Clinical and Molecular Characteristics of Eight Israeli Families with Thyroid Hormone Receptor Beta Mutations

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ABSTRACT: Background: Reduced sensitivity to thyroid hormone (RSTH) syndrome describes a group of rare heterogeneous genetic disorders. Precise diagnosis is essential to avoid unnecessary treatment.

Objectives: To identify and characterize patients previously undiagnosed with RSTH in Israel.

Methods: Patients with suspected RSTH throughout Israel were referred for study. After clinical evaluation, genomic DNA was obtained and all coding exons of the thyroid hormone receptor beta (THRB) gene were sequenced. If mutations were found, all available blood relatives were evaluated. The common polymorphism rs2596623, a putative intronic regulatory variant, was also genotyped. Genotype/phenotype correlations were sought, and the effect of mutation status on pregnancy outcome was determined.

Results: Eight mutations (one novel, two de-novo, six dominant) were identified in eight probands and 13 family members. Clinical and genetic features were similar to those reported in other populations. Previous suggestions that rs2596623 predicts clinical features were not confirmed. There was no evidence of increased risk of miscarriage or fetal viability. Mothers carrying a THRB mutation tended to have increased gestational hypertension and low weight gain during pregnancy. Their affected offspring had increased risk of small-for-gestational age and poor postnatal weight gain.

Conclusions: Clinical heterogeneity due to THRB mutations cannot be explained by the variant rs2596623. Mothers and newborns with THRB mutations seem to be at increased risk for certain complications, such as gestational hypertension and poor intrauterine and postnatal growth. However, these issues are usually mild, suggesting that routine intervention to regulate thyroid hormone levels may not be warranted in these patients.

KEY WORDS: genetic disease of the thyroid, monogenic thyroid disease, thyroid hormone receptor, thyroid hormone receptor beta (THRB) mutations, thyroid hormone resistance

Reduced sensitivity to thyroid hormone (RSTH) syndrome is a heterogeneous group of defects characterized by impaired biological activity of intact thyroid hormones (THs). To date, three distinct clinical syndromes have been identified, each associated with defects in a different molecular process. The most common syndrome is resistance to thyroid hormone (RTH), which is attributed to mutations in the thyroid receptor-beta (RTH-β) gene. The syndrome is characterized by decreased sensitivity of target tissues to TH action, leading to elevated TH levels, accompanied by normal or high thyroid-stimulating hormone (TSH) values, with variable clinical manifestations [1].

The molecular mechanism responsible for the clinical heterogeneity seen in RTH-β is unknown, but genetic or yet-to-be-discovered environmental factors modifying the mutant/normal allele expression ratio in various target tissues may be responsible. One possible mechanism could be related to the general observation that genes that are specifically expressed in different tissues may use alternative regulatory regions to fine-tune tissue-specific expression. Cis-regulatory non-coding variants can reduce or increase allele-specific transcription in a particular cell type, modifying disease risk or phenotype and thus contributing to the diversity in human genetic diseases.

The thyroid hormone receptor beta (THRB) gene has two isoforms, THRBI, which is widely distributed, and THRBI2, which is limited to the cochlea, retina, and pituitary gland. These two splice variants differ in their amino-terminal ends, but both include the same functional ligand-binding domain in the carboxyl-terminal region, where most of the disease-causing mutations that have been identified to date are located [1]. Previous in vitro studies using pituitary cell lines defined a basal promoter region in the THRBI2 isoform. While assessing the promoter's function in vivo, Jones and co-workers [2] identified a conserved 600 base-pair intronic region that appears to act as an enhancer, modifying expression of the THRBI2 isoform in pituitary and retinal cells. Alberobello and colleagues [3] pro-
provided evidence to support their hypothesis that polymorphisms in this intronic enhancer region (ICR) could modulate pituitary expression of the mutated THRB allele, thus affecting clinical presentation.

Abnormal thyroid function is known to have deleterious effects when present in either the mother or the fetus [4]. In early gestation, the fetus is solely dependent on transplacental delivery of maternal thyroid hormones, which play an important role in normal fetal development, particularly of the central nervous system. Hence, in a family carrying a THRB mutation, mismatch in maternal and fetal mutation status could have a negative impact on fetal growth and pregnancy outcome since an affected (i.e., THRB mutation carrier) fetus of an unaffected mother could be exposed to relatively insufficient TH levels, whereas a normal fetus of an affected mother could be exposed to TH excess. Data addressing the particular issue of THRB mutations and pregnancy outcome are sparse and further studies are needed to determine the clinical impact of fetal and maternal mutation status [5,6].

The primary goal of our study was to identify additional families with RSTH in Israel, particularly those with RTH-β, which is the most common subtype of RSTH, with more than 2400 patients identified to date [7]. Secondary goals were to determine whether a common variant in the ICR could explain the observed heterogeneity in RTH-β clinical symptoms and to evaluate the outcomes of pregnancies in families with THRB mutations.

Our study comprised 21 patients from eight different families harboring eight different THRB mutations, one of them novel (p.His435Arg). We discuss the impact of a specific ICR variant on the RTH-β clinical phenotype and the effects of THRB mutation status on pregnancy outcome.

PATIENTS AND METHODS

PATIENT RECRUITMENT

Between August 2011 and May 2013, endocrinologists throughout Israel were asked to refer patients suspected of having RSTH. Thirteen endocrinologists responded and referred potential participants: eight from Jerusalem, three from Tel Aviv, and one each from northern and southern areas of Israel. Prior to inclusion in the study, clinical history and medical record of each candidate was re-evaluated by the study team (EZ, BG). Only those patients with results consistent with the diagnosis of RSTH (namely, persistent inappropriately elevated free-T4 levels with non-suppressed TSH) were invited for further assessment. At the time of recruitment, after written informed consent was obtained, physical examination was performed and blood samples were taken. THRB mutation analysis was performed on patients who presented with THs levels consistent with the diagnosis of RTH-β. After a mutation was identified, all available blood relatives were recruited and evaluated using the same procedure. The Hadassah Medical Center ethics review committee approved the study.

LABORATORY STUDIES

Peripheral blood samples were obtained for DNA extraction and hormone determination. At the time of recruitment, hormonal data were supplemented by results obtained from patient medical records. In all cases, hormone levels were evaluated by fully certified automated assays. Whenever possible, free T3 (FT3), free T4 (FT4), and TSH levels were verified at the clinical laboratory of Hadassah Medical Center using the ADVIA Centaur system (Siemens Healthcare, Bayswater, Australia), catalogue numbers 03154228, 06490106, and 06491080 respectively. If plasma was not available at time of testing, the last available values were recorded from the medical records. Because of the high prevalence of autoimmune thyroid disease in RTH-β, thyroid autoantibodies were not measured and their presence on previous tests was not an exclusion criteria.

Genomic DNA was extracted from peripheral blood leukocytes using the Flexi-Gene DNA Extraction Kit (Qiagen, Germany, catalog number 51206). In infants, buccal swabs were used (DNA Genotek Inc., Canada, catalog number YGT50). All seven THRB coding exons (exons 4–10, NG_009159.1, ENST00000356447) were Sanger sequenced (primer sequences available on request) in one clinically affected individual per family (proband). Each identified variant was evaluated using the NCBI dbSNP, 1000 Genomes and Human Gene Mutation Database (HGMD). We reviewed the literature to determine the strength of evidence supporting causality for each identified mutation. Family members were genotyped by sequencing the relevant exon.

In all patients who tested positive for an established THRB mutation, the 600 base-pair intronic control region (ICR), previously reported to regulate allele-specific THRB2 expression in the pituitary, was also sequenced [2]. A common single nucleotide polymorphism (SNP) in this region, rs2596623, has been implicated as a cis-acting, allele-specific modifier of pituitary expression, thus potentially influencing the clinical phenotype [3]. In probands who were heterozygous for both a THRB mutation and for this variant, we genotyped parents (if available) and children for both. Haplotype was determined assuming complete linkage disequilibrium between the mutation and the variant. Clinical characteristics of individuals in whom the mutation and the rs2596623 variant co-segregated on the same allele were compared with those in whom the mutation and the variant were located on different alleles.

PREGNANCY, LABOR, AND THE INFANT’S FIRST MONTHS OF LIFE

A telephone questionnaire addressing key issues related to pregnancy and perinatal outcomes was designed and administered to all mothers recruited to the project. Given the limitations associated with retrospective data collection primarily based on maternal recall, all questions were designed to maximize reli-
ability. Whenever possible, data were corroborated by review of medical records.

The questionnaire was divided into two main sections. The first included questions related to pregnancy, such as miscarriages, complications, thyroid-directed treatments, gestational week, and birth weight. Infants with birth weight below the 10th percentile for gestational age were considered small for gestational age (SGA) [8]. The second part addressed main complications in the neonatal period and early childhood, including phototherapy suggesting significant jaundice, and the need for evaluation or intervention due to developmental delay.

RESULTS

THRB MUTATIONS

Based on hormone analysis and clinical findings reported in the patient referrals, 19 probands were invited for evaluation for possible RTH. In eight, a careful review of all available data, including that obtained during evaluation for this study, excluded this diagnosis. In three, the diagnosis of subclinical hypothyroidism was confirmed, with normalization of FT4, FT3, and TSH following thyroid hormone replacement. In five, previous thyroid function tests were reported to be within the normal range. One patient elected not to participate in the study.

In the 10 patients included in the study, all THRB coding exons and intron-exon boundaries were sequenced. No mutations were found in two patients, and further investigation of both showed clear evidence of a pituitary tumor on pituitary-directed magnetic resonance imaging, strongly suggesting TSHoma. Neither consented to surgery and both were treated with somatostatin analog. In the eight remaining probands, eight different THRB mutations were identified, seven previously reported and one novel (p.His435Arg) [Table 1]. Two of the mutations were de-novo (p.Leu328Ser and p.Pro453Ser). The remaining six were autosomal dominantly inherited. All mutations were located within the previously described mutation clusters 1 and 2 [Figure 1A] [1]. These eight families came from diverse ethnic backgrounds and included two Arab Muslim families and six Jewish families. The latter were from several different ethnic groups: Ashkenazi (2 families), Moroccan (1), Georgian (1), and diverse ethnic combinations (2).

NOVEL THRB GENE Mmissense Mutation (p.His435Arg)

The proband of family 7, a 30 year old Arab Muslim woman, was referred after 5 years of follow-up with repeated TFTs showing persistent elevation of serum FT4 and FT3 levels along with non-suppressed TSH (data not shown). At the time of evaluation, the patient had a goiter and sinus tachycardia, partially controlled by propranolol (160 mg/day).

Sanger sequencing identified a single nucleotide change, which predicted in an amino acid substitution (histidine to arginine) in codon 435, (p.His435Arg). This change was not predicted in an amino acid substitution (histidine to arginine) in codon 435, (p.His435Arg). This change was not

Table 1. THRB gene mutations identified in eight patients

<table>
<thead>
<tr>
<th>Mutation name</th>
<th>Exon number</th>
<th>DNA sequence</th>
<th>Family symbol</th>
<th>Inheritance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.Arg320Cys</td>
<td>9</td>
<td>...cttgcccgtggcagctgT/C-Tgcctagaccaggac...</td>
<td>8</td>
<td>AD</td>
<td>Adams et al. [22]</td>
</tr>
<tr>
<td>p.Arg320Leu</td>
<td>9</td>
<td>...cttgcccgtggcagctgT/C-Tgcctagaccaggac...</td>
<td>4</td>
<td>AD</td>
<td>Adams et al. [22]</td>
</tr>
<tr>
<td>p.Leu328Ser</td>
<td>9</td>
<td>...cagaaagtgagacttT/C-acctctgaaaggggac...</td>
<td>5</td>
<td>de-novo</td>
<td>Grace et al. [23]</td>
</tr>
<tr>
<td>p.Arg338Trp</td>
<td>9</td>
<td>...gaaatggcagtgacacG/CTtgggccagctgctaa...</td>
<td>2</td>
<td>AD</td>
<td>Adams et al. [22]</td>
</tr>
<tr>
<td>(p.His435Arg)*</td>
<td>10</td>
<td>...tgatagagccgcc-A/G-tgccagccgccgtt...</td>
<td>7</td>
<td>AD</td>
<td>Adams et al. [22]</td>
</tr>
<tr>
<td>p.Arg438Trp</td>
<td>10</td>
<td>...cttgcccgtggcagctgA/C-tctgctgacagtga...</td>
<td>6</td>
<td>AD</td>
<td>Adams et al. [22]</td>
</tr>
<tr>
<td>p.Pro453Ser</td>
<td>10</td>
<td>...acaagacttcccc-C/T-cttgctcttgagag...</td>
<td>1</td>
<td>de-novo</td>
<td>Adams et al. [22]</td>
</tr>
<tr>
<td>p.Pro453Thr</td>
<td>10</td>
<td>...acaagacttcccc-C/A-cttgctcttgagag...</td>
<td>3</td>
<td>AD</td>
<td>Adams et al. [22]</td>
</tr>
</tbody>
</table>

AD = autosomal dominant inheritance

*The novel THRB gene mutation is in bold

Figure 1. A] Graphic representation of the coding exons of the thyroid hormone receptor-beta gene (THRB) showing the alternatively spliced regions (β1 and β2), the three mutations clusters and the approximate location of the eight mutations identified in this study. The figure is modified from Dumitrescu AM, Refetoff S. The syndromes of reduced sensitivity to thyroid hormone. Biochim Biophys Acta 2013; 1830: 3987-4003 [1] B] Pedigree of family 7. Circles indicate females, squares indicate males. Diagonal line through symbols indicates that the subject was deceased at the time of the study. Double lines connecting married couples indicate a first-cousin marriage. Thick arrow indicates the proband. Roman numerals indicate generation. Individuals who were included in the study are numbered consecutively in each generation (number within the symbol). THRB genotype is given below relevant symbols (M = mutant; WT = wild-type allele). Shaded symbols indicate mutation carrier. Hatched symbol indicates an obligate heterozygote who died a few years before this study was initiated.
found in any of the relevant databases, although three different missense mutations were previously reported in this codon in patients with clinical and biochemical evidence supporting RTH-β (p.His435Leu, p.His435Gln, and p.His435Tyr) [9,10].

Additional family members were recruited. Fifteen agreed to participate and six of these, one adult and five children, were found to be mutation carriers [Figure 1B]. The mutation co-segregated with classic laboratory phenotype, including elevated TSH and non-suppressed TSH, as well as typical and variable clinical characteristics [Table 2]. Taken together, these findings strongly suggest p.His435Arg is causal and results in RTH-β syndrome.

CLINICAL PRESENTATION OF THRB MUTATION CARRIERS

Further evaluation of all eight families identified 13 additional THRB mutation carriers and 25 non-carrier family members. Thus, a total of 21 mutation carriers – 13 affected adults (3 males, 10 females) and 8 affected children (5 males, 3 females) – were evaluated.

In general, the clinical characteristics of our patients were similar to those reported in the literature and included goiter (48%), growth disorder (43%), and sinus tachycardia (40%) [Table 2]. There was no discernable genotype/phenotype relationship and marked clinical diversity was noted between individuals, even within the same family.

EFFECT OF INTRON CONTROL REGION VARIANT RS2596623 ON CLINICAL RTH-β PHENOTYPE

To test the hypothesis that variants in the putative intron control region (ICR) could explain phenotypic variability [3], we evaluated the clinical characteristics of double heterozygotes in

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**Table 2. Clinical presentation of THRB gene mutation carriers**

<table>
<thead>
<tr>
<th>Mutation*</th>
<th>Family code</th>
<th>Individual code**</th>
<th>Age</th>
<th>Gender</th>
<th>Thyroid function tests TSH / FT4 / FT3 / FT3***</th>
<th>Goiter*</th>
<th>Tachycardia**</th>
<th>Neurologic disorder***</th>
<th>Growth disorder**</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.Arg320Xys</td>
<td>8</td>
<td>II-1</td>
<td>61 years</td>
<td>Male</td>
<td>2.2 / 33.9 / 2.6</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>II-2</td>
<td>36 years</td>
<td>Female</td>
<td>2.3 / 25.1 / 2.7</td>
<td>No</td>
<td>No</td>
<td>Mild-to-Moderate</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>II-3</td>
<td>33 years</td>
<td>Female</td>
<td>2.2 / 26.7 / 3.1</td>
<td>No</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>p.Arg320Leu</td>
<td>4</td>
<td>II-1</td>
<td>33 years</td>
<td>Female</td>
<td>4.6 / 7.9 / 11.3</td>
<td>Yes</td>
<td>No</td>
<td>Mild</td>
<td>No</td>
</tr>
<tr>
<td>p.Leu326Ser</td>
<td>5</td>
<td>II-1</td>
<td>2 years</td>
<td>Male</td>
<td>6.1 / 50.4 / (15.3)</td>
<td>No</td>
<td>N/A (11)</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>p.Arg338Trp</td>
<td>2</td>
<td>I-1</td>
<td>44 years</td>
<td>Male</td>
<td>2.07 / 39.6 / (11)</td>
<td>Yes</td>
<td>No</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>I-2</td>
<td>44 years</td>
<td>Male</td>
<td>2.43 / 76.8 / (16.8)</td>
<td>Yes</td>
<td>No</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
<td>(p.His435Arg) (Novel)</td>
<td>7</td>
<td>III-3</td>
<td>30 years</td>
<td>Male</td>
<td>2.1 / 28.4 / (7.2)</td>
<td>Yes</td>
<td>Yes</td>
<td>Mild</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>IV-7</td>
<td>5 years, 7 months</td>
<td>Male</td>
<td>4.5 / 25.7 / 4.1</td>
<td>Yes</td>
<td>Yes</td>
<td>Mild</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>IV-8</td>
<td>1 year, 7 months</td>
<td>Male</td>
<td>4.6 / 31 / 3.9</td>
<td>Yes</td>
<td>Yes</td>
<td>Mild</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>IV-9</td>
<td>5 months</td>
<td>Male</td>
<td>N/A</td>
<td>No</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>III-2</td>
<td>38 years</td>
<td>Male</td>
<td>2.5 / 27.9 / 2.5</td>
<td>Yes</td>
<td>Yes</td>
<td>Mild</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>IV-1</td>
<td>14 years</td>
<td>Male</td>
<td>2.3 / 40.5 / 3.6</td>
<td>Yes</td>
<td>Yes</td>
<td>Mild</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>IV-4</td>
<td>9 years</td>
<td>Male</td>
<td>2.0 / 24.7 / 4.4</td>
<td>Yes</td>
<td>Yes</td>
<td>Mild</td>
<td>Yes</td>
</tr>
<tr>
<td>p.Arg436His</td>
<td>6</td>
<td>II-1</td>
<td>38 years</td>
<td>Female</td>
<td>2.7 / 24.1 / 2.1</td>
<td>No</td>
<td>Yes</td>
<td>Moderate</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>II-2</td>
<td>43 years</td>
<td>Female</td>
<td>N/A</td>
<td>No</td>
<td>Yes</td>
<td>Moderate</td>
<td>Yes</td>
</tr>
<tr>
<td>Pro453Ser</td>
<td>1</td>
<td>II-1</td>
<td>26 years</td>
<td>Male</td>
<td>2.8 / 31 / 8.3</td>
<td>No</td>
<td>Yes</td>
<td>Moderate</td>
<td>No</td>
</tr>
<tr>
<td>Pro453Thr</td>
<td>3</td>
<td>II-1</td>
<td>36 years</td>
<td>Female</td>
<td>3.3 / 40.4 / (8)</td>
<td>No</td>
<td>No</td>
<td>Mild</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>II-2</td>
<td>10.5 years</td>
<td>Female</td>
<td>4.2 / 25.9 / 3.9</td>
<td>No</td>
<td>No</td>
<td>Mild</td>
<td>None</td>
</tr>
<tr>
<td>Study Prevalence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10/21 (49%)</td>
<td>8/20 (40%)</td>
<td>9/17 (53%)</td>
<td>9/21 (43%)</td>
</tr>
<tr>
<td>Reported Prevalence(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>66-68%</td>
<td>33-75%</td>
<td>4-60%</td>
<td>~30%</td>
</tr>
</tbody>
</table>

Key clinical categories were adapted from Refetoff S, Dumitrescu AM [24]

*Mutation names are listed in order of coding sequence

**Generations are labeled with Roman numerals. Arabic numbers indicate the individual in each generation. For all families except for family 7, the proband generation is arbitrarily designated as I and the proband designated individual 1. Siblings of the proband are designated II-2, II-3, etc. The proband's mother is designated generation I, individual 1 (I-1). For family 7, generations and individuals are indicated as shown in Figure 1B

***Results for free T3 (FT3) are given in parenthesis. Reference ranges TSH (0.35-5.5 mU/L, FT4 (10-20 pmol/L), total T3 (1.2-3 mmol/L), FT3 (3.1-6.8 pmol/L)

1Physician examination, post-thyroidectomy status, or imaging

2For adults, resting pulse > 100 beats per minute or current related medical treatment (beta-blockade or calcium-channel blockade). For children, resting heart-rate greater than age-adjusted normal range, adapted from Fleming et al. [25].

3Emotional disturbances (or relevant medical treatment), hyperkinetic behavior, attention deficit hyperactivity disorder, learning disability and/or mental retardation [IQ < 70] [24]

4For adults, height < 5th percentile for height distribution of the Israeli population [24], or body mass index < 18 kg/m². For children, height < 5th percentile for height distribution of the Israeli population and/or weight < 5th percentile for weight distribution of the Israeli population

5Currently under treatment with T3, 80 mcg/day

6TSH values were determined by a different laboratory; reference range 0.4-7 mU/L

N/A = not available, TSH = thyroid-stimulating hormone
whom the ICR variant rs2596623 coding mutation haplotype could be determined. We identified six individuals with the variant on the mutant \textit{THRB} allele (\textit{in-Cis}) and three individuals with the variant on the wild type allele (\textit{in-Trans}). Patients in whom both alleles had the same ICR genotype were defined as concordant and served as controls (12 patients). These three small subgroups lacked statistical power to identify anything but a very major effect. However, review of the data did not reveal any suggestion of association between specific clinical symptoms and this variant (data not shown).

**EFFECT OF THRB MUTATIONS ON PREGNANCY OUTCOME**

Abnormal thyroid hormone function is known to have deleterious effects on pregnancy outcome when present in either the mother or fetus [4]. To evaluate the effect of maternal mutation status on miscarriage rate, we included 14 mothers with known mutation status and sufficient clinical data, even if the offspring mutation status was unknown. One additional mother could not be reached to respond to the questionnaire. Miscarriage rate, defined as spontaneous abortion occurring at any stage of pregnancy, was calculated for each mother and the mother or fetus [4]. To evaluate the effect of maternal mutation status on miscarriage rate, we included 14 mothers (both 0%). Interestingly, affected infants of affected mothers appeared more likely to be born SGA (50%) and have weight gain problems during infancy (63%) than any of the other groups (9–33%). Moreover, all four affected SGA offspring born to affected mothers had birth weights below the 5th percentile for gestational age; whereas, the weight of the unaffected SGA infant of the affected mother was in the 10th percentile. One normal infant of an unaffected mother was severely SGA (<1st percentile), possibly related to maternal hypertension, which was diagnosed during the first month of gestation. One of two de-novo affected offspring had intrauterine growth restriction, which was diagnosed during the second trimester, and was born SGA (~3rd percentile).

Only one mother received thyroid-related treatment during pregnancy. This mother (family 4), previously thought to have pituitary T4-monodeiodinase deficiency [11], was treated with T3 throughout two pregnancies. She gave birth to two offspring: a male carrying her mutation, born SGA (2.4 kg at term) and his unaffected sister, who was born appropriate to gestational age (2.8 kg at term), albeit on the lower end of the birth weight distribution in Israel.

**DISCUSSION**

We identified and recruited as many Israeli patients as we could who presented with evidence supporting the diagnosis of RSTH. During the 20 month recruitment period, 19 potential RSTH patients were recruited; however, only eight of those were subsequently shown to have clinical and biochemical findings fully consistent with RSTH. One novel (p.His435Arg) and seven previously reported \textit{THRB} mutations were identified in eight probands. After expanding these families to include all available relatives, a total of 21 heterozygous \textit{THRB} mutation carriers were identified. In addition to the patients reported in the table, we also explored the potential impact of maternal \textit{THRB} mutations on other pregnancy outcomes. We then compared the pregnancy and perinatal complications in all four possible combinations of maternal/fetal mutation status. The results are summarized in Table 3 and are presented as percentages as well as absolute numbers, to account for missing data. Although the numbers are too small to allow statistical analysis, our findings suggest possible increased risk of gestational complications when the mother carried a \textit{THRB} mutation regardless of fetal genotype. Specifically, affected mothers may have a higher rate of gestational hypertension (44%) and an increased incidence of inadequate weight gain during pregnancy, defined as less than 8 kg weight gain throughout the duration of the pregnancy (16%), when compared to unaffected mothers (both 0%).

### Table 3. Pregnancy outcomes in \textit{THRB} mutation carriers

<table>
<thead>
<tr>
<th></th>
<th>Affected mother (n=7)</th>
<th>Unaffected mother (n=6)</th>
<th>Overall (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term birth</td>
<td>7/8 (94)</td>
<td>2/6 (33)</td>
<td>9/14 (64)</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>4/8 (50)</td>
<td>1/6 (17)</td>
<td>5/14 (36)</td>
</tr>
<tr>
<td>Fallon</td>
<td>2/8 (25)</td>
<td>3/6 (50)</td>
<td>5/14 (36)</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>8/8 (100)</td>
<td>7/7 (100)</td>
<td>15/15 (100)</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>4/8 (50)</td>
<td>4/6 (67)</td>
<td>8/14 (58)</td>
</tr>
<tr>
<td>Maternal weight gain</td>
<td>4/8 (50)</td>
<td>3/6 (50)</td>
<td>7/14 (50)</td>
</tr>
<tr>
<td>Low maternal weight gain (€8 kg)</td>
<td>1/5 (20)</td>
<td>1/7 (14)</td>
<td>2/12 (17)</td>
</tr>
</tbody>
</table>
| Miscarriage rate calculated in 10 affected and 4 unaffected mothers, subsequent analyses were performed on 7 affected and 4 unaffected mothers as indicated

We calculated the miscarriage rate for each mother and the mother or fetus [4]. To evaluate the effect of maternal mutation status on miscarriage rate, we included 14 mothers (both 0%). Interestingly, affected infants of affected mothers appeared more likely to be born SGA (50%) and have weight gain problems during infancy (63%) than any of the other groups (9–33%). Moreover, all four affected SGA offspring born to affected mothers had birth weights below the 5th percentile for gestational age; whereas, the weight of the unaffected SGA infant of the affected mother was in the 10th percentile. One normal infant of an unaffected mother was severely SGA (<1st percentile), possibly related to maternal hypertension, which was diagnosed during the first month of gestation. One of two de-novo affected offspring had intrauterine growth restriction, which was diagnosed during the second trimester, and was born SGA (~3rd percentile).
here, to the best of our knowledge, only two previously published case reports described patients in Israel with genetically proven RSTH [12,13].

Sixty-two percent of affected individuals were females, which is not significantly different from expected if both genders are equally affected (P = 0.27), as previously reported [1]. The ethnic background of affected families, two Arab Muslim and six Jewish, reflects the population make-up of the country (20% Arab Muslim) and does not suggest any ethnic bias in disease prevalence. Two of the eight THRB mutations were de-novo (p.Pro453Ser, p.Leu328Ser), consistent with previous reports [12]. Each of the inherited mutations was limited to a single extended family, excluding a founder mutation in any of these ethnic groups. Consistent with most previous reports, and despite the fact that some Israeli populations have a high prevalence of consanguinity, inheritance was autosomal dominant in all multi-generational families. Recessive inheritance has been reported, but is exceedingly rare and typically associated with very severe phenotypes [1].

All previously reported causal mutations are located in the THRB gene region coding for the ligand-binding domain of the receptor, clustered into three hot spots, which are rich in CpG nucleotides and thus prone to single nucleotide substitutions [1]. In our study, all of the mutations, including the novel mutation (p.His435Arg), were located within two of these three clusters [Figure 1A].

We identified one novel mutation (p.His435Arg) in an Arab Muslim family. Although functional studies were not performed, it is highly likely that this mutation was causal, since three different mutations in the same codon have been previously reported in RTH-β patients [9,10,14] and the mutation co-segregated perfectly with diagnostic clinical findings in this large family [Table 2, Figure 1B]. While the precise mechanism by which this specific mutation causes receptor dysfunction is not known, codon 435 is located in the gene region coding for the ligand-binding domain of the receptor where virtually all THRB mutations described so far are located. Mutations in this region have been reported to cause either reduced affinity or abnormal interaction with one or more cofactors involved in TH action [1]. More specifically, Hassan and co-authors [15] reported that codon 435 is particularly critical for receptor function since it creates an essential functional component of the protein referred to as the His-Phe switch. Mutations that disrupt this specific codon severely inhibit T3-mediated activation of the receptor.

Although most common, THRB mutations are not the only cause of reduced sensitivity to thyroid hormone. THR alpha (THRA) mutations have been reported but are far more rare, with only a few cases to date reported since 2011. Also described are TH cell membrane transporter (MCT8) defects. One Israeli patient has been described with a deletion in this gene [13]. Impaired intracellular 5'-deiodination of T4 to T3 has also been described, although these appear to be exceedingly rare.

All three syndromes share laboratory features of inappropriate serum levels of THs with non-suppressed TSH, although levels of the different THs differ considerably between the different syndromes. Dumitrescu and Refetoff [1] and Refetoff et al. [7] and colleagues presented a comprehensive review of these various syndromes. Patients with all forms of RSTH would be expected to meet our initial criteria for inclusion. Our failure to identify additional cases with RSTH syndromes other than RTH-β supports the findings that these defects are also extremely rare in the general Israeli population.

DOES THE INTRON CONTROL REGION VARIANT (RS2596623) MODIFY THE RTH-β CLINICAL PHENOTYPE?

Consistent with previous findings [1], the clinical presentation of THRB gene mutation carriers in our cohort varied considerably in patients, even among individuals within the same family [Table 2]. The importance of genetic variation within non-coding regulatory regions for disease causation and modulation of disease phenotype is being increasingly recognized [16]. Alberobello et al. [3] proposed that cis-acting variants within a putative regulatory intron region could explain some of this phenotypic heterogeneity. However, we failed to identify any suggestion of an effect of the ICR variant that we evaluated on disease phenotype. Thus, although our double heterozygotes sample size is too small to draw definitive conclusions, our findings do not support the hypothesis of Alberobello and colleagues.

PREGNANCY AND RESISTANCE TO THYROID HORMONE SYNDROME

We determined the incidence of pregnancy and post-natal complications when the mother, the fetus, or both carry a THRB mutation. We did not observe increased miscarriage rates in any of the groups. Our observed miscarriage rate in mutation-carrying mothers (16%) is like that recently reported in the United States national pregnancy analysis series [17]. The somewhat greater rate observed in the unaffected mothers (36%) is probably skewed due to the small numbers (n=4) and the relatively high miscarriage rate in two of the unaffected mothers.

Affected mothers appeared to have an increased incidence of gestational hypertension and poor weight gain during pregnancy. Only those pregnancies in which both the mother and fetus carried the THRB mutation appeared to be associated with an increased incidence of SGA at birth and poor postnatal weight gain. Our data imply that this impact on birth weight may be clinically important since SGA in this sub-group was relatively severe (< 5th percentile for Israel's newborn population [8]).

These findings were unexpected, since mutation mismatch between the mother and fetus was predicted to cause relative hypothyroidism in affected fetus of a normal mother and relative hyperthyroidism in a normal fetus of an affected mother.
In other clinical settings, maternal or fetal hypo- or hyperthyroidism has been associated with gestational and postnatal complications [4], as demonstrated, for example, in a recent large Dutch cohort correlating high maternal TH levels during pregnancy with higher incidence for low birth weights and SGA newborns [18]. When both mother and fetus have the same THRB mutation genotype, physiologically appropriate TH levels are predicted, leaving unexplained our observation of increased incidence of SGA and poor postnatal growth in affected infants of affected mothers.

Our findings appear to contradict those reported by Anselmo and co-authors [19], which described pregnancy outcomes in a large Azorean family with a known THRB gene mutation (p.Arg243Gln). Affected mothers (n=9) were compared to normal mothers married to affected fathers (n=9), and unaffected first-degree relatives (n=18) served as controls. Affected mothers were noted to have an increased obstetric risk, with threefold to fourfold increased chance for miscarriage, an observation suggested by the authors as being due to relative intrauterine hyperthyroidism in unaffected fetuses. In addition, normal infants carried by affected mothers were significantly smaller for gestational age and had undetectable serum levels of TSH at birth. The differences between our findings and the findings of Anselmo’s group might be explained by the fact that, by studying a single large family, Anselmo was able to better define disease-related phenotypes by eliminating variances related to different mutations and limiting environmental variation. However, we believe this to be unlikely since our study population also included a large, extended family (family 7) that enabled comparison between two nuclear families carrying the same mutation, one maternally and the other paternally inherited [Figure 1B]. As with our entire study population, no excess obstetric risk was observed and only affected infants born to an affected mother appeared to have more gestational and neonatal complications than the other group (data not shown). The findings in both our study and that of Anselmo must be interpreted with caution since in both clinical and genetic data is missing for a relatively large percentage of individuals. Sarkissian et al. [20] also reported multiple miscarriages in a RTH-β affected female. In contrast to the findings in these two reports, our findings are more consistent with previous studies that describe benign outcomes of pregnancies involving THRB mutations, albeit frequently associated with some degree of intrauterine growth retardation [5,6].

Our findings are more consistent with studies in a mouse model of RSTH described by Alonso and colleagues [21], who observed no increased intrauterine mortality in any of the groups and also demonstrated that pregnancies of mothers with THRB gene deletions result in low birth weights regardless of fetal genotype. In their recent review of the topic, Weiss and co-authors [5] concluded that there are insufficient data to determine the risk associated with RSTH and pregnancy and that more data are needed before management recommendations can be made. A subsequent article reiterated this recommendation [6].

CONCLUSIONS
We identified eight Israeli families with RTH-β, each with a different THRB mutation. Clinical features were variable, even among affected family members. Our findings do not confirm a previous report suggesting that a specific intronic genetic variant affects the clinical phenotype. Although, in general, pregnancy outcomes in our cohort were good and without the need for thyroid-related treatment, our findings also suggest that mothers with RSTH may be at risk of gestational hypertension and their infants may be at risk for SGA at birth and later poor weight gain regardless of fetal genotype.

As with all rare diseases, no single study can be large enough to present conclusive, statistically validated results. More data are needed to develop clear and well-supported recommendations for treatment and follow-up of patients with THRB mutations, particularly during pregnancy, which is an issue that is addressed in only a small number of reports. This undertaking could be accomplished by the formation of an international consortium aimed at collecting and analyzing data from a large number of cases.

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References
1. Dumitrescu AM, Refetoff S. The syndromes of reduced sensitivity to thyroid hormone. Biochim Biophys Acta 2013; 1830: 3987-4003.


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**Capsule**

**Killing tumors by targeting their neighbors**

Pancreatic cancer is infamous for its bad prognosis as well as for its dense stroma. Most therapies target the tumor cells themselves rather than the stroma. Zhou et al. identified a therapeutic target called DKK3, which is produced by pancreatic stellate cells. This protein was present in the majority of human pancreatic tumors sampled. Ablating DKK3, either by genetic means or with a monoclonal antibody, provided a potentially effective treatment. Antibody treatment reduced tumor growth and extended survival in mouse models, especially when combined with an immune checkpoint inhibitor. *Sci Transl Med* 2018; 10: eaat3487 Eitan Israeli

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**Capsule**

**Long-term outcomes of silicone breast implants**

Coroemos and co-authors analyzed the long-term safety and efficacy outcomes of patients with breast implants. Long postapproval study (LPAS) prospectively have monitored long-term implant-related outcomes and systemic harms for silicone/saline implants from two manufacturers (Allergan and Mentor) placed for primary/revision augmentation/reconstruction. Systemic harms, self-harm, and reproductive outcomes were compared with normative data. Implant-related complications were analyzed by implant composition and operative indication in the short and long terms. LPAS data included 99,993 patients, 56% of implants were silicone for primary augmentation. Long-term magnetic resonance imaging surveillance was under 5%. Compared with normative data, silicone implants are associated with higher rates of Sjögren syndrome (standardized incidence ratio [SIR] 8.14), scleroderma (SIR 7.00), rheumatoid arthritis (SIR 0.96), stillbirth (SIR 4.50), and melanoma (SIR 3.71). One case of BI-ALCL was reported. There was no association with suicide. In the short term, rupture was higher for saline (2.5% vs. 0.5%, P < 0.001), and capsular contracture was higher for silicone (5.0% vs. 2.8%, P < 0.001). At 7 years, reoperation rate was 11.7% for primary augmentation, and 25% for primary/revision reconstruction. Capsular contracture (III/IV) occurred in 7.2% of primary augmentations and 12.7% primary reconstructions, and is the most common reason for reoperation among augmentations. *Ann Surg* doi:10.1097/SLA.0000000000002990 Eitan Israeli