Clinical Endocrinology (2009) **70**, 623–628 doi: 10.1111/j.1365-2265.2008.03367.x

ORIGINAL ARTICLE

Expanding clinical spectrum of non-autoimmune hyperthyroidism due to an activating germline mutation, p.M453T, in the thyrotropin receptor gene

Vichit Supornsilchai*, Taninee Sahakitrungruang*, Nattakarn Wongjitrat*, Suttipong Wacharasindhu*, Kanya Suphapeetiporn† and Vorasuk Shotelersuk†

**Division of Pediatric Endocrinology and †Division of Medical Genetics and Metabolism, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

Summary

Objective To describe clinical and genetic features of a Thai family with non-autoimmune hyperthyroidism (NAH) caused by an activating germline mutation in the *thyrotropin receptor* (*TSHR*) gene.

Patients Three affected individuals from the same family (a father and his two children) were studied. Clinical and imaging findings were reviewed and compared.

Genetic analysis Genomic DNA was extracted from peripheral blood leukocytes and mutation analysis of the entire coding sequence of the *TSHR* gene was performed in both children and their parents by direct DNA sequencing.

Results A heterozygous germline T to C transition in exon 10 of the *TSHR* gene (c.1358T→C) resulting in the substitution of methionine (ATG) by threonine (ACG) at codon 453 (p.M453T) was identified in the father and his two children. They presented with different clinical severity and variable age of onset. In addition to hyperthyroidism, ventriculomegaly and bilateral shortening of the fifth metacarpal bones and the middle phalanges of the fifth fingers were consistently found in all affected individuals.

Conclusions Ventriculomegaly and bilateral shortening of the fifth metacarpal bones and the middle phalanges of the fifth fingers might be characteristic features of NAH because of an activating *TSHR* germline mutation. In addition, the shortening of the middle phalanges of the fifth fingers has never been previously described, expanding the phenotypic spectrum of the disease.

(Received 28 February 2008; returned for revision 7 March 2008; finally revised 23 April 2008; accepted 21 July 2008)

Correspondence: Kanya Suphapeetiporn, Division of Medical Genetics and Metabolism, Department of Pediatrics, Sor Kor Building 11th floor, King Chulalongkorn Memorial Hospital, Bangkok 10330, Thailand.
Tel.: +662-256-4989; Fax: +662-245-4911; E-mail: kanya.su@chula.ac.th

Introduction

Hereditary non-autoimmune hyperthyroidism (NAH), a rare autosomal dominant disorder, is caused by activating germline mutations in the thyrotropin receptor (*TSHR*) gene.[1] There have been 16 families with NAH because of different activating germline mutations in the *TSHR* gene reported to date.[1–14] *De novo* activating *TSHR* germline mutations have also been identified in 11 individuals with sporadic NAH.[15–24] The clinical manifestations of NAH are highly variable, which include the age of onset, severity of hyperthyroidism and goitre size.[20] Hyperthyroidism is typically refractory to antithyroid drugs. Thyroid ablation by total thyroidectomy or radiotherapy is therefore recommended.[25]

Genotype-phenotype correlation has not been clarified.[9] Several clinical entities have been found to be associated with this condition regardless of the type of mutations. These include advanced bone age,[2,16,17,19,21] craniosynostosis,[18,23,26] exophthalmos,[16,27] jaundice and hepatosplenomegaly,[16] mitral valve prolapse,[5] preterm delivery and low birth weight,[11] psychomotor and speech retardation,[2,15,17,26] shortening of fifth metacarpal bones,[23] thrombocytopenic purpura,[16] and ventriculomegaly.[23]

In this study, we report a family with three members affected with NAH because of an activating *TSHR* mutation, p.M453T. They presented with different clinical severity and variable age of onset. However, similar brain structural anomalies and skeletal deformities were identified in all affected individuals. We also compared the phenotype seen in this family with that of two previously reported sporadic cases with the same mutation.

Subjects and methods

The proband (III-2, Fig. 1) was a 4-year-old-girl referred to our hospital at 24 months of age because of uncontrollable hyperthyroidism and developmental delay. She was born prematurely at 30 weeks gestation with a birth weight of 1800 g and was observed in the hospital for one week without any serious complication. Retrospectively, her mother reported that diarrhoea, and poor weight gain had been observed since 4 months of age. She was, however, diagnosed

Table 1. Assessment of thyroid function in the proband and the younger brother

Subject	Age	TSH (mIU/ml)	FT3 (pmol/l)	FT4 (pmol/l)	Therapy
Proband	18 months	< 0·003	25·93	20·80	PTU 75 mg/day
					Propranolol 10 mg/day
	2 years	0·01	12·83	12·10	PTU 75 mg/day
					Propranolol 15 mg/day
	2·3 years	0·05	15·32	11·97	PTU 75 mg/day
	2·8 years	< 0·005	16·14	11·02	PTU 75 mg/day
	3·2 years	0·011	12·72	8·87	PTU 100 mg/day
	3·4 years	0·005	10·89	9·63	PTU 125 mg/day
	4 years	0·02	14·94	24·84	PTU 150 mg/day
					Subtotal thyroidectomy
					PTU 125 mg/day after surgery
	4·4 years	4·2	6·44	7·34	PTU 125 mg/day
Brother	2 months	0·006	8·64	35·39	PTU 25 mg/day
					Propranolol 0·7 mg/day
	3·5 months	< 0·005	8·53	28·06	PTU 37 mg/day
					Propranolol 1·5 mg/day
	8 months	0·018	10·15	10·94	PTU 38 mg/day
Normal range		0·3–4·1	4·57–7·99	10·30–23·17	

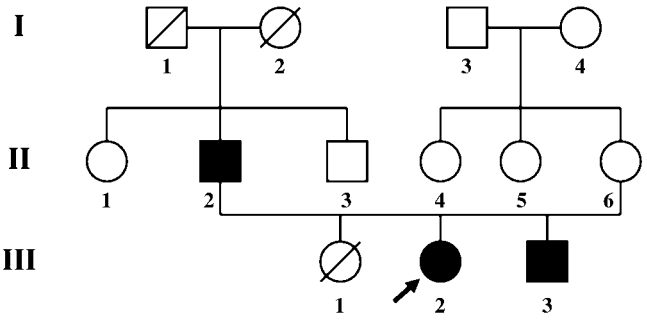

Fig. 1 Pedigree of the family (the proband; III-2, the father; II-2 and the brother; III-3). Circle, women; boxes, male; arrow, proband; closed circles or boxes, affected cases.

with hyperthyroidism at the age of 8 months. Physical examination at 24 months of age revealed frontal bossing, scaphocephaly, exophthalmos with lid lag and retraction, midfacial hypoplasia and goitre. Her weight was 8 kg (< 3rd percentile) and her height was 81 cm (10th percentile). Her head circumference was 47 cm, which was on the mean. Thyroid function tests (TFTs) are shown in Table 1. Antithyroglobulin and antithyroid peroxidase antibodies were negative. Thyroid ultrasound showed diffuse enlargement of thyroid gland with heterogeneous echogenicity. Her bone age was significantly advanced (chronological age 2 years old; bone age 6 years and 10 months old). The patient was initially treated with propylthiouracil (PTU) and propanolol (Table 1). In addition to hyperthyroidism, she had gross motor delay and speech delay, for which she received an early intervention program. On a follow-up visit at 4 years of age, physical examination revealed similar findings (Fig. 2a). Radiography of the hands showed bilateral shortening of the fifth metacarpal bones and the middle phalanges of the fifth fingers (Fig. 2d). The magnetic resonance imaging (MRI) of the brain revealed partial obstruction of the cerebral aqueduct and ventriculomegaly (Fig. 2g). Because of uncontrollable hyperthyroidism despite high doses of PTU, she underwent subtotal thyroidectomy at the age of 4 years. Histology showed diffuse hyperplasia of the thyroid gland.

The patient's father (II-2, Fig. 1) was 36 years old when he was first seen by us. He was diagnosed with hyperthyroidism at the age of 1 month. He had no perinatal complications and achieved normal milestones for age. He was treated with high doses of PTU for several years. He subsequently underwent thyroidectomies at the age of 8 and 18 years. Because of uncontrollable hyperthyroidism, he eventually underwent three radioiodine therapies at the age of 18, 21 and 28 years, respectively. Since then, his thyroid function tests have been within the normal range without any additional treatment. He has normal intelligence and is currently employed. His physical examination revealed scaphocephaly, exophthalmos, small hands and feet (Fig. 2b). Radiography of the hands showed bilateral shortening of the fifth metacarpal bones and the middle phalanges of the fifth fingers (Fig. 2e). The computer tomography examination (CT) of the brain was performed and demonstrated ventriculomegaly with normal brain parenchyma (Fig. 2h).

The younger brother (III-3, Fig. 1) was born prematurely at 34 weeks gestation with a birth weight of 2350 g. At 2 days old, he developed cholestatic jaundice with generalized petechial hemorrhage. Scaphocephaly, mild craniosynostosis and exophthalmos were noted (Fig. 2c). He also had hepatic and splenic enlargement. An infectious aetiology was suspected but subsequently excluded. Because of a strong family history and awareness of nonautoimmune hyperthyroidism, thyroid function tests were performed and confirmed the diagnosis (Table 1). Antithyroglobulin and antithyroid peroxidase antibodies were negative. PTU and propanolol were immediately started. CT of the brain showed ventriculomegaly without any evidence of increased intracranial pressure (Fig. 2i). During a follow-up, cholestatic jaundice completely resolved at the age of 6 months. His bone age was advanced (chronological age 8 months; bone age 2 years). Radiography of the hands at 8 months of age

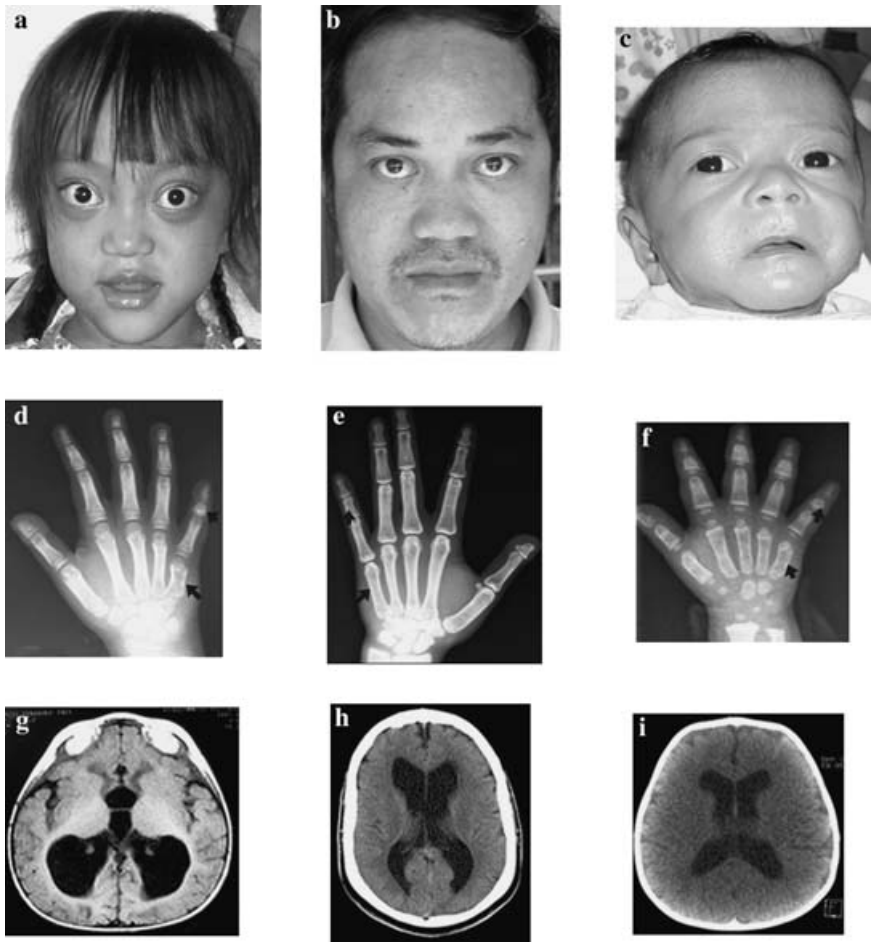

Fig. 2 The facial appearance and the radiological findings of the proband (a, d and g), the father (b, e and h) and the brother (c, f and i) at the age of 4 years, 38 years and 1 month, respectively. Arrows indicate the affected bones.

revealed mild shortening of the fifth metacarpal bones and the middle phalanges of the fifth fingers (Fig. 2f). His developmental milestone at 8 months olds remained appropriate.

Laboratory evaluations of thyroid function

Thyrotropin (TSH), serum free thyroxine (FT4) and free triiodothyronine (FT3) were measured by electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics, Indianapolis, IN). Antithyroglobulin and antithyroperoxidase antibodies were measured with immunochemiluminescent assay (Abbott Laboratories, Abbott Park, IL).

Sequence determination

After informed consent was obtained, genomic DNA was extracted from peripheral leukocytes according to standard protocols. Exons 1–10 of the *TSHR* gene were amplified by polymerase chain reaction (PCR). The sets of primers and reaction conditions are available upon request. We used 100 ng of genomic DNA, 1 × PCR buffer (Promega, Madison, WI), 1·875 mM $MgCl_2$, 0·2 mM dNTPs, 0·25 μM of each primer and 0·5 U Taq DNA polymerase (Promega) in a total volume of 20 μl. The PCR products were verified for correct size on ethidium bromide-stained 1·5% agarose gel. The PCR products were then treated with ExoSAP-IT (USP Corporation, Cleveland, OH) according to the manufacturer's recommendations, and sent for direct sequencing at the Macrogen Inc., Seoul, Korea. The sequence was analyzed using Sequencher (version 4·2; Gene Codes Corporation, Ann Arbor, MI).

Results

Analysis of the *TSHR* gene by PCR-sequencing identified a heterozygous T to C transition in exon 10 at nucleotide position 1358 (c.1358T→C) from the proband, her brother and the father (data not shown). The mutation was not present in their unaffected mother. The heterozygous mutation results in the substitution of methionine (ATG) by threonine (ACG) at codon 453 (p.M453T) in the second transmembrane domain of the TSHR.

Discussion

We describe the clinical and genetic features of a family with three members affected with nonautoimmune hyperthyroidism caused by an activating *TSHR* germline mutation. The p.M453T mutation has previously been reported in two sporadic cases, one presented with severe neonatal hyperthyroidism and the other presented with

Table 2. Clinical findings of patients with nonautoimmune hyperthyroidism due to p.M453T germline mutation in the *TSHR* gene

Features	III-2 (proband)	II-2 (father)	III-3 (proband's brother)	Patient reported by De Roux *et al.*	Patient reported by Lavard *et al.*
Age at diagnosis	8 months	1 month	At birth	At birth	7 months
Gestation at delivery* (weeks)	30	N/A	34	32·5	36
Birth weight (g)	1800	N/A	2350	1690	3040
Craniosynostosis	Yes	Yes	Yes	No	No
Exophthalmos	Yes	Yes	Yes	Yes	Yes
Ventriculomegaly	Yes	Yes	Yes	N/A	N/A
Hepatomegaly	No	No	Yes	Yes	No
Splenomegaly	No	No	Yes	Yes	Yes
Jaundice	No	No	Yes	Yes	No
Thrombocytopenic purpura	No	No	Yes	Yes	No
Advanced bone age	Yes	Yes	Yes	N/A	Yes
Shortening of fifth metacarpi and middle phalanges	Yes	Yes	Yes	N/A	N/A
Psychomotor development	Delay	Grossly normal	N/A	N/A	Grossly normal

*Premature birth (37 weeks or less).
NA, not available.

thyrotoxicosis at age 7 months (Table 2).[16,27] Functional studies of this mutation have demonstrated a sevenfold enhancement in basal cAMP accumulation by the mutated receptor compared with the wild-type receptor.[16]

A considerable phenotypic variation has been observed in different patients with identical *TSHR* mutations even within the same family, suggesting a role of other genetic, epigenetic or environmental factors.[9,10,20] All three affected individuals in our studied family had a different age of onset with the earliest in the neonatal period. The last affected family member presented with hyperthyroidism, exophthalmos, hepatic and splenic enlargement, thrombocytopenic purpura and jaundice. These clinical features were consistent with those of a previous report of a sporadic congenital case with the same mutation.[16] Interestingly, additional features, craniosynostosis, ventriculomegaly and digital abnormalities were identified in our patient. These features were also observed in his affected father and older sister, but not in his unaffected mother.

Both children in this family with an activating p.M453T germline mutation in the *TSHR* gene were born prematurely with appropriate birth weight. Previous reports demonstrated that premature birth and low birth weight were also important features of the disorder.[11,12] Premature delivery was also found in mothers with Graves' disease and was probably due to placental-transferred TSH stimulating antibodies acting on the chorionic gonadotropin (CG) receptor.[11,28] However, the mechanism underlying premature delivery in patients with activating *TSHR* germline mutations remains unknown at present. There is increasing evidence of the TSHR expression in extra-thyroid tissues, such as adipocytes, fibroblasts, bone, and placental tissues (http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist = Hs.160411).[29,30] Therefore, it remains possible that activating TSHR in the placenta derived from foetal tissues might have an effect on the timing of delivery. How TSHR or its downstream signalling pathway regulates this process is still unclear. Further studies in a mouse model for NAH would help to elucidate the underlying mechanism.

Craniosynostosis associated with NAH was previously identified in three sporadic cases with congenital autoimmune hyperthyroidism, both of whom required neurosurgical intervention because of hydrocephalus.[17,18,23] Our three patients had craniosynostosis without any sign of increased intracranial pressure and did not require any surgical intervention. As craniosynostosis was rarely found in NAH, there was no guideline regarding management of this particular condition. In addition, it might not progress with the remission of hyperthyroidism after medical or surgical treatment. A careful follow-up is therefore required before any neurosurgical intervention is performed. Our three patients also developed venticulomegaly, a recognized effect of thyroid hormones on the brain. With high levels of thyroid hormones, brain parenchyma will become thinner leading to relative ventriculomegaly.[31] The pathogenic mechanism underlying an effect of inappropriately high levels of thyroid hormones on the brain remains unclear. Nevertheless, it is interesting to note that ventriculomegaly, found in patients with activating *TSHR* germline mutations, is irreversible as seen in our adult patient.

Exophthalmos, previously thought to be specific to Graves' disease (GD) and thought to be mediated through an immune mechanism, was present in all three patients. Exophthalmos in GD was characterized by an increase in the volume of the orbital fat, connective tissues and extraocular muscles within the orbit. A recent study has revealed that adipogenesis within the orbit with enhanced expression of TSHR in orbital adipocytes may be a central player in the pathogenesis of exopthalmos seen in patients with GD.[32] The TSHR is expressed in the orbital adipose tissues and acts as an autoantigen, playing a role in initiation or progression of the autoimmune process. How increased expression of TSHR leading to stimulation of orbital adipogenesis remains unclear. Various cytokines or immune mediators present in the orbits may be involved. Alternatively, it remains possible that TSHR

can directly activate the process of adipogenesis. This latter mechanism might explain the exophthalmos seen in some of the patients with NAH caused by an activating germline mutation in the *TSHR* gene. Further investigations of genes involved in adipogenesis or adipocyte-specific genes in orbital tissues from patients with NAH or a mouse model for NAH are needed to verify this hypothesis.

Recognition of this disease and further investigation by genetic testing are required for accurate diagnosis. The diagnosis of NAH resulted from an activating *TSHR* mutation has important implications for both management and genetic counselling. Hyperthyroidism associated with this condition is characteristically aggressive and often requires thyroid ablation for long-term remission. Most patients suffering from nonautoimmune hyperthyroidism are associated with impaired neurological function including mental retardation and speech disturbance.[2,15,17,26] Early and effective control of hyperthyroidism is therefore essential to prevent permanent psychomotor retardation and other complications. The proband had developmental delay, which might be partly due to unawareness of this condition and thus the delay of treatment. After the definite diagnosis was made in this family, her brother who also inherited the causative mutation was immediately treated with high doses of antithyroid drugs after hyperthyroidism was detected. Other features found in this patient including jaundice, hepatosplenomegaly and thrombocytopenic purpura gradually resolved. In addition, his developmental milestones remained appropriate for age.

There have been limited studies regarding the effect of thyroid hormones and bone deformity. Accelerated skeletal maturation, craniosynostosis and brachydactyly identified in neonatal hyperthyroidism were first reported by Riggs *et al.*[33] A recent study found an individual with sporadic congenital hyperthyroidism caused by an activating p.L512N mutation in the *TSHR* gene.[23] In addition to craniosynostosis, this patient also had bilateral shortening of the fifth metacarpal bones as well as the third and fourth metatarsi. All our patients with the p.M453T mutation had skeletal deformities particularly in the acral part of extremities, bilateral shortening of the fifth metacarpal bones and the middle phalanges of the fifth fingers, which might be characteristic findings in patients with activating *TSHR* gene mutation. The bone abnormalities seemed to be irreversible as they still presented in our adult patient. Studies of patients with NAS which represent naturally occurring human models of increased thyroid hormone action could give insights into the perplexing effect of central and peripheral thyroid status on human skeletal growth and development. Thyroid hormones are crucial for normal skeletal growth and the maintenance of bone mass as evidenced by thyrotoxicosis resulting in advanced bone age and decreased bone mass whereas hypothyroidism leading to impaired bone formation and growth retardation. It was previously believed that bone loss identified in thyrotoxicosis was a direct consequence of thyroid hormone excess. However, an unexplored consequence of decreased levels of TSH due to hyperthyroidism was started to be elucidated by a subsequent study in *TSHR* null mice showing that TSH might be a direct negative regulator of bone turnover acting through the TSH receptor on both osteoblasts and osteoclasts.[30] The *TSHR*–/– mice were runted, hypothyroid, and died by 10 weeks of age. Severe osteoporosis with focal osteosclerosis was also observed. The heterozygous *TSHR*+/– mice, however, have normal levels of T3, T4, and TSH with a significant decrease in bone density. These findings indicate an important role of *TSHR* and its downstream signalling pathway in bone homeostasis. Interestingly, supplementation of the *TSHR* null mice (*TSHR*–/–) with thyroid hormones normalizes body weight, but not bone mass. This observation suggested that the osteoporosis arose from *TSHR* deficiency rather than altered thyroid hormone levels. However, this was challenged by a recent study demonstrating a role of two thyroid hormone receptor isoforms, TRα and TRβ on skeletal development.[34] TRα deficiency induces osteosclerosis in adult mice with normal circulating thyroid hormone and TSH levels while TRβ deficiency with elevated TSH and thyroid hormone levels causes osteopaenia in mice. Bone loss in hyperthyroidism therefore could be mediated mostly by TRα independently of TSH levels. Thus, evidence from human and mouse studies suggest that both abnormal thyroid hormone and TSH levels could have an unfavourable effect on skeletal homeostasis. While advanced bone age seen in our patients was likely due to elevated thyroid hormones, other bone abnormalities might be caused by either low TSH levels or thyroid hormone excess or combination of both during the foetal growth. A mouse model for NAH caused by an activating germline mutation in the *TSHR* gene if generated would help explore the effect of the thyroid hormone axis on different tissues during development. As both low TSH levels and thyroid hormone excess could contribute to bone loss seen in patients with hyperthyroidism, it remains possible that patients with NAH might later develop osteopaenia. Long-term follow-up of these patients is essential to monitor and could probably prevent the occurrence of this condition.

In conclusion, we described a Thai family with hereditary nonautoimmune hyperthyroidism due to a p.M453T mutation in the *TSHR* gene. Even though phenotypic variations including age of onset and clinical severity were observed, ventriculomegaly and bilateral shortening of the fifth metacarpal bones and the middle phalanges of the fifth fingers, in addition to hyperthyroidism, were consistently found in all affected family members. These features might be characteristics of hyperthyroidism caused by an activating mutation in the *TSHR* gene. This study also demonstrates that the *TSHR* gene is responsible for NAH across different populations and emphasizes an important role of genetic testing for definite diagnosis as well as genetic counselling.

Acknowledgements

We would like to thank the patients and their family for participation in this study. We are grateful to Siraprapa Tongkobpetch for technical assistance. This study was supported by the Ratchadapiseksompotch Fund, Faculty of Medicine, the Research Unit Grant from Chulalongkorn University, and the Thailand Research Fund.

References

1 Duprez, L., Parma, J., Van Sande, J. *et al.* (1994) Germline mutations in the thyrotropin receptor gene cause non-autoimmune autosomal dominant hyperthyroidism. *Nature Genetics*, **7**, 396–401.

2 Schwab, K.O., Sohlemann, P., Gerlich, M. *et al.* (1996) Mutations of the TSH receptor as cause of congenital hyperthyroidism. *Experimental Clinical Endocrinology and Diabetes*, **104**(Suppl. 4), 124–128.

3 Tonacchera, M., Van Sande, J., Cetani, F. *et al.* (1996) Functional characteristics of three new germline mutations of the thyrotropin receptor gene causing autosomal dominant toxic thyroid hyperplasia. *Journal of Clinical Endocrinology and Metabolism*, **81**, 547–554.

4 Fuhrer, D., Wonerow, P., Willgerodt, H. *et al.* (1997) Identification of a new thyrotropin receptor germline mutation (Leu629Phe) in a family with neonatal onset of autosomal dominant nonautoimmune hyperthyroidism. *Journal of Clinical Endocrinology and Metabolism*, **82**, 4234–4238.

5 Khoo, D.H., Parma, J., Rajasoorya, C. *et al.* (1999) A germline mutation of the thyrotropin receptor gene associated with thyrotoxicosis and mitral valve prolapse in a Chinese family. *Journal of Clinical Endocrinology and Metabolism*, **84**, 1459–1462.

6 Fuhrer, D., Warner, J., Sequeira, M. *et al.* (2000) Novel TSHR germline mutation (Met463Val) masquerading as Graves' disease in a large Welsh kindred with hyperthyroidism. *Thyroid*, **10**, 1035–1041.

7 Alberti, L., Proverbio, M.C., Costagliola, S. *et al.* (2001) A novel germline mutation in the TSH receptor gene causes non-autoimmune autosomal dominant hyperthyroidism. *European Journal of Endocrinology*, **145**, 249–254.

8 Biebermann, H., Schoneberg, T., Hess, C. *et al.* (2001) The first activating TSH receptor mutation in transmembrane domain 1 identified in a family with nonautoimmune hyperthyroidism. *Journal of Clinical Endocrinology and Metabolism*, **86**, 4429–4433.

9 Arturi, F., Chiefari, E., Tumino, S. *et al.* (2002) Similarities and differences in the phenotype of members of an Italian family with hereditary non-autoimmune hyperthyroidism associated with an activating TSH receptor germline mutation. *Journal of Endocrinological Investigation*, **25**, 696–701.

10 Lee, Y.S., Poh, L. & Loke, K.Y. (2002) An activating mutation of the thyrotropin receptor gene in hereditary non-autoimmune hyperthyroidism. *Journal of Pediatric Endocrinology and Metabolism*, **15**, 211–215.

11 Vaidya, B., Campbell, V., Tripp, J.H. *et al.* (2004) Premature birth and low birth weight associated with nonautoimmune hyperthyroidism due to an activating thyrotropin receptor gene mutation. *Clinical Endocrinology*, **60**, 711–718.

12 Nwosu, B.U., Gourgiotis, L., Gershengorn, M.C. *et al.* (2006) A novel activating mutation in transmembrane helix 6 of the thyrotropin receptor as cause of hereditary nonautoimmune hyperthyroidism. *Thyroid*, **16**, 505–512.

13 Ferrara, A.M., Capalbo, D., Rossi, G. *et al.* (2007) A new case of familial nonautoimmune hyperthyroidism caused by the M463V mutation in the TSH receptor with anticipation of the disease across generations: a possible role of iodine supplementation. *Thyroid*, **17**, 677–680.

14 Nishihara, E., Nagayama, Y., Amino, N. *et al.* (2007) A novel thyrotropin receptor germline mutation (Asp617Tyr) causing hereditary hyperthyroidism. *Endocrine Journal*, **54**, 927–934.

15 Kopp, P., van Sande, J., Parma, J. *et al.* (1995) Brief report: congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. *New England Journal of Medicine*, **332**, 150–154.

16 de Roux, N., Polak, M., Couet, J. *et al.* (1996) A neomutation of the thyroid-stimulating hormone receptor in a severe neonatal hyperthyroidism. *Journal of Clinical Endocrinology and Metabolism*, **81**, 2023–2026.

17 Holzapfel, H.P., Wonerow, P., von Petrykowski, W. *et al.* (1997) Sporadic congenital hyperthyroidism due to a spontaneous germline mutation in the thyrotropin receptor gene. *Journal of Clinical Endocrinology and Metabolism*, **82**, 3879–3884.

18 Gruters, A., Schoneberg, T., Biebermann, H. *et al.* (1998) Severe congenital hyperthyroidism caused by a germ-line neo mutation in the extracellular portion of the thyrotropin receptor. *Journal of Clinical Endocrinology and Metabolism*, **83**, 1431–1436.

19 Esapa, C.T., Duprez, L., Ludgate, M. *et al.* (1999) A novel thyrotropin receptor mutation in an infant with severe thyrotoxicosis. *Thyroid*, **9**, 1005–1010.

20 Fuhrer, D., Mix, M., Wonerow, P. *et al.* (1999) Variable phenotype associated with Ser505Asn-activating thyrotropin-receptor germline mutation. *Thyroid*, **9**, 757–761.

21 Tonacchera, M., Agretti, P., Rosellini, V. *et al.* (2000) Sporadic nonautoimmune congenital hyperthyroidism due to a strong activating mutation of the thyrotropin receptor gene. *Thyroid*, **10**, 859–863.

22 Borgel, K., Pohlenz, J., Koch, H.G. *et al.* (2005) Long-term carbimazole treatment of neonatal nonautoimmune hyperthyroidism due to a new activating TSH receptor gene mutation (Ala428Val). *Hormone Research*, **64**, 203–208.

23 Nishihara, E., Fukata, S., Hishinuma, A. *et al.* (2006) Sporadic congenital hyperthyroidism due to a germline mutation in the thyrotropin receptor gene (Leu 512 Gln) in a Japanese patient. *Endocrine Journal*, **53**, 735–740.

24 Pohlenz, J., Pfarr, N., Kruger, S. *et al.* (2006) Subclinical hyperthyroidism due to a thyrotropin receptor (TSHR) gene mutation (S505R). *Acta Paediatrica*, **95**, 1685–1687.

25 Corvilain, B., Van Sande, J., Dumont, J.E. *et al.* (2001) Somatic and germline mutations of the TSH receptor and thyroid diseases. *Clinical Endocrinology*, **55**, 143–158.

26 Kopp, P., Jameson, J.L. & Roe, T.F. (1997) Congenital nonautoimmune hyperthyroidism in a nonidentical twin caused by a sporadic germline mutation in the thyrotropin receptor gene. *Thyroid*, **7**, 765–770.

27 Lavard, L., Sehested, A., Brock Jacobsen, B. *et al.* (1999) Long-term follow-Up of an infant with thyrotoxicosis due to germline mutation of the TSH receptor gene (Met453Thr). *Hormone Research*, **51**, 43–46.

28 Gunji, K., Endo, T., Ohno, M. *et al.* (1993) Recombinant thyrotropin stimulates cAMP formation in CHO-K1 cells expressing recombinant chorionic gonadotropin receptor. *Biochemical and Biophysical Research Communications*, **197**, 1530–1535.

29 Davies, T., Marians, R. & Latif, R. (2002) The TSH receptor reveals itself. *Journal of Clinical Investigation*, **110**, 161–164.

30 Abe, E., Marians, R.C., Yu, W. *et al.* (2003) TSH is a negative regulator of skeletal remodeling. *Cell*, **115**, 151–162.

31 Delong, G.R. (1996) *The Neuromuscular system and brain in Thyrotoxicosis*. In: L.E. Braverman and R.D. Utiger, eds. *Werner and Ingbar's the Thyroid a Fundamental and Clinical Text*, pp. 645–652. Lippincotte-Raven Publishers, Philadelphia, pp. 645–652.

32 Kumar, S., Coenen, M.J., Scherer, P.E. *et al.* (2004) Evidence for enhanced adipogenesis in the orbits of patients with Graves' ophthalmopathy. *Journal of Clinical Endocrinology and Metabolism*, **89**, 930–935.

33 Riggs, W. Jr, Wilroy, R.S. Jr & Etteldorf, J.N. (1972) Neonatal hyperthyroidism with accelerated skeletal maturation, craniosynostosis, and brachydactyly. *Radiology*, **105**, 621–625.

34 Bassett, J.H., O'Shea, P.J., Sriskantharajah, S. *et al.* (2007) Thyroid hormone excess rather than thyrotropin deficiency induces osteoporosis in hyperthyroidism. *Molecular Endocrinology*, **21**, 1095–1107.

