CLINICAL STUDY

Retarded bone growth in thyroid hormone resistance. A clinical study of a large family with a novel thyroid hormone receptor mutation

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Abstract

Objective: Thyroid hormone resistance (RTH) is characterised by variable tissue hyporesponsiveness to thyroid hormone. The disorder is usually caused by mutations in the thyroid hormone receptor beta (TR β). We describe a large family with this disorder.

Subjects and measurement: We identified 36 family members with RTH in four generations by screening relatives of patients with the diagnosis. The diagnosis was verified by identification of a mutation in the thyroid hormone receptor beta (TR β) gene. Symptoms, clinical findings and laboratory tests of 29 affected individuals were compared with those of 16 first-degree relatives.

Results: Bone maturation in children with RTH was delayed. The height was lower both in children and in adults with RTH than in the controls. Children with RTH had lower birth weight than the controls, particularly when the condition was inherited from the father. We did not observe increased prevalence of neuropsychological symptoms associated with RTH in this family. Palpitations and increased pulse rate indicated mild cardiac hyperthyroidism. Direct sequence analysis of the TR β gene revealed a novel point mutation, a heterozygous transition c.1031G > C in exon 9 theoretically substituting Gly344Ala.

Conclusions: We found evidence of skeletal tissue hypothyroidism that resulted in permanent growth retardation from prenatal to adult life. We found substantial variations in thyroid hormone levels and clinical presentation, but most individuals were without symptoms of thyroid disorder.

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Introduction

Thyroid hormone resistance (RTH) is a rare autosomal dominant disorder characterised by reduced sensitivity to thyroid hormone (TH) in the pituitary gland and in peripheral tissues. The hallmarks of the disorder are high levels of free thyroxine (FT4) in serum, and paradoxically normal or elevated levels of thyroid-stimulating hormone (TSH) (1). Most mutations are found in three exons in the thyroid hormone receptor-beta (TR β) gene on chromosome 3, coding for the hormone-binding domain in the receptor (2–4). More than a hundred different mutations in the TR β gene have been characterised (5). TH also exerts its effects through thyroid hormone receptor-alfa (TR α), but mutations in this gene have not been identified. About 10% of individuals with classical RTH do not have mutations in the TR α or TR β genes. A defect in the cofactors of the TH-signalling pathway has been postulated (5).

The clinical picture varies from no symptoms, indicating generalised resistance, to manifest thyrotoxicosis, as

would be seen in selective pituitary resistance. Variable resistance in different organs due to differences in the distribution of TR α and TR β can cause a mosaic of hyper- and hypothyroid signs in the same patient. The most common findings are growth retardation, attention deficit and hyperactivity disorder (ADHD), goitre and hyperthyroid cardiac symptoms (1, 3, 6).

We describe symptoms, clinical findings and laboratory data from four generations of a large family with a novel TR β mutation.

Patients and methods

The family

Altogether, 36 persons with RTH were identified (Fig. 1). Eleven individuals were known to have RTH prior to this investigation, as diagnosed by elevated FT4 levels without suppression of TSH. These persons and their siblings and children were invited to participate in the study. Forty-five persons were thus included, of whom

Figure 1 Pedigree of a family with thyroid hormone resistance. Closed symbols represent affected members; symbols with a central dot, unaffected members; open symbols, untested members. Diamonds indicate undetermined sex and number of persons. Arrow indicates index case. Diagonal line across a symbol means the person is dead. Persons that participated in the investigation are numbered. In those with an asterisk, a gene test was done.

29 had elevated FT4 levels, and 16 had values in the normal reference range. The medical records of another seven family members with elevated thyroxine levels, who were dead or refused further investigations, were reviewed. The diagnosis of RTH was considered correct if FT4 exceeded the normal reference range, with normal or elevated TSH, since the biochemical findings were consistent with the results of the genetic analysis in 29 persons who were tested.

The index case, IV-25 in the pedigree (Fig. 1), a 6-year-old girl, presented with remarkable restlessness and a high resting pulse at a consultation for asthma. The work-up revealed hyperthyrotoxaemia. The presenting symptoms in those who had already been diagnosed were also restlessness, asthenia or palpitations. Five patients had erroneously received thyreostatic treatment for suspected Graves' disease before the diagnosis of RTH was made. One of these persons eventually underwent hemithyroidectomy after developing marked goitre, possibly due to the treatment.

Clinical assessment

Symptoms of hypothyroidism or hyperthyroidism were recorded. The persons were asked about hearing loss and major psychiatric symptoms. For children, records of height, weight and audiometric measurements were provided by the local mother-and-child health clinics. Standard deviation scores (SDS) for height and weight were estimated from Norwegian reference data (7–9). Bone age was determined by radiography of the left hand according to a standard atlas, and scored according to the Brush foundation study (10). Parents were interviewed about symptoms of ADHD in their child, according to DSM-IV criteria. The answers were quantified and SDS was calculated (11). They also answered the Conners 48-point questionnaire (CPSQ-48) (12).

Adult ADHD was evaluated according to the Utah criteria (13). The level of education was scored from 0 (none) to 3 (college and university). All the individuals underwent a full clinical examination by one of the investigators (the children by PHK, who is experienced in paediatric endocrinology; the adults by KL, who is experienced in adult endocrinology). The presence of goitre was determined by palpation. Heart rate abnormalities were defined according to established norms (3, 14). Night-time (0200–0400 h) and daytime (1300–1500 h) heart rate was measured by 24-h ECG recordings in 15 adults.

Laboratory analyses

Blood samples were taken from all 45 family members and eight spouses. The samples were frozen at -70°C and analysed in one batch. Free thyroxine (FT4), total thyroxine (T4), triiodothyronine (T3), thyroxine-binding globulin (TBG), TSH, sex hormone-binding globulin (SHBG) and ferritin were analysed by established methods. SHBG and ferritin were used as markers of thyroid hormone action in the liver (15, 16).

DNA was extracted from EDTA blood. PCR amplification was carried out by standard procedures using Amplitaq GOLD (ABI, Foster City, CA, USA) or Taq polymerase (Boehringer-Mannheim, Germany). Following amplification, the PCR products were treated with SAP/exonuclease (Amersham, UK), and both strands were sequenced using the ABI PRISM Big Dye terminator sequencing kit, version 2.0, and analysed on an ABI 310 Genetic Analyser. The sequence variant identified here introduces a new *AccI* (New England Biolabs, Beverly, MA, USA) recognition site in exon 9, forming the basis for restriction enzyme testing of family members.

Statistics and ethics

Student's *t*-test was used for comparison of continuous distributions, whereas the chi-square test or Fisher's exact test was used for comparison of proportions, with a 5% significance level. Standard deviation scores (SDS) (that is, Z-score) represent actual value minus mean value divided by standard deviation.

The study protocol was approved by the Regional Committee for Ethics in Medical Research and by the Centre for Registers in Medicine.

Results

Growth and somatic development

The birth length of the individuals with RTH was lower, although not significantly so, than their family controls. However, at the time of this study, they were significantly shorter than their unaffected relatives, as shown in Table 1. There were similar findings for adults. Women and men with RTH were respectively 4.4 and 4.7 cm shorter than their relatives. Twenty per cent of the RTH patients, but none of the controls, had heights below mean minus 1.5 s.d. (approximately the 5th percentile). If their mother had RTH, the children were less growth retarded, as shown in Fig. 2. The subgroups of non-RTH children were too small for statistical evaluation. Seven of eight children with RTH, but only one of six controls, had bone age below mean minus 2 s.d. The RTH children had lower birth weight than their unaffected siblings. None of the eight RTH children of mothers with RTH had birth weight below mean minus 2 s.d., in contrast to three of four children who inherited the condition from their father. Mean age of menarche for girls with RTH was 11.5 years, 1.9 years earlier than their unaffected relatives.

Other clinical findings

ADHD was diagnosed in only one boy with RTH. The levels of ADHD symptom scores were low both in adults and in children, and there were no significant differences between the RTH and the non-RTH groups. Depression and anxiety were equally frequent in the groups. The level of education was similar in the groups (RTH vs non-RTH, score 2.8 [0.9] vs 2.9 [0.8]). The resting pulse and recorded heart rate were only non-significantly increased in the adults with RTH (Table 1). No adults reported reduced hearing. One girl had severe sensorineuronal hearing loss, and one boy had unilateral severe conductive hearing loss, possibly due to juvenile otosclerosis. Three persons in the RTH group had had abnormal bowel function in childhood, regarded as 'toddler diarrhoea', but none in the control group. Five of nine children with RTH had problems with dry skin, including four with atopic eczema. However, two of five children without RTH had similar findings. Most of the persons with RTH were clinically euthyroid. Six persons (22%) with RTH, and one without RTH, had palpable goitre. These were equally distributed in the subfamilies.

Laboratory results

The results of the hormone analyses are given in Fig. 3. SHBG was lower in the group with RTH than in the non-RTH group (47.5 [21.2] vs 80.6 [57.3] nmol/l, $P < 0.05$). Ferritin was not statistically different (63 [43] vs 99 [66] µg/l, $P > 0.2$). TSH and thyroid hormone levels examined in eight spouses of the family members were not significantly different from the unaffected adult group.

DNA analysis in one patient showed a mutation in the T3-binding domain in one of the two TRβ alleles.

Table 1 Age and gender distribution, and clinical findings in 29 family members with thyroid hormone resistance (RTH) and 16 family controls (non-RTH). Mean values and s.d. (in square brackets).

	Children			Adults		
	Non-RTH (n = 7)	RTH (n = 10)	P value	Non-RTH (n = 9)	RTH (n = 19)	P value
Age (years)	6.1 [4.5]	9.6 [5.1]		32.7 [5.8]	36.7 [12.1]	
(range)	(0.4–13)	(1–18)		(23–43)	(23–57)	
Females no.	3	5		4	14	
Birth length SDS*	−0.09 [0.82]	−0.69 [0.98]	0.19			
Length at examination SDS	0.34 [0.83]	−0.67 [0.85]	0.03	0.14 [0.60]	−0.66 [1.07]	0.05
Bone age SDS	−0.24 [1.77]	−2.3 [1.45]	0.04			
Birth weight SDS§	−0.14 [0.58]	−0.91 [0.78]	0.04			
Weight/length SDS	−0.02 [0.67]	−0.85 [0.73]	0.04			
Body-mass index (BMI) SDS				0.47 [1.56]	−0.88 [0.66]	0.29
Heart rate at examination				66.5 [10.5]	78.4 [15.9]	0.06
Heart rate daytime (1300–1500 h)**				79.5 [14.6]	88.1 [17.5]	0.34
Heart rate night-time (0200–0400 h)**				62.0 [13.9]	70.1 [9.3]	0.20

§ Referred to age.

* Birth length and birth weight from 12 individuals with RTH.

** 24-h ECG recordings from six non-RTH and nine RTH individuals.

SDS: For definition, see text, *Statistics and ethics*.

Figure 2 Birth length and weight, and length at examination in children with thyroid hormone resistance; mean standard deviation scores (Z-scores) with s.d. Children presumed to be exposed to higher levels of thyroid hormones in intrauterine life from mother with hyperthyroxinaemia have less growth retardation.

The normal guanine in nucleotide 1031 in exon 9 was replaced by cytosine, which theoretically replaces glycine by alanine in position 344 of the receptor protein. All 16 persons with elevated FT4 who were tested had the same mutation. The mutation was not found in any of the 13 family members with normal FT4 levels who were tested.

Discussion

This is the largest family with thyroid hormone resistance yet reported. Neither have so many unrelated people with identical TRβ mutation previously been described. In this investigation, elevated FT4 levels without suppression of TSH defined RTH with 100%

Figure 3 Hormone values in a family with thyroid hormone resistance. N = unaffected first grade family members to persons with resistance (RTH). Closed symbols represent adults; open symbols, children; circular symbols, females; square symbols, males. Dotted lines show normal ranges.

sensitivity and specificity, as verified by gene analysis. We studied a representative sample of RTH individuals, since most of our subjects were not previously registered patients, but were included through screening of family members. In contrast to studies of small families including many index cases, our approach reduces the risk of overestimating symptoms and clinical findings associated with the gene defect.

Thyroid hormone is necessary for normal bone growth. It has both direct effects on bone cells and indirect effects by stimulation of growth hormone release (17, 18). We found evidence of marked thyroid hormone resistance in bone, with significantly delayed skeletal maturation and growth. Other investigators have found similar results (1, 3, 18). Few authors, however, have used healthy family member controls, and no one has evaluated birth data in this way. Birth *weight* is generally regarded as a more accurate indicator of body size than birth *length*. Our data indicate that maturation is influenced by RTH even in prenatal life, resulting in slight intrauterine growth retardation in some RTH individuals. The children with RTH who were born by mothers without RTH were smaller than those of mothers with the condition. A possible explanation is that excess intrauterine transmission of TH from mothers with the same condition prevents prenatal growth retardation (3). In contrast to other studies (18), we also found reduced height in the RTH adults. The growth pattern in the children with RTH resembles that of other children with intrauterine growth retardation, who often have persistent short stature (19). Low final height in our study could also be explained by early puberty. The influence of RTH on time of pubertal start has not been described before, and whether there is a casual relationship is unknown. Interference with hypothalamic maturation and/or influence on the hypothalamic-pituitary-gonadal axis are possible. It is known that long-lasting hypothyreosis can cause early puberty.

A lower ratio of weight to height, or BMI, is also mentioned in an earlier study (3). This is probably not due to higher motor activity in RTH cases, but possibly to higher basal metabolic rate.

We did not identify major neuropsychological symptoms associated with RTH in this family. High frequency of ADHD has been reported in RTH (1, 3, 20, 21). Only one child in our study had ADHD. Nor did we find indications of increased minor impairment of attention or hyperactivity. We did not formally test intelligence, but the persons with RTH managed well in school, with a level of education similar to that of their unaffected relatives. Many studies have described a lower intelligence level than in control groups (3, 6). Depression and anxiety have been associated with hyperthyroidism (22), but neither others (20) nor we have found increased frequency of such symptoms in RTH. The RTH subjects in this study reported frequent palpitations. Enhanced sensitivity to catecholamine

stimulation has been demonstrated in hyperthyroidism (23, 24), and mild cardiac hyperthyroidism could thus be a feature of RTH in this family, although the resting heart rate was not significantly increased.

Surprisingly few had goitre. This is one of the main findings in RTH, reported in 65–90% of cases (1, 3). Sensorineuronal deafness has been described in some patients with RTH (1, 3), and the TR β is expressed in cochlear cells (25). Our patient with sensorineuronal hearing loss had several neonatal risk factors, but the receptor aberration might have contributed to her deafness. Juvenile otosclerosis was not mentioned earlier, but conductive hearing loss is a common finding. This has most often been associated with recurrent ear infections (3), which we did not find in this study. We found transient signs of intestinal hyperactivity in some children, which has not been mentioned by others. These findings could be hyperthyroid signs, but the numbers are small and of uncertain significance. The findings of dry skin in some children might reflect cutaneous TH resistance. Normal ferritin and reduced SHBG levels with high thyroid hormones reflect hyporesponsiveness in liver tissue, and this is also found in most other studies.

As in other studies, we have found large individual variation in clinical and hormonal results. This may be explained by individual differences in the distribution of TR α and TR β in the tissues (2, 3, 26). Moreover, variations in expression of the mutant TR β and the extent to which it inhibits the wild-type TR β (4, 27) or other nuclear receptors (28) may contribute to this diversity. Our study demonstrated no significant difference in FT4 and T4 levels between unaffected family members and unrelated spouse controls. Therefore, there is no reason to suspect that other hereditary factors influence the phenotype of RTH in this family, as suspected in another family investigated (29).

In conclusion, this is the largest family with thyroid hormone resistance reported so far in the medical literature. We found evidence of hypothyroidism in skeletal tissue with persistent growth and bone maturity retardation from intrauterine life. There were no important neurological disturbances. Palpitations and slightly increased heart rate point to moderate cardiac hyperthyroidism. We found substantial variations in thyroid hormone levels and clinical presentation, but most individuals were healthy and asymptomatic.

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