CBG Santiago: A Novel CBG Mutation

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Context: Corticosteroid-binding globulin (CBG; *SERPIN A6*) gene mutations are rare; only four mutations have been described, often in association with fatigue and chronic pain, albeit with incomplete penetrance.

Patient: We report a kindred with a novel *SERPINA6* mutation. The proband, a 9-yr-old male, had excessive postexertional fatigue, weakness, and migraine.

Main Outcome Measures and Results: Investigations revealed low morning and ACTH-stimulated peak cortisol levels. *SERPIN A6* sequencing detected a novel exon 2 single base deletion (c.13delC) leading to a frameshift generating a stop codon within the signal peptide coding region (p.Leu5CysfsX26) and 50% reduced CBG levels in heterozygotes. The patient's father and two sisters share the mutation. Symptom expression within the family may have been modified by a polymorphic CBG allele (c.735G>T). Exogenous hydrocortisone had no effect on the fatigue.

Conclusion: This report documents the fifth CBG gene mutation in humans and the second causing major effects on CBG levels. Individuals with low CBG levels may be misdiagnosed as having secondary hypocortisolism. The association with fatigue and idiopathic pain is again noted and may relate to altered stress system function. Variability of the phenotype may relate to other genetic variations of the CBG gene or environmental factors. **(*J Clin Endocrinol Metab* 97: E151–E155, 2012)**

Corticosteroid binding globulin (CBG) is the 50- to 60-kDa high- affinity plasma transport glycoprotein for cortisol (1). CBG is secreted after cleavage of a 22-amino acid signal peptide and circulates at concentrations of 450–650 nmol/liter (1). The 19-kb five exon (four coding) gene *SERPINA6* (MIM no. 122500) is located at 14q32.1, among several contiguous highly homologous genes (2). Circulating CBG levels are increased by estrogen and pregnancy and decreased by insulin or glucocorticoids (1). In the nonstressed state, 80% of cortisol is bound to CBG, 10–15% is loosely bound to albumin, and 5–8% is unbound or free (1, 3). CBG levels fall markedly with sepsis or severe stress, amplifying rises in free cortisol due to cleavage of CBG at inflammatory sites (2) and inhibition of synthesis by inflammatory cytokines (4–7).

CBG may have other roles in stress system regulation and the risk of development of fatigue/pain disorders, conditions associated with relatively low cortisol levels (8, 9). Human studies have shown a relation between specific CBG haplotypes and chronic widespread pain

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Abbreviations: CBG, Corticosteroid-binding globulin; SDS, SD score; SNP, single nucleotide polymorphism.

TABLE 1. Old and new nomenclature for known mutations and polymorphisms in *CBG*

Mutations and polymorphisms	Old nomenclature coding DNA	New nomenclature coding DNA	Old nomenclature protein	New nomenclature protein
Leuven	T433A	c.344T>A	Leu93His	p.Leu115His
Lyon	G1254A	c.1165G>A	Asp367Asn	p.Asp389Asn
Null	G121A	c.32G>A	Trp-12X	p.Trp11X
Non-cortisol binding		c.776G>T	p.Gly237Val	p.Gly259Val
Santiago		c.13delC		p.Leu5CysfsX26
p.Ala246Ser polymorphism	c.825G>T	c.736G>T	Ala-Ser224	p.Ala246Ser

(10). Animal models of genetic CBG deficiency show altered activity, immune responses, and behavioral responses to stress (11, 12). A study of chronic fatigue syndrome patients showed a trend toward increased prevalence of a common CBG gene polymorphism (c.736G>T, AL-Ser224), with increased plasma CBG and lower plasma cortisol levels (13).

Four major function-altering mutations of the CBG gene have been described in humans. These include CBG Leuven, CBG Lyon, CBG Null/Adelaide, and a CBG non-cortisol binding variant. CBG Leuven (c.344T>A, p.Leu115His) reduces CBG:cortisol binding 3-fold (14). CBG Lyon has been described in three kindreds and reduces cortisol binding affinity 4-fold (c.1165G>A, p.Asp389Asn) (15–17). CBG Adelaide (c.32G>A, p.Trp11X) prevents CBG synthesis, and homozygotes are completely CBG deficient (17). Previously used and new nomenclature for the mutations according to den Dunnen *et al.* (18) are summarized in Table 1. Both CBG Lyon and Null are associated with fatigue and chronic pain and were described together in a single kindred where the phenotype was similar (17). The description of a kindred with a non-cortisol binding variant of CBG included an index case with fatigue (19).

This report summarizes the presentation of a novel CBG variant, the second to have a profound effect on circulating CBG levels, where the proband presented with fatigue and headaches, but clinical features were absent in other family members, although only the proband had the Ser224 allele.

Subjects and Methods

Case report

A 9-yr and 2-month-old boy of Spanish descent in Santiago, Chile, was referred for long-standing relatively short stature. His height was 129.7 cm [−0.79 SD score (SDS)], and weight was 27.3 kg (body mass index, 0 SDS). Maternal height was 164 cm, and paternal height was 185 cm. General examination was normal with no dysmorphic features, genitalia were prepubertal, and blood pressure was 106/59 mm Hg. Pregnancy and delivery were uneventful, and birth dimensions at 38 wk gestation included weight of 2930 g and length of 50 cm. Developmental milestones were normal. The patient had been investigated for headaches with a magnetic resonance imaging scan that was normal. Nonclassical migraine had been diagnosed, and he had been prescribed a nonsteroidal antiinflammatory for acute headaches. The patient's mother reported that he became excessively tired after playing sport. He seemed to have a reduced appetite. Despite fatigue, he participated in school sport several times a week. He performed well in his school classes.

Wrist x-ray revealed a delayed bone age relative to chronological age at 7 yr. Measurements of renal, liver, kidney, and thyroid function; lipids as well as complete blood count; IGF-I; IGF binding protein-3; prolactin; LH; FSH; and dehydroepiandrosterone sulfate were normal. In view of fatigue, a morning cortisol was obtained and was 92 nmol/liter (range, 300–650 nmol/liter). A 250-μg ACTH (1-24) stimulation test revealed a baseline cortisol of 108 rising to 378 nmol/liter (normal peak, >550 nmol/liter). Baseline ACTH level was normal at 17 pg/ml, and plasma renin activity was normal at 3.2 ng/ml·h. A repeat ACTH stimulation test showed a baseline cortisol of 100 rising to 405 nmol/liter at 30 min. The patient was treated with hydrocortisone 12 mg/m^2 body surface area for 3 months. There was no increase in energy levels or appetite, so that hydrocortisone was suspended.

The patient continued to grow in the same percentile, but his mother insisted he had a decreased level of energy compared with his two older siblings. By 12 yr and 2 months of age, an increase in testicular size to 6 ml was noted, height was 145.6 cm (−0.64 SDS), weight was 32 kg, and body mass index was in the fifth percentile. At this time, CBG deficiency was suspected, and a urinary free cortisol was requested, which was within the normal range (189 nmol/d). At 15 yr and 10 months, he reached a height of 171 cm (−0.28 SDS) and weight of 55 kg (24th percentile). The patient's father had a history of pubertal delay and migraine. Pubertal development had been apparently normal in the patient's mother and sisters. His mother was taking T_4 for Hashimoto's thyroiditis. His older sister at age 20 was well, her height was 171 cm, weight was 57.5 kg, and menarche was at age 13; she was taking an oral contraceptive. The younger sister at age 18 was also well, had a height of 164 cm and weight of 54 kg, menarche was at age 13, and she was not taking regular medications.

DNA sequencing

Using the genomic DNA sequence of *SERPINA6* from the University of California, Santa Cruz Genome browser (Human GRCh37/hg19 assembly chromosome position14q32, 94768948-9478386), PCR primers were designed to amplify the five exons/splice sites using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) from extracted blood leukocyte DNA. PCR used standard protocols and AmpliTaq Gold

(Roche, Indianapolis, IN). To ensure both entire *SERPINA6* alleles had been analyzed, exons 1 to 3 and 3 to 5 were amplified by the Expand Long template PCR system (Roche) and nested primers. Heterozygosity was confirmed by sequencing of long-PCR products and analysis of known intronic single nucleotide polymorphisms (SNP) throughout the gene. Purified PCR products were sequenced by Sanger capillary sequencing and analyzed with Mutation Surveyor (SoftGenetics, State College, PA) with RefSeq NM_001756.3 as the reference sequence.

CBG immunoassay

Plasma CBG was measured using an in-house method, a two-site noncompetitive monoclonal antibody-based ELISA, as previously described (4).

Results

The pedigree, individual plasma CBG levels, and DNA sequencing data are shown in Fig. 1. Sequencing in the proband revealed a single base deletion, c.13delC, within exon 2 inherited from the father and a substitution in exon 3 (c.736G>T) inherited from the mother. The exon 2 deletion (c.13delC) should produce a frameshift at position 5 in the coding sequence and a premature stop codon at amino acid 26 (p.Leu5CysfsX26). Exon 2 encodes the signal peptide of the CBG molecule, hence no mature CBG is expected. The proband, his sisters, and his father were all heterozygous for this mutation. Plasma CBG levels were reduced approximately 50% by the mutation (168–280 nmol/liter; reference range, 450–650 nmol/liter), as expected from the mutation, except in one individual (II.1) who was taking an estrogen-containing oral contraceptive preparation that elevated CBG levels to the normal range. The SNP (c.736G>T) detected in exon 3 has been identified in the SNP database as rs2228541:G>T. The mother and proband were heterozygous for this SNP, whereas the other family members were homozygous for G at this position. This SNP leads to an amino acid change p.Ala246Ser and has previously been associated with chronic fatigue syndrome, higher plasma CBG, and lower cortisol levels (13). The symptomatic proband had a serine allele of this polymorphism, but the nonsymptomatic father and siblings with the CBG Santiago mutation had the Ala allele.

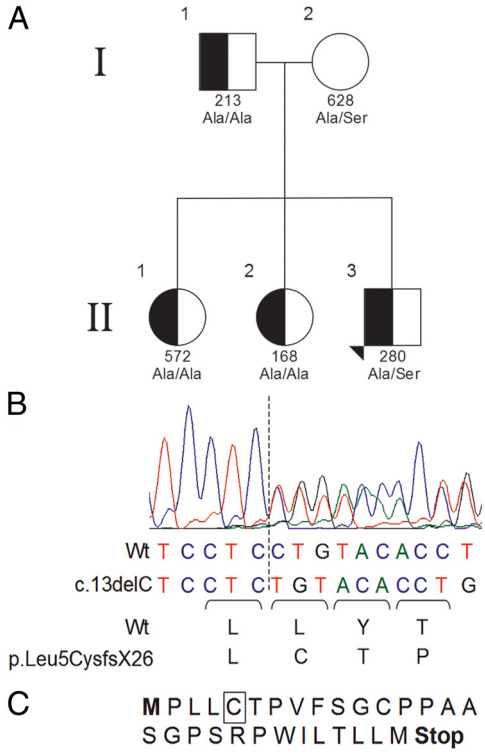

FIG. 1. A, The CBG Santiago kindred. The father and all children are heterozygous for the Santiago mutation. Plasma levels of CBG in nanomoles per liter are indicated by the *numbers under the symbol* for each individual. The genotype for the p.Ala246Ser polymorphism is shown under the symbol for each individual. The proband is marked by an *arrow*. B, Gene analysis of exon 2 of *CBG* in the proband identified a heterozygous mutation. The deletion of a C on one of the alleles at c.13 is indicated by the *dotted line*. The wt sequence is RefSeq NM_001756.3. The deletion leads to a frameshift at position 5 in the protein as indicated for p.Leu5CysfsX26. The wt sequence is NP_001747.2. C, The predicted sequence for the protein with the Santiago mutation. The first amino acid change resulting from the frameshift is marked by a *square*, the following amino acids all differ from the original protein, and the mutated protein is terminated at position 26 after the frameshift.

Discussion

We report the fifth discovered CBG gene mutation "CBG Santiago" in humans, a point mutation leading to a frameshift and CBG nonsynthesis, with a 50% reduction in plasma CBG levels in heterozygotes. The importance of this observation is 2-fold. First, using standard endocrine procedures for evaluation of suspected hypoadrenalism, serum cortisol levels are reduced and ACTH levels are normal, as are renin-aldosterone levels, so that a misdiagnosis of isolated secondary hypocortisolism is possible in patients with CBG mutations that alter CBG levels or CBG:cortisol binding. Second, the data contribute to a body of knowledge suggesting a subtle phenotype involving fatigue and/or unexplained pain, a finding that may have implications for both the role of CBG and for etiology of common idiopathic fatigue/pain syndromes.

The proband's presentation was marked by a persistent complaint of physical fatigue, especially after exercise, although his father did not complain of fatigue. His sisters, both of whom were also heterozygotes, had no fatigue or pain. Developmentally, there were no apparent differences between the individuals to account for the phenotypic differences. Interestingly, the symptomatic proband was heterozygous for the serine allele of the c736G>T

polymorphism, an allele previously associated with chronic fatigue syndrome, whereas his asymptomatic father and siblings had the Ala allele. Hence, the serine allele may act as a "hypermorph" increasing the clinical expression of the CBG Santiago mutation. However, it must be acknowledged that the association between the serine allele and chronic fatigue syndrome was weak, and there is no direct known mechanism for an effect of the serine allele or other coinherited genetic variation of CBG on the fatigue phenotype.

These findings reflect those in previous reports of fatigue and pain in probands and members of families with CBG mutations such as CBG Lyon (three kindreds), CBG Adelaide (one kindred), and CBG nonbinding (one kindred). In all cases, the proband and in some cases other family members had fatigue and/or pain, although the findings were not universal among those with the mutation. The largest family reported, which segregated for both Adelaide and Lyon mutations, showed many individuals with fatigue and/or chronic pain symptoms from both mutations whether in heterozygous (both mutations), homozygous (null), or compound heterozygotes (17). It is common for individuals with single gene mutations to express phenotypic variation, even within kindreds, due to the influence of other genes or the prenatal or postnatal environment acting via epigenetic or other mechanisms.

We recently attempted to control for the potential for ascertainment bias in descriptions of the effects of CBG mutations, given that probands are often discovered after investigations prompted by unexplained fatigue. We completed a blinded study of an isolated population with an increased frequency of CBG Adelaide and Lyon mutations (3.6%) and found an association between the CBG mutations and chronic pain rather than fatigue, relative to controls, suggesting an environmental effect on the phenotypic expression of CBG mutations (20).

Many studies have associated low cortisol secretion with a range of common clinical syndromes associated with unexplained chronic fatigue and/or pain with either diffuse (chronic fatigue syndrome, fibromyalgia) or localized (migraine, irritable bowel syndrome) descriptions (8, 9). Exogenous glucocorticoids are generally ineffective, and the pathogenesis of the hypothalamic-pituitary-adrenal axis defect is unknown, although a CRH neuron defect is postulated (9). Recently, a study identified two CBG haplotypes, established on SNP typing, as conferring the risk of developing chronic widespread pain, but no association with six other genes integral to hypothalamic-pituitary-adrenal axis function (10). In addition, one murine model of genetic CBG deficiency revealed reduced activity levels, perhaps a corollary to fatigue (11). A second murine model revealed a depression-like neurobehavioral abnormality in response to severe stress (footshock) described as learned helplessness (12). These studies together suggest a hitherto unrecognized role for CBG in antinociceptive function and stress-induced behavior, perhaps at the central nervous system level.

In summary, there are four CBG mutations (CBG Adelaide-Null, CBG Lyon, CBG non-cortisol binding, now CBG Santiago) that have been associated with fatigue and/or chronic pain in kindreds and populations. Perhaps variable expression is a result of coinherited polymorphisms such as the CBG Ser/Ala polymorphism. These clinical observations and recent work in murine models suggest a role for CBG beyond cortisol transport, involving a stress system function.

Acknowledgments

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