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Generalized resistance to thyroid hormone associated with a mutation in the ligand-binding domain of the human thyroid hormone receptor β

(triiodothyronine/polymerase chain reaction/transcription/translation)

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ABSTRACT **The syndrome of generalized resistance to thyroid hormone is characterized by elevated circulating levels of thyroid hormone in the presence of an overall eumetabolic state and failure to respond normally to triiodothyronine. We have evaluated a family with inherited generalized resistance to thyroid hormone for abnormalities in the thyroid hormone nuclear receptors. A single guanine → cytosine replacement in the codon for amino acid 340 resulted in a glycine → arginine substitution in the hormone-binding domain of one of two alleles of the patient's thyroid hormone nuclear receptor β gene. *In vitro* translation products of this mutant human thyroid hormone nuclear receptor β gene did not bind triiodothyronine. Thus, generalized resistance to thyroid hormone can result from expression of an abnormal thyroid hormone nuclear receptor molecule.**

The thyroid hormones, thyroxine and triiodothyronine, control development, growth, and the metabolic rate. Their deficiency causes mental retardation in infants, stunted growth in children, and myxedema, characterized by sluggish behavior, low metabolic rate, low temperature, and slow pulse in the adult. Thyroid hormone excess (thyrotoxicosis) increases catabolism and metabolic rate, producing weight loss, restlessness, sweating, and rapid pulse (1, 2). Pituitary thyrotropin [thyroid-stimulating hormone (TSH)] stimulates thyroid hormone synthesis and secretion. Serum TSH is, in turn, regulated through feedback inhibition by thyroxine and triiodothyronine. Thyroid hormones function predominantly through binding to nuclear receptors that regulate the transcription of responsive genes (3–7). Recently cDNAs encoding several different human thyroid hormone receptors have been isolated and characterized; all are members of the *ERBA* superfamily. Human thyroid hormone nuclear receptor β (HTRB) type 1 (and possibly another product, HTRB type 2) is the product of a gene located on chromosome 3 (8, 9). Human thyroid hormone nuclear receptor α (HTRA) types 1 and 2 are derived from the same gene on chromosome 17 through alternative splicing of the mRNA (10–12).

Recognition in 1967 of a sibship of three children with high serum levels of normal and bioavailable (free) thyroid hormone, in the absence of increased metabolism or TSH suppression, suggested the existence of a syndrome of generalized resistance to thyroid hormone (GRTH) (13, 14). About 200 similar individuals have since been described (15–17). There is considerable variation in the reported clinical features of the syndrome, and both dominant and recessive inheritance patterns are found. A recent study suggested linkage of GRTH to the gene for THRB in one family (18). We now report a mutation in one of two alleles of the *THRB* gene of an affected father and son. A hormone-binding defect of the receptor expressed by the mutant cDNA appears to explain the manifestations of the syndrome.

MATERIALS AND METHODS

Clinical Features of Patients. The proband was 6.5 years old when first suspected of having GRTH. Delayed verbal expression led to diagnosis of attention-deficit hyperactivity disorder at age 3. At age 5.5, thyroid gland enlargement and elevated serum thyroxine and triiodothyronine levels were interpreted as hyperthyroidism. Specific treatment for this condition was not successful. As a matter of fact, normalization of the thyroid hormone levels in blood slowed his growth rate and strikingly increased TSH concentration (175 microunits/ml). Physical examination when off therapy revealed a child with poor attention span and immature speech, diffuse thyroid gland enlargement (35 g), and tachycardia but no other physical stigmata of thyrotoxicosis or hypothyroidism. While he showed expressive deficit [verbal intelligence quotient (IQ) 85%], his performance IQ was 120% of the standard score.

The father also had a history of delayed speech development, hyperactivity, and learning disability but finally graduated from high school and later obtained a master's degree. Physical examination was normal except for minimal thyroid gland enlargement (20 g). Thyroid tests are presented in Table 1. The proband and his father had no siblings. The mother and paternal grandmother were normal by examination and had normal hormone levels (Table 1).

Sequences of Primers. The following primers were synthesized according to the reported cDNA sequence (8) and the known intervening sequence of *THRB* (A. Sakurai, unpublished data): A, 5′-ACATGGGCTTCGGTGACAGT-3′; B, 5′-ATGAGAATGAATCCAGTCAG-3′; C, 5′-CCGATGGACTTCTGCAGCTC-3′; D, 5′-GGGTGCCAAGTTCCACACAT-3′; E, 5′-TGGCAACAGATTTGGTGCTG-3′; F, 5′-ACCAGCAATTACCAGAGTGG-3′; G, 5′-TCTGACACCACCCCAAGACC-3′; H, 5′-TCTGACACCACCCCAAGACg-3′; and I, 5′-GGAATTCTGCTGACATGAACTGGTTCT-3′. The underlined nucleotides in primer B indicate the nucleotide errors in the reported sequences of *THRB*

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Abbreviations: GRTH, generalized resistance to thyroid hormone; THRA and THRB, human thyroid hormone nuclear receptors α and β, respectively; PCR, polymerase chain reaction; TSH, thyroid-stimulating hormone.

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Table 1. Tests of thyroid function

Subject	Age, yr	Serum T4, μg/dl	FT4I	Serum T3, ng/dl	TG, ng/ml	TBG, ng/dl	TSH, μU/ml	MCHA/ TGHA, titer
Proband	6.5	24.7	25.1	369	118	2.1	2.3	Neg./neg.
Father	39	17.9	21.7	240	22	1.5	1.2	Neg./neg.
Mother	37	7.2	7.6	111	11	1.5	1.1	Neg./neg.
Paternal grandmother	65	9.3	9.1	120	9	1.7	1.3	Neg./neg.
Normal range		5.0–12.0	6.5–10.5	85–180	1–20	1.1–2.1	0.5–4.0	Neg./neg.

Serum hormone levels for thyroglobulin (TG), thyroxine (T4) binding globulin (TBG), and TSH were determined by specific RIAs. Antithyroid microsomal antibodies (MCHA) and antithyroglobulin antibodies (TGHA) were measured by hemagglutination assay, and the free thyroxine index (FT4I) was calculated as described (19–22). T3, triiodothyronine. Neg., negative; μU, microunits.

(8),†† and the lowercase letter in primer H is the nucleotide substitution in the mutant gene reported herein.

Amplification and Cloning of THRB cDNA. Primers A and B were separately hybridized to 20 μg of total fibroblast RNA, isolated from cultured skin fibroblasts (23), and single-stranded cDNAs were synthesized by using avian myeloma virus reverse transcriptase in the presence of 0.5 mM of each dNTP. After precipitation and resuspension in distilled water, they were used as templates for the synthesis of double-stranded cDNA and amplification by the polymerase chain reaction (PCR) with 100 pmol of each primer, *Thermus aquaticus* (*Taq*) polymerase, and Perkin–Elmer/Cetus thermal cycler (24). Each cycle included 1 min at 94°C for denaturation, 2 min at 50°C for hybridizing, and 3 min at 65°C for extension. For the 3′ cDNA fragment, primers B and E and 40 cycles were used. The 5′ cDNA fragment was first amplified for 40 cycles with primers A and D, followed by an additional 25 cycles with primers C and D (Fig. 1). All amplified fragments were cloned into either M13mp18 or mp19 vectors and sequenced by the dideoxy chain-termination method (25).

Allele-Specific Amplification of Genomic DNAs (26, 27). High molecular weight DNA was extracted from peripheral blood leukocytes (28) and precipitated with ethanol. One hundred picomoles each of two primers, I plus G or I plus H, were hybridized to 1.5 μg of DNA and amplified for 25 cycles with PCR. Primer I is complementary to the intervening sequence distal to the exon containing the mutation. Temperature cycles identical to those described above were used. After PCR, samples were precipitated with ethanol, redissolved in water, and submitted to electrophoresis in 2% GTC-agarose (NuSieve; FMC) and stained with ethidium bromide.

***In Vitro* Expression of Normal and Abnormal *THRB*.** The *Eco*RI insert from pheA12, containing the wild-type *THRB* (8), was cloned into the *Eco*RI site of pGEM3Z (Promega Biotec) to yield phTRβ-W. A 677-base pair (bp) fragment of the mutant allele, containing the substituted nucleotide, was amplified by PCR as described above using an M13 clone of the mutant allele as template and primers B and F. phTRβ-W and the amplified mutant fragment were digested with *Bst* XI and *Bgl* II. The resulting 436-bp *Bst* XI–*Bgl* II fragment of the amplified mutant clone and the fragment of phTRβ-W lacking the corresponding 436-bp sequence were collected from 1% low-melting-temperature agarose (Sea Plaque Agarose; FMC) and were ligated to each other to produce phTRβ-Mf. The structures of both plasmids were confirmed by sequencing. Both phTRβs were linearized with *Hin*dIII, transcribed *in vitro* using T7 polymerase, and translated in rabbit reticulocyte lysates. For the triiodothyronine-binding study, 7 μl of each lysate was mixed with [^{125}I]triiodothyronine (13.8 pM, final concentration) in triiodothyronine-binding buffer (0.3 M KCl/1 mM $MgCl_2$/20 mM Tris·HCl, pH 8.0/1 mM dithiothreitol) at room temperature for 2 hr in a final volume of 300 μl. Bound and free hormone was separated by Dowex 1-X8, Cl^-, 200- to 400-mesh anion-exchange resin (29).

RESULTS

Examination of the Responses of Patients to Thyroid Hormone. Evidence for resistance to thyroid hormone action was obtained by clinical studies during triiodothyronine treatment. Doses of triiodothyronine that achieved highly elevated serum values, ranging from 600–1100 ng/dl (normal range, 85–180 ng/dl) in both the proband and control, completely suppressed the TSH response to thyrotropin-releasing hormone in the normal subject (<0.1 microunits/ml in all serum samples) but only partially suppressed TSH in the proband (Fig. 2), as found in other subjects with GRTH (30). During triiodothyronine treatment basal prolactin concentration paradoxically increased without suppression of its response to thyrotropin-releasing hormone. Results obtained in the father were virtually identical (data not shown). Increasing amounts of triiodothyronine given to the normal subject produced, as expected, incremental changes of the sleeping pulse, basal metabolic rate, serum sex hormone-binding globulin and ferritin, and urea nitrogen excretion in urine, whereas body weight and serum cholesterol levels appropriately declined (Fig. 3*A*). In contrast, the proband showed no significant changes of sleeping pulse, body weight, basal metabolic rate, serum sex hormone-binding globulin, or cholesterol; ferritin paradoxically decreased. Reduction of urinary nitrogen in the proband suggested an anabolic action of triiodothyronine. An identical study on the father yielded similar results.

Tissue response to triiodothyronine was also tested on skin fibroblasts grown in tissue culture. Triiodothyronine has been shown (31, 32) to produce a dose-dependent decrease in

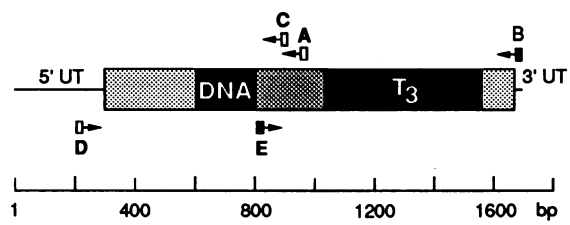

FIG. 1. Schematic representation of THRB cDNA and the cloning strategy. The shaded areas represent the coding region and its DNA and ligand-binding domains. Open boxes and filled boxes indicate primers used for amplification of 5′ half and 3′ half, respectively; arrows indicate positions of primers A–E and direction of elongation. UT, untranslated region; T_3, triiodothyronine.

††The pheA12 clone (8) was sequenced completely in both directions using the dideoxy chain-termination method. Thirteen nucleotide errors were found in comparison with the reported *THRB* sequence.

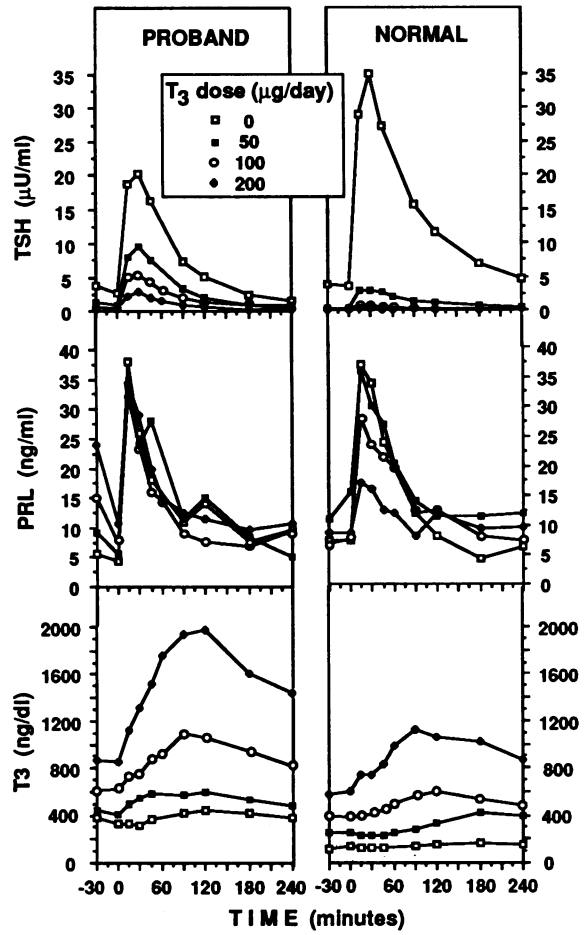

FIG. 2. Basal TSH and prolactin (PRL) and responses to the administration of TRH to the proband and a normal subject on constant diet before and after treatment with graded doses of triiodothyronine. Measurements were obtained before treatment and in the morning 15 min before administration of the last incremental triiodothyronine dose level. Triiodothyronine was given every 12 hr, and each dose was administered for 3 days. TRH (400 μg/1.75 m^2 of body surface area) was given i.v. 15 min later (t_0).

fibronectin synthesis in normal fibroblasts, and this response is diminished in fibroblasts cultured from patients with GRTH. In the present study, triiodothyronine added to the medium to a concentration of 10^{-11} and 10^{-9} M free hormone produced little suppression of fibronectin synthesis in fibroblasts from the affected proband and his father and normally suppressed fibronectin synthesis in fibroblasts from the mother (Fig. 3*B*).

Identification of a Mutation in the Ligand-Binding Domain of One Allele of *THRB*. Because fibroblasts obtained from the affected proband and his father demonstrated *in vitro* an abnormal response to triiodothyronine characteristic of GRTH, we presumed that these cells should express an abnormal receptor if this receptor was responsible for the defect. Both *THRA1* and *THRB* have been shown to be expressed at the mRNA level in normal human fibroblasts (A.S., unpublished observations). cDNAs to the complete coding sequences of fibroblast THRB mRNAs, synthesized as two overlapping fragments by PCR, were sequenced. These sequences were compared to those published for the THRB clone pheA12 (8), as well as partial sequences of the coding region of THRB, which we have derived from three different human genomic DNA libraries, all of which were identical. A single guanosine → cytosine substitution in codon 340 was found in 6 of 11 M13 clones from the affected proband, 12 of 19 clones from the affected father, and 0 of 8

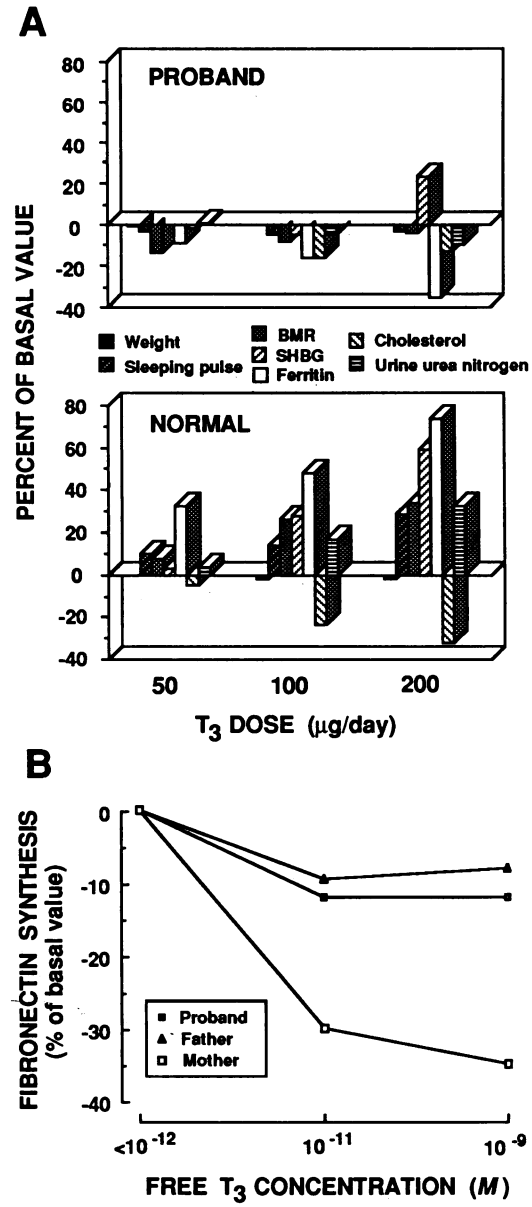

FIG. 3. Tissue responses to thyroid hormone *in vivo* and *in vitro*. (*A*) Subjects received graded doses of triiodothyronine (T_3) according to the schedule outlined in the legend to Fig. 2. Averaged results for each treatment period were expressed as percent increment or decrement from the mean basal value. (*B*) Effect of triiodothyronine on the synthesis of fibronectin by cultured skin fibroblasts obtained from the affected proband and his father and his normal mother. Methods have been described (31, 32). The inhibiting effect of 10^{-9} M free triiodothyronine in fibroblasts from seven normal individuals was 30 ± 5% (mean ± SD) and ranged from 25% to 38%. SHBG, serum sex hormone-binding globin; BMR, basal metabolic rate.

clones from the normal mother (Fig. 4). This nucleotide change resulted in a glycine (codon GGT)-to-arginine (codon CGT) substitution in the hormone-binding domain of the THRB protein. We also found random misincorporation in ≈25% of the clones sequenced.

To confirm that the observed mutation was contained in one allele of the affected subject and to test whether this mutation was present in a subject with GRTH from another family, genomic DNAs were submitted to allele-specific amplification. The primers used in the PCR were either primer I and primer G, both containing the normal sequences, or primer I and primer H, the latter containing the mutant nucleotide at the site of elongation (3′ end). Both pairs primer

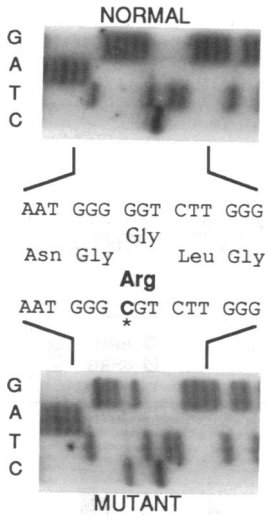

FIG. 4. Sections of sequencing gels showing the nucleotide substitution (*) in the mutant cDNA (Mf) compared with the normal type cDNA. The resulting amino acid substitution at residue 340 is also indicated in boldface letters.

I plus primer G and primer I plus primer H produced a single DNA fragment of the expected 200 bp when used to amplify DNA from the proband and his father. In contrast, only primer pair I plus G amplified the DNA from the unaffected mother and an unrelated subject with GRTH (Fig. 5).

Triiodothyronine Binding Activity of Normal and Mutant *THRB*. Because the mutation was found in the hormone-binding domain of THRB protein, binding of triiodothyronine to the mutant *THRB* allele was evaluated by using gene products transcribed and translated *in vitro*. The *Eco*RI insert from pheA12 (8), which contains the whole coding sequence of *THRB*, was subcloned into pGEM3Z. A fragment of the mutant cDNA containing the substitution was amplified by PCR and used to replace the corresponding portion of normal *THRB* (Fig. 6*A*). The wild (phTRβ-W) and mutant (phTRβ-Mf) plasmid constructs were transcribed using T7 polymerase and translated *in vitro* by using reticulocyte lysates. The [^{35}S]methionine-labeled products, separated by SDS/PAGE, displayed the expected 55- to 52-kDa products (Fig. 6*B*). The difference in size is believed to be due to initiation at Met-1 (55 kDa) or Met-27 (52 kDa). In the triiodothyronine-binding assay, the phTRβ-W product exhibited high triiodothyronine

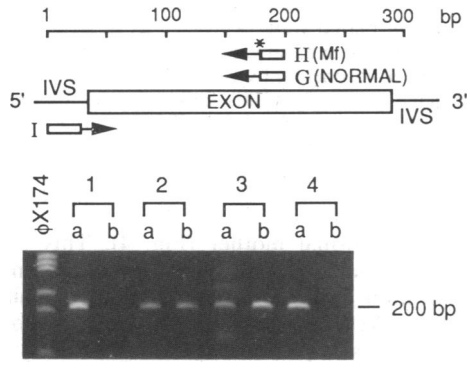

FIG. 5. Detection of normal (primer G) and mutant (primer H; Mf) *THRB* alleles in genomic DNA by allele-specific amplification. DNA samples were hybridized with primers I and G (lanes a) and primers I and H (lanes b). After PCR, samples were submitted to electrophoresis in 2% GTC-agarose and stained with ethidium bromide. DNA samples 1–4 are as follows: 1, mother; 2, proband; 3, father; 4, unrelated subject with familial GRTH; φX174 is a size marker. IVS, intervening sequence.

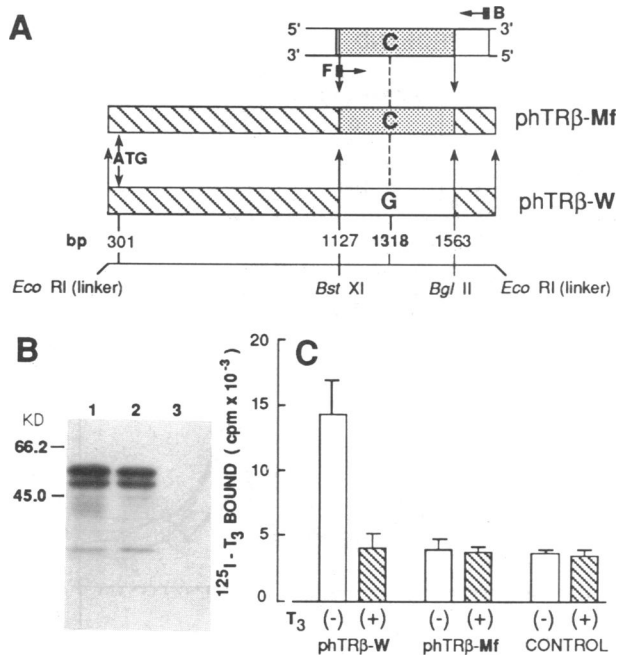

FIG. 6. Triiodothyronine binding activity of the *in vitro*-synthesized normal and mutant THRB proteins. (*A*) Strategy of plasmid construction for *in vitro* translation. Filled boxes and arrows indicate the positions of primers B and F and direction of elongation used for the amplification of M13 clone. The nucleotide sequence numbers follow those of Weinberger *et al.* (8). (*B*) *In vitro* translation of normal and mutant *THRB*. The [^{35}S]methionine-labeled products were separated by electrophoresis on 10% SDS/polyacrylamide gel that was fluorographed. Lanes: 1, phTRβ-W; 2, phTRβ-Mf, and 3, no added RNA. Protein size markers were bovine serum albumin, 66.2 kDa and ovalbumin, 45.0 kDa. (*C*) ^{125}I-labeled triiodothyroxin binding to *in vitro* translation products of normal and mutant *THRB*. *In vitro* translation products of phTRβ-W, phTRβ-Mf, and rabbit reticulocyte lysate without mRNA (control) were incubated with 13.8 pM ^{125}I-labeled triiodothyronine without (−) or with (+) 10^4-fold excess unlabeled triiodothyronine. Data displayed represent the mean ± SD of three independent duplicate experiments.

binding activity (with a K_a of 8.3×10^9 M^{-1}; data not shown), but the mutant phTRβ-Mf product did not bind triiodothyronine (Fig. 6*C*).

DISCUSSION

The affected family members in this sibship clearly have GRTH, as shown by their high serum thyroxine and triiodothyronine levels and normal TSH, lack of stigmata of thyrotoxicosis, failure of triiodothyronine to normally suppress TSH or produce the expected changes in multiple parameters measuring thyroid hormone action, and the attenuated effect of triiodothyronine on their cultured fibroblasts. The mutation we found in *THRB* on one allele of the affected family members appears reponsible for their thyroid hormone resistance. The glycine-to-arginine substitution at codon 340 results in a charge change of the protein that prevents, or greatly reduces triiodothyronine binding to the receptor, as shown by the studies on the *in vitro*-transcribed and translated mutant gene.

Although the defect we have identified is in one of two alleles, both are transcribed and presumably translated. Expression of the abnormal allele should result in decreased levels of normal *THRB*, the activity of which may be further reduced by the abnormal receptor competing for binding to thyroid hormone response elements. Indeed rat *erbAα2*, which is homologous to the functional rat thyroid hormone α 1 nuclear receptor but does not bind triiodothyronine, is

reported to inhibit the response to triiodothyronine induced by rat *erbAβ* or rat *erbAα1* (33). The same mechanism could be involved in the inhibition of normal receptor function by rat *erbAα2* and abnormal *THRB*. This inhibition might theoretically be overcome, as observed in our subjects, by increased triiodothyronine saturation of normal receptors at higher hormone levels. It is also possible that the excess occupancy of the normal receptors produced by the elevated circulating levels of hormone could induce hyperthyroid responses in some tissues. The clinical abnormality we have found was aggravated by an attempt to normalize the elevated thyroid hormone levels. Protection against hypothyroidism was presumably afforded by increased thyroid hormone secretion in the presence of one normal *THRB* allele and normal *THRA1*.

Our study also suggests that normal allelic forms of *THRB* are not present in high frequency. We have completely sequenced three normal alleles from the affected sibship, much of the coding sequences from three human genomic libraries, and the original clone derived from placenta by Weinberger *et al.* (8). All had an identical sequence.

GRTH is associated with a spectrum of phenotypic abnormalities. The diverse clinical presentation, different inheritance patterns, and presence of a family of receptors for triiodothyronine suggests that this syndrome is probably genetically diverse. We have seen receptors with either diminished affinity for triiodothyronine or diminished maximum triiodothyronine binding capacity in families with GRTH (34). A subject with GRTH from another family did not carry the mutation described herein (Fig. 5). The analysis of this and other families will likely lead to the identification of additional mutations in *THRB* as well as in the *THRA* gene. Although GRTH is a relatively rare syndrome caused by the expression of a severely impaired receptor, more subtle defects may also exist and could play an important role in abnormal human psychological or physiologic functions.

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1. McKenzie, J. M. & Zakarija, M. (1989) in *Endocrinology*, ed. DeGroot, L. J. (Saunders, Philadelphia), Vol. 1, pp. 646–682.
2. Utiger, R. D. (1989) in *Endocrinology*, ed. DeGroot, L. J. (Saunders, Philadelphia), Vol. 1, pp. 702–721.
3. Spindler, S. R., Mellon, S. H. & Baxter, J. D. (1982) *J. Biol. Chem.* **257**, 11627–11632.
4. Koenig, R. J., Brent, G. A., Warne, R. L., Larsen, P. R. & Moore, D. D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5670–5674.
5. Glass, C. K., Franco, R., Weinberger, C., Albert, V. R., Evans, R. M. & Rosenfeld, M. G. (1987) *Nature (London)* **329**, 739–741.
6. Oppenheimer, J. H., Schwartz, H. L., Mariash, C. N., Kinlaw, W. B., Wong, N. C. W. & Freake, H. C. (1987) *Endocr. Rev.* **8**, 288–308.
7. Samuels, H. H., Forman, B. M., Horowitz, Z. D. & Ye, Z.-S. (1988) *J. Clin. Invest.* **81**, 957–967.
8. Weinberger, C., Thompson, C. C., Ong, E. S., Lebo, R., Gruol, D. J. & Evans, R. M. (1986) *Nature (London)* **324**, 641–646.
9. Hodin, R. A., Lazar, M. A., Wirtman, B. I., Darling, D. S., Koenig, R. J., Larsen, P. R., Moore, D. D. & Chin, W. W. (1989) *Science* **244**, 76–79.
10. Benbrook, D. & Pfahl, M. (1987) *Science* **238**, 788–791.
11. Nakai, A., Seino, S., Sakurai, A., Szilak, I., Bell, G. I. & DeGroot, L.J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2781–2785.
12. Nakai, A., Sakurai, A., Bell, G. I. & DeGroot, L. J. (1988) *Mol. Endocrinol.* **2**, 1087–1092.
13. Refetoff, S., DeWind, L. T. & DeGroot, L. J. (1967) *J. Clin. Endocrinol. Metab.* **27**, 279–294.
14. Refetoff, S., DeGroot, L. J., Benard, B. & DeWind, L. T. (1972) *Metabolism* **21**, 723–756.
15. Lamberg, B.A. & Liewendahl, K. (1980) *Ann. Clin. Res.* **12**, 243–253.
16. Weintraub, B. D., Gershengorn, M. C., Kourides, I. A. & Fein, H. (1981) *Ann. Int. Med.* **95**, 339–351.
17. Refetoff, S. (1986) in *The Thyroid* eds. Ingbar, S. H. & Braverman, L. B. (Lippincott, New York), pp. 1292–1306.
18. Usala, S. J., Bale, A. E., Gesundheit, N., Weinberger, C., Lash, R. W., Wondisford, F. E., McBride, O. W. & Weintraub, B. D. (1988) *Mol. Endocrinol.* **2**, 1217–1220.
19. Sarne, D., Barokas, K., Scherberg, N. H. & Refetoff, S. (1983) *J. Clin. Endocrinol. Metab.* **57**, 665–667.
20. Refetoff, S., Murata, Y., Vassart, G., Chandramuli, V. & Marshall, J. S. (1984) *J. Clin. Endocrinol. Metab.* **59**, 269–277.
21. Amino, N., Hagen, S. R., Yamada, N. & Refetoff, S. (1976) *Clin. Endocrinol.* **5**, 115–119.
22. Robin, N. I., Hagen, S. R., Collaso, F., Refetoff, S. & Selenkow, H. A. (1971) *Hormones* **2**, 266–279.
23. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
24. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–494.
25. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. A. (1980) *J. Mol. Biol.* **143**, 161–178.
26. Newton, C. R., Graham, A., Heptinstall, L. E., Powell, S. J., Summers, C., Kalscheker, N., Smith, J. C. & Markham, A. F. (1989) *Nucleic Acids Res.* **17**, 2503–2516.
27. Nichols, W. C., Liepnicks, J. J., Benson, M. D. & MuKusick, V. A. (1989) *Genomics* **5**, 535–540.
28. Bell, G. I., Karam, J. H. & Rutter, W. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5759–5763.
29. Torresani, J. & DeGroot, L. J. (1975) *Endocrinology* **96**, 1201–1209.
30. Refetoff, S., Salazar, A., Smith, T. J. & Scherberg, N. H. (1983) *Metabolism* **32**, 822–834.
31. Murata, Y., Ceccarelli, P., Refetoff, S., Horwitz, A. L. & Matsui, N. (1987) *J. Clin. Endocrinol. Metab.* **64**, 334–339.
32. Ceccarelli, P., Refetoff, S. & Murata, Y. (1987) *J. Clin. Endocrinol. Metab.* **65**, 242–246.
33. Koenig, R. J., Lazar, M. A., Hodin, R. A., Brent, G. A., Larsen, P. R., Chin, W. W. & Moore, D. D. (1989) *Nature (London)* **337**, 659–661.
34. Ichikawa, K., Hughes, I. A., Horwitz, A. L. & DeGroot, L.J. (1987) *Metabolism* **36**, 392–399.