Familial thyroxine-binding globulin deficiency: search for linkage with Xg blood groups*

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Summary. Serum thyroxine-binding globulin (TBG), thyroxine, and thyrotrophin were estimated in members of a family with TBG deficiency. Xg^a red cell antigen status was also studied. TBG deficiency appeared to be inherited as an X-linked condition. Four male members of the family showed absent TBG and seven heterozygous women showed intermediate TBG levels. Five informative phase known members of the family showed no recombination between Xg and TBG.

During the past decade, several families with abnormal concentrations of serum thyroxine-binding globulin (TBG) have been described. Most have shown absent or reduced serum TBG (Nicoloff, Dowling, and Patton, 1964; Moloshok *et al*, 1969), but families with elevated TBG levels have also been described (Jones and Seal, 1967). These TBG abnormalities have not been associated with thyroid disease and have usually come to light when abnormal serum thyroxine or protein-bound iodine (PBI) results have been found in patients with normal thyroid function.

In many families, abnormal serum TBG levels

have shown X-linked inheritance, but searches for linkage with the X-borne marker Xg have been informative in only three families (Race and Sanger, 1968; Fialkow, Giblett, and Musa, 1970; Bode, Rothman, and Danon, 1973). The results in these families indicate that the TBG and Xg loci are too

TABLE I
SERUM TBG, THYROXINE, AND THYROTROPHIN RESULTS AND Xg BLOOD GROUP FINDINGS IN FAMILY WITH TBG DEFICIENCY

Subject	Serum TBG (% reference serum)*	Serum Thyroxine (μg/100 ml.)	Serum TSH (μU/ml.)	Xg ^a Antigen
I.1	ND	2.4	1.1	+
I.2	105	8.4		+
II.1	134	7.4		-
II.2†				
II.3	43	5.7	1.0	+
II.4	99	7.5		+
II.5	93	8.4		+
II.6	25	3.5	2.8	+
III.1	116	9.3		+
III.2	53	5.8	1.3	+
III.3	ND	0.6	< 1.0	+
III.4	105	7.4		-
III.5	51	4.6	< 1.0	+
IV.6	50	2.8	< 1.0	+
IV.7	43	4.1	1.3	+
III.8	107	9.9		+
III.9	88	6.6		+
III.10	114	9.1		+
IV.1	102	9.5		-
IV.2	44	5.4	2.3	+
IV.3	101	9.3		+
IV.4	ND	1.9	1.8	+
IV.5	ND	2.4	< 1.0	+
IV.6	110	8.1		-

FIG. 1. Pedigree of the family with TBG deficiency. Twelve consanguineous relatives who were not tested have not been included.

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* ND = none detected.
† Deceased.

FIG. 2a

FIG. 2. Autoradiographs from Laurell electrophoresis of serum from II.4 (Fig. 2a) showing normal thyroxine binding globulin (TBG) and from I.1 (Fig. 2b, opposite with no demonstrable TBG). In both samples, ^{125}I -thyroxine has been bound by thyroxine-binding pre-albumin (TBPA), albumin, α_1 -lipoprotein ($\alpha_1\text{LP}$), and β -lipoprotein (βLP).

far apart on the chromosome for linkage to be measurable. The present paper describes a further family in which TBG deficiency and the Xg groups segregate informatively.

Subjects

The clinical features of the proband, a boy with reduced serum PBI due to TBG deficiency, have been given elsewhere (Raiti and Newns, 1972). The other members of his family who were studied are shown in Fig. 1. None had a history of thyroid disease. Twelve further consanguineous relatives, eight of whom were young children, could not be investigated and have not been included in Fig. 1.

Methods

Serum TBG was measured by a quantitative immunoelectrophoretic method (Freeman and Pearson, 1969), modified so that the radio-labelled material was added to

the samples before electrophoresis. A solution containing approximately $5\mu\text{g/ml}$ of L-thyroxine (T_4) labelled with ^{125}I (approximately $40\mu\text{Ci}/\mu\text{g}$; Radiochemical Centre, Amersham) was mixed with the serum samples (1:10 v/v). After 1 hour's incubation at room temperature, $4.4\mu\text{l}$ of the mixture was submitted to Laurell electrophoresis. The gels were then washed in 0.9% saline, dried, and applied unstained to x-ray film (Kodirex®; Kodak Ltd) for 4 weeks. The autoradiographs obtained showed binding of labelled T_4 to an α_1 -globulin previously identified as TBG (Freeman and Pearson, 1969), together with binding to pre-albumin, albumin, and the lipoproteins (Figs. 2a and 2b). Serum TBG concentration was estimated by comparing the area of the TBG peak with that obtained for a freeze-dried reference serum included in each assay. The results for a control serum run with the samples on 10 occasions showed a coefficient of variation of 8%.

Serum thyroxine was estimated by competitive binding (Ryess, 1972). The normal range in healthy adults is $6.0\mu\text{g}/100\text{ ml}$ to $11.2\mu\text{g}/100\text{ ml}$.

FIG. 2b

Serum thyrotrophin (TSH) was estimated by a double-antibody radioimmunoassay based on the method described by Hall, Amos, and Ormston (1971).

Xg blood groups were determined by the MRC Blood Group Unit. Linkage was analysed by the lod score method of Morton (Maynard-Smith, Penrose, and Smith, 1961).

Results

The results are given in the Table. TBG and Xg status are also indicated in Fig. 1.

TBG was not detected in serum samples from the proband (IV.4) and three of his male relatives (I.1; III.3; IV.5). Serum thyroxine values below $2.5 \mu\text{g}/100 \text{ ml}$ were obtained in these four subjects.

Seven female members of the family (II.3, II.6, III.2, III.5, III.6, III.7, and IV.2) showed reduced serum TBG concentrations with levels approximately half that given by the control serum.

Serum thyroxine values between $2.8 \mu\text{g}/100 \text{ ml}$ and $5.8 \mu\text{g}/100 \text{ ml}$ were obtained in these subjects.

The serum TBG levels were normal in eight consanguineous and four non-consanguineous relatives. In these individuals the serum thyroxine ranged from $6.6 \mu\text{g}/100 \text{ ml}$ to $9.9 \mu\text{g}/100 \text{ ml}$.

Normal TSH values were obtained in all the subjects with reduced or absent TBG.

TBG and Xg linkage information is provided by five members of the family (IV.1, IV.3, IV.5, and IV.6) who are all phase known and are all non-recombinants. The lod score for the family is 1.505 at $\theta = 0.0$, which is suggestive of measurable linkage, but not significantly so.

Discussion

In the present family, TBG deficiency appears to have been inherited as an X-linked characteristic.

The abnormality was not inherited by the son of an affected father and although TBG was not detected in affected males, intermediate levels were found in the heterozygous females. These findings are very similar to those described by others. Nikolai and Seal (1966; 1967) described two extensive pedigrees which indicated that TBG deficiency was inherited as an X-linked condition and a similar pattern of inheritance has been described in several other families (Marshall, Levy, and Steinberg, 1966; Torkington *et al.*, 1970; Bode *et al.*, 1973). As in the present family, TBG was absent in affected males and carrier females usually had levels below the normal range. In some families, low levels of TBG have been detected in affected males, indicating partial TBG deficiency (Moloshok *et al.*, 1969). This abnormality also appears to show X-linked inheritance (Malvaux and De Nayer, 1972).

As in previously reported families, TBG deficiency in the present family was not associated with thyroid disease or other clinical disorders. The low serum TSH values obtained in affected individuals indicate that sub-clinical hypothyroidism was not present.

It is still uncertain whether TBG deficiency represents absence of a specific serum protein or whether it is due to synthesis of an abnormal protein which has a low affinity for thyroxine. As the methods used in most studies have depended on the binding of isotope-labelled thyroxine to identify TBG, a protein with low affinity for thyroxine would not have been detected. Marshall and Pensky (1969), using immunodiffusion methods, found that both normal and TBG-deficient serum contained two proteins which reacted with an antiserum raised against a preparation of serum TBG. However, in subsequent studies using a more specific radioimmunoassay method, Levy, Marshall, and Velayo (1971) were unable to detect TBG in 'TBG-deficient' serum and concluded that the earlier findings may have been due to lack of specificity of the antiserum. The results described above are in keeping with the latter observation. Although ^{125}I -thyroxine was used to identify the location of TBG, the serum concentration was estimated from the area under the TBG peak. If an abnormal protein with a very close immunochemical similarity to TBG had been present, the TBG peaks in affected females would have been expected to be lightly labelled but of normal size. As indicated above, all the affected females showed TBG peaks which were reduced in area.

The Xg linkage results given above differ from those given by others. Race and Sanger (1968) found no evidence of linkage (1 non-recombinant:7 recombinants and z_1 2:2, e_1 2:2) between Xg and

TBG in a family previously described by Nikolai and Seal (1966). Bode *et al.* (1973) also found no evidence of linkage (z_1 3:2, e_1 3:2) in a further family with TBG deficiency. In a family with elevation of serum TBG reported by Fialkow *et al.* (1970) there was again no evidence of linkage (2 recombinants and z_1 1:1, e_1 2:0; z_1 1:1, e_1 2:0; z_1 2:1, e_1 2:1). The suggestion of linkage of Xg and TBG in the present family is not in keeping with these previous findings. If this apparent linkage were to be confirmed by further studies on the younger members of the family, it would strongly suggest that more than one X-borne locus can cause deficiency of TBG.

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