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# A Chinese family with familial dysalbuminaemic hyperthyroxinaemia

## 一個患有家族性血蛋白異常甲狀腺素過高的華人家庭的病例

We report the results of biochemical and genetic studies in a Chinese family with familial dysalbuminaemic hyperthyroxinaemia. Total thyroxine levels were 1.2 to 1.7 times the upper limit of the reference range and free thyroxine levels were 1.2 to 1.6 times the upper reference limit. Concentrations of thyroid-stimulating hormone (thyrotropin) and free tri-iodothyronine were normal in all family members tested. Overall, thyroid function tests showed high total thyroxine levels in five males and two females over two generations in the family. The diagnosis of familial dysalbuminaemic hyperthyroxinaemia was confirmed by the detection of a guanine to adenine missense mutation in the second nucleotide of codon 218 of the gene encoding human serum albumin, showing that the mutation in this family is the same as that previously found in Caucasian populations.

本文報告一個家族性血蛋白異常甲狀腺素過高的華裔家庭，對其進行的生化學和遺傳學研究。接受測試的家族成員，其總甲狀腺素水平為正常範圍上限的 1.2 至 1.7 倍，游離甲狀腺素水平為正常值上限的 1.2 至 1.6 倍。所有家族成員的促甲狀腺激素和游離三碘甲狀腺原氨酸的濃度均屬正常。總體來說，各種甲狀腺功能測試顯示，兩代家族成員中，有五名男性和兩名女性的甲狀腺素屬於高水平。偵測到人體血清白蛋白基因第218號密碼子的第二個核苷酸，出現一個鳥嘌呤變為腺嘌呤的錯義突變，從而確定家族性血蛋白異常甲狀腺素過高的診斷。此家族出現的基因突變，與過往在白人口中發現的突變一樣。

### Introduction

Familial dysalbuminaemic hyperthyroxinaemia (FDH) is an autosomal dominant condition in which abnormal binding of thyroxine ( $T_4$ ) to variant human serum albumin (HSA) leads to euthyroid hyperthyroxinaemia. The disease is characterised by an increased level of serum total thyroxine ( $TT_4$ ) relative to that of total tri-iodothyronine ( $TT_3$ ), mainly because of the much higher binding affinity of the variant HSA for  $T_4$  than  $T_3$ .

The prevalence of FDH is different in different populations.<sup>1-3</sup> In all reports of Caucasian patients with FDH, the molecular basis for FDH was found to be a guanine (G) to adenine (A) missense mutation in the second nucleotide of codon 218 of the *HSA* gene, resulting in the replacement of arginine (coded by CGC) with histidine (CAC).<sup>4,5</sup> The condition has been reported infrequently in Asians. In a Japanese family, Wada et al<sup>6</sup> detected a guanine (G) to cytosine (C) mutation at codon 218 of the *HSA* gene, resulting in the replacement of arginine with proline. In this article, we report on the biochemical and genetic characteristics of a large Chinese family in Hong Kong, some members of which had FDH.

### Case report

A 21-year-old woman was referred to the Thyroid Clinic at the Queen Elizabeth Hospital in July 1997 because of abnormal thyroid function test results during investigations for anxiety and palpitation performed at a private laboratory. Previous laboratory results were as follows:  $TT_4$ , 238 nmol/L (reference range, 58-154 nmol/L); free  $T_4$  ( $FT_4$ ), 47 pmol/L (reference range, 9-24 pmol/L); thyroid-stimulating hormone (TSH [thyrotropin]), 2.3 mIU/L (reference

#### Key words:

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 Serum albumin;  
 Thyroxine

#### 關鍵詞：

華人；  
 甲狀腺素過高；  
 突變；  
 血清白蛋白；  
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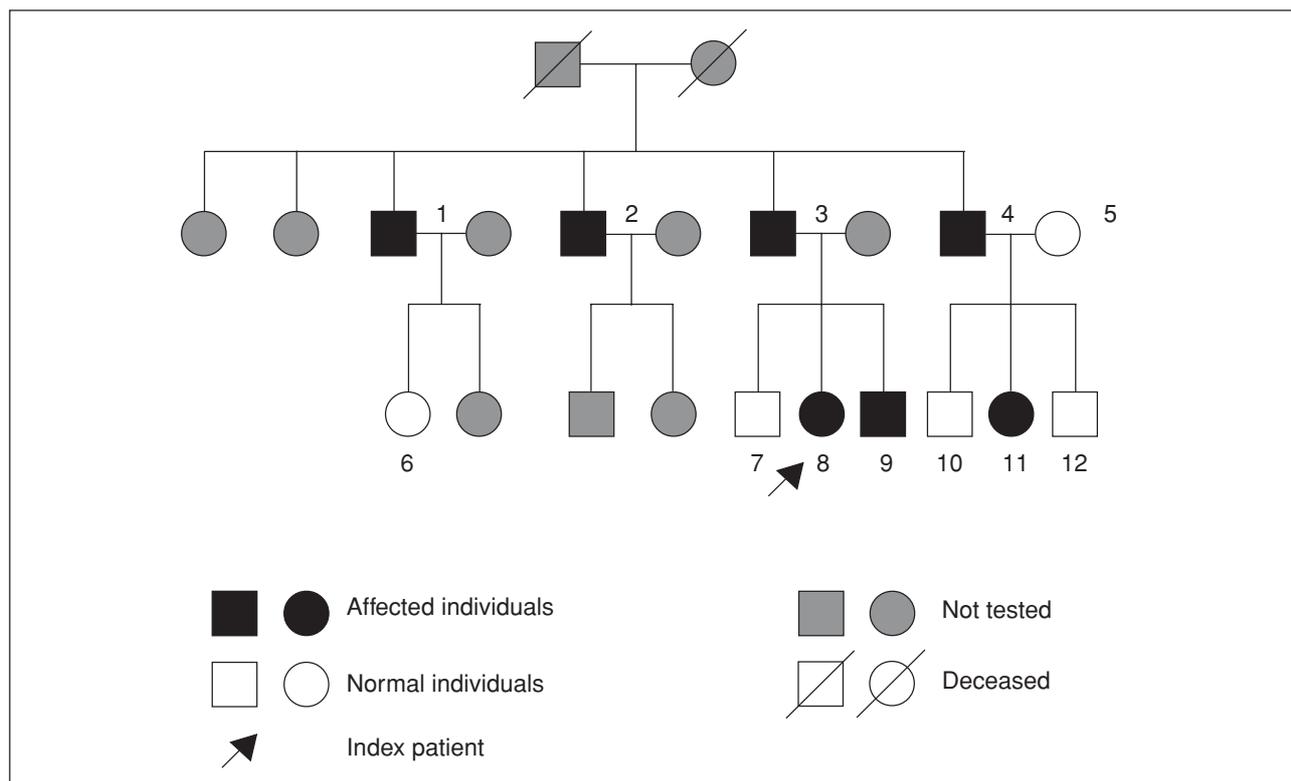


Fig. Pedigree of a family with familial dysalbuminaemic hyperthyroxinaemia

range, 0.3-5.0 mIU/L); T<sub>3</sub> resin uptake, 0.37 (reference range, 0.25-0.35); and FT<sub>4</sub> index, 6.9 (reference range, 1.2-4.0).

The index patient's father had a history of presumed thyrotoxicosis with multiple asymptomatic relapses, and had received several courses of antithyroid drugs during the previous 10 years, despite feeling more lethargic and tired with treatment than without. Thyroid function test results (more than 1 year after discontinuing antithyroid therapy) from the same private laboratory were as follows: TT<sub>4</sub>, 214 nmol/L; FT<sub>4</sub>, 44 pmol/L; TSH, 2.31 mIU/L; T<sub>3</sub> resin uptake, 0.38; and FT<sub>4</sub> index, 6.3. In addition, two paternal uncles had a history of 'thyroid problems'.

All three relatives were euthyroid clinically and had no goitre when assessed in our clinic. In view of the index patient's clinically euthyroid state and her family history, the provisional diagnosis of FDH was made. Family screening was offered, and a total of 12 family members gave informed consent for further biochemical and genetic studies.

The family pedigree chart is shown in the Fig. Among the family members who consented to testing, TT<sub>4</sub> and FT<sub>4</sub> results could be classified into two distinct patterns: hyperthyroxinaemia (n=7) and euthyroxinaemia (n=5) [Table]. In the family members with hyperthyroxinaemia, TT<sub>4</sub> levels according to a competitive mouse antibody test (IMX analyser; Abbott Laboratories, Illinois, US) and FT<sub>4</sub>

levels according to a one-step analog rabbit antibody test (ACS 180 analyser; Bayer Corporation, Tarrytown, US) were 192-257 nmol/L and 28-37 pmol/L, respectively; these values were considerably higher than the upper limit of the reference ranges: 1.2 to 1.7 times for TT<sub>4</sub> and 1.2 to 1.6 times for FT<sub>4</sub>. The FT<sub>4</sub> levels, as measured with a labelled antibody-sheep antibody test (Elecys; Boehringer Mannheim, Roche Diagnostics GmbH, Mannheim, Germany) and a two-step sheep antibody test (IMX analyser) were also elevated in some cases: 18-29 pmol/L, and above the upper reference limit in two of seven affected family members; and 21-28 pmol/L, and above the upper reference limit in four affected members, respectively. The TSH and FT<sub>3</sub> values were normal in all individuals. The abnormal TT<sub>4</sub> and FT<sub>4</sub> values were obtained in assays using antibodies from different animal species, thus excluding heterophilic antibodies as the cause of the abnormality. Overall, thyroid function tests showed high TT<sub>4</sub> and normal FT<sub>3</sub> and TSH levels in five males and two females over two generations in the family.

In addition, we performed mutation analysis on DNA extracted and purified from peripheral blood. A 110-bp DNA fragment from exon 7 of the *HSA* gene (which contains codon 218) was amplified by the polymerase chain reaction using forward and reverse primers of 5'-GTATTTGCCTAGTGTTCAT-3' and 5'-CTCAGCTTTGGGAAATCTCTGCACCAGG-3', respectively, as previously described.<sup>4</sup> The reverse primer

**Table. Results of thyroid function tests among members of a family with familial dysalbuminaemic hyperthyrotoxaemia\***

Family member No.	TT <sub>4</sub> <sup>†</sup> by IMX (nmol/L)	FT <sub>4</sub> <sup>‡</sup> by ACS 180 (pmol/L)	FT <sub>4</sub> by Elecsys (pmol/L)	FT <sub>4</sub> by IMX (pmol/L)	FT <sub>3</sub> <sup>§</sup> (pmol/L)	TSH <sup>  </sup> (mIU/L)
1	192	28	22	21	4.5	1.9
2	257	37	27	28	5.2	0.4
3	217	31	22	23	5.0	1.9
4	202	36	29	24	5.6	1.3
5	82	15	15	13	4.5	1.4
6	81	16	15	12	4.5	1.5
7	88	20	19	17	4.9	1.1
8	214	31	18	21	4.8	1.2
9	241	36	21	26	6.2	2.1
10	86	16	16	14	5.4	1.3
11	234	28	22	22	6.2	1.7
12	80	16	15	13	3.8	1.3
Reference range <sup>¶</sup>	58-154	13-23	12-23	12-23	4.0-7.8	0.3-4.2

\* Shaded rows are cases of hyperthyrotoxaemia; non-shaded rows are cases of euthyrotoxaemia

<sup>†</sup> TT<sub>4</sub> total thyroxine

<sup>‡</sup> FT<sub>4</sub> free thyroxine

<sup>§</sup> FT<sub>3</sub> free tri-iodothyronine

<sup>||</sup> TSH thyroid-stimulating hormone

<sup>¶</sup> Based on reference ranges supplied by the manufacturers

contained three degenerate bases (underlined) that were unmatched with the normal sequence, so that a unique *Dra*III restriction enzyme site would occur if there were a G-to-A mutation at codon 218. The amplified DNA was then digested with *Dra*III and the digestion products analysed by gel electrophoresis. The mutant allele produced an 86-bp band. When amplified DNA was analysed after *Hph*I digestion, the presence of the mutation resulted in a 72-bp band.<sup>5</sup> In this way, the mutation at codon 218 was detected in all affected family members. DNA sequencing of the amplified DNA confirmed the G-to-A mutation at codon 218 (corresponding to a change in arginine to histidine) in affected individuals.

## Discussion

This study reports the biochemical and genetic characteristics of a Chinese family with FDH. Thyroid function test results (high TT<sub>4</sub>, normal FT<sub>3</sub> and TSH in five males and two females over two generations) indicated the autosomal dominant mode of transmission of FDH. The diagnosis of FDH was confirmed by the G-to-A missense mutation in codon 218 of the *HSA* gene. This mutation is the same as that reported in Caucasians<sup>4,5</sup> and in a single Chinese FDH patient in whom genetic studies were performed.<sup>7</sup> However, the mutation is different from that in a Japanese family.<sup>6</sup> There is evidently genetic heterogeneity among individuals with FDH, which is perhaps related to their ethnicity. Among patients with FDH who have a G-to-A mutation in codon 218 of *HSA* gene, serum TT<sub>4</sub> levels were elevated two to three times.<sup>4,8</sup> On the other hand, the G-to-C mutation reported by Wada et al<sup>6</sup> was associated with TT<sub>4</sub> levels of 11 to 17 times the reference level, suggesting that the variant HSA in the latter case had a much higher affinity for T<sub>4</sub>. Affected individuals in the family in our case had the G-to-A mutation, and their serum TT<sub>4</sub> was 1.2 to 1.7 times the reference level.

The TT<sub>4</sub> level might provide a clue to the site and nature of the mutation in the *HSA* gene, but the degree of genotype-phenotype correlation has not been systematically studied.

Although FT<sub>4</sub> levels should theoretically be normal in patients with FDH, in practice, FT<sub>4</sub> levels are elevated with most assays, as illustrated by the biochemical findings in our study. This effect is related to the conjugated T<sub>4</sub> analogs used in one-step assays for FT<sub>4</sub>.<sup>6,9-11</sup> Analogs from different manufacturers tend to bind to the mutant HSA with different affinities, leading to misleading FT<sub>4</sub> results. In contrast, by removing the variant albumin before addition of the radiolabelled tracer, two-step FT<sub>4</sub> assays are expected to yield normal levels in patients with FDH.<sup>12</sup> Our study, however, illustrates that two-step assays can also give falsely elevated results. Removal of the serum in the two-step assay may therefore not completely correct assay interference by a mutant HSA. The reason for this observation has not been studied. One possible mechanism is non-specific binding of the variant HSA to the solid phase anti-T<sub>4</sub> antibody, thereby leaving fewer unoccupied binding sites for the labelled hormone. Another is the incomplete removal of the variant albumin in the first washing, which leads to its interaction with the labelled hormone during the second step of the incubation. Our results with the Elecsys FT<sub>4</sub> assay demonstrated that assays using labelled antibodies also showed interference with FDH samples, although to a lesser degree than did the analog assays.

The diagnosis of FDH is important because controlling the T<sub>4</sub> levels may unnecessarily expose the patient to the adverse effects of surgery, radioactive iodine, or antithyroid drug therapy. In view of the unreliability of most commercial FT<sub>4</sub> assays in excluding FDH biochemically, a high degree of clinical suspicion is required. The most obvious clues are the family history, the absence of thyrotoxic clinical

features despite elevated  $T_4$  levels, and a normal TSH level when the  $TT_4$  or  $FT_4$  levels are high. The diagnosis of FDH can be confirmed rapidly if known genetic mutations can be identified.<sup>13</sup> Results from our genetic studies in this Chinese family suggest that the *HSA* mutation among at least some Chinese families with FDH is the same as that among Caucasians. If known mutations cannot be identified, the presence of a variant HSA with a high affinity for  $T_4$  can be detected by isoelectric focusing of serum for albumin in the presence of labelled  $T_4$ .<sup>14</sup>

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### References

1. Croxson MS, Palmer BN, Holdaway IM, Frengley PA, Evans MC. Detection of familial dysalbuminaemic hyperthyroxinaemia. *Br Med J (Clin Res Ed)* 1985;290:1099-102.
2. Fleming SJ, Applegate GF, Beardwell CG. Familial dysalbuminaemic hyperthyroxinaemia. *Postgrad Med J* 1987;63:273-5.
3. Weiss RE, Sunthornthepvarakul T, Angkeow P, et al. Linkage of familial dysalbuminemic hyperthyroxinemia to the albumin gene in a large Amish kindred. *J Clin Endocrinol Metab* 1995;80:116-21.
4. Sunthornthepvarakul T, Angkeow P, Weiss RE, Hayashi Y, Refetoff S. An identical missense mutation in the albumin gene results in familial dysalbuminemic hyperthyroxinemia in 8 unrelated families. *Biochem Biophys Res Commun* 1994;202:781-7.
5. Petersen CE, Scottolini AG, Cody LR, Mandel M, Reimer N, Bhagavan NV. A point mutation in the human serum albumin gene results in familial dysalbuminaemic hyperthyroxinaemia. *J Med Genet* 1994;31:355-9.
6. Wada N, Chiba H, Shimizu C, Kijima H, Kubo M, Koike T. A novel missense mutation in codon 218 of the albumin gene in a distinct phenotype of familial dysalbuminemic hyperthyroxinemia in a Japanese kindred. *J Clin Endocrinol Metab* 1997;82:3246-50.
7. Tang KT, Yang HJ, Choo KB, Lin HD, Fang SL, Braverman LE. A point mutation in the albumin gene in a Chinese patient with familial dysalbuminemic hyperthyroxinemia. *Eur J Endocrinol* 1999;141:374-8.
8. DeCosimo DR, Fang SL, Braverman LE. Prevalence of familial dysalbuminemic hyperthyroxinemia in Hispanics. *Ann Intern Med* 1987;107:780-1.
9. Stockigt JR, Stevens V, White EL, Barlow JW. 'Unbound analog' radioimmunoassays for free thyroxin measure the albumin-bound hormone fraction. *Clin Chem* 1983;29:1408-10.
10. De Nayer P, Malvaux P, Beckers C. Familial dysalbuminemic hyperthyroxinemia (FDH): inadequacy of the 'analog' methods for assaying free-T4 levels. *Eur J Nucl Med* 1984;9:284-5.
11. Wood DF, Zalin AM, Ratcliffe WA, Sheppard MC. Elevation of free thyroxine measurements in patients without thyrotoxicosis. *Q J Med* 1987;65:863-70.
12. Sapin R, Gasser F, Schlienger JL. Familial dysalbuminemic hyperthyroxinemia and thyroid hormone autoantibodies: interference in current free thyroid hormone assays. *Horm Res* 1996;45:139-41.
13. AvRuskin TW, Juan CS, Weiss RE. Familial dysalbuminemic hyperthyroxinemia: a rare example of albumin polymorphism and its rapid molecular diagnosis. *J Pediatr Endocrinol Metab* 2002;15:801-7.
14. Flechner I, Aranoff G, Reifen R, Landau H. Detection of albumin binding abnormalities in sera of patients with familial dysalbuminaemic hyperthyroxinaemia using isoelectric focusing. *Endocr Res* 1992;18:229-40.