X-Chromosome Linked Inheritance of Decreased Thyroxine-binding Globulin

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Malvaux, P., and De Nayer, Ph. (1972). *Archives of Disease in Childhood*, **47**, 635. **X-chromosome linked inheritance of decreased thyroxine-binding globulin.** Partial thyroxine-binding globulin (TBG) deficiency was observed in a euthyroid boy. A family study was conducted in order to trace the defect. Of the 15 members, 3 male subjects were found to be affected, whereas 6 female subjects were shown to be carriers of the trait. The inheritance pattern of the partial TBG deficiency was found to meet the criteria for X chromosome linked transmission. No association with other X-linked characters (colour blindness, glucose-6-phosphate dehydrogenase activity) was present. Karyotype analysis in the female subjects did not show any abnormality. TBG deficiency does not impair thyroid function. No difference was noted in free thyroxine levels in normal and affected subjects.

A low protein-bound iodine level (PBI) is almost always suggestive of a deficient thyroid secretion. There are however other circumstances where a low PBI level is associated with normal thyroid function but with a thyroxine-binding globulin (TBG) deficiency. A familial incidence of TBG deficiency has been recognized and the mode of inheritance of this trait has been proven to be X-linked (Kraemer and Wiswell, 1968; Marshall, Levy, and Steinberg, 1966; Nikolai and Seal, 1966, 1967; Nusynowitz *et al.*, 1971; Refetoff and Selenkow, 1968; Torkington *et al.*, 1970). Recently attention has been drawn to the fact that a low PBI level in clinically euthyroid patients could be due, not to a complete absence of TBG, but to a low level of this protein (Bayley *et al.*, 1969; Heinonen, Lamberg, and Virtamo, 1970; Moloshok *et al.*, 1969; Roberts, Nikolai, and Lohrenz, 1970). The importance of recognizing this deficiency is to be stressed: discrepancies between the PBI level and the T_3 resin uptake should be further investigated in order to avoid treating these patients for hypothyroidism.

This paper reports the study of a family exhibiting a low TBG level. The inheritance of the trait is compatible with an X-linked mode of transmission.

Methods

The thyroxine (T_4) binding capacity of thyroxine-binding globulin (TBG) and of thyroxine-binding

prealbumin (TBPA) was measured by reverse flow paper electrophoresis in glycine acetate buffer at pH 8.6. To prepare 1 litre of glycine acetate buffer at pH 8.6, 7.4 g glacial acetic acid, 15 g glycine, and 5.8 g NaOH pellets were dissolved in glass-distilled water. The final pH was adjusted with 1N NaOH. Electrophoresis was performed on Whatman No. 3 paper at 110 V for 16 hours. The binding capacity of TBG and of TBPA was assessed at a concentration of 150 $\mu\text{g } T_4\%$ and 600 $\mu\text{g } T_4\%$ of added T_4 . Earlier experiments have shown that under these conditions saturation of binding sites was reached. A tracer dose of $^{125}\text{I}-T_4$ (Abbott) was added to the stock solutions of stable T_4 . The mixture of serum and thyroxine was allowed to equilibrate for at least 30 minutes at room temperature before application of the sample on the paperstrip. Mean values for normal adults (age 21 to 65) were for TBG-binding capacity 22.5 $\mu\text{g } T_4\%$ (range 18 to 30 $\mu\text{g } T_4\%$), and for TBPA binding capacity 281.7 $\mu\text{g } T_4\%$, (range 225 to 350 $\mu\text{g } T_4\%$).

The serum T_4 determinations were performed using a commercial version (Byk, Mallinckrodt) of the competitive binding assay (normal range 3.0 to 7.5 $\mu\text{g } \text{I}$ per cent), the T_3 resin uptake (T_3 RU) using the Abbott Triosorb kits (normal range 25 to 35%). The free thyroxine (T_4 f) and the dialysable fraction (DF) were estimated by the dialysis method of Oppenheimer *et al.* (1963). Briefly, $^{125}\text{I}-T_4$ (specific activity 50 to 90 mCi/mg, Abbott) was added to serum (addition of less than 1 $\mu\text{g } T_4/100$ ml). The serum was diluted 1:25 with phosphate buffer pH 7.4, ionic strength 0.15; samples of 5 ml were pipetted into cellulose film bags and dialysed for 20 hours at 37 °C against 25 ml of the same buffer. The fraction of unbound T_4 in the system (dialysable fraction or DF) was computed from the

FIG. 1.—Pedigree of family under study. The *propositus* is subject III-2.

concentration of radioactive T_4 in the outer and the inner compartment. The dialysable T_4 —an approximation for T_4 f—is obtained by multiplying the DF times the total T_4 in the system, i.e. the serum T_4 and the added radioactive T_4 . Determinations were always performed in duplicate. Data obtained with the same method in normal subjects have been previously reported (Malvaux *et al.*, 1966). The range of normal values for T_4 f in this laboratory was between 1 and 3×10^{-11} moles T_4 /l.

Case Report

A low PBI value was found in a 13-year-old boy presenting with moderate obesity. The patient was referred to us for further endocrinological investigation. The physical examination, except for general obesity (height 143.5 cm, weight 47.2 kg), was unremarkable. No signs of hypothyroidism were noted. The thyroid data are presented in Table I. The discrepancy be-

TABLE I
Thyroid Data of Propositus

Total T_4	1.7 μg I/100 ml
PBI	2.5 μg /100 ml
T_3 resin uptake	62%
^{131}I uptake, 24 hr	46.9%
DF	0.0583
Free T_4	1.298×10^{-11} M

tween the high T_3 RU value and the low T_4 level prompted us to analyse the thyroxine-binding proteins. The discovery of low thyroxine-binding capacity of TBG led us to investigate the patient's family.

Results

The pedigree of the family under study is shown in Fig. 1.

The individual data are presented in Table II. Low but detectable values for TBG were found in male patients I-1, III-1, and III-2. Female subjects II-2, II-3, III-3, III-4, III-5, and IV-1 exhibited intermediate values. The TBG value of subject IV-2 was high but within the normal range for a child; on the other hand the TBG value of subject IV-1 must be considered as an intermediate value in reference to the high TBG values normally observed at this age.

TBPA-binding capacities were within the normal range in respect to the age. As expected a high DF was found in subjects with a low TBG-binding capacity. Free T_4 levels were normal in all subjects.

Discussion

The subjects of the family under study were classified in three groups according to their TBG value: normal subjects, affected subjects, and carriers. The three affected subjects—all males—had a very low but detectable TBG value. The presence of a TBG band was confirmed by autoradiography of the electrophoregram (Fig. 2). We may, therefore, assume that this low level is not due to an artefact; the more so in that the reverse flow technique eliminates the spilling of T_4 from albumin during the electrophoretic run.

The carrier group—which included only females—

TABLE II

Subject	Sex	Age (yr)	T ₄ (μg I/100 ml)	TBG	TBPA	DF (fraction)	Free T ₄ (× 10 ⁻¹¹ M)
				(μg T ₄ /100 ml)	(μg T ₄ /100 ml)		
Normal							
I-2	F	63	3.9	23.4	301.1	0.0214	1.096
II-1	M	45	3.0	19.3	294.7	0.0308	1.210
II-4	M	44	4.3	21.7	319.1	0.0284	1.599
II-5	M	21	4.3	24.3	260.0	0.0232	1.306
III-6	M	24	6.9	24.2	354.6	0.0255	2.304
IV-2	F	4	4.8	27.5	168.4	0.0243	1.537
Affected							
I-1	M	65	2.1	3.7	337.5	0.0447	1.217
III-1	M	17	2.3	1.3	304.8	0.0461	1.376
III-2	M	13	1.7	3.3	246.7	0.0583	1.298
Carriers							
II-2	F	42	3.0	14.5	258.4	0.0267	1.049
II-3	F	44	3.0	6.7	287.0	0.0380	1.493
III-3	F	15	2.5	11.2	247.8	0.0321	1.042
III-4	F	20	3.5	12.5	269.6	0.0278	1.260
III-5	F	23	4.0	7.9	318.7	0.0398	2.085
IV-1	F	5	3.5	17.2	162.6	0.0354	1.632

had a mean TBG value of 11.7 μg T₄/100 ml, which is exactly half the mean value of the normal group (23.4 ± 2.75 (SD) μg T₄/100 ml).

It has to be noted that sex influence would have the opposite effect on TBG. Braverman, Foster, and Ingbar (1967) and Malvaux *et al.* (1966) have shown that females tend to have higher TBG values than males. Subject IV-1, aged 5 (TBG 17.2 μg T₄/100 ml), was included in the carrier group; the TBG value found is abnormally low for her age. High TBG values have

been shown to occur during infancy (De Nayer, van den Schrieck, and de Visscher, 1965). In agreement with this interpretation are the values in another subject of this family (IV-2), 4 years old, exhibiting a TBG value of 27.5 μg/100 ml, a normal value for children of this age.

It is likely that thyroxine-binding globulin deficiency, as evidenced by the measurement of the thyroxine-binding capacity in this family, reflects a decrease in TBG concentration. Recent reports indicate indeed that this defect is not due to a decrease in the affinity of T₄ for TBG, but rather to an actual decrease in the serum concentration of this protein (Levy, Marshall, and Velayo, 1971; Refetoff, Robin, and Alper, 1970; Roberts *et al.*, 1970).

The inheritance of the complete absence of TBG has been shown to follow an X-linkage pattern (Marshall *et al.*, 1966; Nikolai and Seal, 1966, 1967; Nusynowitz *et al.*, 1971; Refetoff and Selenkow, 1968; Torkington *et al.*, 1970). A similar mode of inheritance has been proposed for the partial TBG deficiency (Bayley *et al.*, 1969; Moloshok *et al.*, 1969). In some cases however an autosomal transmission of the partial TBG deficiency was found (Heinonen *et al.*, 1970; Roberts *et al.*, 1970; Nicoloff, Dowling, and Patton, 1964).

Thyroxine-binding globulin deficiency was described by Refetoff and Selenkow (1968) in a patient with Turner's syndrome (XO). In order to exclude this possibility, chromosome karyotypes were performed in all female subjects: no abnormality was detected.

In the family of this study, the mode of trans-

FIG. 2.—Autoradiography of the electrophoregram of a normal subject and of subject III-2. A faint radioactive band is located at the TBG level.

mission is compatible with an X-linkage mechanism. There is no male-to-male transmission, and both daughters of an affected father are carriers. Affected subjects are offspring of a carrier, and carriers are offspring of an affected male or a carrier female.

The mean TBG value in the group of heterozygous females is half the normal value. Random and permanent inactivation of one of the X-chromosomes in somatic cells, resulting in a cellular mosaic with regard to the X-chromosomes of paternal origin, would lead to the same result.

Colour blindness tests and measurement of the glucose-6-phosphate dehydrogenase activity performed in the affected subjects and the carriers did not show any deficiency.

Extensive evidence has been presented that the thyroid status is related to the level of serum free T_4 rather than to the serum total T_4 level (see Robbins and Rall, 1967). It is likely indeed that the entry of the hormone in the cell is restricted to the free form of the hormone. In the serum, the free T_4 form is in equilibrium with the protein bound T_4 form. The free T_4 concentration is in direct proportion to the total T_4 and in inverse relation to the concentration of thyroxine-binding proteins. In patients with TBG deficiency, serum T_4 levels are adjusted so as to normalize the free T_4 concentration. In this case the steady state reached with a depressed total T_4 level, but with a normal free T_4 level, ensures a normal supply of hormone to the peripheral cells.

No precise role has been found for the thyroxine-binding proteins. Through their binding affinity for T_4 they may act as buffer against rapid changes in T_4 production. Though the binding affinity of T_4 for TBPA is lower than for TBG, it is likely that in TBG deficient subjects, TBPA may present a major contribution to this function. As expected from the foregoing considerations the TBG deficient subjects of this study were euthyroid. No differences were noted in the free T_4 levels in normal and affected subjects.

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