Homozygous Mutation in Human Serum Albumin and Its Implication on Thyroid Tests

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An individual with familial dysalbuminemic hyperthyroxinemia (FDH) due to a homozygous mutation (c.653G>A, p.R218H) in the *human serum albumin* (*HSA*) gene is reported. The patient was identified during evaluation of abnormal thyroid tests in a large family with multiple levels of consanguinity. He showed a greater increase in total thyroxine (T4) relative to that observed in heterozygous family members. The higher affinity of mutant HSA for T4, together with the large molar excess of HSA relative to thyroid hormones in serum, results in preferential association of T4 with the mutant rather than wild-type HSA in heterozygous individuals. The twofold greater amount of T4 bound to the mutant HSA in the homozygote, relative to heterozygotes, is an adaptive requirement to maintain a normal free T4 concentration.

Keywords: familial dysalbuminemic hyperthyroxinemia (FDH), thyroid hormone regulation, human serum albumin (*HSA*), thyroid hormone, albumin (*ALB*)

Introduction

FAMILIAL DYSALBUMINEMIC HYPERTHYROXINEMIA (FDH) is an autosomal dominant condition that is caused by heterozygous gain-of-function mutations in the *human serum albumin* (*HSA*) gene that result in mutant HSA with increased binding affinity for T4 (1–4). Herein, the first report of thyroid test abnormalities in an individual homozygous for mutant *HSA* in a large consanguineous family of Anatolian Turkish descent is given.

Patients

The homozygous individual was an uncle of the proband, a 23-year-old woman who presented to one of the authors (A.K.) for evaluation of hirsutism. She was otherwise asymptomatic. Thyroid tests showed a high free thyroxine (T4) of 2.82 ng/mL (reference range [RR] 0.58–1.25 ng/mL) and triiodothyronine (T3) of 5.36 pg/mL (RR 2.3–4.2 pg/mL), with a normal thyrotropin (TSH) of 2.1 mIU/L (RR 0.4–4.0 mIU/L), raising suspicion for resistance to thyroid hormone beta (RTH- β).

Results

Written consent was obtained in accordance with an Institutional Review Board–approved protocol. As shown in Figure 1A, 9/14 relatives tested had elevated serum total T4 (TT4) with normal free T4 index (FT4I) and total T3 (TT3),

except individual IV-2. These results, in the presence of non-suppressed TSH, suggested FDH. Four individuals (II-5, III-4, IV-2, and IV-5) had mildly elevated TSH consistent with subclinical hypothyroidism, and two (II-5 and IV-5) had positive thyroid autoantibodies. Individual IV-2, a 10-year-old boy with obesity, had FT4I and TT3 at 8% and 32% above the adult reference range, respectively (Fig. 1A), consistent with RTH- β . However, sequencing of exons 7–10 of *THRB* was negative for a mutation.

Sequencing of DNA from peripheral leukocytes revealed a mutation in the *HSA* gene (c.653G>A; Fig. 2A), resulting in substitution of the normal arginine-218 with histidine (p.R218H) in the nine hyperthyroxinemic family members. Interestingly, individual III-2, born to heterozygous parents, was homozygous for the R218H mutation (Fig. 2A). His TT4 was 37% and 161% above the mean TT4 of heterozygous and wild-type (WT) family members, respectively (Fig. 2B). Isoelectric focusing (IEF) of this individual's serum, visualized with radiolabeled T4, identified the mutant HSA protein (Fig. 1B, lane 2). Notably, labeled T4 bound the mutant protein to the exclusion of WT HSA present in sera of a heterozygous sibling (Fig. 1B, lane 3).

Non-denaturing polyacrylamide gel electrophoresis of sera from individuals of each genotype with radiolabeled T4 was performed, as described in the legend to Figure 2, to determine the proportion of T4 bound to the three T4-binding serum proteins (Fig. 1C). The effect of sample preparation on

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FIG. 2. (A) Representative chromatograms of sequences from family members WT (c.653G, p.R218), heterozygous (c.653G>A, p.R218H monoallelic substitution), and homozygous (c.653G>A, p.R218H biallelic substitution) for the HSA mutation. (B) Scatter and box plots of total T4 concentrations in WT, heterozygous, and homozygous individuals. Line represents the median, and whiskers represent the range, when applicable. (C) Quantification of relative T4 content in bands representing TBG, HSA, and TTR separated by non-denaturing PAGE shown in Figure 1C. The table below the graph shows the corresponding measured concentrations of T4, TBG, and HSA in sera from these individuals, and calculated quantity of T4 bound to HSA and TBG, adjusted for the serum concentrations of these proteins. PAGE was carried out as follows: 1 μ L containing 20,000 counts per minute (cpm; or 16 pg) of 125 I-labeled T4 (specific activity 1250 μ Ci/ μ g) was added to 10 μ L of serum from each individual and incubated for 30 min at room temperature. The added 125 I-T4 thus represented 0.6–2.2% the endogenous T4 measured in the serum samples. Following dilution in buffer (250 mM of Tris•Cl, pH 8.5), 6 μ L was loaded for separation by 8% non-denaturing PAGE at 60 V for 4 h at room temperature. The gel was subsequently exposed to film at -80°C with an intensifying screen for 1–4 days and scanned into an electronic format, and relative quantification of the bands was performed using the publically available NIH ImageJ software.

is the most common of five HSA variants causing FDH (1). Substitution of R218 for a smaller residue electrostatically stabilizes the T4-binding site and thereby promotes increased affinity for T4 (6). Individuals with *R218H* have elevated serum TT4 and, to a lesser degree, reverse T3 due to increased mutant HSA binding affinity for these iodothyronines, but normal dialyzable free T4 and a non-suppressed TSH. Mutant HSA can interfere with indirect methods of measuring free T4 and T3, producing falsely elevated values (7), leading to inappropriate treatment and explaining the elevated free T4 and T3 levels in the proband at presentation.

To the authors' knowledge, this is the first report of a biallelic *HSA* mutation causing FDH. As HSA is present in

6000-fold molar excess relative to T4, T4 binds exclusively to mutant HSA, as shown by IEF, despite representing only half the total HSA in heterozygotes. Quantification of the T4 bound to HSA showed that the homozygote had a two-fold increase in T4 binding per gram of HSA compared with heterozygotes (Fig. 2C), including subject IV-2 (1.3 μ g T4/g HSA). Thus, biallelic expression of the mutant HSA requires twice the amount of bound T4 to maintain a normal free T4.

Acknowledgments

This study was supported in part by grants from the National Institutes of Health, R37DK15070 to S.R. and R01DK110322

FIG. 3. Radioautographs and quantification of relative T4 associated with TBG, HSA, and TTR separated by non-denaturing PAGE in serum from (A) a WT individual with different amounts of added 125 I-labeled T4 and (B) serially diluted sera from a WT individual or an individual homozygous for the HSA mutation. (A) Sera are from aliquots containing 20,000 cpm (lane 1), 10,000 cpm (lane 2), or 5000 cpm (lane 3) of 125 I-labeled T4. (B) Sera was undiluted (lanes 1 and 4), diluted 1:2 (lanes 2 and 5), or diluted 1:4 (lanes 3 and 6), and 10,000 cpm (or 8 pg) of 125 I-labeled T4 was added to each sample. The relative amounts of T4 added with the 125 I-labeled T4 were 1.1–4.4% of the endogenous T4 for the WT and 0.3–1.2% for the mutant.

to A.M.D. M.S.M. was supported by a Ruth L. Kirschstein National Research Service Award for Individual Postdoctoral Fellows T32DK007011. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Diabetes and Digestive and Kidney Diseases or the National Institutes of Health. The authors wish to thank Quest Diagnostics for performing serum quantification of TBG and HSA. Thanks are due to Miles Tracy for the determination of thyroid tests and to Prof. Roy E. Weiss for useful discussion. A.K. acknowledges Dr. Isilay Taskaldiran for the referral of the proband and her mentor Prof. Dr. Nese Ersoz Gulcelik for advice.

Author Disclosure Statement

M.S.M. declares that her spouse receives salary and stock from AbbVie, Inc. The authors declare no competing financial interests exist.

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