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Autosomal Dominant Resistance to Thyrotropin as a Distinct Entity in Five Multigenerational Kindreds: Clinical Characterization and Exclusion of Candidate Loci

Helmut Grasberger, Aviva Mimouni-Bloch, Marie-Christine Vantyghem, Guy van Vliet, Marc Abramowicz, Daniel L. Metzger, Hussein Abdullatif, Catherine Rydlewski, Paolo E. Macchia, Neal H. Scherberg, Jacqueline van Sande, Marc Mimouni, Roy E. Weiss, Gilbert Vassart, and Samuel Refetoff

Departments of Medicine (H.G., N.H.S., R.E.W., S.R.) and Pediatrics (S.R.) and Committee on Genetics (S.R.), The University of Chicago, Chicago, Illinois 60637; Schneider Children's Medical Center (A.M.-B., M.M.), Sackler School of Medicine, Petah-Tiqva 49202 Israel; Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (C.R., M.A., J.v.S., G.V.) and Department of Genetics (G.V.), Campus Erasme, Universite Libre de Bruxelles, 1070 Brussels, Belgium; Clinic Marc Linquette (M.-C.V.), Lille University Hospital, 59000 Lille, France; Sainte-Justine Hospital (G.v.V.), University of Montreal, Montreal, Quebec, Canada H3T 1C5; Endocrinology and Diabetes Unit (D.L.M.), British Columbia's Children's Hospital, Vancouver, British Columbia, Canada V6H 3V4; Department of Pediatrics (H.A.), University of Alabama at Birmingham, Birmingham, Alabama 35203; and Universita degli Studi di Napoli (P.E.M.), Federico II, 80131 Naples, Italy

Context: Resistance to TSH (RTSH) is an inherited disorder of variable hyposensitivity to TSH. The metabolic consequences can range from euthyroid hyperthyrotropinemia to severe congenital hypothyroidism with thyroid hypoplasia. Although subclinical and mild hypothyroidism fitting the RTSH phenotype is common in the population, the role of genetic factors is far from being understood. Only in rare cases has RTSH been attributed to *TSHR* or *PAX8* gene mutations.

Objective, Setting, and Participants: Toward the identification of novel RTSH genes, we studied five large, unrelated families comprising 102 individuals, 56 of whom were affected.

Results: Inheritance of RTSH in these families followed an autosomal dominant pattern without evidence for incomplete penetrance, yet expressivity was variable. Considering only fully phenotyped generations, 64% of the progeny was affected, with a 1:1.4 male-to-female ratio. Of 18 affected individuals tested in the neonatal period, two were undetected because of borderline results. The thyroid phenotype was indistinguishable from that observed with *PAX8* and *TSHR* defects. In four families, untreated affected subjects of all ages had elevated serum thyroglobulin levels, consistent with a defect in the thyroid follicle cells. Linkage of RTSH to *TSHR* and *PAX8* was excluded in all five families. For the largest families, we likewise excluded a contribution of genes previously only associated with syndromic forms of RTSH, namely *TITF1, GNAS*, and *FOXE1*.

Conclusions: These kindreds represent a distinct etiological entity of autosomal dominant RTSH. According to the clinical presentation of these families, genetic causes of mild hyperthyrotropinemia in the general population may be more common than currently appreciated.
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PITUITARY TSH IS the principal regulator of growth, differentiation, and function of the thyroid follicular cells. TSH exerts its effect by binding to the ectodomain of a specific heptahelical G protein-coupled receptor (TSHR). TSHR activates the cAMP cascade via the stimulatory guanine nucleotide binding protein ($G_s\alpha$) and, at higher concentrations of TSH, also through G_q, the phospholipase C pathway.

Resistance to the action of TSH (RTSH) is an inherited condition of variable hyposensitivity to a biologically active TSH molecule. RTSH was first recognized as a clinical entity in patients with congenital hypothyroidism by Stanbury *et al.* (1) and subsequently described in several similar cases (2–4). The clinical hallmarks are: 1) normal to low levels of free thyroid hormones (TH), depending on the degree of TSH hyposensitivity; 2) elevated serum levels of biologically active TSH; and 3) presence of a normal-sized or hypoplastic eutopic thyroid gland despite high TSH concentrations (5). The most common cause of elevated TSH with normal free TH levels is early-stage autoimmune thyroid disease. These individuals are distinguished from subjects with RTSH by 1) postnatal onset of abnormalities, 2) presence of thyroid autoantibodies, and 3) often enlarged thyroid gland.

At the molecular level, RTSH can arise from the inability to receive the TSH signal or to transmit the TSHR-originated signals to the genes controlling thyroid cell activity and proliferation. *TSHR* gene mutations, first found to be the cause of RTSH in congenitally hypothyroid *hyt/hyt* mice, have been identified in over a dozen human families (reviewed in Ref. 5). In these cases, RTSH is inherited in a recessive fashion. Heterozygotes for one defective allele are usually euthyroid but may have mild hyperthyrotropinemia (6, 7). *TSHR* mutations, however, account for only a minority of cases with RTSH (7, 8).

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Abbreviations: B/I ratio, Ratio of biological to immunological activity; FT_4, free T_4; RTSH, resistance to TSH; SH, subclinical hypothyroidism; TG, thyroglobulin; TH, thyroid hormones; TSHR, TSH receptor.

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Inability of thyroid tissue to adequately respond to the TSHR-generated signal is also the common feature of the few reported mutations in *PAX8* and *TITF1* (*Nkx-2.1*), transcription factors regulating the expression of thyroid-specific genes including *TSHR*. Their thyroid phenotype is comparable to that found in *TSHR* mutations, with variable severity even within the same family (reviewed in Ref. 9).

We previously reported two small families with mild RTSH not genetically linked to *TSHR*, suggesting locus heterogeneity (10). Furthermore, the pattern of inheritance was compatible with a dominant mode of transmission. The most powerful approach for gene mapping in dominant, Mendelian inherited diseases is parametric linkage analysis of extended multigenerational families. The latter have proven to be an invaluable resource in elucidating pathophysiological pathways in other genetically heterogenous disorders, such as familial lipodystrophy (11), and those seemingly complex or polygenic in their sporadic forms, such as non-insulin-dependent diabetes (12). We report the clinical and genetic investigation of five multigenerational kindreds, two of which have individually enough predictive power to detect genome-wide significant linkage.

Subjects and Methods

Diagnostic criteria and assignment of affection status

Families were evaluated with the aim of collecting multigenerational pedigrees with dominantly inherited RTSH, amenable for eventual genome-wide screen. Subjects live in the United States (families 25 and 30), Israel (family 25), France (family 24), and Canada (families 26 and 35). No genealogical link was found between the five kindreds. The studies were approved by the Institutional Review Board of the University of Chicago and all other participating institutions, and written informed consent was obtained from all participating subjects. The index cases are presented below, together with pertinent information on other family members. Minimal diagnostic tests in all subjects included measurements of serum TSH and either free T_4 (FT_4) or FT_4 index. For subjects on L-T_4 replacement, a definitive affection status could be assigned based on records of pretreatment TSH levels or its increase after temporary interruption of hormone treatment. Otherwise, these individuals are marked as presumptive affected (*gray filled symbols* in the pedigrees) and, for the purpose of linkage analysis, assigned unknown or positive affection status depending on the absence or presence of affected progeny, respectively. The presence of autoantibodies to thyroid peroxidase, thyroglobulin (TG), or TSHR (TSHR-binding Igs) was excluded for all affected subjects, except for those with positive neonatal TSH screening, which were not all routinely tested for autoantibodies. Apart from subject III-9 of family 25, who reportedly had goiter on clinical examination (13), and I-2 of family 30 with large multinodular goiter of unknown etiology, all affected subjects had normal-sized or hypoplastic thyroid glands of normal shape and in normal position. Subjects are identified in the text by generation number and chronological order of birth.

Family 24 (Fig. 1)

The proposita (I-8) was suspected of being acromegalic or hypothyroid because of coarse facial features at age 57 yr. She had a high serum TSH of 14.5 mU/liter with normal levels of FT_4, FT_3, and GH (0.3 μg/liter). Pituitary glycoprotein hormone α-subunit level was 1.4 μg/liter (molar ratio of α-subunit to TSH of 0.9; normal, <1). TSH increased to 56 mU/liter 15 min after the administration of 200 μg TRH iv. A small thyroid gland with small cystic nodules was found on ultrasound. Magnetic resonance imaging showed a normal pituitary gland and stalk.

One of her daughters (II-1) had thyroidectomy at the age of 30 yr for a benign thyroid nodule and has been on L-T_4 replacement ever since. Before thyroidectomy, but reportedly already on L-T_4 therapy, serum TSH was 16.6 mU/liter with FT_4 of 3.2 pmol/liter (normal, 7–17). The inherited nature of the disorder was not recognized until the realization that three hyperthyrotropinemic children of the last generation (III-1, III-4, and III-8) were cousins. All three had high TSH levels on neonatal screening, but total T_4 levels were at the lower limit of normal. Thyroid scans showed normally located and appearing glands.

Subject III-3 had delayed mental development and was found to have mild hyperthyrotropinemia on separate occasions at the age of 7 yr (mean TSH of 7 mU/liter). Importantly, TSH at neonatal screening was not reported as abnormal. FT_4 concentration and thyroidal radioiodide uptake (RAIU) were within the normal range.

Blood samples were subsequently obtained from 16 additional members of the family originally from the Hainaut Francais region of northern France. Seven were hyperthyrotropinemic, but only one (II-2) had symptoms of hypothyroidism consisting of hair loss, asthenia, and cold intolerance. Pertinent information included a low normal thyroid volume for age on ultrasound in the proposita's other daughters (II-2, II-3, and II-4), all of whom had one or two small thyroid nodules. Except for I-4, I-5, and I-11, who were first tested after initiation of this study, all affected individuals were given replacement doses of L-T_4, which brought the serum TSH levels to normal. Pretreatment TG levels were above the upper limit of normal in all affected individuals tested but not the unaffected subjects. A total of 13 affected members in three generations were identified.

Family 25 (Fig. 2)

Eleven affected subjects of kindred 25 were briefly reported previously (13). The propositus (III-12), born to unrelated Ashkenazi Jews, was referred at 3.8 yr of age for the investigation and treatment of hypothyroidism. FT_4 was 8.0 pmol/liter (normal, 11–26), TSH was 100 mU/liter, and RAIU at 24 h was 11% (normal, 8–35). Weight and height were at the 30th percentile, and bone age was retarded at 1.3 yr. Treatment with L-T_4 was initiated, and the subject, now age 27 yr, appears healthy.

Thyroid function test results for 37 family members are shown in Fig. 2. For most receiving L-T_4, results of tests before or off treatment could be obtained. None had apparent sequelae of congenital hypothyroidism. Among the affected adults, except for subjects II-8 and II-11, none had history, signs, or symptoms of hypothyroidism at time of initial diagnosis. Subject II-8 was on L-T_4 replacement from the age of 6 yr. The TSH response to TRH was exaggerated in the three affected adults that were tested (I-5, II-9, and II-10) but not in the four normal relatives (III-7, III-10, III-13, and III-14). Thyroid glands were in the normal anatomic position in the five individuals (II-10, III-5, III-6, III-9, and III-11) on whom radioiodide scans were performed, but RAIU at 24 h was low, from 3.8–12%.

A total of 17 affected family members, spanning four generations, were identified. Three other subjects (I-6, II-7, and II-11) were on continuous L-T_4 treatment because of high serum TSH concentrations, but records of their pretreatment TSH values could not be traced.

Family 26 (Fig. 3)

The propositus (IV-3), born at term, had a high blood TSH (74 mU/liter) with normal total T_4 when screened at 3 d of age. At 16 d of age, the knee epiphyses were normally ossified, and ^{99m}pertechnetate scan revealed slightly diminished uptake in a eutopic thyroid gland. Hyperthyrotropinemia with normal FT_4 was confirmed on a serum sample, and treatment with L-T_4 was started. After stopping treatment for 1 month at the age of 3 yr, serum TSH rose from 0.46 to 25.2 mU/liter concomitant with a drop of FT_4 from 19.4 to 8.4 pmol/liter (normal, 9–27). The thyroid gland was of normal size and echo pattern on ultrasound. At 4 yr of age, sensorineural deafness was diagnosed. The mother (III-4) had taken L-T_4 since the age of 14 yr for the treatment of nongoitrous hypothyroidism with no detectable thyroid autoantibodies.

Subject IV-1, born at term to a maternal cousin of III-4, had a blood spot TSH of 66 mU/liter and normal total T_4 at 3 d of age. At 16 d, he had dry skin, a 0.5-cm umbilical hernia, and no ossification of the proximal tibial epiphyses. ^{99m}Pertechnetate scan showed normal uptake into a eutopic thyroid gland. Treatment with L-T_4 was initiated after confirmation of hyperthyrotropinemia on a serum sample. After discontinuation of L-T_4 treatment for 1 month at the age of 3 yr, TSH rose from 0.12 to 10.4 mU/liter with FT_4 decreasing from 24.5 to 10.4 pmol/liter (normal, 9–27). RAIU was 4.7% at 3 h (normal, 2–6). Nine months

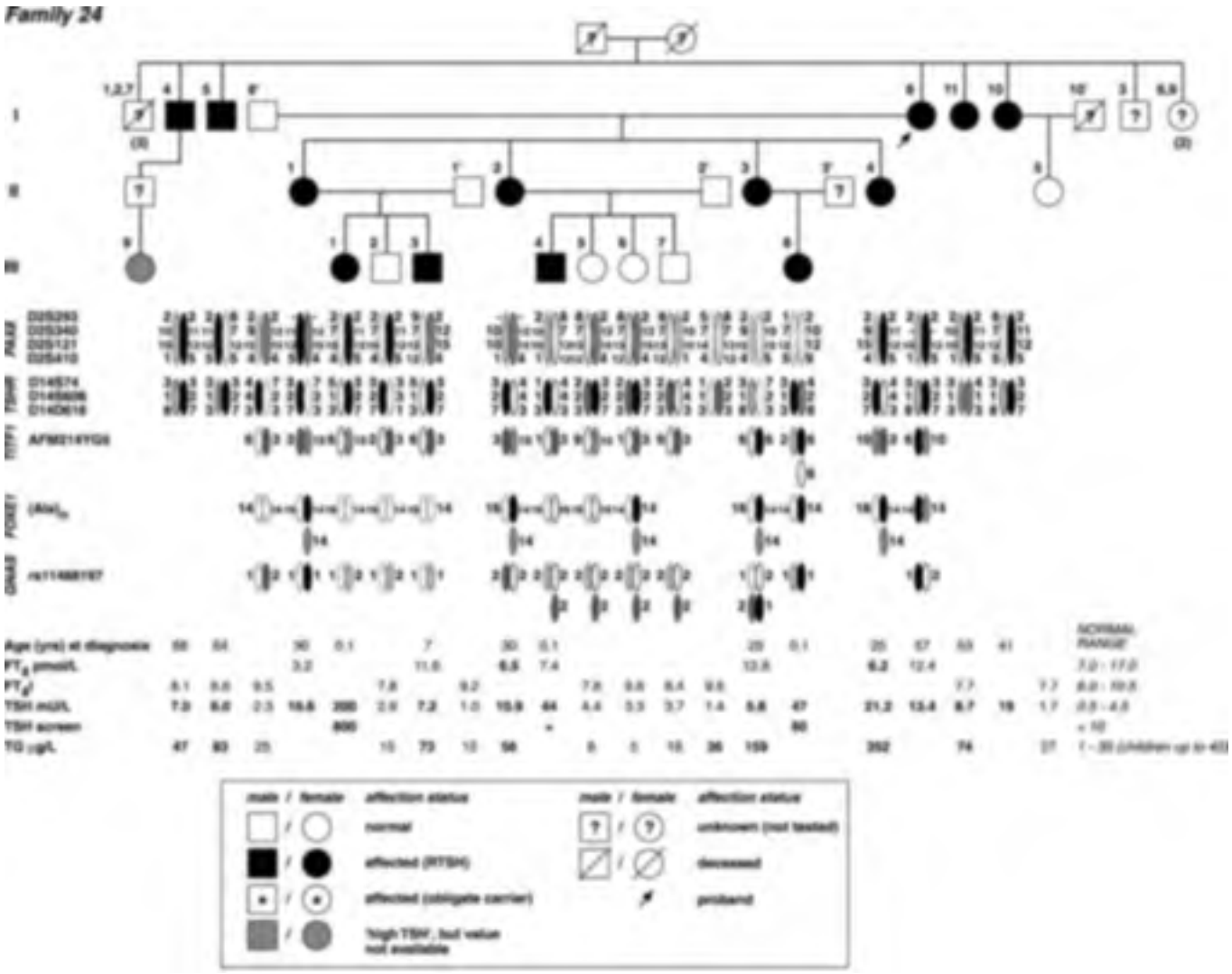

FIG. 1. Pedigree of family 24 with hereditary RTSH. The *symbols* used are explained on the figure. Results of thyroid function tests are aligned with each *symbol*, representing a family member. Values outside the normal range are in *bold numbers*, and those obtained under L-T_4 therapy are in *parentheses*. When multiple tests were obtained, values were averaged. Subjects were tested for the indicated polymorphic markers corresponding to five RTSH candidate genes. The haplotypes are also shown below each *symbol*, and *shading* is provided to help trace the inheritance of the different alleles. +, Positive neonatal TSH screen (high TSH value).

after the birth of IV-1, his mother (III-1) was found to have a TSH of 23 mU/liter with normal FT_4 and total T_3 concentrations. RAIU was only 5% at 24 h (normal, 10–30). Fine needle aspiration of a single, scintigraphically cold nodule yielded benign follicular cells.

Five members from three generations of this French Canadian family, with Amerindian admixture, were found to have RTSH. Subjects II-5 and III-4 had been on L-T_4 therapy for years, but records of pretreatment TSH levels were not available. Because II-5 is the mother of III-4, who has an affected child (IV-3), they are both obligate carriers of the putative genetic defect.

Family 30 (Fig. 4)

The index case (III-2) was the second child of nonconsanguineous parents and the product of a term gestation. He had a positive neonatal TSH screen, confirmed at 2 and 3 wk of age (30 and 21 mU/liter, respectively), with a normal thyroid gland in size, shape, and position on ^{99m}pertechnetate scan. L-T_4 replacement was initiated. An umbilical hernia resolved spontaneously at 8 wk of age. There was neither prolonged jaundice nor constipation, and his mental and physical development proceeded normally. At the age of 5 yr, discontinuation of L-T_4 for 1 month increased the TSH to 25 mU/liter, but FT_4 remained normal.

The older brother (III-1) of the propositus also had a positive neonatal blood TSH screen, which on retesting was normal. He was not treated and when retested at 8 yr of age was hyperthyrotropinemic with normal FT_4 on three separate occasions (mean TSH of 7.4 mU/liter). In contrast, in the younger brother (III-3), hyperthyrotropinemia detected by newborn screening was confirmed at 4 wk of age (TSH of 50 mU/liter; normal FT_4).

When first seen by one of us, the granduncle of the propositus (I-1) had been on L-T_4 therapy for 2 yr for an approximately 300-g multinodular goiter of unknown etiology. His pretreatment TSH level is unknown. Serum TG concentration was very high at 700 μg/liter, and repeated fine needle aspirations provided poor cellular material. The patient declined surgery. One of his two children (II-1) was found to have a high serum TSH with a normal thyroid gland. Thus, subject I-1 is presumably a carrier of the genetic defect (obligatory affected).

Six members from three generations had the RTSH phenotype. The ethnic origins of the affected grandmother (I-2) were Danish and other Caucasians of European ancestry.

Family 35 (Fig. 5)

The proposita (III-4) had a positive newborn TSH screen, confirmed at 10 d of age (TSH, 45 mU/liter; normal FT_4). A ^{99m}pertechnetate scan

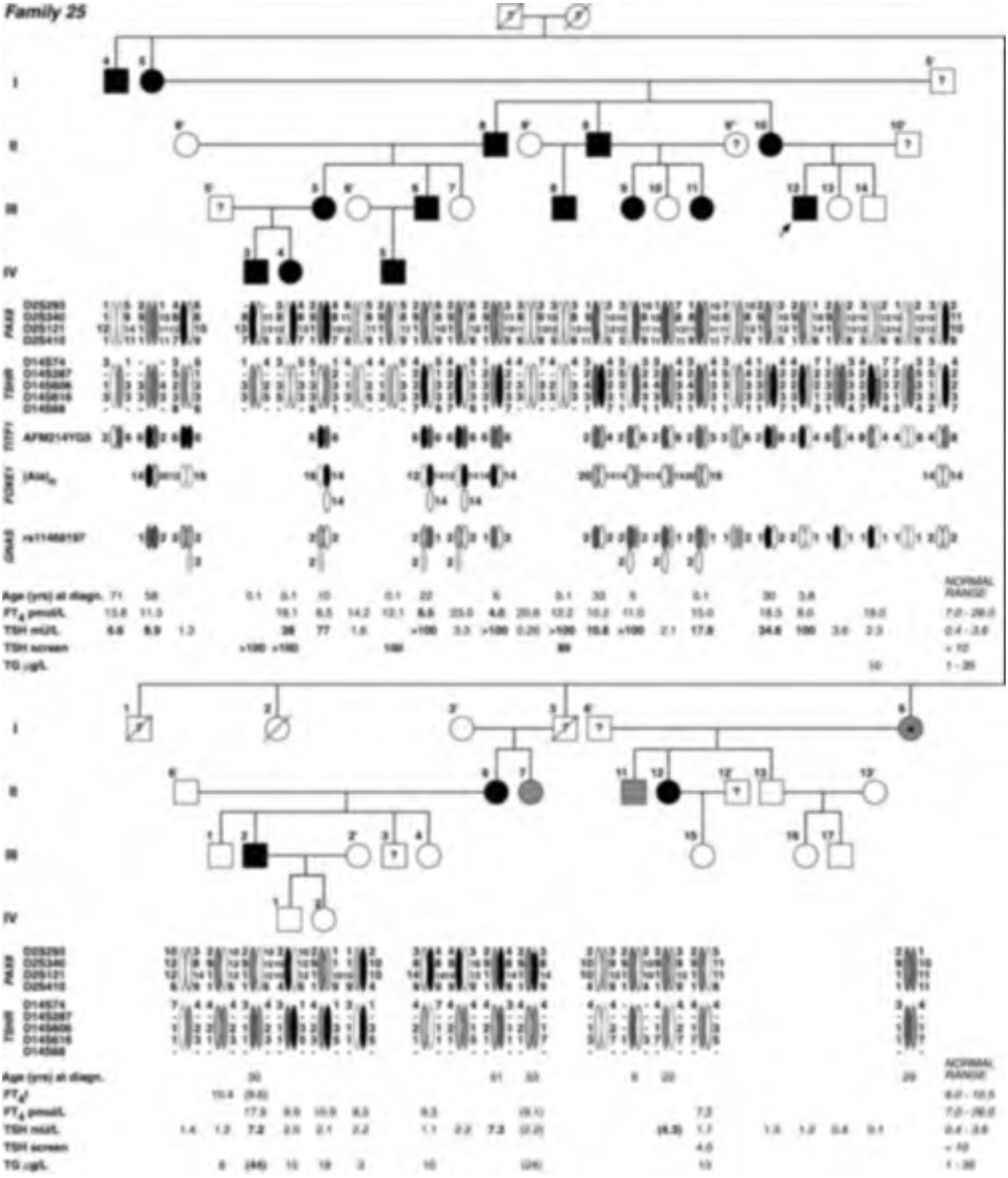

FIG. 2. Pedigree of family 25 with hereditary RTSH. Progeny of I-1 (five subjects) were normal and are not shown. For details, see legend and key to Fig. 1.

showed a eutopic gland with slightly reduced uptake. She was started on L-T_4 therapy, with normalization of serum TSH. The older sister of the proposita (III-3) also had an abnormal newborn TSH screen of 38 mU/liter and 9.0 mU/liter with a normal FT_4 on recall testing. She was not treated and when tested at 4 yr of age, her serum TSH was high at 6.5 mU/liter with normal free TH. Their father (II-3) had been treated intermittently with L-T_4. When tested by us off-treatment, serum TSH was 6.2 mU/liter with normal TH levels.

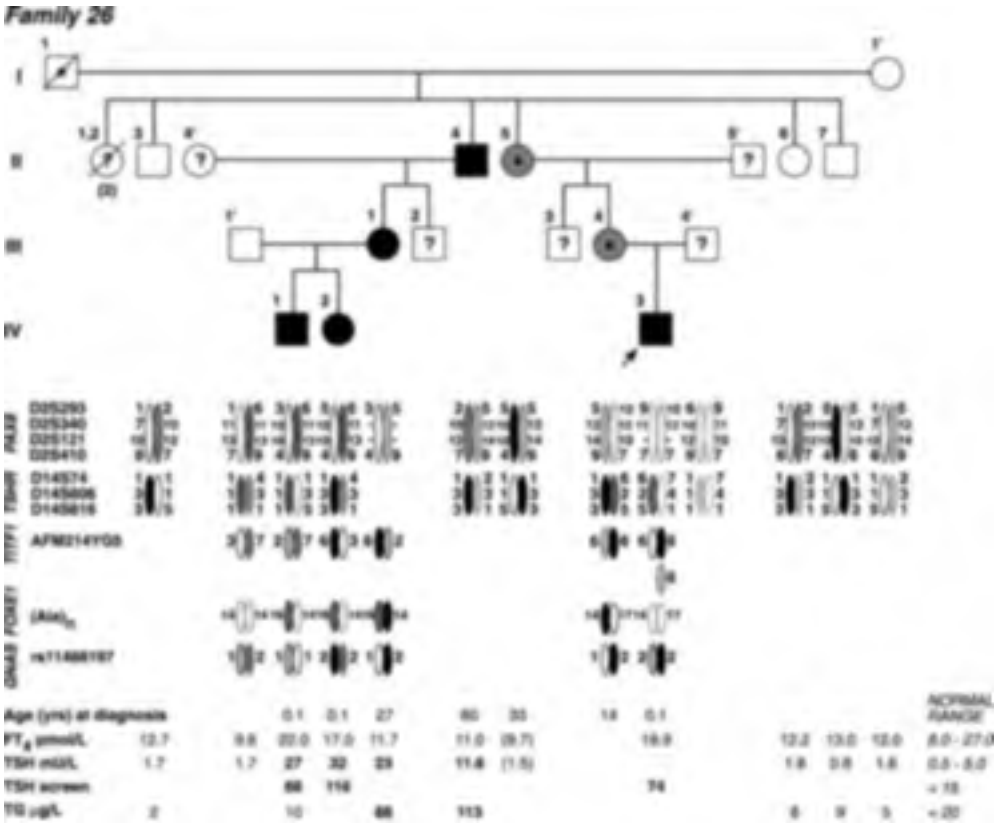

FIG. 3. Pedigree of family 26 with hereditary RTSH. For details, see legend and key to Fig. 1.

The family was of Welsh ancestry, living in Canada. A total of seven affected members in three generations were identified.

Tests of thyroid function

Total and free T_4 and T_3 and TSH were measured using commercial automated immunometric methods. For the studies correlating bioactive to immunoreactive serum TSH, the latter was determined by a third-generation chemiluminescence assay (Elecsys 2010; Roche, Indianapolis, IN). FT_4 index was calculated as the product of the serum total T_4 and the normalized resin T_4 uptake ratio (14). The RIA for serum TG was, in most instances, an in-house assay as previously reported (15). Antithyroid antibodies were measured by passive hemagglutination (Fujirebio, Inc., Tokyo, Japan).

Measurement of TSH bioactivity

Bioactivity of serum TSH was determined by measurement of cAMP generation by serum added to cultured Chinese hamster ovary cells stably transfected with a human TSHR cDNA. The method was as

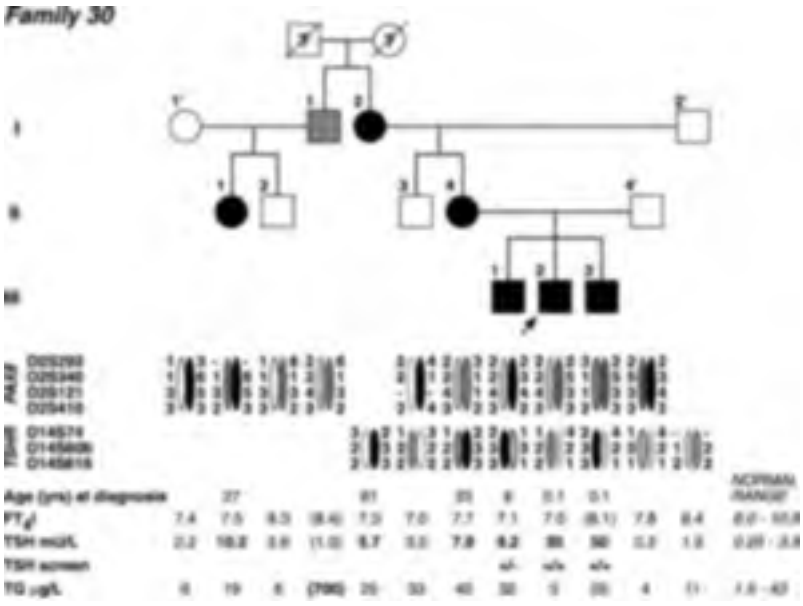

FIG. 4. Pedigree of family 30 with hereditary RTSH. TSH screen indicates initial screen/retesting. For details, see legend and key to Fig. 1.

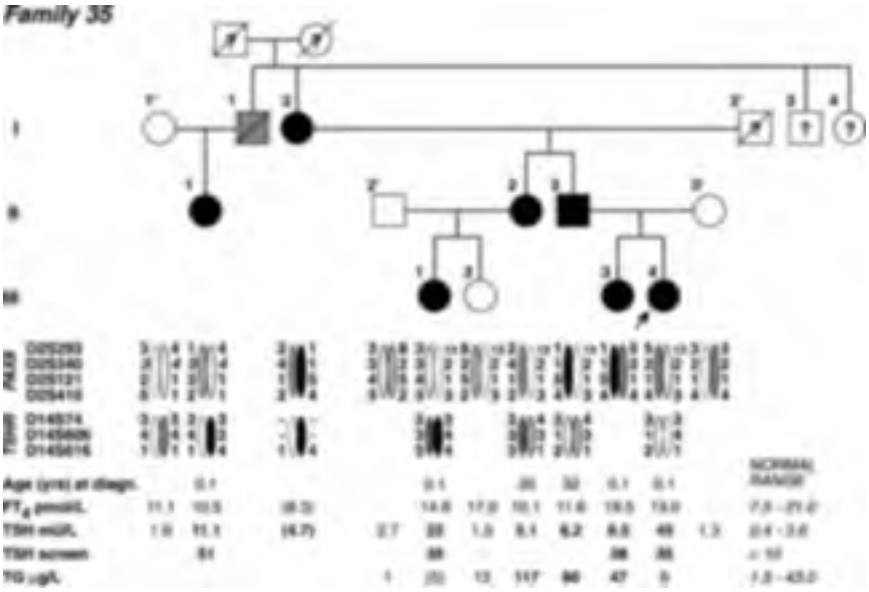

FIG. 5. Pedigree of family 35 with hereditary RTSH. For details, see legend and key to Fig. 1.

described for the measurement of TSH bioactivity in mouse serum (16), except for the use of human rather than mouse TSH-deficient serum. The former was a pool of sera from five subjects, without thyroid autoantibodies, receiving suppressive doses of L-T_4. A similar pool of sera with high TSH was obtained after short-term withdrawal of L-T_4 treatment and used for the construction of a standard curve using as diluent the TSH-deficient serum pool. Serum samples from five individuals with postablative hypothyroidism and immunoreactive TSH levels in the range found in the samples from RTSH subjects served as control. Results of cAMP measurements were correlated to those of the TSH immunoassay by linear regression. To calculate the intrinsic TSH bioactivity, *i.e.* the ratio of biological activity to amount of immunoreactive TSH (B/I ratio), the cAMP results were corrected for TSH-independent cAMP generation by subtracting the y-intercept of the (uncorrected) standard regression line.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes of 94 individuals belonging to the five kindreds. The sequences of PCR primers used for genotyping and mapping information for markers and candidate genes are listed in supplemental Table 1 (published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org/). Fluorophore-labeled PCR products were analyzed on ABI377 DNA sequencers with the GENESCAN software (PE Applied Biosystems, Foster City, CA).

Linkage analysis

Two-point LOD scores were calculated using the FASTLINK (version 4.1P) (17) version of MLINK from the LINKAGE program package (18). Multipoint linkage analysis was performed using SIMWALK2 (version 2.91) (19). All individuals of families 24 and 25 included in the linkage analysis had a definitive phenotype. In families 26, 30, and 35, subjects presumed to be affected were coded as being of unknown affection status. Parametric analysis used an autosomal dominant model of inheritance with a disease gene frequency of 1:10,000 and a penetrance for the affected genotype of 95%. Mild RTSH manifests as subclinical hypothyroidism (SH), which is the most common thyroid disorder with high prevalence in elderly women (20, 21). However, chronic autoimmune thyroiditis, the most common etiology of SH, was excluded as confounding factor in our subjects by the absence of thyroid autoantibodies and characteristic echogenicity on thyroid ultrasound. Idiopathic SH with negative thyroid autoantibodies occurs in less than 6% of all cases (22). Based on this information, we defined four liability classes with phenocopy rates of 0% (females and males 0–17 yr of age at time of diagnosis), 0.24% (females 18–44 yr of age), 0.48% (females >45 yr of age), and 0.1% (males >18 yr of age). For multipoint linkage analysis, the intermarker distances were taken from the Marshfield sex-averaged genetic map (http://research.marshfieldclinic.org/genetics/). Allele frequencies were estimated from our own genotyping data using the combined founder genotype data only. The hypothesis of linkage was rejected when LOD scores were less than −2.0.

Results

Serum TSH bioactivity

To exclude the possibility that the high serum TSH was caused by a molecule of reduced biological activity, the latter was measured in an *in vitro* bioassay by its ability to stimulate cAMP generation over the level of TSH-deficient serum. A bivariate plot of the bioassay results and the amount of immunoreactive TSH determined on the same samples by RIA fits a linear regression (Fig. 6). The intrinsic TSH bioactivity (B/I ratio) in serum of subjects with RTSH (from families 24 and 30) was not significantly different from that found in serum of patients with high TSH caused by postablative hypothyroidism (95% confidence interval for mean B/I of 19.6 ± 2.9 *vs.* 21.6 ± 6.7; $P = 0.44$; two-tailed t test). Normal TSH bioactivity had already been demonstrated for several affected members of family 25 using FRTL-5 cells (13). These results indicate that the defect resides at the level of the thyroid gland, in its responsiveness to a normal TSH molecule.

Segregation analysis of RTSH in five multigenerational kindreds

A total of 102 subjects (48 definitively affected, eight presumed to be affected, and 46 normal) were studied. Of the affected subjects, 33 were women and 23 men, a 1.4:1 ratio. Considering nuclear families with one affected parent and complete information on the progeny's phenotype, 34 were affected and 19 were not. This apparent preponderance of affected of 64% was not significantly different from the expected 50% for a dominant Mendelian distribution. Results of selected thyroid function tests are depicted on the pedi-

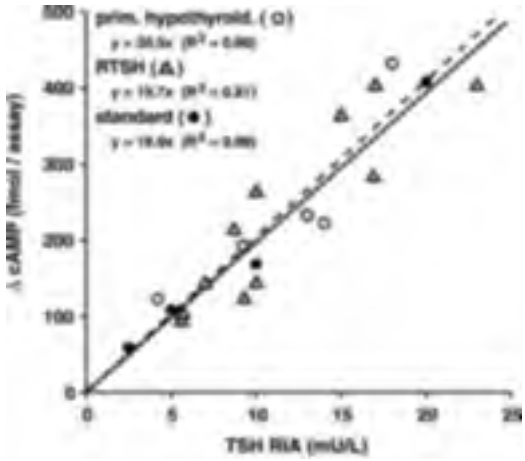

FIG. 6. Correlation of TSH bioactivity with immunological reactivity in serum of patients with RTSH (*gray shaded triangles*; *continuous regression line*) and patients with hyperthyrotropinemia due to primary hypothyroidism (prim. hypothyroid) (*open circles*; *dotted regression line*). TSH serum bioactivity was measured as amount of cAMP generated by incubation of CHO cells stably transfected with a human TSHR cDNA and corrected for the cAMP level produced by incubation with TSH-deficient serum. The immunological amount of TSH in the same serum samples was determined by a third-generation RIA. The intrinsic TSH bioactivity is reflected by the B/I ratio.

grees (Figs. 1–5), with a more detailed description of the phenotype presented in *Subjects and Methods*. In all five families, segregation of the RTSH phenotype was consistent with Mendelian inheritance following an autosomal dominant pattern with high penetrance. There was no evidence of bilineal inheritance of RTSH and all progeny of unaffected family members had normal serum TSH levels, arguing against a high rate of phenocopies or imprinted expression. In families 30 and 35, the pattern of inheritance could also be consistent with X-chromosomal dominant inheritance. However, this mode of transmission typically results in a more severe phenotype in males compared with females, which we did not observe. In family 26, the defect likely originates from the male ancestor I-1 (deceased), because I-1′ is unaffected. Therefore, the finding of an affected male (II-4) and an unaffected female (II-6) in the second generation argues against X-linked inheritance, favoring autosomal dominant inheritance.

Affected children born after initiation of national screening programs generally had a positive test, with two exceptions. One was subject III-1 of family 30, found to be hyperthyrotropinemic at the age of 8 yr, for whom the recall test after an initial positive blood screen test was considered negative. The second was the developmentally delayed subject III-3 of family 24, for whom results from the neonatal screen could not be traced but who was found to be hyperthyrotropinemic at the age of 7 yr. Thus, RTSH is clearly a congenital condition in these families. There was no hint toward incomplete penetrance, phenocopies, or parental imprinting, because unaffected parents never had affected progeny.

Linkage analysis of the TSH receptor gene

All three *TSHR* markers were informative for linkage in all five families, except marker D14S74 in family 35 (for the complete results of the two-point linkage analysis, see supplemental Table 2, which is published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org/). For each pedigree, inheritance of the inferred haplotypes was discordant with linkage of RTSH to the *TSHR* gene. Multipoint analysis formally excluded the *TSHR* locus for all five families (Fig. 7, C and D). The maximum LOD scores within the three-markers interval comprising *TSHR* were −6.37 (0.89 cM qter from D14S74) for family 24; −17.72 and −3.16 (both 1.78 cM qter from D14S74) for families 25 and 26, respectively; −2.1 (2.22 cM qter from D14S74) for family 30; and −4.50 (at the uninformative marker D14S74) for family 35.

Linkage analysis of the PAX8 gene

All four *PAX8* markers were informative for linkage in all five families, except marker D2S293, which was not informative in family 24. For each pedigree, the segregation pattern of the inferred haplotypes was inconsistent with linkage between RTSH and the *PAX8* locus. Multipoint analysis of the four-markers interval containing *PAX8* revealed maximum LOD scores of −3.81, −7.74, −3.30, and −5.15 (all at D2S293) for families 24, 26, 30, and 35, respectively (Fig. 7, A and B). The maximum multipoint LOD score for family 25 was −18.29 (0.65 cM qter D2S293) (Fig. 7A). Thus, for all five families, linkage of RTSH to the *PAX8* locus has been excluded.

Linkage analysis of other candidate genes (TITF1, FOXE1, and GNAS)

For families 24, 25, and 26, two-point LOD scores (at a recombination fraction $\theta = 0$) for markers within *TITF1*, *GNAS*, and *FOXE1* were all less than −2.0 (Table 1). Thus, under the specified inheritance model, linkage of RTSH to any of these genes was excluded in the three families tested.

Discussion

We present here the clinical and genetic investigation of five multigenerational families with RTSH. Segregation of RTSH in each family followed an autosomal dominant mode of inheritance with high penetrance consistent with a single major genetic defect. Affected members had TSH of normal bioactivity and, in all five kindreds, the *TSHR* and *PAX8* genes were excluded as the source of the defect responsible for RTSH by linkage analysis, strongly inferring the role of novel gene(s) in nonsyndromic RTSH.

High penetrance but variable expressivity of RTSH is also a typical feature of familial *PAX8* and *TSHR* mutations in other subjects we have investigated. The foregoing manifests as thyroid hypoplasia, but not ectopy or agenesis, in the most severely affected subjects. Because these genes play an important role in the function of the developed gland, we expect that sensitive thyroid function tests will likewise generally reveal abnormalities in carriers of a genetic defect associated with RTSH. It is to be stressed that this inheritance pattern is fundamentally different from that in thyroid dysgenesis because of ectopy or athyreosis, where very low penetrance rates have been proposed to account for the segregation

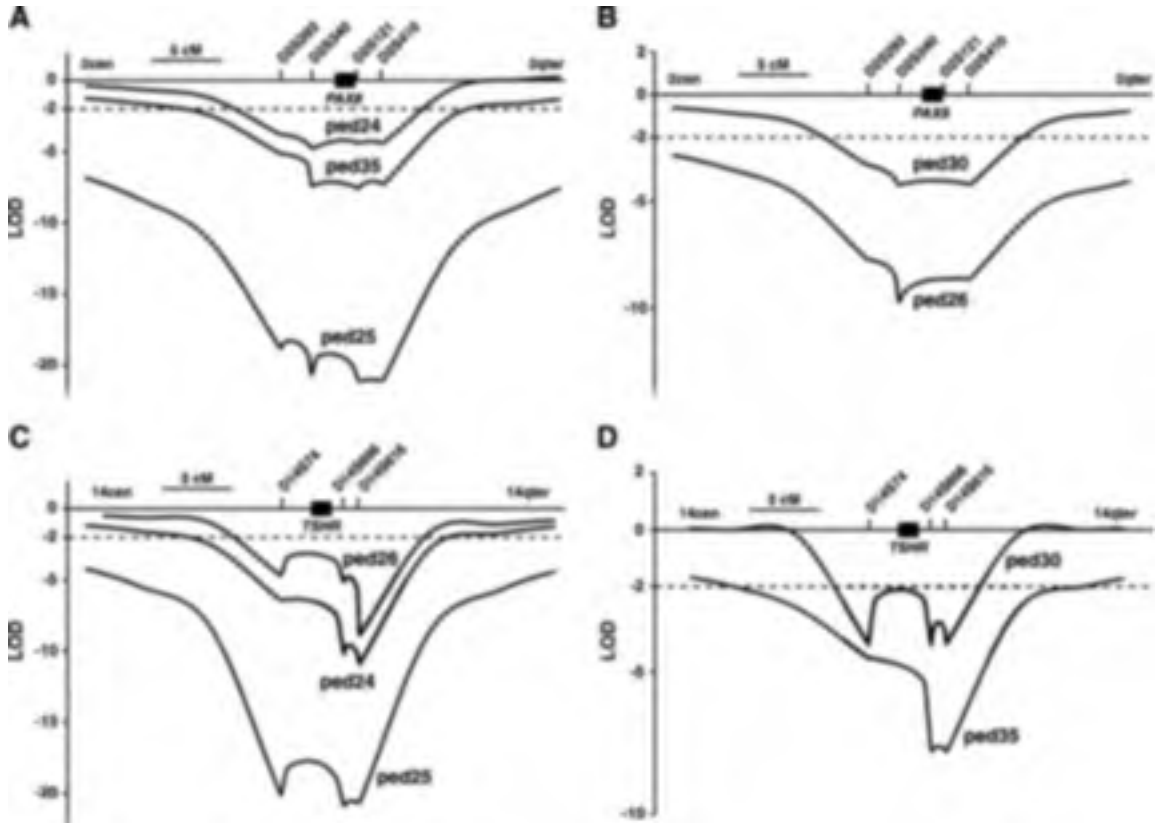

FIG. 7. Multipoint linkage exclusion maps for the *PAX8* (A and B) and *TSHR* (C and D) loci. The criterion for exclusion of linkage (LOD of −2.0) is indicated by the *dotted lines*.

pattern in familial clustered cases (23). Because ectopy and athyreosis have been observed within the same family (24), arrested migration of the thyroid anlage followed by either survival or involution of the ectopic tissue is presumably the common pathogenetic mechanism. Given the predominance of apparently sporadic cases, these forms of dysgenesis may involve threshold and epistatic effects of genes crucial for migration. The genes involved in ectopy/athyreosis, in contrast to those implicated in RTSH/hypoplasia, may not be crucial for function of the migrated gland in nonpenetrant carriers of the defect. This is supported by the observation that, in familial clustered cases of thyroid ectopy and/or athyreosis, all presumptive carriers of the genetic defect with orthotopic gland had normal thyroid function, although some of those may have minor developmental thyroid anomalies (23).

In all five families, affected individuals screened at birth had elevated TSH levels. In two subjects, the newborn TSH screen was not judged to be abnormal based on the results of recall testing. Nevertheless, they were found to be hyperthyrotropinemic later in childhood. It should be noted that 60–70% of newborns with elevated screening TSH are classified as false positives after short-range recall examination, but half of them are found to be subclinically hypothyroid when followed-up at 16–44 months of age (25). Thus, elevated TSH at screening may be a clinically relevant marker for mild persistent thyroid abnormalities.

The metabolic abnormalities of RTSH in our families are

TABLE 1. Two-point parametric LOD scores for linkage between RTSH and *TITF1*, *GNAS*, and *FOXE1* markers for a range of recombination fractions ($0.0 \leq \theta \leq 0.4$)

Marker name (locus)	Family no.	θ					
		0.0	0.05	0.1	0.2	0.3	0.4
AFM214YG5 (*TITF1*)	24	−11.10	−2.14	−1.22	−0.43	−0.10	0.01
	25	−3.09	−0.04	0.37	0.63	0.58	0.36
	26	−4.26	−0.61	−0.36	−0.15	−0.06	−0.01
rs11468197 (*GNAS*)	24	−3.07	−0.72	−0.44	−0.19	−0.08	−0.02
	25	−7.46	−3.64	−2.47	−1.28	−0.63	−0.23
	26	−4.46	−0.76	−0.47	−0.21	−0.08	−0.02
$(Ala)_n$(*FOXE1*)	24	−5.34	−1.56	−1.07	−0.55	−0.26	−0.09
	25	−5.72	−1.18	−0.65	−0.20	−0.02	0.04
	26	−4.38	−0.46	−0.24	−0.09	−0.05	−0.03

reminiscent of those caused by *TSHR* or *PAX8* mutations, yet one observation deserves mentioning: TG levels were above the upper limit of normal in 14 of 20 affected subjects tested, but only in one of 27 unaffected. The association of elevated TG levels with the RTSH phenotype becomes even more striking if we exclude family 30, in which only one of six affected subjects had a high TG level. In patients with *PAX8* or *TSHR* mutations, serum TG levels are variable, from undetectable to normal. In three neonates with severely hypoplastic thyroid glands secondary to biallelic *TSHR* mutations, TG levels were in the normal range, possibly disproportionately high relative to thyroid size (26–28). Follow-up TG levels were normal and did not increase with the rise in TSH when L-T_4 treatment was interrupted. It has been speculated that incomplete polarization of thyrocytes, misrouting of TG as a result of aggregation of mutant TSHR in the endoplasmic reticulum, or intercellular leakage from dysplastic follicles may account for this phenomenon (26). As in mice (29, 30), expression of TG in the neonatal period may also be independent of TSH signaling. In our families, however, high serum TG levels were documented in infants as well as adults, suggesting a permanent defect in thyroid differentiation rather than delayed maturation.

Although only *TSHR* and *PAX8* are established candidate genes for nonsyndromic RTSH, specific defects in genes implicated in syndromic forms of RTSH (*TITF1* and *GNAS*) or thyroid-specific transcriptional regulation (*FOXE1*) could theoretically cause nonsyndromic RTSH. Such defects, *e.g.* in *cis*-regulatory elements crucial for thyroid-specific expression, were, however, likewise excluded in the three families tested by linkage analysis. Numerous genes could be considered as hypothetical candidates based on their biochemical function, but the complexity of TSH signaling makes a comprehensive evaluation using a candidate gene approach daunting. The molecular level at which the genetic defect impairs positive TSH signaling could be further defined by biopsies of the patients' thyroids, which are not only difficult to obtain from hypoplastic glands but also considered unethical. Other than those excluded by linkage, there are currently no other known mediators of the TSH response expected to produce a thyroid-restricted phenotype.

We believe that the role of genetic defects in the etiology of mild thyroid abnormalities in the general population may be underestimated for several reasons. First, age-independent ethnic differences in the median serum TSH levels, not explained by the prevalence of autoimmune thyroid disease or confounding environmental factors (21), is a well known phenomenon (21, 31). This suggests distinct genetically determined thyroid-pituitary set-points. Second, a significant proportion of patients with mild hypothyroidism without detectable autoantibodies does not progress to overt hypothyroidism. Genetic factors have to be considered in this subset of patients, especially if born before implementation of newborn screening. Third, although current neonatal screening protocols are highly efficient in the detection of congenital hypothyroidism, mild, yet permanent, hyperthyrotropinemia is likely to be missed by screening, as indicated by follow-up studies of children with false positive or borderline test results (25, 32). This notion is also supported by the failure of neonatal screening to identify two affected subjects of our family material. Because the postnatal TSH surge requires a high TSH cutoff level to maintain specificity of the screening test, subtle RTSH may go unnoticed in the neonatal period. Thus, the diagnostic difficulties in newborns and the high prevalence of thyroid autoimmunity in the general population may both obscure the inherited nature of RTSH.

By exclusion of linkage to all genes previously associated with RTSH, this study provides the most convincing evidence for an unidentified genetic component in isolated RTSH. RTSH in our families is inherited in an autosomal dominant fashion and frequently associated with elevated serum TG levels. Identification of the underlying genetic defect(s) should provide fresh insights into pathways important for development and normal physiological function of the thyroid gland. Via epigenetic mechanisms, the very gene(s) responsible for familial RTSH may well be involved in the etiology of seemingly sporadic forms of RTSH and thyroid hypoplasia. Two of the kindreds reported here, families 24 and 25, individually have enough power to detect linkage that genome-wide screens have been undertaken in an effort to map the culprit gene(s).

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Address all correspondence and requests for reprints to: Samuel Refetoff, The University of Chicago, MC3090, 5841 South Maryland Avenue, Chicago, Illinois 60637. E-mail: refetoff@uchicago.edu.

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