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HYPERTHYROXINEMIA AND HYPERCORTISOLEMIA DUE TO FAMILIAL DYSALBUMINEMIA

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BRIEF REPORT

HYPERTHYROXINEMIA AND HYPERCORTISOLEMIA DUE TO FAMILIAL DYSALBUMINEMIA

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RUNNING TITLE: Hyperthyroxinemia, hypercortisolemia in R218P FDH

KEY TERMS: Familial dysalbuminemic hyperthyroxinemia (FDH); assay interference; discordant thyroid function tests; hypercortisolemia; albumin

Abstract

A 23 year old man and his grandmother with hyperthyroxinemia and hypercortisolemia were heterozygous for an *ALB* mutation (p. Arg218Pro), known to cause Familial Dysalbuminemic Hyperthyroxinemia (FDH). However, serum free cortisol levels in these individuals were normal and total cortisol concentrations fell markedly following depletion of albumin from their serum. We conclude that binding of steroid as well as iodothyronines to mutant albumin causes raised circulating cortisol as well as thyroid hormones in euthyroid, euadrenal individuals with R218P FDH, with potential for misdiagnosis, unnecessary investigation and inappropriate treatment.

Introduction

Familial dysalbuminemic hyperthyroxinemia (FDH), a dominantly-inherited condition due to circulating mutant albumin with altered binding affinity for thyroid hormones (TH), is a recognised cause of raised serum T4 in euthyroid individuals. Heterozygous *ALB* mutations (R218H, R218P, R218S, R222I) change sidechains of aminoacids around a T4 binding pocket (Site 1) in the protein, thereby reducing steric hindrance and increasing its affinity for iodothyronines (1, 2, 3). This T4 binding site in albumin is also known to interact with steroids *in vitro* (4). Here, we report two individuals with FDH due to R218P mutant albumin who exhibit hypercortisolemia and hyperthyroxinemia, likely due to increased binding of steroid as well as iodothyronines to mutant albumin.

Patient History

A 23 year old male Swiss army recruit (P1), on no medication or supplements, was investigated following collapse during endurance training. He was tachycardic (pulse rate 100/min), without other hyperthyroid features and overweight (BMI 31kg/m^2) without stigmata of Cushing's syndrome. His circulating total and free thyroid hormones were elevated [Total T4 >300nmol/L (NR 66-181); Free T4 77 pmol/L (NR 9-19.1); Free T3 6.1pmol/L (NR 2.63-5.7)], but with normal TSH levels (TSH 1.4 mU/L). Measurements of serum total cortisol in P1, using either immunoassay (1610 nmol/L, NR 170-500) or by tandem mass spectrometry (MS) (1920 nmol/L, NR 280-650), indicated that he was also markedly hypercortisolemic (Table 1). His circulating 17-hydroxyprogesterone (17-OHP) [immunoassay 12.5nmol/L (NR 1.9-6.5); MS 8.1nmol/L (NR<5)], cortisone (175 nmol/L, NR 34-91) and 11-deoxycorticostrone (0.35 nmol/L, NR <0.25) levels were also raised.

His grandmother (P2), who is otherwise well, was also hyperthyroxinemic with relatively high, late daytime, serum total cortisol levels (Table 1).

Results

Both individuals were investigated further, either under clinical auspices or with informed consent as part of an ethically-approved protocol (Cambridgeshire LREC 98/154).

Elevated total and free T4 concentrations with normal thyroxine binding globulin levels (20.8 μg/L, NR 14-31) in P1 prompted an alternative TH binding protein abnormality to be considered. *ALB* sequencing showed that P1 and his

hyperthyroxinemic relative (P2) were heterozygous for a mutation (R218P) (Supplementary Figure 1), known to cause FDH.

Given the discordance between hypercortisolemia and absence of features of Cushing's syndrome in P1, we measured free cortisol levels in his urine (342 nmol/L, NR 99-378) and serum (16.7 nmol/L, NR 12.3-44.2) and found them to be within the normal range (Table 1). We reasoned that his hypercortisolemia could be due to abnormal association of steroid with a circulating binding protein. Measurement of serum corticosteroid binding globulin (CBG) concentrations in P1 were normal (Table 1). However, after immunodepletion of circulating albumin from his serum, the total cortisol concentration fell markedly (to 46% of baseline) compared to minimal changes following albumin depletion of serum from age and gender-matched healthy control subjects (106-131%) (Fig 1A).

His grandmother (P2) also showed discordance between relatively high, late daytime, serum total cortisol measurement (479 nmol/L) and low-normal serum free cortisol (8.5 nmol/L, NR 11-38) concentrations (Table 1). Serum CBG concentration in P2 was normal (Table 1) but there was a greater fall in serum total cortisol (to 69% of baseline) following albumin immunodepletion than observed in healthy control subjects (97-141%) (Fig 1A).

Serum cortisol levels in unrelated subjects with FDH due to other ALB mutations (R218H, R222I) were in the normal range (Supplementary Figure 2).

Discussion

In two individuals with hyperthyroxinemia due to an *ALB* mutation (R218P) known to cause FDH, we have documented hypercortisolemia with euadrenal

status and shown that this discordance is due to abnormal binding of steroid to circulating albumin.

A previous study supports this, showing that steroids (including corticosterone, and 17-OHP) bind to the Site 1 T4 binding pocket of albumin (2). In this context, we note that, in addition to hypercortisolemia, P1 also exhibited elevated circulating 17-OHP, cortisone and 11-deoxycorticosterone levels.

We have modelled binding of cortisol to wild type or mutant albumin and find that the Arginine to Proline substitution at residue 218 reduces steric hindrance, enabling cortisol to be easily accommodated within the Site 1 pocket of the R218P mutant protein (Fig 1B), providing a structural basis for our observations. Our finding of normal cortisol levels in individuals R218H or R2221 FDH (Supplementary Fig 1) correlates with modelling showing that mutation of Arg 218 to Histidine or Arg222 to Isoleucine would not increase the width of the Site 1 pocket to accommodate cortisol.

We have documented hypercortisolemia as well as hyperthyroxinemia in individuals with R218P dysalbuminemia, likely due to interaction of steroid as well as iodothyronines with mutant albumin, with spurious elevation of this combination of hormones providing potential for misdiagnosis of an apparent, new, endocrine entity.

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Author Disclosure Statement

The authors have nothing to declare.

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Table 1 Biochemical measurements in patients

	ALB **Geno-type**	**Total T4 nmol/L**	**FT4 pmol/L**	**FT3 pmol/L**	**TSH mU/L**	**CBG ug/ml**	**Total Cortisol (Immuno-assay) nmol/L**	**Total Cortisol (LCMS) nmol/L**	**Urine Free Cortisol nmol/L**	**Serum Free Cortisol nmol/L**
P1	**R218P**	>300 (69-141)	>80 (10-19.0)	10.9 (3.5-6.5)	3.9 (0.35-5.5)	14 (10-25)	1610 (145-619)	1920 (145-619)	342 (99-378)	16.7 (12.3-44.2)
P2	**R218P**	>300 (69-141)	>154 (10.5-21)	10.6 (3.5-6.5)	0.93 (0.35-5.5)	10 (10.5-16)	479 (95-462)	480 (95-462)	N/A	8.5 (11-38)

Reference ranges in brackets; N/A, not available

Figure 1A

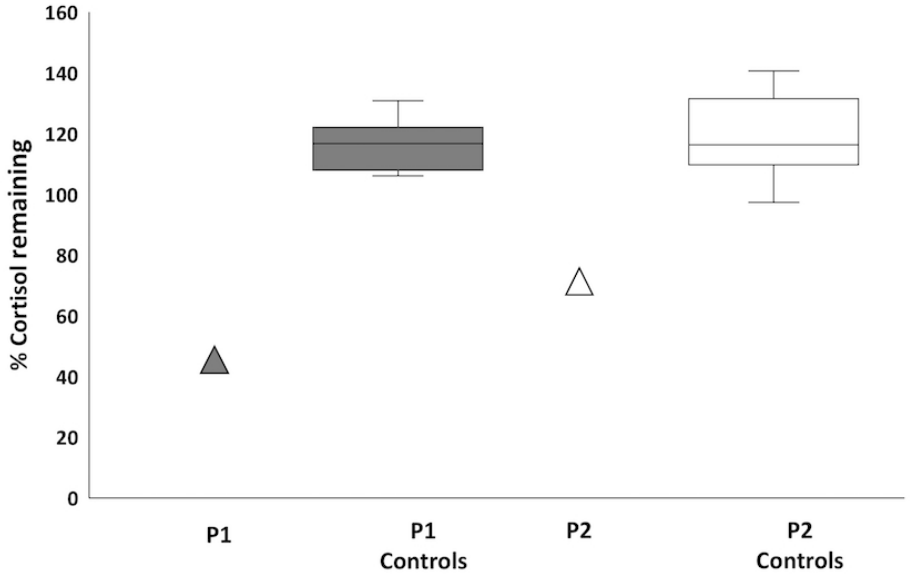

Figure 1A

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Figure 1B

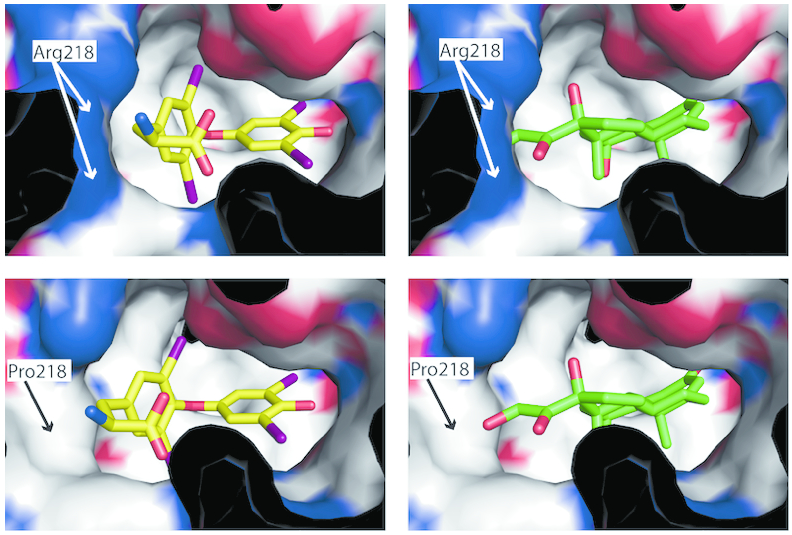

Figure 1B

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Figure 1A

Percentage of total cortisol remaining following immunodepletion of albumin from serum of individuals with R218P FDH (P1, P2) compared with that of age and gender matched healthy controls (n=10). Box indicates interquartile range, horizontal line within box indicates median, whiskers indicate range.

Figure 1B

Top left: Structure of thyroxine (T4) bound to site 1 of wild type albumin with the molecule positioned so that its polar ends are complementary with polar residues lining the binding pocket.

Bottom left: Substitution of Proline at position 218 reduces steric hindrance within the pocket, enabling T4 to be accommodated more easily whilst maintaining polar contacts.

Top right: Cortisol, a longer molecule, is less easily accommodated within the site 1 pocket of wild type albumin.

Bottom right: Substitution of Proline at position 218 increases the width of the site 1 pocket to accommodate cortisol easily.

SUPPLEMENTARY MATERIAL

METHODS

Biochemical measurements

Hormone measurements were undertaken using automated immunoassay systems (FT4, FT3, TSH, Cortisol:Advia Centaur, TBG:Immulite; Siemens, Germany; Total T4: AutoDelfia, Perkin Elmer, Belgium; 17-OHP Roche) or by liquid chromatography mass spectrometry (Cortisol, Cortisone, 11-Deoxycorticosterone). Serum corticosteroid binding globulin in patients (P1, P2) and controls (P1, 10 males age 18-23 yrs; P2, 10 females, age 57-65 yrs) was measured by ELISA (BioVendor).

Serum free cortisol was assayed by overnight dialysis of serum at 37^{o}C in buffer (1), from patients (P1, P2) and controls (P1, 10 males age 18-23 yrs; P2, 10 females, age 57-65 yrs), with measurement of cortisol in dialysate by liquid chromatography-tandem mass-spectrometry as described previously (2).

Cortisol was measured using liquid chromatography-tandem mass-spectrometry in serum samples from patients (P1, P2) and controls (P1, 10 males age 18-23 yrs; P2, 10 females, age 57-65 yrs) before and after depletion of albumin using antibody (PureProteome™ Albumin Magnetic Beads, Millipore, UK) as specified by the manufacturer. As the protocol involved significant dilution of samples, the results were adjusted for recovery of creatinine to correct for this.

Albumin gene sequencing

Exons of the human albumin gene were PCR amplified from genomic DNA using specific primers and analysed by Sanger sequencing as described previously (3).

Molecular modelling

Wild type and mutant albumins were modelled (Pymol) using previously described wildtype albumin (1bm0) albumin-T4 (1hk1), R218H FDH mutant albumin-T4 (1hk2), and R218P FDH mutant albumin-T4 (1hk3) crystal structures (4, 5), selecting the rotamer with the fewest clashes.

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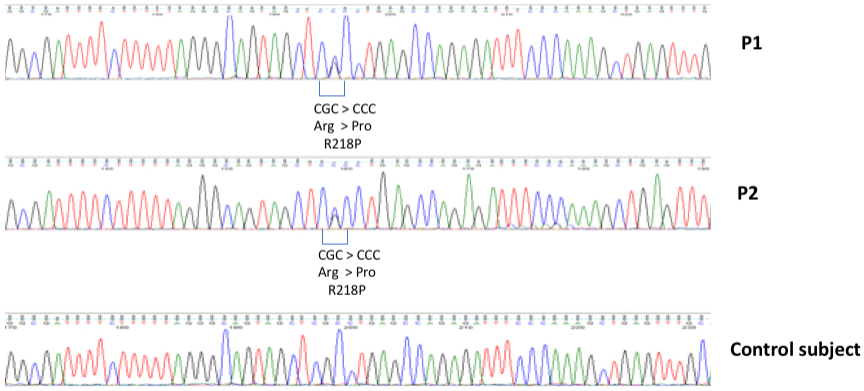
Supplementary Figure 1



Supplementary figure 1

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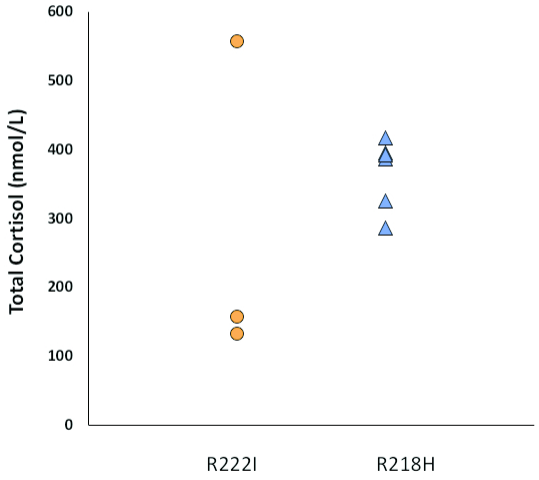
Supplementary Figure 2



Supplementary Figure 2

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