DEFECTS OF THYROID HORMONE TRANSPORT IN SERUM

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ABSTRACT

Inherited abnormalities of thyroid hormone-binding proteins are not uncommon and can predominate in some ethnic groups. They alter the amount of iodothyronines present in serum and, although the concentration of free hormones remains unaltered, routine measurement can give erroneous results. With a single exception, inherited defects in thyroxine-binding globulin (TBG), are X-chromosome linked and thus, the full phenotype is expressed mostly in males. Partial TBG deficiency is more common than complete efficiency. High frequency of variants TBGs have been identified in African Blacks, Australian Aborigine and Eskimos. Most defects producing TBG deficiency are caused by mutations in the structural gene. However, inherited X-linked partial deficiency can occur as the consequence of mutations of a gene enhancer. Inherited forms of TBG excess are all cases by gene duplication or triplication. Mutations in the transthyretin (TTR) gene producing a molecule with increased affinity for T₄ are relatively rare. A variant TTR produces transient hyperthyroxinemia during non-thyroidal illness. Mutations of the human serum albumin (HSA) gene produce increased concentration of serum T₄, a condition known as familial dysalbuminemic hyperthyroxinemia (FDH). They are relatively more common in individuals of Hispanic origin. They cause an increase in serum T₄ owing to increased affinity for this iodothyronine but high concentrations in free T₄ observed in direct measurement by some commercial methods are erroneous. A variant with increased affinity for T₃ has been also identified.

INTRODUCTION

Abnormalities in the serum proteins that transport thyroid hormone do not alter the metabolic state and do not cause thyroid disease. However, they do produce alterations in thyroid hormone concentration in serum and when unrecognized have lead to inappropriate treatment. When the abnormality is the consequence of altered synthesis, secretion or stability of the variant serum protein, the free thyroid hormone level estimated by most of the clinically available techniques remains
within the range of normal. In contrast, when the defect results in a significant alteration of the affinity of the variant protein for the hormone, estimates of the free thyroid hormone level give often erroneous results and thus, it is prudent to measure the free hormone concentration by more direct methods such as equilibrium dialysis or ultrafiltration. This is also true in cases of complete TBG deficiency, in whom the estimation of free thyroid hormone level in serum by indirect methods, or using iodothyronine analogs as tracers, can also give erroneous results.

The existence of inherited defects of serum transport of thyroid hormone was first recognized in 1959 with the report of TBG-excess by Beierwaltes and Robbins (1). Genetic variants for each of the three major thyroid hormone transport proteins have been since described and in recent years, the molecular basis of a number of these defects has been identified (2). Clinically, these defects usually manifest as either euthyroid hyperthyroxinemia or hypothyroxinemia and more rarely, isolated hypertriiodothyroninemia (3). Associated abnormalities such as thyrotoxicosis, hypothyroidism, goiter and familial hyperlipidemia are usually coincidental (4). However, individuals with thyroid disorders are more likely to undergo thyroid testing leading to the fortuitous detection of a thyroid hormone transport defect.

THYROXINE-BINDING GLOBULIN (TBG) DEFECTS

Familial TBG abnormalities are inherited as X-chromosome linked traits (5,6), compatible with the location of the TBG gene on the long arm of the X-chromosome (Xq22.2) (7,8). This mode of inheritance also suggests that the defects involve the TBG gene proper, rather than the rate of TBG disposal, as long ago postulated (5). The normal, common type TBG (TBG-N or TBG-C), has a high affinity for iodothyronines [affinity constants (Ka): $10^{-10}$ M$^{-1}$ for T$_4$ and $10^{-9}$ M$^{-1}$ for T$_3$] and binds 75% of the total T$_4$ and T$_3$ circulating in blood. Thus, with a single exception [HSA R218P (9,10), see below], among the inherited abnormalities of thyroid hormone transport proteins, those involving the TBG molecule produce usually more profound alterations of thyroid hormone concentration in serum.

Clinically TBG defects are classified according to the level of TBG in serum of affected hemizygotes (XY males or XO females, that express only the mutant allele): complete TBG deficiency (TBG-CD), partial TBG deficiency (TBG-PD) and TBG excess (TBG-E). In families with TBG-CD, affected males have no detectable TBG and carrier females (mothers or daughters) have on the average half the normal TBG concentration (4). In families with partially TBG deficient males, the mean TBG concentration in heterozygous females is usually above half the normal. Serum TBG concentration in males with TBG-E is 2 to 4-fold the normal mean and that in the corresponding carrier females, is slightly higher than half that of the affected males. These observations indicate an equal contribution of cells expressing the normal and mutant TBG genes. On rare occasions, selective
Inactivation of the X-chromosome has been the cause of the manifestation of the complete defect (hemizygous phenotype) in heterozygous females (11).

Inherited TBG defects can be further characterized by the level of denatured TBG (dnTBG) in serum and the physicochemical properties of the molecule. The latter can be easily determined without the need of purification. These properties are: (a) immunologic identity; (b) isoelectric focusing (IEF) pattern; (c) rate of inactivation when exposed to various temperatures and pH; and (d) affinity for the ligands, T4 and T3. More precise identification of TBG defects requires sequencing of the TBG gene.

MiP a subject with TBG-CD

The proposita, a phenotypic female, was 13 years old when first seen because of retarded growth, amenorrhea and absence of secondary sexual traits. She was the first sibling of a second marriage for both parents. The family included a younger brother and four older half-siblings, two maternal and two paternal. The proposita was born to her 30-year-old mother after full-term, uncomplicated pregnancy. Infancy and early childhood development were normal until 4 years of age when it became apparent that she was shorter than her peers. She was 12 years of age when a low protein bound iodine (PBI, then a measure of T4) of 2.2 µg/dl (normal range 4.0–8.0) was noted and treatment with 120 mg of desiccated thyroid (equivalent to 200µg L-T4) daily was initiated. Since, during the ensuing 6 months, no change in her growth rate occurred and because PBI remained unchanged (2.0 µg/dl), the dose of desiccated thyroid was increased to 180 mg/day. This produced restlessness, perturbed sleep and deterioration of school performance necessitating discontinuation of thyroid hormone treatment. No family history of thyroid disease or short stature was elicited and the parents denied consanguinity.

On physical examination, the patient appeared younger than her chronological age, was short (137 cm) and showed no signs of sexual development. She had a webbed neck, low nuchal hairline, bilateral eyelid ptosis, shield-shaped chest, increased carrying angle and short 4th metacarpals and metatarsals. The thyroid gland was normal in size and consistency.

Buccal smear was negative for Barr bodies and karyotyping revealed 45 chromosomes consistent with XO Turner’s syndrome. No chromosomal abnormalities were found in lymphocytes from the mother and father. Bone age was 12 years and X-ray of the scull showed a mild degree of hypertelorism. PBI and butanol extractable iodine were low at 2.0 and 1.8 µg/dl, respectively. Resin-T3 uptake was high at 59.9% (normal range 25-35%) indicating reduced TBG-binding capacity. A 24-hour thyroidal radioiodide uptake was normal at 29%, basal metabolic rate was +20% (normal range -10 to +20) and TG autoantibodies were not present. Serum cortisol was normal as were the responses to ACTH and
metyrapone. Basal growth hormone concentration was normal at 8.0 ng/ml which rose to 32 ng/ml following insulin hypoglycemia.

Studies were carried out in all first degree relatives and the propositus was treated cyclically with diethylstilbestrol which produced withdrawal uterine bleeding and gradual breast development.

Five family members, in addition to the proposita had thyroid function tests abnormalities. Two were males and three females. The two males (maternal grand father and maternal half-brother) and the proposita had the lowest PBI levels and undetectable T₄-binding to serum TBG. In contrast, the three females (mother, maternal aunt and maternal half-sister) had a lesser reduction of their PBI and T₄-binding capacity to TBG approximately one-half the normal mean value. The two sons of the affected grandfather (maternal uncles to the proposita) had normal PBI and T₄-binding to TBG. No interference with T₄-binding to TBG or other serum protein abnormalities were found in affected members of the family. In vivo T₄ kinetic studies revealed a rapid extrathyroidal turnover rate but normal daily secretion and degradation, compatible with their euemetic state.

**Interpretation**

The incidental identification of thyroid tests abnormalities in the propositus is typical for most subjects with TBG deficiency as well as TBG excess. So is the initial unnecessary treatment; though less frequent with the routine measurement or estimation of free T₄. The inherited nature of the defect is suspected by exclusion of factors known to cause acquired TBG abnormalities and should be confirmed by the presence of similar abnormalities in members of the family. The absence of male to male transmission and the carrier state of all female offspring of the affected males is a typical pattern of X-chromosome linked inheritance. This is further supported by the complete TBG deficiency in individuals having a single X chromosome (males and the XO female) and only partial TBG deficiency in carrier XX females.

Since the publication of this family in 1968 (12), the cause of the TBG defect was identified. From the mutation identified in the TBG gene of this family [TBG Harwichport (TBG-CD H)], it can be deduced that the molecule is truncated, missing 12 amino acids at the carboxyl terminus (13).

Forty nine TBG variants have been so far identified and in 41 the precise defect has been determined by gene analysis. Their primary structure defect, some of their physical and chemical properties and the resulting serum T₄ concentrations are summarized in Table 1 and figure 1.
Complete Deficiency of TBG (TBG-CD)

TBG-CD is defined as undetectable TBG in serum of affected hemizygous subjects or a value lesser than 0.03% the normal mean; the current limits of detection using the most sensitive radioimmunoassay (RIA) being 5ng/dl (24). The prevalence is approximately 1:15,000 newborn males. Twenty five TBG variants having this phenotype have been characterized at the gene level. These are shown in table 1 that also contains references to the original publications. Eighteen of the 25 TBG-CDs have truncated molecules. Early termination of translation of these variants is caused in 4 by a single nucleotide substitution (TBG-CDP1, TBG-CDP2, CD5, TBG-CDB and TBG-CDT2) or by a frame shift due to one nucleotide deletion (TBG-CDY, TBG-CDN, TBG-CDNi, TBG-CD6, CD-PL, TBG-CD7, TBG-CD8, and TBG-CDJ, TBG-CDPe) or deletion of 19 nucleotides (TBG-CDH). In 5 variants mutations occurred in introns close to splice sites (TBG-CDMi, TBG-CDK, TBG-CDH, TBG-CDL and TBG-CDJa). A mutation at the acceptor splice junction caused also a frame shift producing early termination of translation in TBG-CDK (22). In contrast a nucleotide substitutions in the 5' donor splice site of intron IV (TBG-CDL and TBG-CDJa), resulted in a complete splicing of exon 3, also producing a truncated molecule (28) and personal observation. A similar mechanism is likely responsible for CD in TBG-CDMi, though direct experimental prove was not provided (14). Single amino acid substitution was the cause of CD in five families (TBG-CDT1, TBG-CDPa, TBG-CD5, TBG-CDP3 and TBG-CDKo). In TBG-CD5 Leucine-227 with a proline was shown to cause aberrant post-translational processing (42). One TBG variant (TBG-CDNI), with two nucleotides deleted close to the carboxyl terminus, the resulting frame shift predicts an extension of the molecule by the addition of 7 nonsense residues (33). TBG-CDJ has been so far identified only in Japanese but its allele frequency in the population remains unknown (30,52) (Table. 1).

Partial Deficiency of TBG (TBG-PD)

This is the most common form of inherited TBG deficiency having a prevalence of 1:4,000 newborn. Identification of heterozygous females by serum TBG measurement may be difficult because levels often overlap the normal range. In contrast to variants with complete TBG deficiency, all TBG-PDs have missense mutations. It is possible that three of the five variants with single amino acid substitutions included in the category of TBG-CD have also partial deficiency which was not identified owing to the low sensitivity of routine assays for the measurement of TBG. Twelve different mutations, producing a variable degree of reduction of TBG concentration in serum, have been identified, 11 of which involve mutations in the TBG gene proper. They are listed in table 1. In addition, some of these variants are unstable (TBG-PDG, TBG-PDA, TBG-PDSD, TBG-PDM TBG-PDQ and TBG-PDJ) or have lower binding affinity for T4 and T3 (TBG-PDG, TBG-PDA, TBG-PDS TBG-PDSD, TBG-PDM and TBG-PDQ), impaired intracellular transport and
secretion (TBG-PDJ and TBG-CDJ) and some exhibit an abnormal migration pattern on IEF electrophoresis (TBG-PDG, TBG-PDM, and TBG-PDO) (Fig. 1). Variants with decreased affinity for T4 and T3 have a disproportionate reduction in hormone concentration relative to the corresponding serum TBG level (Fig. 2) and estimations of the free hormone levels by any of the index methods gives erroneous results (37,59). One of these variants, TBG-PDA, is found with high frequency in Australian Aborigines (allele frequency of 51%) (44).

Table 1. TBG Variants and Gene Mutations

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<tr>
<th>TBG NAME</th>
<th>Abbreviated name</th>
<th>Intron Exon</th>
<th>COD ON*</th>
<th>AMINO ACID</th>
<th>NUCLEOTIDE</th>
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<td>fs</td>
<td>5’ DS SS</td>
<td>unk known</td>
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*Note: CD refers to the chromosome, IVS to intron, 5' to 5' splice site, 3' to 3' splice site, and X to the exon.*
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Polymorphism | Poly | 3 | 283 | L (Leu) | F (Ph) | TTG | TT | (24,52)
---|---|---|---|---|---|---|---|---
Chicago | CH or Cgo | 3 | 309 | Y (Tyr) | F (Ph) | TAT | TT | (53,54)

* Codon numbering from first amino acid of the mature protein. The 20 amino acids of the signal peptide are numbered -1 to -20, from N- to C-terminus. The codon at the site of mutation is followed by the codon at the site of termination of translation.

† coexistence of TBG Poly

§ complete deficiency is uncertain as the TBG assay used was unable to detect values <10% the mean normal

¶ Also a silent mutation at codon 55: GCA -> GCG

de, delete; add, addition; aa, amino acid; fs, frame shift

Pt, patent; fam, family

IVS, intervening sequence or intron; ASS, acceptor splice site; DSS, donor splice site

Figure 1. Properties of some TBG variants causing partial TBG deficiency (TBG-PD). The TBG variants are: -SD, San Diego; -G, Gary; -M, Montreal, -S, slow; -A, Aborigine; -Poly, polymorphic; -Cgo, Chicago; and -Q, Quebec. For detailed description, see (1) Sarne et al (37) and Bertenshaw et al (35); (2) Murata...
et al (32), Mori et al (40) and Kambe et al (55); (3) Takamatsu et al (41) and Janssen et al (42); (4) Takamatsu et al (50) and Waltz et al (51); (5) Murata et al (56) and Takeda et al (44); (6) Mori et al (24) and Takeda et al (52); (7) Takamatsu et al (54) and (53); (8) Takamatsu et al (41) and Bertenshaw et al (46).

Figure 2. Serum T4-bound to TBG and the concentration of TBG and denatured TBG (dnTBG) in hemizygous subjects expressing the different TBG variants. Results, graphed as mean ± SD, were normalized by expressing them as % of those for the common type TBG (TBG-C). For abbreviations used in the nomenclature of the TBG variants, see legend to figure 1. [Adapted from Janssen et al (58)]

A unique family with TBG-PD has been described in which inheritance of the partial deficiency was autosomal dominant with transmission of the phenotype from father to son (60). The concentration of TBG in affected males and females was about one half the normal mean value. The TBG had normal affinity for T4, normal IEF and heat lability. No sequence changes were found in the entire coding arias of the gene or in the promoter region. Although the mechanism of TBG-CD in this family is unknown an abnormality in one of the factors regulating TBG gene transcription is a distinct possibility.

In 5% (4 or 74) families with X-chromosome lined TBG deficiency, studied in the author’s laboratory, no mutations were identified in the entire TBG gene, including all exons, introns, untranslated regions and the promoter region of the gene, covering a total of 9.2 kb. Next-generation sequencing identified a novel single nucleotide substitution 20 kb downstream of the TBG gene in all four families. In
silico analysis predicted that the variant resides within a liver-specific enhancer. In vitro studies confirmed the enhancer activity of a 2.2-kb fragment of genomic DNA containing the novel variant and showed that the mutation reduces the activity of this enhancer. The affected subjects share a haplotype of 8 Mb surrounding the mutation. Three were of known Arab ethnicity and in all four families the most recent common ancestor was estimated to be 19.5 generations ago (95% confidence intervals). This is first report of an inherited endocrine disorder caused by a mutation in an enhancer region (61).

**TBG Excess (TBG-E)**

TBG-E has a lower prevalence than TBG deficiency, with values obtained from neonatal screening programs from 1:6,000 to 1:40,000 (62,63). Considering that some newborn may have non-inherited, transient TBG excess, a conservative overall estimate of inherited TBG-E would be 1:25,000 (64). Early sequencing of the coding and promoter regions of subjects with TBG-E failed to show any defects (65). However, in 1995, Mori et al (66) found that gene amplification was the cause of TBG-E in two families. Gene triplication and duplication were demonstrated by gene dosage studies using HPLC measurements of the PCR-amplified product. As expected, hemizygous affected males had approximately 3- and 2-fold the average normal serum TBG concentration, respectively. The presence of multiple TBG gene copies in tandem was confirmed by in situ hybridization of prometaphase and interphase chromosomes from an affected male.

**TBG Variants with Unaltered TBG Concentrations in Serum**

Five TBG variants have been identified that present with normal or slight and clinically insignificant alterations in their concentration in serum. Four occur with high frequency in some population groups and thus, can be considered as polymorphic. TBG-Poly (Fig. 1), with no alterations of its physical or biological properties, has been detected in 16% and 20% of the French Canadian and Japanese populations, respectively (24,52). TBG-S exhibits a slower mobility on polyacrylamide gel electrophoresis and cathodal shift on IEF (49,50), owing to the loss of a negative charge due to the replacement of the normal Asp\textsuperscript{171} by Asn (51) (Figs. 1 and 3). It has an allele frequency of 5 to 16% in Black populations of African origin and 2 to 10% in Pacific Islanders. The molecular structure of two other polymorphic TBG variants has not been identified. TBG-F has an allele frequency of 3.2% in Eskimos residing on the Kodiak and St. Lawrence islands. It has a slight anodal (fast) mobility on IEF (67). TBG-C1 has been identified in subjects inhabiting two Mali village (68). It has a small cathodal shift on IEF and an allele frequency of 5.1%. TBG-Cgo, resistant to high temperatures (54), has normal affinity for T\textsubscript{4} and T\textsubscript{3}. All SERPINs except human TBG have a Phe at a position corresponding to Tyr\textsuperscript{309}. Structure modeling suggests that the replacement of the
normal Tyr$^{309}$ by Phe in TBG-Cgo, ties the internal L-helix hI1 to the molecule, thus stabilizing its tertiary structure (53). However, more recent studies using recombinant TBG–Cgo showed that the molecule exists in loop expelled conformation. However, when exposed at 37°C, the protein readily converts to a more stable loop inserted conformation explaining its subsequent enhanced heat stability, as observed in vivo (69).

Figure 3. Microheterogeneity of TBG. Tracer amounts of I25I T4 were added to serum prior to submission to isoelectric focusing and radioautography. TBG C (common type) exhibits 6 bands spanning from pH 4.18 to 4.58. Three of the six are major and shown here between pH 4.35 and 4.50. TBG-Slow (TBG S) from a hemizygous male shows a cathodally shifted pattern. A mixed pattern occurs in heterozygous females expressing both TBG-C and TBG-S. [Reproduced from Waltz et al (51)]
Biological Consequences of Structural Changes Caused by Mutations in the TBG Gene

The mechanisms whereby structural abnormalities of the TBG molecule produce the variant phenotypes have been investigated by expression of some of these molecules in living cells. Contrary to previous speculation, increased extracellular degradation due to instability is a rare cause of reduced concentration of the variant TBG in serum (36). More commonly, intracellular retention and degradation of the defective TBG molecules is responsible for their presence in low concentrations in serum (42,48,55,70). Of note is the full intracellular retention of TBG-CD5 despite synthesis in normal quantities. A single amino acid substitution in TBG-CD5 is sufficient to alter its tertiary structure and thus prevent export. The same finding in the case of TBG-CDJ has been traced to its retention within the endoplasmic reticulum. Furthermore, the increased amount of GRP78 mRNA in cells transfected with TBG-PDJ suggests that association of this TBG variant with the GRP78 molecular chaperon is responsible for its impaired secretion (48). The variant TBG-AL is unique and important as it provides information about the function of the signal peptide. The resulting variable decrease in the serum TBG concentration associated with diminished in vitro secretion is compatible with impaired cotranslational processing (34).

Several speculations regarding the properties of variant TBGs have been confirmed based on the recent elucidation of the TBG structure by X-ray crystallography (71). The reduced ligand-binding of TBG-SD (36,37) can be explained by the direct proximity of the amino acid substitution to the binding pocket. Indeed, the methyl group of the side chain of Thr23, replacing the normal Ser, will sterically hinder the binding of $T_4$. Similarly, in TBG-A, the replacement of Ala191 by Thr (44) perturbs the H-bonds that stabilizes the binding pocket, leading to the reduced $T_4$ binding.

In contrast, the loss of His331 in TBG-Q (H331Y) (41,46) allows unrestricted loop insertion in the upper half of the A-sheet, accounting for the increased in serum dnTBG and reduced $T_4$ binding.

Recently TBG deficiency was found to coexist in the same family with resistance to thyroid hormone beta (RTHß) (72). Both TBG (P50fs51X) and THRBP (P453A) gene mutations have been previously described in unrelated families (14,73) but not in the same family. The mother harboured both gene mutations, whereas the proband and his siter had only the THRBP gene mutation and a brother only the TBG gene mutation. This famil illustrates the difficulty that might be encountered in the interpretation of thyroid function tests when different genetic defects, having opposite effect on thyroid function tests, coexist in the same family, and especially the same individual.
TRANSTHYRETIN (TTR) DEFECTS

Sequencing of the TTR gene, formerly known as thyroxine-binding prealbumin (TBPA) on chromosome 18 (18q11.2-q12.1), has uncovered mutations that produce variant TTR molecules with or without alterations in the binding affinity for iodothyronines (2,74). Only those known to affect iodothyronine binding are listed in Table 2. Some of the TTR variants are responsible for the dominantly inherited familial amyloidotic polyneuropathy (FAP), causing multiple organ failure and death in early adulthood.(74). Because TTR has a relatively lower affinity for T₄ (about 100-fold lesser than that of TBG), it plays a minor role in thyroid hormone transport in blood. Accordingly, changes in the TTR concentration in serum and variant TTRs with reduced affinity for T₄ have little effect on the concentration of serum T₄ (75,87). Only variant TTRs with a substantially increased affinity for iodothyronines produce significant elevation in serum T₄ and rT₃ concentrations and account for 2% of subjects with euthyroid hyperthyroxinemia (86).

Table 2. TTR variants with altered affinity for T₄ and potentially an effect on tests of thyroid function in serum

<table>
<thead>
<tr>
<th>AFFINITY FOR T₄</th>
<th>TTR CONCENTRATION</th>
<th>CODON NUMBER</th>
<th>AMINO ACID (NORMAL - VARIANT)</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant / Normal</td>
<td>HETERO*</td>
<td>HETER O*</td>
<td>DECREASED</td>
<td></td>
</tr>
<tr>
<td>&lt;0.1</td>
<td>0.17 - 0.41</td>
<td>N</td>
<td>30</td>
<td>Val - Met</td>
</tr>
<tr>
<td></td>
<td>0.54</td>
<td>58</td>
<td>Leu - His</td>
<td>(76)</td>
</tr>
<tr>
<td></td>
<td>0.45</td>
<td>77</td>
<td>Ser - Tyr</td>
<td>(76)</td>
</tr>
<tr>
<td></td>
<td>0.19 – 0.46</td>
<td>N</td>
<td>84</td>
<td>Ile - Ser</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>~1.0</td>
<td>0.44</td>
<td></td>
<td>Val - Ile</td>
<td>(76)</td>
</tr>
<tr>
<td><strong>INCREASED</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.35</td>
<td>N</td>
<td>6</td>
<td>Gly - Ser</td>
</tr>
<tr>
<td>8.3-9.8</td>
<td>3.2 - 4.1</td>
<td>N</td>
<td>109</td>
<td>Ala - Thr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>109</td>
<td>Ala - Val</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inc or N</td>
<td>119</td>
<td>Thr - Met</td>
</tr>
</tbody>
</table>

* HOMO, homozygous; HETERO, heterozygous.

† Probably overestimated since the subjects harboring this TTR variant have normal serum TT4 concentrations.

‡ Affinity of recombinant TTR Thr\(^\text{109}\) is 9-fold that of the normal TTR (82).

Variant TTR tested and shown not to have altered affinity to T4 are: Ala\(^{60}\), (hetero) (75,76).

N, normal; Inc, increased

Endonucleases useful in the identification of TTR variants: Msp I -ve for Ser\(^6\) in exon 2 associated PHA; BsoFI -ve and Fnu 4H +ve for Thr\(^\text{109}\); BsoFI -ve for Val\(^\text{109}\) and Nco I +ve for Met\(^\text{119}\), all in exon 4.

A family with elevated total T\(_4\) concentration which was predominantly bound to TTR was first described in 1982 by Moses et al (88). The inheritance was autosomal dominant and affected members were clinically euthyroid with normal free T\(_4\) levels measured by equilibrium dialysis. The variant TTR has a single nucleotide substitution replacing the normal Ala\(^{109}\) with a Thr which increases its affinity for T\(_4\), rT3 and tetraiodothyroacetic acid and to a lesser extent T3 and triiodothyroacetic acid (80,82). Crystallographic analysis of this variant TTR revealed an alteration in the size of the T\(_4\)-binding pocket (89). Another TTR gene mutation involving the same codon has been more subsequently described (81). This mutant TTR with Val\(^{109}\) has an increased affinity for T\(_4\) that is of similar magnitude as TTR Thr\(^\text{109}\), about 10-fold higher than that of wild-type TTR.
A more common defect found in subjects with **prealbumin associated hyperthyroxinemia** (PAH) is a point mutation in exon 4 of the TTR gene replacing the normal Thr\textsuperscript{119} with Met (86). First described in a single individual with normal serum total and free T\textsubscript{4} levels (85), the majority of subsequently identified heterozygous subjects harboring the TTR Met\textsuperscript{119} had an increase in the fraction of T\textsubscript{4} and rT\textsubscript{3} associated with TTR, but only few had serum T\textsubscript{4} levels above the upper limit of normal. Furthermore, their hyperthyroxinemia appears to be transient, usually in association with non-thyroidal illness (86). The variant TTRs associated with PAH are not amyloidogenic.

**Variant TTRs without Known Biological Effects**

Several TTR variants have been found that do not alter the properties of the molecule, nor cause FAP, and are thus of no clinical significance. Of interest is a TTR variant found in the rhesus monkey, *Macaca mulatta*, but not in man (90,91). This variant has a slower electrophoretic mobility resulting in three phenotypes which exhibit: (a) a single rapidly migrating band similar to that found in human and other primates (PA\textsuperscript{FF}); (b) a single slowly migrating band cathodal to albumin (PA\textsuperscript{SS}); and (c) a five banded form corresponding to the various tetrameric recombinants present in the heterozygous state possessing the two different subunits (PA\textsuperscript{FS}). This finding was important because the variant rhesus PA-S could be hybridized in vitro with human TTR yielding a five-banded pattern hence, demonstrating that human TTR is also a tetramer. All naturally occurring and hybrid polymorphic variants show no detectable alteration in the binding of either T\textsubscript{4} or retinol binding protein (92).

**HUMAN SERUM ALBUMIN (HSA) DEFECTS**

Another form of dominantly inherited euthyroid hyperthyroxinemia, later to be linked to the albumin gene on chromosome 4 (4q11-q13), was first described in 1979 (93,94). Known as **familial dysalbuminemic hyperthyroxinemia** (FDH) (95), it is the most common cause of inherited increase in total T\textsubscript{4} in serum in the Caucasian population, producing on the average a 2-fold increase in the serum total T\textsubscript{4} concentration. In a study of 430 subjects suspected of having euthyroid hyperthyroxinemia 12% were proven to have FDH (86). The prevalence varies from 0.01 to 1.8%, depending on the ethnic origin, with the highest prevalence in Hispanics (96-99). This form of FDH has not been reported in subjects of African origin and the isolated occurrence in a Chinese (100) was possibly brought by Hispanic travelers (see below). The euthyroid status of subjects with FDH has been confirmed by normal TSH response to TRH, normal free T\textsubscript{4} concentration measured by equilibrium dialysis using appropriate buffer systems, normal T\textsubscript{4} production rate and normal serum sex hormone-binding globulin concentration
Nevertheless, the falsely elevated free T4 values, when estimated by standard clinical laboratory techniques, has often resulted in inappropriate thyroid gland ablative or drug therapy (103-105). A recent survey of commonly used commercial tests for measurement of free T4 indicates that equilibrium or symmetric dialysis are the only tests that will consistently provide accurate values in subjects with FDH (106).

FDH is suspected when serum total T4 concentration is increased without proportional elevation in total T3 level and non suppressed serum TSH. Half of affected subjects have also rT3 values above the normal range (107) (Table 3). Since the same combination of test results are found in subjects with the Thr109 TTR variant, the diagnosis of FDH should be confirmed by the demonstration that an increased proportion of the total serum T4 migrates with HSA on non denaturing electrophoresis or precipitates with anti-HSA serum.

A tight linkage between FDH and the HSA gene (lod score 5.25) was found in a large Swiss-Amish family using two polymorphic markers (107). This was followed by the identification of a missense mutation in codon 218 of the HSA gene replacing the normal arginine with a histidine (R218H) (108,112). Furthermore, the same mutation was present in all subjects with FDH from 11 unrelated families. Its association with a Sac I+ polymorphism, suggest a founder effect and is compatible with ethnic predilection of FDH (108). The coexistence of FDH and a TTR variant with increased affinity for T4 in the same individual (78,79) and FDH with TBG-PD in another (113) have been reported. In both instances these individuals were the product of parents each heterozygous for of one of the two defects.

Another mutation in codon 218, with increased affinity to iodothyronines, was first identified by Wada et al (9). The mutation, a replacement of the normal Arg218 with a Pro (R218P), initially believed to be unique for Japanese was also identified in a Swiss family with no Asian ancestry (Fig. 4) (10). In this form of FDH, serum total T4 levels are 14-20-fold the normal mean, a level confirmed by measurements in serum extracts by HPLC. Total rT3 and T3 concentrations are 7- and 2-fold above the mean, respectively. Thus, in order to maintain a normal free T4 level, the calculated affinity constant (Ka) of HSA R218P should be about 16-fold higher than that of HSA R218H. Surprisingly, the Kas measured at saturation were similar, 5.4 x 10^6 M^-1 and 6.4 x 10^6 M^-1 for HSA R218H, respectively (10,114,115) (Table 3). However, at T4 concentrations equivalent to those found in subjects with HSA R218P, the dialysable FT4 concentration was 11-fold higher in serum of subjects with HSA R218H and 49-fold higher in serum with the common type HSA only (10).
Figure 4. A Swiss family with HSA R218P: genotyping, pedigree and thyroid function tests. A, Genotyping for the mutation HSA R218P. Results are aligned with each subject depicted on the pedigree in B. Amplification of a segment of the HSA gene containing the mutation with a mismatched oligonucleotide primer creates a new restriction site for Ava II only in the presence of the mutant nucleotide (CGC -> CCC). Affected subjects expressing proline 218 (CCC) show a 122 bp DNA fragment produced by enzymatic digestion of the mutant allele. Note that all affected subjects are heterozygous and that the 153 bp fragment amplified from DNA of the two normal subjects, expressing arginine 218 (CGC) only, resists enzymatic digestion. B, Pedigree of the family. Roman numerals indicate each generation and numbers below each symbol identify the subject. Individuals expressing the FDH phenotype are indicated by half filled symbols. C, Thyroid function tests. Results are aligned with each symbol. Values outside the normal range are in bold numbers. Note the disproportionate increase in serum T4 concentration as compared to that of T3 in the affected individuals. Subject I-1, a
year old man, had diabetes mellitus with multiple organ complications and mild subclinical hypothyroidism, explaining the relatively lower serum T4 and T3 but not rT3 levels. [Adapted from Pannain et al (10)].

Recently two additional HSA gene mutations have been identified. One in the same codon resulting in a different amino acid substitution (R218S) (110) and another in a different amino acid (R222I) (111) in the proximity of the same iodothyronine-binding pocket (Fig. 5). While both manifest increased affinity for T4 and rT3, it is considerably higher for T4 in the former and for rT3 in the latter (Table 3). It is of note that the two amino acids, 218 and 222, involved in the gain-of-function mutations are located in the main predominantly hydrophobic pocket where T4 is bound in a cisoid conformation (116).

A fifth gain-of-function mutation, a replacement of the normal Leu66 with a Pro (L66P) has been identified in a single Thai family (3). It produces a 40-fold increase in the affinity for T3 but only 1.5-fold increase in the affinity for T4 (Table 3). As a consequence, patients have hypertriiodothyroninemia but not hyperthyroxinemia. In this FDH-T3, serum T3 concentrations are falsely low, or even undetectable, when T3 is measured using an analog of T3 as a tracer rather than a radioisotope. It has resulted in the inappropriate treatment with thyroid hormone (3).

Table 3. Albumin variants with increased affinities for iodothyronines, their effect on the serum concentrations of and affinities to these hormones

<table>
<thead>
<tr>
<th>VARIANT</th>
<th>SERUM CONCENTRATION</th>
<th>BINDING AFFINITY (Ka) of the variant albumins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T4 µg/dl</td>
<td>T3 ng/dl</td>
<td>rT3 ng/dl</td>
</tr>
<tr>
<td></td>
<td>(fold the normal mean)</td>
<td>(fold the normal mean)</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>8.0 ± 0.2</td>
<td>125 ± 4</td>
<td>22.5 ± 0.9</td>
</tr>
<tr>
<td>R218H</td>
<td>16.0 ± 0.5</td>
<td>154 ± 3</td>
<td>33.1 ± 1.1</td>
</tr>
<tr>
<td>R218</td>
<td>135 ±</td>
<td>241 ±</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>P</td>
<td>17</td>
<td>25</td>
<td>± 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>± 13</td>
</tr>
<tr>
<td>R218S</td>
<td>70</td>
<td>159</td>
<td>55.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.3)</td>
</tr>
<tr>
<td>R222I</td>
<td>21±1.4</td>
<td>135±18</td>
<td>1417±107</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.2)</td>
</tr>
<tr>
<td></td>
<td>8.7</td>
<td>320</td>
<td>22.3</td>
</tr>
<tr>
<td>L66P</td>
<td>320</td>
<td></td>
<td>22.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22.3</td>
</tr>
</tbody>
</table>

Values reported are means ± standard error, and the number of subjects per genotype are indicated under “N.”

* Determined at saturation. Affinities are higher at the concentrations of T₄ and T₃ found in serum.

NM, not measured

All data were generated in the Chicago laboratory except for 4 of the 8 individuals with ALB R218P and hose with ALB R222I, provided by Nadia Schoenmakers, University of Cambridge, UK.
Bisalbuminemia and Analbuminemia

Variant albumins, with altered electrophoretic mobility produce "bisalbuminemia" in the heterozygotes (117). T4 binding has been studied in subjects from unrelated families with a slow HSA variant. In two studies only the slow moving HSA bound
T₄ (118,119) and in another, both (120). The differential binding of T₄ to one of the
components of bisalbumin may be due to enhanced binding to the variant
component with charged amino acid sequence. Bisalbuminemia does not seem to
be associated with gross alterations in thyroid hormone concentration in serum.

Analbuminemia is extremely rare, occurring in less than 1 in a million individuals
(121). The first case was reported in 1954 (122) but the HSA gene mutation was
identified 56 years later (123). The less than 50 cases so far reported have
nonsense mutations causing premature termination of translation or splicing defects
(124). Despite the complete lack of such an important substance, symptoms are
remarkably mild owing to the a compensation by an increase in non-albumin serum
proteins. Studies with respect to T₄-transport showed no clear effect or slight
increase total serum iodothyronines, associated with increased levels of TBG and
TTR. (124,125). The latter two normalized when serum HSA was restored to
normal by multiple transfusions (125)

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