state; the DNA change consisted this time of a substitution of an aspartic acid at position 312 with an asparagine in the AIRE gene region coding for the PHD (D312N in exon 8). The authors comment on the possible evolution of the clinical manifestations of this patient to APS1. Alternatively, this may reinforce the hypothesis of the contribution of heterozygous AIRE mutations to the pathogenesis of autoimmune conditions other than autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. This could only be unravelled through extended epidemiological investigations in patients and controls and may provide valuable insights as to whether the combination of genetic and immunological markers may contribute to the development of autoimmunity.

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doi: 10.1111/j.1365-2265.2010.03930.x

References

1 Fierabracci, A. (2010) Recent insights into the role and molecular mechanisms of the autoimmune regulator (AIRE) gene in autoimmunity. *Autoimmunity Reviews*, doi:10.1016/j.autrev.2010.98.019.
2 Cetani, F., Barbesino, G., Corsari, S. *et al.* (2001) A novel mutation of the autoimmune regulator gene in an italian kindred with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, acting in a dominant fashion and strongly cosegregating with hypothyroid autoimmune thyroiditis. *Journal of Clinical Endocrinology and Metabolism*, **86**, 4747–4752.
3 Su, M.A., Giang, K., Zuuumer, K. *et al.* (2008) Mechanisms of an autoimmunity syndrome in mice caused by a dominant mutation in AIRE. *The Journal of Clinical Investigation*, **118**, 1712–1726.
4 Chentoufi, A.A. & Polychronakos, C. (2002) Insulin expression levels in the thymus modulate insulin-specific autoreactive T-cell tolerance: the mechanism by which the IDDM2 locus may predispose to diabetes. *Diabetes*, **51**, 1383–1390.
5 Fan, Y., Rudert, W.A., Grupillo, M. *et al.* (2009) Thymus-specific deletion of insulin induces autoimmune diabetes. *EMBO Journal*, **28**, 2812–2824.
6 Pugliese, A., Zeller, M., Fernandez Jr, A. *et al.* (1997) The insulin gene is transcribed in the human thymus and transcription levels correlate with allelic variation at the INS VNTR-IDDM2 susceptibility locus for Type 1 diabetes. *Nature Genetics*, **15**, 293–297.
7 Vafiadis, P., Bennet, S.T., Todd, J.A. *et al.* (1997) Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nature Genetics*, **15**, 289–292.
8 Cavadini, P., Vermi, W., Facchetti, F. *et al.* (2005) AIRE deficiency in thymus of 2 patients with Omenn syndrome. *The Journal of Clinical Investigation*, **115**, 728–732.
9 Cervato, S., Morlin, L., Albergoni, M.P. *et al.* (2010) AIRE gene mutations and autoantibodies to interferon omega in patients with chronic hypoparathyroidism without APECED. *Clinical Endocrinology*, **73**, 630–636.
10 Toth, B., Wolff, A.S.B., Halasz, Z. *et al.* (2010) Novel sequence variation of AIRE and detection of interferon-ω antibodies in early infancy. *Clinical Endocrinology*, **72**, 641–647.

A new compound heterozygous for c.886C>T/c.2206C>T [p.R277X/p.Q717X] mutations in the thyroglobulin gene as a cause of foetal goitrous hypothyroidism

The identification of thyroglobulin (TG) mutations has contributed significantly to the understanding of the molecular pathogenesis of congenital hypothyroidism because of dyshormonogenesis, an autosomal recessive inherited disorder characterized by congenital goitre. Fifty-one germline mutations have been identified in the human TG.[1,2] Although the incidence of TG mutations is still unknown in France, previous studies have reported four TG mutations in two compound heterozygous patients.[2,3] In the first, a foetal goitrous hypothyroidism, the paternal mutation consisted of a cytosine deletion at nucleotide 1143 in exon 9 (c.1143delC), resulting in a frameshift which generated a stop codon at position 382 (p.G362fsX382), and the maternal mutation was a c.6725G>A in exon 38, creating the p.R2223H missense mutation in the acetylcholinesterase (ACHE)-homology domain of the TG.[3] The second patient was heterozygous for a nonsense mutation because of a c.4588C>T at exon 22 [p.R1511X] (father's mutation) and for a c.5386C>T at exon 27 [p.Q1777X] (mother's mutation).[2]

The aim of the present study was to characterize a novel compound heterozygous for TG mutations in a French family with foetal goitrous hypothyroidism.

In a 27-year-old white women, a foetal goitre was diagnosed coincidentally by ultrasound at the 22th week of gestation during her first pregnancy. The ultrasound examination showed enlargement of the goitre (thyroid circumference 105 mm; >99th percentile for gestational age),[4] but foetal vitality and amniotic fluid volume appeared normal. There was no historical evidence of iodine deficiency in the family, and the parents were nonconsanguineous. The thyroid parameters of the mother when foetal goitre was diagnosed were in the normal range, with no circulating autoantibodies (anti-TPO, anti-TG and anti-TSH receptor). Percutaneous umbilical vein blood sampling under ultrasound guidance was performed at the 25th week of gestation, showing important foetal hypothyroidism with high serum TSH (384 mU/l; reference values 10.2 ± 3.8 mU/l) and low free T_4 (5.2 pmol/l; reference values 13.10 ± 1.60 pmol/l) levels. An intra-amniotic injection of 180 μg L-thyroxine was performed at the end of the 27th week, at the beginning of the 30th week and at the end of the 32th week of gestation. At the 33th week, thyroid circumference decreased to 76 mm. The woman had a caesarean delivery at the 34th week of gestation for foetal suffering and delivered a female infant with a weight of 1980 g, a body height of 40.5 cm and a cranial circumference of 30 cm with an Apgar score of 10 at 1 min. The neonate had a moderate asymmetric thyroid goitre with right predominance. Scintigraphy showed a normally located thyroid gland with a goitre which was confirmed by ultrasound imaging. The right lobe measured 22 × 15 × 8 mm and the left lobe 20 × 10 × 8 mm. The lack of a complete reduction of the foetal goitre after intra-amniotic treatment in our case is in perfect agreement with the observed in a recently published cohort of 12 treated foetuses.[5] Intra-amniotic treatment led to a reduction in the size of the goitre in 90% of the cases, but complete reduction was never observed with L-thyroxine

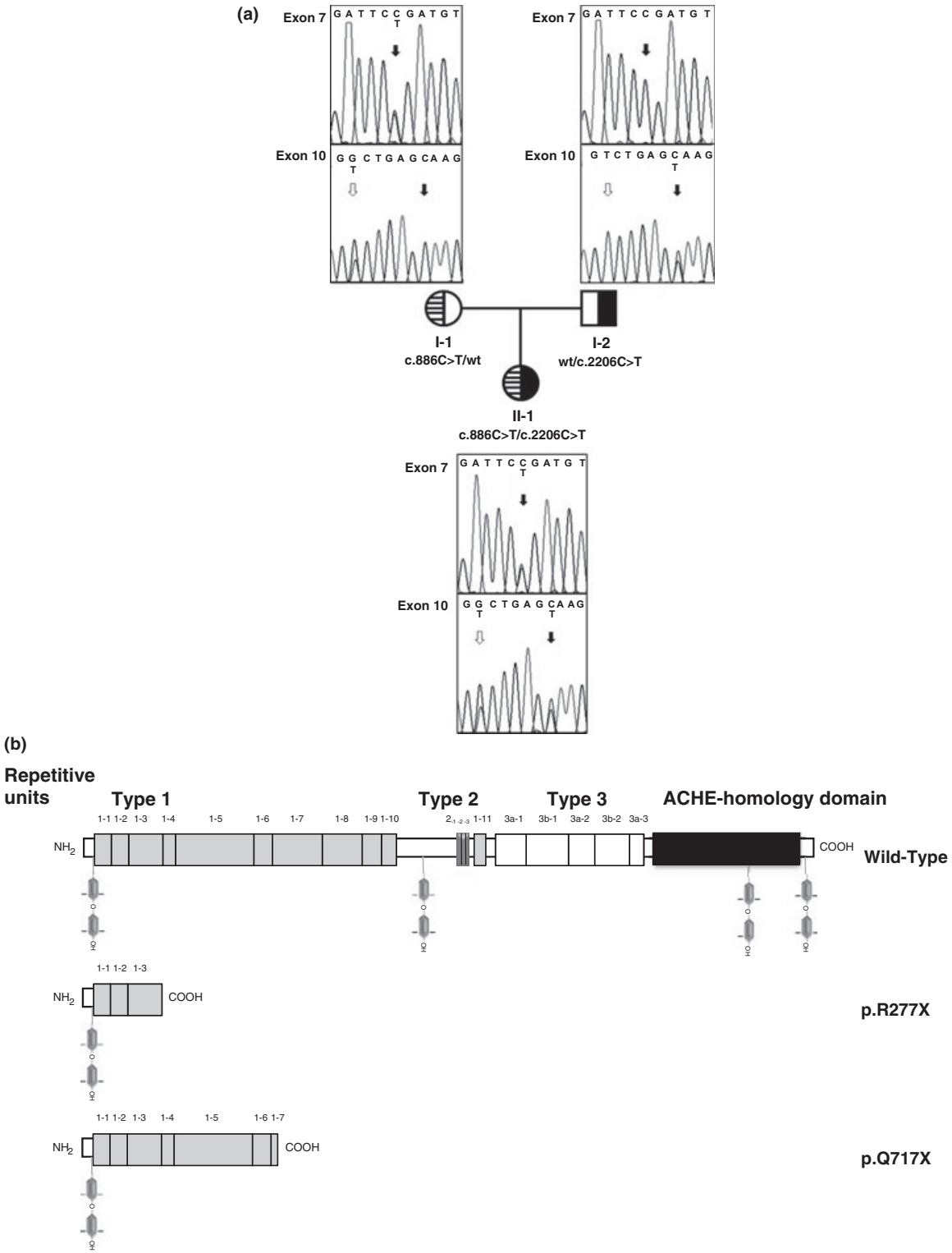

Fig. 1 Mutations in the thyroglobulin gene in index patient and their parents. (a) Sequencing chromatograms of genomic DNA are shown. The pedigree shows the pattern of inheritance of the mutant thyroglobulin alleles. Squares represent men and circles women. Filled symbols denote affected individuals and half-filled symbols, unaffected heterozygote individuals. The solid symbols indicate the c.2206C>T mutated allele and the hatched symbols the c.886C>T mutated allele. Sense strand is shown. Black arrows denote the positions of identified mutations, white arrows indicate polymorphism (c.2200T>G [p.S715A]), single chromatogram peaks denote homozygosity and two overlapping peaks at the same locus, heterozygosity. (b) Schematic representation of the repetitive, acetylcholinesterase-homology and hormonogenic domains in the wild-type and putative mutant thyroglobulin proteins (p.R277X and p.Q717X). The repetitive units (Type 1, 2 and 3) and the acetylcholinesterase (ACHE)-homology domain are represented by boxes. The N-terminal limit of repeat Type 1–5 is ambiguous. The positions of T_4 and T_3 are shown.

dosage ranging from 200 to 800 μg per injection and number of injections from 1 to 6, thus indicating that the optimal management of this disorder was still to be determined. Thyroid functions tests in our patient indicated elevated TSH (359 mUI/l; reference values: 0.5–5.0 mUI/l), low free T_4 (5.8 pmol/l, reference values: 10–25 pmol/l) and low normal free T_3 (4.2 pmol/l, reference values: 4–7 pmol/l) levels and anti-TPO negative. The serum TG concentration was low (0.3 μg/l, reference values: >30 μg/l) suggesting that dyshormonogenetic goitre could be related to defective TG synthesis. Treatment with L-thyroxine drops was started at 4 days (15 μg/kg per day). She had a very good compliance, and her growth was normal. She had been evaluated at the age of 4 using the Battery for Rapid Evaluation of Cognitive Functions (BREV), and her results were in normal range.

Written informed consent was obtained from the parents, and the research project was approved by the Institutional Review Board.

Sequence analysis of the TG gene revealed that the index patient was heterozygous for a previously documented nonsense mutation owing to a cytosine to thymine transition at nucleotide 866 in exon 7 (c.886C>T, mother's mutation) in one allele and for a novel also cytosine to thymine transition at nucleotide position 2206 in exon 10 (c.2206 C>T, father's mutation) in the other allele (Fig. 1). The c.886C>T and c.2206 C>T mutations resulted in premature stop codons at amino acids 277 [p.R277X] and 717 [p.Q717X], respectively (Fig. 1).

The p.R277X mutation is the most frequently reported mutation in the TG gene, and affected individuals are either homozygous or compound heterozygous.[1] The clinical spectrum of the homozygous patients ranges from moderate to severe goitrous hypothyroidism. This putative truncated protein of 276 amino acids eliminates eight repetitive units Type 1, from Type 1–4 to 1–11, all elements of Type 2 and Type 3 repetitive, and ACHE-homology domain of TG (Fig. 1). These domains are also eliminated in the putative truncated protein p.Q717X, with the difference that the deletion of Type 1 domain is localized between repeat motifs Type 1–7 and Type 1–11 (Fig. 1). ACHE-homology domain may function as an intramolecular chaperone for the efficient transport of the TG to extracellular space.[1] It has been demonstrated that the presence of a stop codon in the position 175 in the mouse TG still allows the secretion of a truncated protein.[1] This observation suggests that the intracellular transport and the secretion capacity continue to work despite the complete deletion of ACHE-like region, as observed in the p.R277X and p.Q717X peptides. In our reported case, some foetal L-T_4 are present at the 25th week of gestation, indicating that both early truncated TG proteins could be externalized to the thyroid follicles where the enzymatic iodinating machinery could iodinate the truncated TG proteins, which still harbour both the hormonogenic acceptor tyrosine 5 and the donor tyrosine 130.

In conclusion, we here describe a French family in whom a new compound heterozygous constellation (p.R277X/p.Q717X) was identified in the TG gene as the underlying molecular defect for a foetal goitrous hypothyroidism. The prevalence of a limited number of mutations in each population will facilitate greatly the molecular genetic testing.

Acknowledgements

C.E. Citterio is a research fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). C.M. Rivolta and H.M. Targovnik are established investigators of the (CONICET). This work was supported by grants from Universidad de Buenos Aires (B 078/2008 to HMT), CONICET (PIP 112-2000801-0054/2009 to CMR and HMT) and Fundación Mutua Madrileña to RG-S.

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doi: 10.1111/j.1365-2265.2010.03932.x

References

1 Targovnik, H.M., Esperante, S.A. & Rivolta, C.M. (2010) Genetics and phenomics of hypothyroidism and goiter due to thyroglobulin mutations. *Molecular and Cellular Endocrinology*, **322**, 44–55.

2 Targovnik, H.M., Souchon, P.F., Machiavelli, G.A. *et al.* (2010) Congenital goitre with hypothyroidism caused by a novel compound heterozygous mutations in the thyroglobulin gene. *Clinical Endocrinology*, **72**, 716–718.

3 Caron, P., Moya, C.M., Malet, D. *et al.* (2003) Compound heterozygous mutations in the thyroglobulin gene (1143delC and 6725G>A[R2223H]) resulting in fetal goitrous hypothyroidism. *Journal of Clinical Endocrinology and Metabolism*, **88**, 3546–3553.

4 Ranzini, A.C., Ananth, C.V., Smulian, J.C. *et al.* (2001) Ultrasonography of the fetal thyroid: nomograms based on biparietal diameter and gestational age. *Journal of Ultrasound in Medicine*, **20**, 613–617.

5 Ribault, V., Castanet, M., Bertrand, A.-M. *et al.* (2009) Experience with intraamniotic thyroxine treatment in nonimmune fetal goitrous hypothyroidism in 12 cases. *Journal of Clinical Endocrinology and Metabolism*, **94**, 3731–3739.