Clinical, Pathological, and Molecular Studies of Two Families with Iodide Organification Defect

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Abstract

Two unrelated families (CA and NA) in which an iodide organification defect (IOD) was present in two siblings of each family were studied. These patients had congenital goiters with hypothyroidism and a positive perchlorate discharge test. Examination of the thyroid tissue revealed no thyroid peroxidase (TPO) activity. Histologic findings were consistent with a microfollicular pattern of hyperplasia. Moderate cellular atypia was present, characterized by nuclear pleomorphism and hyperchromatism. Full length thyroglobulin was purified by gel filtration, but was not iodinated. Immunohistochemical studies using a polyclonal anti-human thyroid peroxidase (hTPO) antibody confirmed the presence of immunoreactive TPO protein in the thyroid tissues. Samples of normal and affected individuals were studied with respect to the presence of various fragments using TPO probes of varying sizes. The two affected siblings from family CA were homozygous for fragments 3.9, 4.6, and 7.0 kb (BglII) and 2.3 and 2.9 kb (TaqI), whereas the parents were heterozygous. In the other family (NA), the BglII digestion and TPO-31 hybridization revealed an interesting and informative polymorphism. The parents showed two different polymorphic patterns: the father had a 5.0/4.6 kb pattern and the mother a 4.7/4.5 kb pattern. However, the two affected siblings showed the same heterozygotic allelic pattern at 4.5/4.6 kb. The restriction fragment length polymorphism detected in these two families suggests an association between the TPO gene and an IOD. Results suggest that in these dyshormonogenetic tissues an altered TPO protein molecule is being synthesized, without detectable in vitro activity, but visible by immunostaining techniques in the goitrous tissue. Mutations in the TPO gene sequence are most likely associated with these changes.

Key Words: Thyroid peroxidase; congenital hypothyroidism; dyshormonogenesis; polymorphisms; iodide organification defect; gene mutations.

Introduction

Thyroid peroxidase (TPO) is a membrane-bound glycosylated hemoprotein involved in two important reactions in the biosynthesis of thyroid hormone: it catalyzes the iodination of tyrosine residues on thyroglobulin and the intramolecular coupling reaction of iodinated tyrosines, leading to the formation of thyroxine (T₄) and triiodothyronine (T₃) [1].

Defective expression of TPO function at the clinical level is caused by a group of defects. The common pathophysiological denominator is the discharge of a significant percentage of labeled iodide from the thyroid gland upon administration of perchlorate, indicating a defect in converting accumulated iodide to organically bound iodine. In thyroid tissue obtained at surgery, TPO activity is usually absent, defec-
tive for iodide oxidation, or unable to perform iodotyrosine coupling [1]. Therefore, the affected subjects are usually hypothyroid since birth, and large goiters will develop during postnatal life because of persistent thyroid-stimulating hormone (TSH) stimulation.

Human TPO gene is about 150 kb long, consisting of 17 exons [2] and is located on the short arm of chromosome 2 region p25-24 [3,4]. The complete sequence of the human peroxidase cDNA is about 3 kb long [5-8]. Two other human peroxidase cDNAs of varying sizes were isolated from a thyroid cDNA library and resulted from alternative splicing of the TPO mRNA, giving rise to a 171 nt shorter mRNA lacking exon 10 [5] and a 131 nt shorter mRNA lacking exon 16 [9].

Linkage studies indicated that the total organification defects are caused by TPO gene mutations in a number of families [10-14]. Other recent studies have indicated different mutations in the TPO gene, such as a -GGCC- duplication in exon 8 [15] and a 20-bp duplication in exon 2 [16] generating a frameshift, resulting in a termination sign in exon 9 and exon 3, respectively.

Bikker et al. [17] reported five novel mutations in the TPO gene, related to total defect in the organification of iodide and Ishikawa et al. [18] identified a single point mutation in the TPO gene that does not alter the amino acid residue, but resulted in a TPO protein partially defective for binding iodide. More recently, Bikker et al. [19] identified a single base substitution (C → T) at nucleotide 1708 of the TPO gene in two brothers with total iodide organification defect (IOD). The mutation introduces a premature termination signal in exon 10 of the TPO gene, preventing the synthesis of enzymatically active peroxidase.

In the present article two unrelated families in which IOD was present in four patients, were studied with respect to clinical, pathological, and molecular biological modifications of the TPO protein.

Materials and Methods

Clinical and laboratory studies were conducted in two unrelated families in which an IOD was present in two siblings of each family.

Family CA (Fig. 1)

The propositus (CA, II-3) was first seen when she was 27 yr old. A goiter was noted when she was 12 yr old, and she was sporadically treated with L-thyroxine (T₄). The parents are second degree cousins. During her first pregnancy L-T₄ was suspended. The goiter enlarged considerably during pregnancy and after delivery. L-T₄ substitution therapy was again prescribed, but compliance was poor. At the first admission to the University Hospital a large multinodular asymmetrical goiter, with a fibro-elastic texture was noted. No clinical signs of hypothyroidism were present. She was off thyroid medication for about 6 wk. Serum T₄ was 25.7 nmol/L (2.0 μg/dL), serum T₃ 2.15 nmol/L (140 ng/dL), and serum TSH 6.4 mU/L. A thyrotropin-releasing hormone (TRH) test (200 μg IV) increased the basal serum TSH level of 6.2 mU/L to a peak level of 102.0 mU/L. Serum thyroglobulin (Tg) was 63.4 μg/L and increased to 270 μg/L after 10 UI of bovine TSH. Thyroid autoantibodies (anti-TPO and anti-Tg) were repeatedly negative. After oral administration of radioactive iodide the 2 h thyroid uptake was 70% of the administered dose declining to 55% at 24 h. Thyroid scan indicated a pattern suggestive of heterogeneous accumulation of iodide in the glandular tissue. Ultrasonographic studies of the thyroid confirmed the multinodular...