In the current issue of the JCEM, Torpy et al. (1) describe the case of a 9-yr-old boy from Chile with recurring, severe headaches and postexertion fatigue. The boy’s complaints did not prevent him from participating in regular school activities, which is a hint for a seasoned pediatrician to look for true pathology (sick kids often want to attend school, whereas children with psychosomatic complaints display school avoidance) (2). The boy’s mother and his two older sisters were healthy, whereas the father had a history of migraine headaches. Parental concerns about his growth and stature triggered a referral to a pediatric endocrinologist (V. Mericq; Ref. 1), who assured the patient and his family that he would achieve normal adult height based on her general assessment and, more specifically, based on his delayed bone age in combination with his father’s history of constitutional delay of growth and development. However, the previous diagnosis of migraines (a head magnetic resonance imaging had been normal) and the complaint of fatigue raised the physician’s suspicion to go beyond a basic work-up; a low plasma morning cortisol and inappropriately normal plasma ACTH levels were followed up with an ACTH stimulation test, which showed an insufficient plasma cortisol response. Thus, the overall picture was consistent with secondary adrenal insufficiency. Lack of clinical response to steroid replacement and continued parental worries led to another consultation and consideration of an exceedingly rare condition. The latter brought Mericq and Torpy (now on opposite sides of the world) together again, both having received further training at the National Institutes of Health Clinical Research Center in Bethesda, Maryland, where rare conditions are common and common conditions are rare. In collaboration with their colleagues, they identified a single base mutation in the gene encoding corticosteroid binding globulin (CBG). This mutation in exon 2, which they named “Santiago,” caused a frameshift and premature stop codon at amino acid 26. As expected, CBG plasma levels were approximately 50% of normal. The father and both daughters were heterozygous for the same mutation; however, this boy’s other allele was affected, too. He and his asymptomatic mother shared an amino acid substitution (Ala246Ser) reported to be possibly associated with chronic fatigue and somewhat elevated CBG levels (3).

Could the frameshift on one allele and/or the single amino acid change on the other allele account for the clinical symptoms? Given that the two male family members (father and son) with one copy of the newly discovered Santiago mutation had a history of severe headaches, whereas the two female heterozygotes (daughters) were asymptomatic, this mutation may in fact result in a clinical phenotype only in males. More likely, considering the frequency of migraine headaches in the general adult population and its heritability, the two phenomena may be entirely unrelated. However, due to the uncommon combination of migraine headaches and postexertion fatigue in children, the compound heterozygosity of the boy may have been responsible for the symptoms. Of note, the correlation between plasma levels of CBG and clinical symptoms is weak; low CBG levels are observed in asymptomatic patients, and conversely somewhat elevated levels can occur in symptomatic patients (4). Also, symptoms associated with CBG mutations typically cannot be explained by low free plasma cortisol concentrations. Instead, most studies show cortisol measurements in the normal range when determined as free hormone levels in plasma, urine, or saliva.

Abbreviations: CBG, Corticosteroid binding globulin; HPA, hypothalamic-pituitary-adrenal.
CBG Mutations in Mice and Men

In 2006, Petersen et al. (5) reported tissue hypersensitivity to glucocorticoids in the setting of elevated free plasma corticosterone levels in genetically engineered CBG-deficient mice. The animals had high ACTH levels and a reduced expression of known glucocorticoid target genes in the liver (e.g., phosphoenolpyruvate carboxykinase). When exposed to an ip injection of the bacterial endotoxin lipopolysaccharide, they showed an ineffective stress response and increased mortality. Overall, physical activity of these CBG−/− mice was reduced. No abnormalities were observed in glucose metabolism. In a second mouse model, Richard et al. (6) observed normal free corticosterone concentrations at rest but a reduced surge after stress, similar to Petersen’s results. The animals also exhibited altered behavioral responses, e.g., increased immobility in the forced-swimming test and markedly enhanced learned helplessness, which are considered reliable animal models of chronic stress and depression.

In humans, only a few CBG mutations have been identified so far (4). The report of Torpy et al. (1) in this issue of the JCEM contains the fourth heritable CBG variant leading to reduced plasma cortisol binding affinity. Interestingly, two of the three previously identified variants [Leuven (1982) and Null (2001)] are also due to single amino acid changes in exon 2, similar to the Santiago point mutation (7, 8). Both Santiago and Null mutations result in premature stop codons. The fourth one, Lyon (2000), is due to a point mutation in exon 5 (9). Both Lyon and Leuven mutations result in amino acid substitutions associated with 3- to 4-fold reduced CBG-cortisol binding affinities, respectively. The clinical consequences of these mutations are not straightforward, and various factors may account for this variability. Heterozygous individuals with the Lyon or Null mutations may have one or more complaints reminiscent of adrenal insufficiency: chronic fatigue and/or chronic pain, depression, and hypotension. Furthermore, family size was unexpectedly small in a group of affected individuals from Calabria, which may raise the question of increased pregnancy loss and/or decreased fertility (10). Because free cortisol levels are normal in these individuals, it is not surprising that steroid treatment typically does not relieve symptoms.

CBG: Established Physiological Functions

Cortisol transport is considered the main function of CBG (Table 1). Under normal, nonstressed conditions, 80–90% of circulating cortisol is bound to CBG, 10–15% is bound to albumin, and the remaining 5–10% circulate as free and active hormone. Before 1959, cortisol binding capacity was thought to be entirely attributable to albumin. With the advent of radioactive immunoassays came the identification of a protein—"transcortin" as it was then called—that was the functional mirror image of albumin, i.e., low in capacity but high in affinity for corticosteroids (11). In addition to cortisol, CBG binds progesterone, aldosterone, and 11-deoxycorticosterone. It is mostly produced by the liver, up-regulated by estrogens, and suppressed by steroids. Clinically, high levels are observed in pregnancy and low levels in patients with cirrhosis.

Hormones are powerful “stuff” to be handled with care; their appropriate availability and delivery are therefore exquisitely regulated. Some are stored within the hormone-producing cells (e.g., insulin in pancreatic β-cells) ready for immediate secretion and promptly replenished by up-regulated transcription and translation. Others are bound to specific transport proteins that provide a reservoir from which hormones can be released. Examples are CBG, SHBG, IGF binding protein 3, and thyroglobulin, which is structurally similar to CBG. Thyroglobulin carries T₄, which is a prohormone rather than an active hormone different from cortisol. In the case of thyroid hormones, another layer of control consists in the local conversion of T₄ to the biologically active T₃ by various tissue-specific deiodinases. For cortisol and the hypothalamic-pituitary-adrenal (HPA) axis, evolution seems to have scouted a slightly different path. Although cortisol is promptly synthesized (but not stored in significant amounts by the adrenal glands) and secreted into the circulation, CBG binds cortisol in a one-to-one ratio and regulates its delivery to target tissues and inflammatory sites. In fact, cortisol release from CBG is temperature-sensitive within the physiological range of body temperatures (12). Therefore, during hyperthermia, more cortisol is made available.

### TABLE 1. Physiological functions of CBG

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<th>Established functions</th>
<th>Emerging functions and research questions</th>
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<tr>
<td>Transport protein for cortisol, 11-deoxycorticosterone, progesterone, aldosterone (11)</td>
<td>Regulation of body composition (15, 16) and insulin sensitivity (17)</td>
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<tr>
<td>Modulation of chronic pain and fatigue (due to unknown mechanism(s), (10)</td>
<td>Control of intestinal sodium absorption (in rodents) (13)</td>
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CBG—Emerging Functions

Several novel functions for CBG are emerging. CBG may control intestinal sodium (Na⁺) absorption via an aldosterone-dependent mechanism (13). When Na⁺ is restricted in the diet, CBG-deficient mice are unable to reduce fecal Na⁺ excretion. This observation unravels another potential function of CBG and, if confirmed in humans, may contribute to the hypotension and fatigue observed in subjects carrying CBG mutations.

As recently reviewed (4), several clinical studies have reported an inverse association between CBG plasma levels and body mass index, waist-hip ratio, and indices of insulin sensitivity. This is still controversial and may depend on ethnic background. In a population study of 495 adults, we did not find any relationship between CBG levels and blood pressure, waist circumference, and body mass index (10).

The most interesting and least understood role of CBG is related to chronic pain and fatigue, which are the most striking clinical features of subjects carrying CBG mutations, including Torpy’s proband. Support for a direct effect of CBG in the brain stems from neuroanatomical observations. As elegantly reviewed by Henley and Lightman (14), CBG-positive fibers are found in neuronal structures involved in the regulation of pain, such as the periaqueductal gray area, the medulla oblongata, and the dorsal horn of the spinal cord. In addition, CBG immunoreactivity is found in the anterior pituitary, the median eminence, and the magnocellular nucleus of the hypothalamus, where it is coexpressed with oxytocin and vasopressin. Of interest, these neuroendocrine structures are also involved in the central regulation of the stress response and the HPA axis.

Further Unanswered Research Questions

In the realm of basic research, phylogenetic studies of the evolutionary development of CBG would be informative to determine when CBG was co-opted to become a cortisol carrier. We know that the CBG molecule is a member of the clade A serine proteinase inhibitor (serpin) family and shares substantial molecular similarities with α-1 antitrypsin, an important player in the acute phase reaction. Whether CBG has a direct role in the inflammatory response and whether this and other roles are mediated via a specific CBG receptor or in a receptor-independent manner remains to be determined.

From the clinical viewpoint, in-depth characterization of individuals carrying CBG mutations are needed, together with focused use of animal models. We suggest uniting our efforts on a large scale: individuals with known CBG mutations from all over the world should become a cohort participating in prospective, centralized clinical studies using standardized methods to assess them at basal and under stress conditions. This characterization should include circadian rhythmicity and sleep architecture. Experimental paradigms of stress should address the ability to withstand physical, psychological, and nutritional stress. Unaffected siblings could function as appropriate controls coming from a similar environmental and genetic background. Resiliency, the ability to successfully adapt to adversity, displays large individual variability that is likely related to the function of the HPA axis. Understanding the mechanisms that make some individuals resilient and others susceptible to the same amount of stress is of great importance. Further characterization of the function of CBG in the stress response may contribute to that understanding.


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