A Chinese family with familial dysalbuminaemic hyperthyroxinaemia

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.

Introduction

Familial dysalbuminaemic hyperthyroxinaemia (FDH) is an autosomal dominant condition in which abnormal binding of thyroxine (T₄) to variant human serum albumin (HSA) leads to euthyroid hyperthyroxinaemia. The disease is characterised by an increased level of serum total thyroxine (TT₄) relative to that of total tri-iodothyronine (TT₃), mainly because of the much higher binding affinity of the variant HSA for T₄ than T₃.

The prevalence of FDH is different in different populations. 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). The condition has been reported infrequently in Asians. In a Japanese family, Wada et al 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: TT₄, 238 nmol/L (reference range, 58-154 nmol/L); free T₄ (FT₄), 47 pmol/L (reference range, 9-24 pmol/L); thyroid-stimulating hormone (TSH [thyrotropin]), 2.3 mIU/L (reference range, 0.5-4.0 mIU/L).
Familial dysalbuminaemic hyperthyroxinaemia

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: TT₄, 214 nmol/L; FT₄, 44 pmol/L; TSH, 2.31 mIU/L; T₃ resin uptake, 0.38; and FT₄ 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, TT₄ and FT₄ results could be classified into two distinct patterns: hyperthyroxinaemia (n=7) and euthyroxinaemia (n=5) [Table]. In the family members with hyperthyroxinaemia, TT₄ levels according to a competitive mouse antibody test (IMX analyser; Abbott Laboratories, Illinois, US) and FT₄ 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 TT₄ and 1.2 to 1.6 times for FT₄. The FT₄ levels, as measured with a labelled antibody–sheep antibody test (Elecsys; 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 FT₃ values were normal in all individuals. The abnormal TT₄ and FT₄ 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 TT₄ and normal FT₃ 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'-GTATTTGCCTAGTGTTTTCAT-3' and 5'-CTCAGCCTTGGGAATCTCTGCACCAGG-3', respectively, as previously described. The reverse primer
Table. Results of thyroid function tests among members of a family with familial dysalbuminaemic hyperthyroxinaemia

<table>
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<th>Family member No.</th>
<th>TT₄ by IMX (pmol/L)</th>
<th>TT₄ by ACS 180 (pmol/L)</th>
<th>TT₄ by Elecsys (pmol/L)</th>
<th>FT₃ by IMX (pmol/L)</th>
<th>FT₃ by ACS 180 (pmol/L)</th>
<th>FT₃ by Elecsys (pmol/L)</th>
<th>TSH (mIU/L)</th>
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* Shaded rows are cases of hyperthyroxinaemia; non-shaded rows are cases of euthyroxinaemia

The TT₄ 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 FT₄ levels should theoretically be normal in patients with FDH, in practice, FT₄ levels are elevated with most assays, as illustrated by the biochemical findings in our study. This effect is related to the conjugated T₄ analogs used in one-step assays for FT₄. Analogs from different manufacturers tend to bind to the mutant HSA with different affinities, leading to misleading FT₄ results. In contrast, by removing the variant albumin before addition of the radiolabelled tracer, two-step FT₄ assays are expected to yield normal levels in patients with FDH. 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-T₄ antibody, thereby leaving fewer occupied 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 FT₄ 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 T₄ 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 FT₄ 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 symptoms, the higher FT₃ level, and the higher TT₄ level.

Discussion

This study reports the biochemical and genetic characteristics of a Chinese family with FDH. Thyroid function test results (high TT₄, normal FT₃, 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 mutation in codon 218. The amplified DNA was then digested with Dral restriction enzyme and the digestion products analysed by gel electrophoresis. The mutant allele produced an 86-bp band. When amplified DNA was analysed after HphI digestion, the presence of the mutation resulted in a 72-bp band. In this way, the mutation in codon 218 was detected in all affected family members.

DNA sequencing of the amplified DNA confirmed the G-to-A mutation in codon 218 (corresponding to a change in arginine to histidine) in affected individuals.

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features despite elevated T4 levels, and a normal TSH level when the TT4 or FT4 levels are high. The diagnosis of FDH can be confirmed rapidly if known genetic mutations can be identified. 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 T4 can be detected by isoelectric focusing of serum for albumin in the presence of labelled T4.

Acknowledgements

We wish to thank Miss J Lai of the Tuen Mun Hospital and Mr HF Chan of the Kwong Wah Hospital for assistance with some of the thyroid function tests.

References