CASE REPORT

A point mutation in the albumin gene in a Chinese patient with familial dysalbuminemic hyperthyroxinemia

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Abstract

Familial dysalbuminemic hyperthyroxinemia (FDH) is an autosomal dominant disorder characterized by euthyroid hyperthyroxinemia. However, FDH has not been reported in Chinese or African patients. Here, we report the first case of FDH in a Chinese patient. A 69-year-old Chinese man was found to have increased serum total T4 concentrations (198–242 nmol/l; normal range 58–148 nmol/l) and free T4 concentrations (>58 pmol/l; T4 analog method, normal range 9–28 pmol/l). Serum total T3 and TSH concentrations were normal. The patient was misdiagnosed as hyperthyroid and was later suspected to have a TSH-producing tumor by the finding of a pituitary microadenoma, which was eventually proven to be a non-functional pituitary ‘incidentaloma’. Electrophoretic analysis of the patient’s serum proteins demonstrated enhanced albumin binding of [125I]T4. Serum free T4 concentrations were normal (16–19 pmol/l, normal range 9–26 pmol/l) when a two-step method was used. Direct sequencing of the albumin gene showed a guanine to adenosine transition in the second nucleotide of codon 218, resulting in a substitution of histidine (CAC) for the normal arginine (CGC) in one of the two alleles in the patient. The point mutation was further confirmed by HphI digestion of exon 7 of the albumin gene. The patient’s son was not affected. Our studies demonstrated that the point mutation of the albumin gene in a Chinese patient with FDH was similar to that found in western white families, but differed from that in a Japanese family in whom a guanine to cytosine transition at the same position was found.

Introduction

Familial dysalbuminemic hyperthyroxinemia (FDH) is an autosomal dominant syndrome characterized by an increased serum total thyroxine (T4) (T4), and free T4 (fT4) concentrations measured by the index and analog methods, whereas the serum fT4 determined by equilibrium dialysis or two-step methods are normal. The total tri-iodothyronine (T3) (T3) and thyroid-stimulating hormone (TSH) concentrations, usually, are normal (1–6). These discrepancies are due to the presence of an abnormal serum albumin that exhibits enhanced binding of T4, and patients with FDH are clinically euthyroid (7–10). Although FDH has been widely reported in the medical literature and accounts for the most common cause of genetic euthyroid hyperthyroxinemia in western countries, particularly among Hispanics of Puerto Rican origin (11–13), to our knowledge no case has been reported in individuals of Chinese or African origin. Recently, the molecular basis of FDH has been found to be a substitution of histidine (CAC) and proline (CCC) for arginine (CGC) in codon 218 of the albumin gene in unrelated western families and in a Japanese kindred, respectively (14–16). Recombinant human serum albumin with a substitution of His218 for Arg218 has been shown to have an increased affinity for T4, similar to the human serum albumin purified from the FDH patients, confirming that the substitution of His218 for Arg218 generates the phenotype of FDH (17–19). The Kd of FDH albumin-Pro218 for T4 in the Japanese kindred was approximately 80-fold that of albumin in normal individuals and the replacement of arginine by proline is believed to be responsible for this condition (16).

We now report the first case of FDH in a Chinese patient who had increased serum fT4 and fT4 concentrations (analog method) and normal serum TSH. The patient had been misdiagnosed as having thyrotoxicosis, and was suspected to have a TSH-producing tumor. Direct sequencing of the albumin gene revealed
that the arginine (CGC) in codon 218 was replaced by histidine (CAC), which is similar to the genotype reported in western white individuals (14, 15), but differs from that reported in a Japanese family in whom the arginine (CGC) was replaced by proline (CCC) at the same position (16).

Case report
The patient was a Chinese male veteran, who was born in mainland China and moved to Taiwan during the Chinese civil war in 1949. He was accidentally found to have an increased serum fT4 concentration (analog method) in a routine examination in 1988 when he was 61 years old. The thyroid was normal and the patient had no signs of thyrotoxicosis or ophthalmopathy. The patient and his son were the only family members in Taiwan, and they had no significant medical problems. The family history of other members of the family was not available, because the patient’s parents and other family members had passed away in mainland China many years previously.

Antithyroid drugs were prescribed for the suspected hyperthyroidism, but were discontinued by the patient after a few months of treatment. The patient complained of headache in 1992 and serum tT4 and fT4 were again found to be increased, but serum tT3 and TSH were normal. Computed tomography (CT) scan of the sella revealed a low-density, non-enhancing mass in the left pituitary fossa. A thyrotrophin-releasing hormone (TRH) test showed a normal TSH response to intravenous TRH administration, and other pituitary function tests were normal. The patient was diagnosed as having euthyroid hyperthyroxinemia with a non-functional small pituitary ‘incidentoloma’. In 1995, serum fT4 was normal when a fT4 assay was used. The patient was referred to our laboratory for further evaluation, with the suspected diagnosis of FDH. The results of his thyroid function tests are shown in Table 1. Serum fT4 concentrations were high, ranging from 198 to 242 nmol/l, whereas serum tT3 and TSH concentrations were normal, ranging from 2.0 to 2.3 nmol/l and 1.64 to 2.42 mU/l respectively. Serum fT4 concentrations were strikingly increased (>58 pmol/l) determined by a one-step analog method from 1988 to 1991, but were normal (16–19 pmol/l) when measured by a two-step method after 1995. TSH receptor antibody, antimicrosomal and antithyroglobulin antibodies were not detected. Thyroxine-binding globulin (TBG) and thyroid iodine-131 uptake were normal. The serum TSH, fT4, fT3, tT4 and tT3 concentrations of the patient’s son were within the normal ranges (data not shown) on two different occasions in 1996. The distribution of [125I]T4 and [123I]T3 among the serum thyroid hormone-binding proteins was analyzed by 8% PAGE and there were no differences in the distribution of [125I]T4 in the TBG, albumin and transthyretin (TTR) zones in a normal volunteer and the patient’s son. However, the distribution of [125I]T4 was markedly increased in the albumin fraction in the patient’s serum. There were no differences in the

Table 1 Results of thyroid function tests in the patient from 1988 to 1996.

<table>
<thead>
<tr>
<th>Date</th>
<th>tT3 (nmol/l)</th>
<th>tT4 (nmol/l)</th>
<th>fT4 (pmol/l)</th>
<th>TSH (mU/l)</th>
<th>TBDII (%)</th>
<th>AMA</th>
<th>ATA</th>
<th>Albumin (g/l)</th>
<th>TBG (nmol/l)</th>
<th>Thyroid 131I uptake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec 1988</td>
<td>2.2</td>
<td>198</td>
<td>&gt;58†</td>
<td>44</td>
<td>2.05</td>
<td>&lt;3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>37–53</td>
</tr>
<tr>
<td>Feb 1989</td>
<td>2.1</td>
<td>205</td>
<td>&gt;58†</td>
<td>35</td>
<td>2.42</td>
<td>&lt;3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>172–463</td>
</tr>
<tr>
<td>Mar 1992</td>
<td>2.3</td>
<td>242</td>
<td>19‡</td>
<td>364</td>
<td>1.64</td>
<td>&lt;3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>15–40</td>
</tr>
<tr>
<td>Jun 1995</td>
<td>2.3</td>
<td>242</td>
<td>19‡</td>
<td>364</td>
<td>2.39</td>
<td>&gt;3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>15–40</td>
</tr>
<tr>
<td>April 1996</td>
<td>2.0</td>
<td>218</td>
<td>16‡</td>
<td>364</td>
<td>2.39</td>
<td>&gt;3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>15–40</td>
</tr>
<tr>
<td>Normal ranges</td>
<td>1.3–2.5</td>
<td>58–148</td>
<td>0.4–3.1</td>
<td>37–53</td>
<td>172–463</td>
<td>15–40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† One-step method; normal range 9–28 pmol/l; ‡ two-step method; normal range 9–26 pmol/l. TBDII, TSH-binding inhibitory activity; normal range < 10%.
distribution of [125I]T3 among the serum proteins in the patient, normal individuals, and the patient’s son (data not shown). DNA fragments containing all exons of the albumin gene obtained from the patient, the patient’s son and a normal volunteer after PCR amplification were subjected to direct sequencing. The results revealed a guanosine to adenosine transition in the second nucleotide of codon 218 in exon 7 of the patient (Fig. 2). This transition results in the replacement of arginine by histidine. Guanosine was also present at the same position, suggesting that the patient was heterozygous. No mutations were seen in the patient’s son and the sequence was identical to that in normal individuals. DNA sequences of all other exons from the patient’s albumin gene were identical to the albumin gene reported previously (20). The PCR products of exon 7 containing DNA fragments (355 bp) were digested by HphI. Partial digestion was seen in the patient’s DNA fragments, which formed two bands (Fig. 3). The DNA fragment with 355 bp represented the undigested portion, which carried the normal DNA sequence. The thick band seen at position about 180 bp represented the digestion of the mutant exon 7 into an unsolved doublet containing fragments 172 bp and 182 bp. The DNA fragments of the patient’s son and the normal volunteer were not digested by HphI. The results were compatible with the DNA sequencing, suggesting a guanine to adenosine transition in the second nucleotide of codon 218 of the albumin gene in one of the patient’s two alleles.

### Methods

Serum tT4 was measured by GammaCoat [125I]T4 radioimmunoassay kit (Dade Baxter Travenol Diagnostic Inc., Cambridge, MA, USA). Serum fT4 concentrations were measured by a one-step analog method by GammaCoat [125I]T4 radioimmunoassay kit (Dade Baxter Travenol Diagnostic Inc.) before 1995 and by a two-step method by GammaCoat fT4 (two-step) [125I]RIA kit (Incstar Corporation, Stillwater, MN, USA) after 1995. Serum TSH was measured by SPAC T3 RIA kit (Daichi, Tokyo, Japan). Serum TSH was determined by Clinical Assay GammaCoat hTSH [125I] IRMA kit (Incstar Corporation). Antimicrosomal (AMA) and antithyroglobulin antibodies (ATA) were measured by hemagglutination and TBG by a time-resolved fluoroimmunoassay method (Delfia, Wallac Oy, Turku, Finland). The distribution of tracer concentrations of [125I]T4 and [125I]T3 among serum proteins was measured by 8% PAGE in phosphate buffer (pH 9.0) as described previously (3, 21). Genomic DNA was isolated from peripheral blood using the Puregene DNA isolation kit (Gentra System, Inc., MN, USA) (22). DNA fragments containing each exon of the albumin gene were generated by PCR (Gene Amp PCR System 2400, Perkin-Elmer Co., Norwalk, CT, USA) using primers synthesized according to genomic sequences as reported previously (15, 16, 20). The purified PCR products of all exons were sequenced directly using the same primers as PCR with ABI Prism dye terminator cycle sequencing ready reaction kits (Perkin-Elmer Co.) and sequenced on a Perkin-Elmer DNA Sequencer Model 377. The amplified DNA fragments containing exon 7 of the patient, the patient’s son and normal volunteers were digested by HphI (New England Biolabs,
Beverly, MA, USA) or water (for negative control) and subjected to electrophoresis on a 3% agarose gel (15).

**Discussion**

Measuring $\Gamma T_4$ instead of $tT_4$ has the additional advantage of differentiating patients with thyrotoxicosis from a substantial proportion of patients with euthyroid hyperthyroxinemia caused by increased thyroid hormone binding to serum proteins. However, in the one-step analog $\Gamma T_4$ assay, the abnormal albumin in patients with FDH has an increased binding affinity for the [\(^{125}\)I]$T_4$ analogs, which decreases the binding of the analogs to the antibodies, resulting in over-estimation of the $\Gamma T_4$ concentration (4–6). Accordingly, patients with FDH may be misdiagnosed as having hyperthyroidism and be inappropriately treated, as was the case with this patient (1–7). Furthermore, they may also be misdiagnosed as having inappropriate TSH secretion, as the serum TSH is normal (23, 24). In our patient, the finding of a pituitary microadenoma by CT scan, associated with the increased serum $\Gamma T_4$ and $tT_4$ but normal TSH, suggested the possibility of a TSH-producing tumor. However, pituitary enlargement is a common disorder and pituitary ‘incidentalomas’ are present in 10–20% of the population according to autopsy reports and careful imaging studies (25, 26). The normal serum $T_3$ concentration and TRH test in this patient were particularly useful in excluding the possibility of a TSH-producing tumor. Although FDH had not previously been reported in Chinese, the finding of a normal serum $\Gamma T_4$ concentration determined by a two-step method in the later tests raised the distinct possibility of FDH in this patient. The diagnosis was eventually confirmed by demonstrating the point mutation in exon 7 of the albumin gene as described in western white populations (16). The guanidine to adenosine transition in the second nucleotide of codon 218 of the albumin gene, resulting in the replacement of the normal arginine by histidine in one of the two alleles has been confirmed to be responsible for the phenotype of FDH in western white families (18, 19). The guanidine to cysteine transition in the same position, causing the replacement of the arginine by proline in the Japanese kindred, produced a distinct phenotype of FDH that displays greater serum $\Gamma T_4$, $tT_4$ and $rT_3$ concentrations (16). The findings suggest that codon 218 located in subdomain 2A of human serum albumin has an important role in the high binding affinity of $T_4$ (27).

In human serum albumin, the guanidino group of arginine in codon 218 is involved in an unfavorable binding interaction with the amino group of $T_4$ (19). As neither histidine nor proline has the guanidine group, the FDH $H_1^{218}$-albumin or $P_2^{218}$-albumin produces a more favorable binding interaction with $T_4$. Probably, the FDH $P_2^{218}$-albumin may induce conformational changes in the $T_4$-binding site that may have a higher-affinity $T_4$ binding than the normal albumin and FDH $H_2^{218}$-albumin (16).

In conclusion, FDH is present in Chinese, and a mutation of the serum albumin gene that is the same as that described in western white populations, but which differs from the mutation reported in a Japanese family. DNA analysis is a rapid and simple method with which to confirm the diagnosis and etiology of FDH, and is particularly useful in small families for whom there is no detailed pedigree or FDH history.

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