It consists of 15 exons and encodes a protein of 643 amino acids (Smanik *et al*., 1997).

In this paper we describe the first Italian case of ITD with congenital hypothyroidism due to a homozygous deletion of five amino acids located in the 6th intracellular loop of NIS protein between the two transmembrane segments XI and XII.

Case report

The patient, a 28-year-old woman when studied in our institution, was born at term after an uncomplicated pregnancy. Birth weight was 3500 g and length 50 cm. CH was suspected at 3 months of age because of feeding problems, failure to thrive, a hoarse cry, somnolence, a large protruding tongue and an umbilical hernia. Protein-bound iodine was 0·5 mg/dl (normal values 3·5–8). Her parents were first cousins. At the time of her birth, no routine screening for hypothyroidism was available. Treatment with desiccated thyroid was started at 6 months of age, and at 13 years this was changed to synthetic L-T4. At 3 years of age, following a temporary discontinuation of hormone therapy, a ^{131}I scan showed no functional thyroid tissue in the neck while serum TSH levels were high and thyroid hormone concentration low. At 12 years, after 1 month L-T4 discontinuation, FT4 was 0·013 pmol/l (normal range: 8·5–18), FT3 was 0·86 pmol/l (normal range 3·0–8·4) and TSH concentration was 100 mU/l (normal range 0·2–4). On this occasion a treatment trial with iodide only was started (14 mg/day) as described by Wolff *et al*. (1983). Thyroid function tests after 1 month of iodide administration are shown in Table 1.

The patient had mental retardation, psychomotor defects and bilateral deafness. IQ was reported to be 67% of normal. Menstrual periods were normal since menarche at age 12 years.

Current clinical features and investigation

When first seen in our Institution, the patient was 28 years old and was receiving 150 μg L-T4/day. The patient and her parents gave informed consent to undergo studies approved by the local ethical committee. She was 167 cm in height and weighed 67 kg. The patient's face was cretinoid, but the bridge of the nose was not flattened. There was no umbilical hernia and muscles were well developed. The patient had mild mental retardation and a divergent strabismus. Thyroid ultrasound confirmed the presence of a slightly enlarged thyroid gland (volume 16 ml), with a normoechogenic pattern. Solid hypoechogenic nodules measuring $10 \times 13 \times 16$ and $10 \times 12 \times 21$ were found in the right and left lobes, respectively. Fine-needle aspiration showed a pattern of follicular adenoma in the two nodules that could not rule out the presence of a carcinoma. Accordingly, total thyroidectomy was performed. Cardiac two-dimensional echocardiography was normal. Bone age corresponded to the chronological age. Slight hypoplasia of the right hip with bilateral hip- valgus deformity was observed on X-ray. Ultrasonography of the abdomen was normal.

Table 1 Serum thyroid hormones, TSH and Tg measurement before (day 0) and at different times (14, 20, 28, 35 days) following the administration of iodide (14 mg/day)

	Days				
	0	14	20	28	35
T3 (0·92–3·23 nmol/l)	0·98	3·30	3·93	3·58	3·82
T4 (57·9–160·9 nmol/l)	8·02	37·06	65·38	71·56	66·92
FT3 (4·3–8·6 pmol/l)	0·86	4·27	6·80	6·48	6·57
FT4 (8·5–18 pmol/l)	0·01	2·34	7·32	9·07	9·92
TSH (0·21–4 mU/l)	100	82	34	29	20
Tg (< 60 mg/l)	1995	1300	540	450	483

On 150 μg/day L-T4, serum FT4 was 15·70 pmol/l (normal range 8·5–18), FT3 was 3·3 pmol/l (normal range 3·0–8·4), thyroxine binding globulin was 28 mg/l (normal values 8·9–30·5), TSH was 0·3 mU/l (normal range 0·5–5) and Tg was 135 μg/l (normal range 2–30). Urinary iodine excretion was 139 μg/l. Tg and TPO antibodies were not detected. Calcium, phosphorus and alkaline phosphatase concentrations were within normal limits.

Family members

Both parents and her younger brother were clinically euthyroid. While the father had a small nontoxic diffuse goitre, the mother and the brother had glands of normal volume on ultrasound. Serum tests of thyroid function were in the normal range and TPO and Tg antibodies were not detectable.

Dynamic tests performed in vivo

Recombinant human TSH (rhTSH; 0·9 mg; Thyrogen, Genzyme Biotherapeutics, Cambridge, MA, USA) was given. Serial determination of tests of thyroid function are shown in Table 2. Thyroid uptake (Atomlab, Biodex Medical System, New York, New York, USA) as percentage of administered oral ^{131}I (50 μCi, 1·85 MBq) dose was 1·4, 1·2, 1·1, 1·0, 0·8 and 0·3 at 1, 2, 3 4, 5

Table 2 Serum thyroid hormones, TSH and Tg measurement before and after the administration of rhTSH (0·9 mg rhTSH was given intramuscularly on days 0 and 1)

	Days			
	0	1	2	3
FT4 (8·4–21·2 pmol/l)	10·3	13·9	18·1	24·3
FT3 (4·3–8·6 pmol/l)	4·5	4·3	5·9	5·2
TSH (0·4–3·4 mU/l)	0·3	68	> 75	43
Tg (mg/l)	157	183	352	554

and 24 h, respectively. A scan performed with a K545 collimator failed to show thyroid tissue and no iodide accumulated in the salivary glands or stomach. Further, to confirm the diagnosis of an iodide trapping defect, the saliva to plasma (S/P) iodide ratio was measured using a modification of the method described by Harden *et al*. (1968). Saliva was collected without stimulation over a period of 5–10 min, 1 h after the oral administration of 500 μCi of ^{131}I. At the same time, a venous blood sample was obtained and radioactivity measured in equal volumes of these fluids. The S/P ratio was 1·1 (normal 25–140), showing that the inability of the thyroid gland to concentrate I^- was also present in the salivary glands normally expressing this NIS-dependent function.

Materials and methods

Laboratory evaluation of thyroid function

Serum FT4 and FT3 concentrations were measured by radioimmunoassay (RIA) after chromatographic separation of the free hormone (FT4 RIA, FT3 RIA, Lysophase; Technogenetics S.r.l., Milan, Italy). Serum TSH was determined by a third-generation immunometric assay (AutoDelfia hTSH Kit; Pharmacia s.p.a., Milan, Italy). TPO and Tg antibodies were measured by passive agglutination (SERODIA-AMC and SERODIA-ATG, Fujirebio, Tokyo, Japan). Serum Tg was measured with an immunoradiometric assay kit (HTGK, Sorin Biomedia, Saluggia, Italy).

Gene sequencing

Genomic DNA was extracted from peripheral lymphocytes using standard procedures. Each exon of the hNIS gene was amplified by PCR using pairs of primers annealing at flanking introns. Exons 2 and 3, 6 and 7, 9 and 10, 11 and 12 were co-amplified with the intervening introns. All, and at least 15 nucleotides at all exon–intron boundaries, were sequenced in both orientations using AmpliTaqDNA polymerase FS, with an ABI Prism Bigdye terminator cycle sequencing kit (Applied Biosystems, Inc., Foster City, CA, USA) and were analysed on a sequencer (model ABI PRIMS 310 Genetic Analyser, Applied Biosystems). The oligonucleotide primers were as previously described (Tonacchera *et al*., 2002).

cDNA synthesis

Total RNA was isolated from 30 to 40 mg of frozen thyroid tissue using the AquaPure RNA Isolation Kit (Bio-Rad Laboratories, Hercules, CA, USA). The quality of RNA was assessed by electrophoresis through denaturing agarose gel and the amount was quantified spectrophotometrically at 260 nm.

One microgram of total RNA was reverse transcribed for 1 h at 50 °C in a 20-μl reaction volume using 200 units of Superscript II RNase H^- reverse transcriptase (Life Technologies Inc., Gaithersburg, MD, USA) in the presence of 1·5 μM random examers (Pharmacia Biotech, Uppsala, Sweden), 0·01 M dithiothreitol (DTT) and 1 mM dNTP mix. cDNA was sequenced with internal primers.

Construction of expression vectors and functional analysis

Preparation of the wild-type (wt) hNIS in the expression vector pcDNA3 was as described (Pohlenz *et al*., 1997). Mutant, del-(439–443), was generated by site-directed mutagenesis using the GeneTailor site-directed mutagenesis system (Invitrogen Life Technologies, Carlsbad, CA, USA). The accuracy of the recombinant construct was verified by direct sequencing.

COS-7 cells were transfected with 500 ng of wt hNIS, mutant hNIS or with empty vector by the DEAE–dextran method (Lopata *et al*., 1984). To mimic hNIS expression in the parents, who were heterozygous for the mutation, equal amounts of wt and mutant hNIS vectors were transfected into COS-7 cells. As control, co-transfection using wt hNIS together with the expression vector alone was also performed.

2×10^5 COS-7 cells were plated in a 3·5 cm tissue culture plates and cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum (FCS). Forthy-eight hours after transfection, cells were assayed for iodide uptake as described (Tonacchera *et al*., 2001). Briefly, Na^{125}I uptake was determined by incubating cells at 37 °C with 1 ml buffer A [Hanks' balanced salt solution containing 0·5% bovine serum albumin (BSA) and 10 mM HEPES, pH 7·4], 10 μM NaI, containing 200 000 cpm of carrier-free Na^{125}I. After 5 min incubation, cells were quickly washed twice with 4 ml of ice-cold buffer A. Cells were then solubilized with 1 ml of 0·1 M NaOH and radioactivity was measured using a gamma-counter. Data of iodide uptake were expressed as pmoles/well.

Immunohistochemistry

Thyroid tissue was fixed in 10% formalin and embedded in paraffin. Five millimeter sections were stained with haematoxylin–eosin

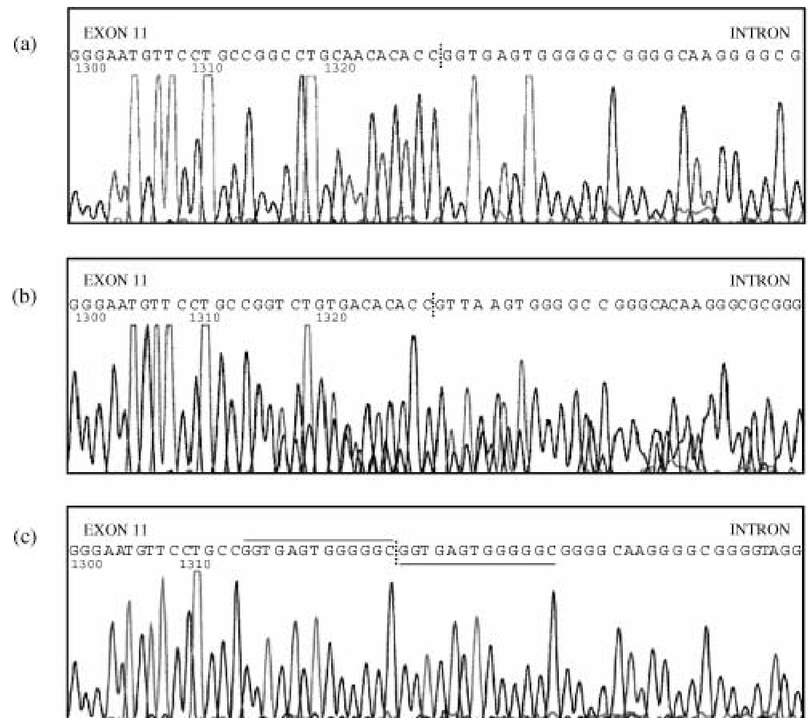

Fig. 1 The nucleotide sequences of the genomic DNAs extracted from the leucocytes of a normal subject (a), the mother (b) and the Patient (c). Both mother and father (not shown) were heterozygous while the patient was homozygous for the insertion/deletion mutation.

and additional 5-µm sections were used for immunohistochemistry. Sections were incubated with a monoclonal antibody recognizing hNIS (dilution 1 : 30) produced by genetic immunization (a kind gift from Dr Costagliola, Bruxelles, Belgium) as described in Pohlenz *et al*. (2000). This antibody was previously shown to work in immunohistochemistry (Tonacchera *et al*., 2002).

Sections were deparaffininated in xylene and rehydrated in alcohol. Endogenous peroxide activity was blocked by incubating the slides in 1% hydrogen peroxide in methanol for 10 min. In order to unmask the antigens, slides were microwave-treated in 10 mM citrate buffer, pH 6, for a total of 10 min. After blocking nonspecific staining with normal serum, the sections were incubated with the primary antibody. Sections were then incubated with biotin-labelled secondary antibody (dilution 1 : 500) and avidin–biotin complex (Vector, Burlingame, CA, USA) for 30 min, respectively. 3-3′-Diaminobenzidine tetrahydrochloride was used as chromogen. Finally, the sections were counterstained with haematoxylin, dehydrated and mounted.

Results

Direct sequencing of genomic DNA revealed the presence of a 15 nt deletion, from nt 1314 through nt 1328, and the insertion of 15 nt which corresponded to the duplication of the first 15 nt of the intron (Fig. 1). The patient was homozygous for this insertion/deletion. Direct sequencing of genomic DNA in both parents showed the same insertion/deletion in the heterozygous state (Fig. 1).

Direct sequencing of cDNA, prepared from thyroid mRNA of the patient, revealed an unique sequence showing the deletion of nt 1314–1328 (Fig. 2). This deletion produces a protein lacking the five terminal amino acids of exon 11 (439–443). These amino acids are located in the 6th intracellular loop between the two transmembrane segments XI and XII.

Pathology and immunohistochemistry

The thyroid gland was 45 g in weight. On cross-section, multiple nodules of variable size were seen. Some were surrounded by a partial capsule. Some nodules were composed by microfollicles while others showed micromacrofollicular pattern of growth. Thyroid parenchyma surrounding the nodules was composed predominantly of macrofollicles lined by flattened cells. The colloid was scant (Fig. 3). Nodules and surrounding parenchyma showed no hNIS immunoreactivity.

Functional studies

COS-7 cells transfected with the mutant del-(439–443) hNIS construct were unable to concentrate $^{125}I^-$, suggesting that the mutation was the direct cause of the ITD in this patient (Fig. 4). Cells co-transfected with both wt and del-(439–443) hNIS showed approximately half the iodide uptake activity as compared to cells transfected with wt hNIS alone, but uptake was similar to that in cells co-transfected with wt hNIS and empty vector (Fig. 4).

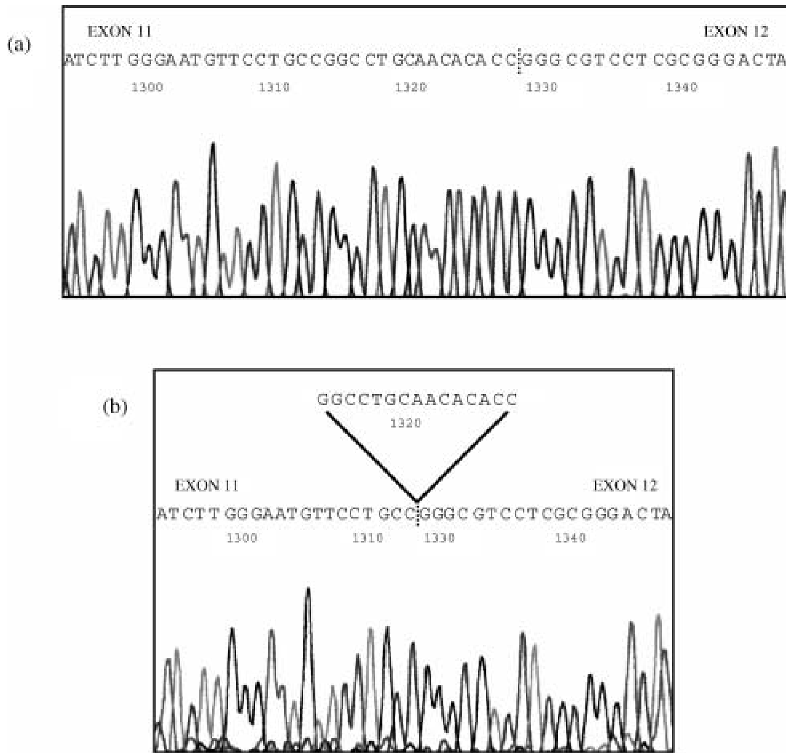

Fig. 2 Sequences of cDNA prepared from the thyroid of a patient with Graves' disease (a) and the thyroid of the patient (b).

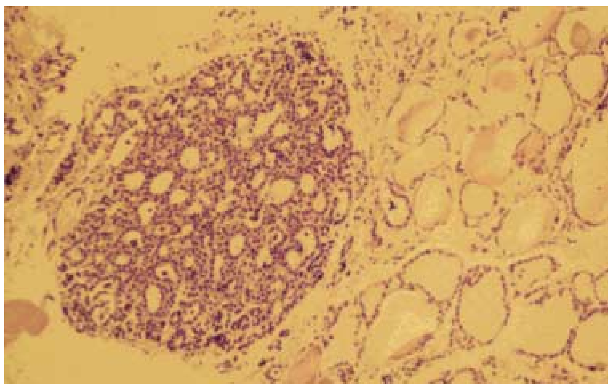

Fig. 3 Histological examination of thyroid tissue of the patient. A small nodule is predominantly formed by microfollicles. Surrounding thyroid parenchyma show macrofollicular pattern of growth with a small amount of colloid.

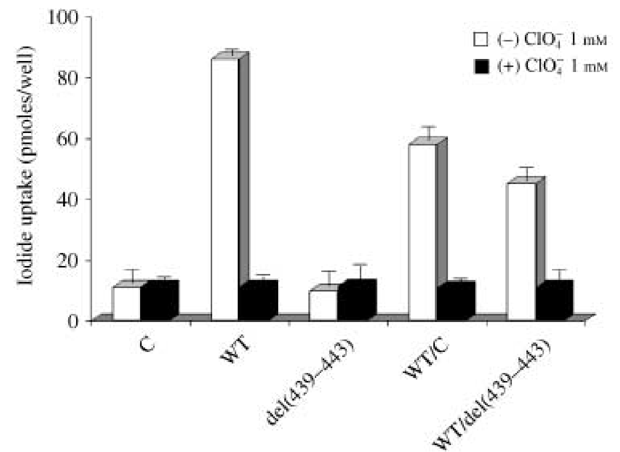

Fig. 4 Iodide uptake activity in COS-7 cells transfected with wt hNIS (wt), mutant NIS cDNA (del-439–443) or control empty vector (C). wt/mutant and wt/control indicate cells co-transfected with equal amounts of the two kinds of DNA constructs. No specific transport of iodide in the presence of 1 mM ClO_4^- is shown. □, (–) ClO_4^- 1 mM; ■, (+) ClO_4^- mM.

$NaClO_4^-$ (1 mM), a known inhibitor of iodide uptake, was used as control. These results suggest that the del-(439–443) hNIS protein does not interfere with the function of wt hNIS.

Discussion

We describe the first Italian case of CH due to failure of the thyroid gland to concentrate sufficient amounts of iodide for the normal synthesis of thyroid hormones. The patient showed signs and symptoms of hypothyroidism at the age of 3 months. Biochemical hypothyroidism was confirmed. The diagnosis of ITD was suspected on the basis of nodular goitre, hypothyroidism and

little if any uptake of ^{131}I in a normally located thyroid gland and the salivary glands. After treatment with iodide, TSH decreased and T4 increased. The genetic analysis of the hNIS gene of the patient revealed a 15 nt deletion from nt 1314 through nt 1328 and the insertion of 15 nt which corresponded to the duplication of the first 15 nt of the adjacent intron (Fig. 1). The patient was homozygous for this insertion/deletion. Direct sequencing of genomic DNA in both parents showed the same insertion/deletion in the heterozygous state (Fig. 1).

The genetic defect identified in our patient is predicted to produce a protein lacking five amino acids located at the 6th intracellular loop. Functional studies showed that cells transfected with the mutant del-(439–443) hNIS construct showed no perchlorate-sensitive iodide uptake, confirming that the mutation was the direct cause of ITD in this patients. When the mutant was co-transfected with the wt hNIS no dominant negative effect was observed. These data are in agreement with the clinical observation that the parents, heterozygous for the mutant allele, have no ITD. In our patient, hypothyroidism was partially corrected by the administration of high doses of iodide. Considering the absence of functional activity of this mutant allele, this effect of iodide overload can be attributed to simple diffusion of the ion. More intriguing is the observation of increase in FT4 and FT3 after the administration of rhTSH. It is possible that TSH stimulated the release of small amounts of stored hormone retained in the thyroid gland because of endogenous TSH suppression by L-T4.

By using immunohistochemistry with a monoclonal antibody directed against NIS, we were unable to reveal the protein in thyroid tissue specimen. Inability to detect the deleted hNIS mutant protein might be due to failure to synthesize the protein or to rapid degradation, or to absence of the epitopes reactive with the monoclonal antibody. The antibody used in immunohistochemistry has been proposed to react with the 6th extracellular loop (Pohlenz *et al*., 2000; Tonacchera *et al*., 2002). However, we cannot exclude that the deletion modifies the three-dimensional structure of the protein so that the antibody can not recognize the epitope any more.

Histological examination of the thyroid gland of our patient showed the presence of a small goitre with multiple partially encapsulated nodules. Most patients with ITD described in literature have goitre (Fujiwara *et al*., 1997, 2000; Matsuda & Kosugi, 1997; Pohlenz *et al*., 1997, 1998; Kosugi *et al*., 1998a, 1998b, 2002), but some cases without goiter have been reported (Fujiwara *et al*., 1998; Kosugi *et al*., 1998b, 1999). The amount of iodide intake has been suggested to influence the difference in goitre development (Kosugi *et al*., 1998b). Interestingly, no ITD patients in the Hutterite family described by Kosugi *et al*. (1999) developed goitre. This could be due to a specific characteristic of the mutation or to the fact of the early diagnosis in these patients who were identified by neonatal screening. It has been proposed that L-T4 treatment from the early neonatal period may prevent the development not only of cretinism but also of goitre (Kosugi *et al*., 1999). Goitre formation in our patient might be due to thyroid growth-stimulating effect of TSH, enhanced by low intrathyroidal iodide concentration (Dumont *et al*., 1992; Uyttersprot *et al*., 1997).

Fifty-five patients with iodide trapping defects from 29 families have been reported to date since the first case published in 1958 (Federman *et al*., 1958). The molecular cloning and characterization of NIS accounted the identification of several hNIS gene mutations in patients with ITD (Fujiwara *et al*., 1997, 2000; Matsuda & Kosugi, 1997; Pohlenz *et al*., 1997, 1998; Kosugi *et al*., 1998a, 1998b, 1999, 2002). Although all resulted in an iodide-trapping defect, the clinical pictures are heterogeneous. Some, like our patient, show severe hypothyroidism and cretinism while others remain euthyroid without mental and developmental disorders. A first homozygous hNIS gene mutation was reported by Fujiwara *et al*. (1997), in a Japanese patient, born to consanguineous parents, who presented with congenital hypothyroidism and nodular goitre caused by an iodide transport defect. In this patient, a T354P missense mutation was identified. *In vitro* expression of the mutated symporter revealed complete loss of iodide transport activity. The T354P mutation has been demonstrated in 14 Japanese patients with iodide-trapping defect (Fujiwara *et al*., 1998; Kosugi *et al*., 1998b). The T354P NIS mutation was shown to be overexpressed at the protein level by western blotting and immunohistochemistry analysis and the protein was properly located at the basal and lateral membranes of the thyrocyte (Kosugi *et al*., 1998a). Other gene mutations of hNIS have been reported in patients with ITD. The majority of gene defects are represented by missense mutations (Q267E, G93R, G543E, G395R) or truncated proteins (C272X). In contrast to hNIS T354P, the two mutant hNIS molecules Q267E and S515X were not found in significant amount on the surface of transfected COS cells indicating a defect of membrane targeting (Pohlenz *et al*., 2000). In one case, a C to G tranversion of nt 1940, produced a stop codon as well as a downstream cryptic 3′ splice acceptor site in exon 13, resulting in a 67 nt deletion, frameshift and premature stop codon (Pohlenz *et al*., 1998). Recently, Kosugi *et al*. (2002) identified a novel and peculiar loss-of-function germline mutation consisting of a large 6192-bp deletion and 431-bp inverted insertion, in two siblings of Spanish origin with total defect of iodide transport. The mechanism by which this deletion and the insertion/deletion we describe in the present manuscript are formed is not known.

Acknowledgements

This work was supported by the following grants: Ministero dell'Università e della Ricerca Scientifica (MURST), Programma

di Ricerca: Le malattie della tiroide: dalle basi molecolari alla clinica. Ministero dell'Università e della Ricerca Scientifica (MURST), Programma di Ricerca: Strategie per la valutazione degli effetti disruptivi dei contaminanti ambientali sul sistema endocrino degli animali e dell'uomo. CNR Progetto Biotecnologie CTB 99·00·224.PF 31: Basi molecolari delle neoplasie benigne e maligne della tiroide. Istituto Superiore di Sanità: Basi Molecolari dell'ipotiroidismo congenito: predizione, prevenzione ed intervento I.S.S. Centro Eccellenza AmbiSEN, Pisa. DK 15070 and RR 00055 from the National Institutes of health, USA.

References

Dumont, J.E., Lamy, F., Roger, P. & Maenhaut, C. (1992) Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. *Physiological Review*, **72**, 667–697.

Federman, D., Robbins, J. & Rall, J.E. (1958) Some observations on cretinism and its treatment. *New England Journal of Medicine*, **259**, 610–613.

Fujiwara, H., Tatsumi, K., Miki, K., Harada, T., Miyaik, K., Takai, S. & Amino, N. (1997) Congenital hypothyroidism caused by a mutation in the Na^+I^- symporter. *Nature Genetics*, **16**, 124–125.

Fujiwara, H., Tatsumi, K., Miki, K., Harada, T., Okada, S., Nose, O., Kodama, S. & Amino, N. (1998) Recurrent T354P mutation of the Na^+/I^- symporter in patients with iodide transport defects. *Journal of Clinical Endocrinology and Metabolism*, **84**, 3248–3253.

Fujiwara, H., Tatsumi, K., Tanka, S., Kimura, M., Nose, O. & Amino, N. (2000) A novel V59E missense mutation in the sodium iodide symporter gene in a family with iodide transport defect. *Thyroid*, **10**, 471–474.

Harden, R.M., Alexander, W.D., Chisholm, C.J.S. & Shimmins, J. (1968) The salivary iodide trap in non toxic goiter. *Journal of Clinical Endocrinology and Metabolism*, **28**, 117–120.

Kosugi, S., Inoue, S., Matsuda, A. & Jhiang, S.M. (1998a) Novel, missense and loss-of-function mutations in the sodium/iodide symporter gene causing iodide transport defect in three Japanese patients. *Journal of Clinical Endocrinology and Metabolism*, **83**, 3373–3376.

Kosugi, S., Sato, Y., Matsuda, A., Ohyama, Y., Fujieda, K., Inomata, H., Kameya, T., Isozaki, O. & Jhiang, S.M. (1998b) High prevalence of T354P sodium/iodide symporter mutation in Japanese patients with iodide transport defect who have heterogeneous clinical pictures. *Journal of Clinical Endocrinology and Metabolism*, **83**, 4123–4129.

Kosugi, S., Bhyayana, S. & Dean, H.J. (1999) A novel mutation in the sodium/iodide symporter gene in the largest family with iodide transport defect. *Journal of Clinical Endocrinology and Metabolism*, **84**, 3348–3253.

Kosugi, S., Okamoto, H., Tamada, A. & Sanchez-Franco, F. (2002) A novel peculiar mutation in the sodium/iodide symporter gene in Spanish siblings with iodide transport defect. *Journal of Clinical Endocrinology and Metabolism*, **87**, 3830–3836.

Levy, O., Vieja, A. & Carrasco, N. (1998) The Na^+I^- symporter (NIS): recent advances. *Journal of Bioenergetic Biomembranes*, **30**, 195–206.

Lopata, M.A., Cleveland, D.W. & Solmer-Wess, B. (1984) High level expression of a chloramphenicol acetyltransferase gene by DEAE–dextran mediated DNA transfection coupled with dimethyl-sulfoxide or glycerol shock treatment. *Nucleic Acids Research*, **12**, 5707–5711.

Matsuda, A. & Kosugi, S. (1997) A homozygous missense mutation of sodium/iodide symporter gene causing iodide transport defect. *Journal of Clinical Endocrinology and Metabolism*, **82**, 3966–3971.

Moreno, J.C., Bikker, H., Kempers, M.J.E., Van Trotsenburg, A.S.P., Baas, F., De Vijlder, J.J.M., Vulsma, T. & Ris-Stalpers, C. (2002) Inactivating mutations in the gene for thyroid oxidase 2 (THOX2) and congenital hypothyroidism. *New England Journal of icine* **347**, 95–102.

Pohlenz, J., Medeiros-Neto, G., Gross, J.L., Silveiro, S.P., Knobel, M. & Refetoff, S. (1997) Hypothyroidism in a brazilian kindred due to iodide trapping defect caused by a homozygous mutation in the sodium/iodide symporter gene. *Biochemistry and Biophysics Research Communications*, **226**, 339–345.

Pohlenz, J., Rosenthal, I.M., Weiss, R.E., Jhiang, S.M., Burant, C. & Refetoff, S. (1998) Congenital hypothyroidism due to mutations in the sodium/iodide symporter: identification of a nonsense mutation producing a downstream cryptic-3′ splice site. *Journal of Clinical Investigations*, **101**, 1028–1035.

Pohlenz, J., Duprez, L., Weiss, R.E., Vassart, G., Refetoff, S. & Costagliola, S. (2000) Failure of membrane targeting causes the functional defect of two mutant sodium iodide symportes. *Journal of Clinical Endocrinology and Metabolism*, **85**, 2366–2369.

Refetoff, S., Vassart, G. & Dumont, J.E. (2001) Thyroid disorders. In: *The Metabolic and Molecular Basis of Inherited Disease.* (eds C.R. Sciver, A.L. Beaudet, W.S. Sly, D. Valle), pp. 2883–2928. McGraw-Hill Inc., New York.

Smanik, P.A., Ryu, K.Y., Theil, K.S., Mazzaferri, E.L. & Jhiang, S.M. (1997) Expression, exon–intron organization, and chromosome mapping of the human sodium iodide symporter. *Endocrinology*, **138**, 3555–3558.

Stanbury, J.B. & Chapman, E.M. (1960) Congenital hypothyroidism with goiter: absence of an iodide-concentrating mechanism. *Lancet*, **1**, 1162–1165.

Tonacchera, M., Agretti, P., Ceccarini, G., Lenza, G., Refetoff, S., Santini, F., Pinchera, A., Chiovato, L. & Vitti, P. (2001) Autoantibodies from patients with autoimmune thyroid disease do not interfere with the activity of the human iodide symporter gene stably transfected in CHO cells. *European Journal of Endocrinology*, **144**, 611–618.

Tonacchera, M., Viacava, P., Agretti, P., De Marco, P., Perri, A., Di Cosmo, C., De Servi, M., Miccoli, P., Lippi, F., Naccarato, A.G., Pinchera, A., Chiovato, L. & Vitti, P. (2002) Benign nonfunctioning thyroid adenomas are characterized by a defective targeting to cell membrane or a reduced expression of the sodium iodide symporter protein. *Journal of Clinical Endocrinology and Metabolism*, **87**, 352–357.

Uyttersprot, N., Pelgrims, N., Carrasco, N., Gervy, C., Maenhaut, C., Dumont, J.E. & Miot, F. (1997) Moderate doses of iodide in vivo inhibit cell proliferation and the expression of thyroperoxidase and Na^+I^- symporter mRNAs in dog thyroid. *Molecular and Cellular Endocrinology* **131**, 195–203.

Vulsma T, de Vijlder J.M. Thyroid disease in newborns, infants and children. In: *Endocrinology and Diabetes.* (eds J.A.H. Wass, S.M. Shalet), pp. 532–541. Oxford Press, Oxford.

Wolff, J. (1983) Congenital goiter with defective iodide transport. *Endocrine Review*, **4**, 240–254.