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Corticosteroid-Binding Globulin Gene Mutations and Chronic Fatigue/Pain Syndromes: An Overview of Current Evidence

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1. Introduction

Several lines of evidence suggest that corticosteroid-binding globulin (CBG), long known as a cortisol-transport glycoprotein, may have broader roles in targeted-tissue hormone delivery and the neurobehavioural responses to stress. These include studies of individual kindreds with rare severe CBG gene (*SERPINA6)* mutations, a study of chronic fatigue patients, a community study of individuals with a relatively high prevalence of two function altering CBG gene mutations in Calabria, Italy, a study of the genetic epidemiology of chronic pain, and, finally, two separate animal CBG gene knockout models.

2. Corticosteroid-binding globulin: Structure and function

CBG circulates as a 383 amino acid (50-55kDa) glycoprotein in blood, and was discovered in the late 1950s[1-6] as a transport glycoprotein for cortisol in human plasma[7,8]. The liver is the main source of circulating CBG, although gene expression is also present in the placenta and kidney, and CBG is differentially expressed according to developmental stage in foetal life[9,10]. CBG is highly glycosylated with six consensus sites for N-glycosylation and sialyation[11]. Each molecule contains a single high-affinity (Ka = 1.7x 10^8) cortisol binding site[12,13], for which glycosylation at Asn[238] appears to be critical, probably due to the effect of this glycosylation site on tertiary structure[14]. Deglycosylation of the mature protein does not alter cortisol binding affinity. CBG is a Clade A member of the serine protease inhibitor (serpin) superfamily, however it lacks intrinsic serine protease inhibitory activity[15,16]. The CBG (SERPINA6) gene is located in a group of other serine protease inhibitor genes, thought to be phylogenetically related, on chromosome 14q31 - q32.1[17].

Approximately 80% of circulating cortisol under basal conditions is bound to CBG. About 5-8% of the cortisol is in a free or an unfractionated state, which is generally thought to be the biologically active form, and the remainder is loosely bound to high capacity albumin[18]. CBG, as part of its biological function, undergoes a so called 'stressed to relaxed' (S→R) conformational change following the cleavage of its surface-exposed loop called the reactive centre loop or RCL[18]. However, the mode of cleavage in CBG differs from other members of

the serpin superfamily[19]. The RCL of CBG is cleaved by human leukocyte elastase (HLE) at sites of inflammation[10,20] rather than by inhibiting proteinases[18]. The HLE cleavage of CBG results in a ten-fold decrease in its binding affinity[21], thus releasing cortisol[10]. In states of stress such as sepsis[22], burns[23] and myocardial infarction[24], the free cortisol percentage increases to up to 20%, due to the saturation of available CBG by increased cortisol and reduced CBG levels (a result of increased CBG cleavage/catabolism and inhibited synthesis)[25,26]. Inflammatory cytokines such as IL-6, glucocorticoids, insulin, hyperthyroidism, nephrotic syndrome, and cirrhosis can also reduce CBG concentrations. On the other hand, oestrogen and pregnancy can increase CBG concentrations[10,27]. It is interesting to note, in this context, that increased production of HLE by neutrophils has been reported in chronic fatigue syndrome[20,28].

3. Corticosteroid-binding globulin: More than just a transport glycoprotein

CBG has traditionally been considered to be a transport vehicle for the water insoluble cortisol[29], with perhaps some role in moderating release of free cortisol in times of cortisol excess or deficiency[30]. This is in keeping with the 'free hormone hypothesis' proposed by Mendel[31], which states that the biological activity of a hormone depends on the free rather than its protein-bound concentrations. The free steroid hormone can cross the plasma membrane of the target cell due to its small size and lipid solubility[32].

However, there is evidence that suggests that CBG-bound cortisol could play a functional role different to unbound cortisol. While a specific CBG cell receptor has not yet been cloned, cell membrane binding sites for CBG, which share many features of a receptor, have been known for some time[33,34]. This has led some to speculate that CBG may act as a hormone and there may be a direct contribution of bound cortisol in glucocorticoid bioavailability via this yet unidentified CBG receptor[34]. Accumulation of cyclic AMP[35] occurs as a result of this CBG:cell receptor interaction. Recently, it has been shown that the NeuAc residues on the N-glycans restrict the binding of CBG to the cell receptor. Removing these NeuAc residues resulted in marked increase in cyclic AMP levels[35]. Dilution of CBG results in release of cortisol and thus suggests, at the very least, an indirect contribution of bound cortisol in glucocorticoid bioavailability[36].

A closely related steroid binding glycoprotein in the human body is sex hormone binding globulin (SHBG), which binds testosterone and oestradiol. A role for SHBG beyond transport has been shown. Sex steroid tissue delivery involves endocytic uptake of SHBG–sex steroid complexes via the LDL receptor–related protein member megalin[37]. Megalin knockout mice exhibit sexual infantilism[37]. While possible a megalin-like mechanism for endocytic uptake of CBG-cortisol complexes has not been demonstrated.

4. Chronic fatigue/pain syndromes, the concept of 'allostasis' and the role of hypothalamo-pituitary-adrenal axis

Chronic fatigue/pain syndromes are common. Epidemiological studies have estimated the point prevalence of chronic widespread pain (CWP) in the community to be up to 11%[38], and about 9% of the total population will experience significant chronic fatigue at any one time[39]. It should be noted, however, that up to two-thirds of these patients complaining of chronic fatigue will not meet the criteria for chronic fatigue syndrome (CFS)[40,41]. A

considerable overlap exists, however, in terms of both clinical and biochemical characteristics, and perhaps the pathogenesis of chronic fatigue and chronic pain[42].

CFS is a clinical diagnosis, the hallmark of which is disabling fatigue for over six months with prolonged (>24hrs) post-exertional exacerbation along with other symptoms which include impaired short-term memory and concentration, sore throat, tender lymph nodes, myalgia, arthralgia, headaches and unrefreshing sleep[43]. Since the term 'chronic fatigue syndrome' was proposed in 1988 to replace the prior 'chronic Epstein Barr virus syndrome' (based on the realization that not all chronic fatigue cases were post-infective in nature)[44], newer aetiological models based on neuroendocrine[45], psychiatric[46], evolutionary[47], immunological[48] and non–infective inflammatory[49] mechanisms have been described. A well-accepted explanation for the development of CFS or even the relative contribution of the different possible mechanisms, however, remains elusive. CFS, a debilitating disease sharing many features with fibromyalgia[50], CWP and similar idiopathic chronic fatigue syndromes, significantly impairs a patient's quality of life[51,52], social[53] and emotional well being[54,55], besides putting considerable economic strain on the community[56]. It is, therefore, imperative that a better understanding of the causation of CFS and related disorders is achieved to enable development of effective therapeutic options, which are currently lacking[57].

A hereditary component to CFS has also been suggested[58-60]. Recently, an analysis of the Utah population database (UPDB) looking at the genetic relationships of CFS patients was published[61]. 811 patients diagnosed with CFS by the US CDC or the Fukuda criteria[43] underwent genealogical analysis. A significant excess in CFS relative risk among first (2.70, 95% CI: 1.56-4.66), second (2.34, 95% CI: 1.31-4.19), and third degree relatives (1.93, 95% CI: 1.21-3.07) was observed.

The human stress system includes the two effector arms, the hypothalamic-pituitary-adrenal axis (HPA axis) and the sympathetic nervous system, with their chief hormonal products, cortisol and noradrenaline/adrenaline, respectively. The stress system responds in a highly coordinated and stress-specific manner to stressors, which may be defined as threats to homeostasis or the stable internal milieu of the organism. Stressors such as infection, inflammation, trauma, and psychic disturbance such as fear or anxiety act via inflammatory cytokines or internal CNS influences to produce a range of physiological responses designed to protect the body from stress, such as elevated blood pressure, redirection of blood flow, mobilization of metabolic substrates and CNS arousal. The stress system has basal tone and it has been proposed that altered chronic stress system activity, which may be produced by excessive stress at key developmental stages such as intrauterine, childhood and adolescence, may be detrimental to health. For example, excessive stress system activity may lead to metabolic deterioration such as hypertension, diabetes, central adiposity, osteoporosis and mental illness, which together comprise a high proportion of modern chronic illnesses. Chronically altered stress system activity may be described as a state of allostasis representing stability but with risk of long term tissue damage[62].

On the other hand, reduced stress system activity, another form of allostasis, may be expected to produce a state of hypo-arousal and lack of anti-nociceptive activity centrally, leading to the many chronic pain and fatigue based disorders (CFS, fibromyalgia, irritable bowel syndrome, migraine and many others). A number of studies have shown relative

hypocortisolism in pain/fatigue disorders[63-65]. In patients with CFS, studies have demonstrated low levels of cortisol in plasma[63,64] (in morning[63] as well as in the evening[66]), urine[63,67-70] and saliva[70-73]. Corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) challenge tests, which test adequacy of the HPA axis also show similar results in CFS patients[63] although not consistently[45]. Hypocortisolism has also been shown in patients with fibromyalgia[74-76] and chronic pain syndromes[77,78]. Chronic fatigue syndrome has a strikingly high female preponderance (up to 75%) and it has been shown that the glucocorticoid sensitivity of pro-inflammatory cytokine production after psychological stress is different among the sexes[79].

5. CBG gene mutation: Kindred studies

Four major function altering mutations of the CBG gene have been described in humans. These include CBG Leuven, CBG Lyon, CBG null and a CBG non-cortisol binding variant. Old and new genetic nomenclature for these mutations is shown in Table 1. CBG Leuven (c.344T>A, p.Leu115His) reduces CBG:cortisol binding three-fold[15,80]. CBG Lyon has been described in three kindreds and reduces cortisol binding affinity 4-fold (c.1165G>A, p.Asp389Asn)[15,81]. CBG null (c.32G>A, p.Trp11X) prevents CBG synthesis and homozygotes are completely CBG deficient[82]. Both CBG Lyon and null are associated with fatigue and chronic pain and were described together in single kindred where the phenotype was similar[82]. The description of a kindred with a non-cortisol binding variant of CBG included an index case with fatigue[83].

Mutations and polymorphisms	Coding DNA (old nomenclature)	Coding DNA (new nomenclature)	Protein (old nomenclature)	Protein (new nomenclature)
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
p.Ala246Ser polymorphism	c.825G>T	c.736G>T	Ala-Ser224	p.Ala246Ser

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

6. CBG null

We have described a 39 member Italian-Australian kindred with a novel null (complete loss-of-function) CBG mutation, an exon 2 mutation causing premature termination codon corresponding to residue-12 (c121G➔A)[82] (Fig. 1). The 48 year-old female proband was found to have low total plasma cortisol levels but normal 24-hour urinary free cortisol. She had an elevated plasma cortisol fraction and undetectable CBG levels. CBG gene sequencing of the family revealed two null homozygotes, 19 null heterozygotes, three Lyon heterozygotes and two compound (Null/Lyon) heterozygotes. CBG levels were also undetectable in the other two CBG null homozygotes. There was a 50% CBG reduction in the null heterozygotes and an even greater reduction in the compound heterozygotes. Five

members of the family, including the female proband, met the United States' Centre for Disease Control (CDC) criteria[43] for chronic fatigue syndrome. In addition, 12 out of the 14 members with heterozygote mutation and two out of three with homozygous mutation were found to have idiopathic chronic fatigue. Pain syndromes were observed in six subjects with null mutation – four were null heterozygotes while two were homozygotes. One of these pain affected null subjects also fulfilled the criteria for CFS. Prior to finding this family with CBG null deficiency it had been thought that complete CBG deficiency was incompatible with life[9,84].

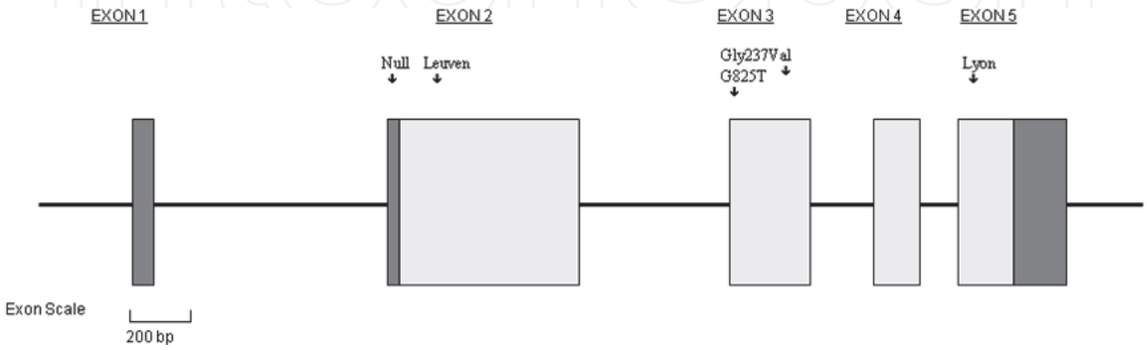


Fig. 1. Schematic diagram of the CBG gene and the location of CBG mutations. Darker shaded portions indicate regions of the exons that are untranslated. The mutations include CBG null (G121A; Trp-12X), transcortin Leuven (T433A; Leu93His), CBG Lyon (G1254A; Asp367Asn) and Ser/Ala224 polymorphism (G825T) and CBG non -binding polymorphism (Gly237Val).

7. CBG Lyon

The CBG variant produced due to a missense mutation, an aspartic acid to aspargine substitution at residue 367 (Asp^{367} → Asn) was first described in a 43 year old woman of northwest African origin in Lyon, France, and is known as the CBG Lyon mutation[81]. Her main symptoms included chronic asthenia, depressive mood and hypotension. She was found to have very low total plasma cortisol but normal ACTH and urine free cortisol levels. A low free cortisol concentration suggested an abnormality in CBG binding and was later confirmed. Her four children were all found to heterozygous for this mutation.

Since then, CBG Lyon has been described in two other kindreds[82,85]. The mutation has been reported in a 40 year-old white Brazilian woman presenting with chronic fatigue and hypotension. The family members screened, including her parents and her children, were found to be heterozygous for the mutation but did not complain of chronic fatigue[85]. We have also reported CBG Lyon mutation in the family members of a proband with CBG null mutation[82]. Three family members were CBG Lyon heterozygotes, while two had co-inheritance of CBG Lyon and CBG null (compound heterozygotes). The family members with co-inheritance had clearly low CBG levels. All available family members with CBG Lyon mutation, both heterozygotes and compound heterozygotes reported significant fatigue[82].

8. CBG non-binding Gly 237 Val

This CBG gene polymorphism, involving a c.776g>t transversion in exon 3 of the *SERPINA6* gene resulting in a p.Gly237Val substitution, was described recently in a 26 year-old Pakistani–British woman presenting with fatigue and hypotension. This CBG variant lacks any steroid binding activity. Two siblings were found homozygous for this mutation and two more family members (including the proband's mother) were found to be heterozygous. The homozygous members had reduced CBG levels (about 50% for the proband) by RIA measurements but undetectable CBG when measured with cortisol-binding capacity assays. However, aside from the proband, none of the family members, including two siblings found homozygous for the mutation, reported symptoms of chronic pain or fatigue. The only biochemical finding that differentiated the proband from the other homozygous members was the increased cortisol pulsatility[83].

9. Genetic epidemiology studies

Given the evidence from the kindred studies, we hypothesized that CBG polymorphisms could act as a genetic risk factor for patients with CFS. Two hundred and forty eight patients with CFS and an equal number of control subjects had full CBG gene sequencing. An exon 3 polymorphism (c.825G-->T, Ala-Ser224) was more commonly observed in CFS patients than expected by chance at the trend level ($P<0.07$), suggesting that homozygosity for the serine allele of the CBG gene may predispose to CFS[86].

We also conducted a population-based study in Calabria, Italy, the region our Italian-Australian null/Lyon kindred originated from, to look at the prevalence of CBG mutations in the local community. The results showed a high prevalence of CBG null and Lyon mutations (3.6%). Chronic widespread pain, but not chronic fatigue, was found to be common in subjects with CBG mutation[87].

Genetic influences have been postulated to account for 50% of the variance as well as the reduced pain thresholds seen in chronic pain syndromes[88,89]. The prospective population–based cohort study EPIFUND (Epidemiology of functional disorders), examined if genetic variation within the HPA axis genes was associated with susceptibility to musculoskeletal pain. The CBG gene (SERPINA6) and six other HPA axis genes CRH, CRH receptor 1 (CRHR1), CRH binding protein (CRHBP), the ACTH precursor pro-opiomelanocortin (POMC) and its receptor (MC2R), the glucocorticoid receptor (NR3C1) were examined. Seventy-five single nucleotide polymorphisms (SNPs) were detected in 164 CWP patients and 172 pain-free controls. Amongst the seven HPA axis genes, the most notable genetic variation was in the *SERPINA6* gene. Two SNPs in *SERPINA6* (rs 941601 and rs 8022616), located within a single haplotype block, were significantly associated with CWP. Moreover, in patients reporting pain, four SNPs of *SERPINA6* were associated with the maximum number of pain sites[88]. This finding assumes significance given that there was no association with SNPs in CRH, CRHR1, CRHBP, POMC or NR3C1 and CWP was observed and only a single SNP in MC2R, rs11661134, was associated with increased odds of having CWP.

CBG gene knockout mice models

The effect of a gene deletion can also be studied in the laboratory setting by producing 'knockout gene' mouse models, achieved by a homologous recombination between DNA sequences in the existing chromosome and the newly introduced DNA into pluripotent

embryo-derived stem cells[90]. In the study reported by Richard et al, the CBG gene knockout mouse was created by 'floxing' - contraction for flanking the lox p sites - exon 2 of the *SERPINA6* gene[91]. The learned helplessness paradigm[92], an animal model of depression, was used to evaluate behavioural changes following intense stress. HPA axis dysregulation has previously been linked to helpless behavior[93,94]. The CBG deficient mice (Cbg -/-) showed increased immobility in the forced-swimming test and markedly enhanced learned helplessness after prolonged uncontrollable stress (footshock) as well as markedly reduced total circulating corticosterone in both rested and stressed states. Responses to milder stressors was not altered. In another CBG knockout mice study[95], Cbg -/- mice had a reduction in CBG levels and a correspondingly ten-fold increase in levels of free cortisol. Despite this, there was no evidence of enhanced glucocorticoid activity, suggesting the role of CBG in mediating corticosteroid functions. More importantly, Cbg -/- mice exhibited a possible fatigue syndrome, characterised by reduced activity levels compared with the control group. The elevated cortisol and reduced activity levels were not seen in the study of Richard et al[91]. Taken together, however, these findings suggest an important hitherto unanticipated role for CBG in the neurobehavioural aspects of stress system function.

10. Conclusion

There is an unequivocal role for CBG as a transport molecule for cortisol, and altered levels of CBG are generally met with unaltered levels of free cortisol, confirming it is free cortisol which is actively regulated in blood.

However, recent studies have linked rare CBG gene mutations, which alter CBG levels or binding affinity, to pain/fatigue syndromes. This association is not universal suggesting that other genetic or environmental factors influence the phenotype. Genetic epidemiology studies point to the CBG gene and its variants as having a role in the risk of developing a chronic pain phenotype. Animal studies have also shown that CBG genetic deletions can produce altered neurobehavioural responses to stress. This mounting evidence suggests a role for CBG in tissue delivery or other elements of stress system function, although the precise mechanisms await elucidation.

11. Acknowledgement

The authors would like to thanks Dr B Ardesjö Lundgren for her expertise in preparing Table 1.

12. References

[1] Daughaday WH. Binding of corticosteroids by plasma proteins. II. Paper electrophoresis and equilibrium paper electrophoresis. *J Clin Invest.* Dec 1956;35(12):1434-1438.
[2] Daughaday WH. Binding of corticosteroids by plasma proteins. I. Dialysis equilibrium and renal clearance studies. *J Clin Invest.* Dec 1956;35(12):1428-1433.
[3] Daughaday WH. Binding of corticosteroids by plasma proteins. IV. The electrophoretic demonstration of corticosteroid binding globulin. *J Clin Invest.* Apr 1958;37(4):519-523.
[4] Daughaday WH. Binding of corticosteroids by plasma proteins. III. The binding of corticosteroid and related hormones by human plasma and plasma protein fractions as measured by equilibrium dialysis. *J Clin Invest.* Apr 1958;37(4):511-518.

[5] Daughaday WH. Binding of corticosteroids by plasma proteins. V. Corticosteroid-binding globulin activity in normal human beings and in certain disease states. *AMA Arch Intern Med.* Feb 1958;101(2):286-290.

[6] Sandberg AA, Slaunwhite WR, Jr., Antoniades HN. The binding of steroids and steroid conjugates to human plasma proteins. *Recent Prog Horm Res.* 1957;13:209-260; discussion 260-207.

[7] Brien TG. Human corticosteroid binding globulin. *Clin Endocrinol (Oxf).* Feb 1981;14(2):193-212.

[8] Ghose-Dastidar J, Ross JB, Green R. Expression of biologically active human corticosteroid binding globulin by insect cells: acquisition of function requires glycosylation and transport. *Proc Natl Acad Sci U S A.* Aug 1 1991;88(15):6408-6412.

[9] Challis JR, Berdusco ET, Jeffray TM, Yang K, Hammond GL. Corticosteroid-binding globulin (CBG) in fetal development. *J Steroid Biochem Mol Biol.* Jun 1995;53(1-6):523-527.

[10] Hammond GL, Smith CL, Underhill CM, Nguyen VT. Interaction between corticosteroid binding globulin and activated leukocytes in vitro. *Biochem Biophys Res Commun.* Oct 15 1990;172(1):172-177.

[11] Hammond GL, Smith CL, Goping IS, et al. Primary structure of human corticosteroid binding globulin, deduced from hepatic and pulmonary cDNAs, exhibits homology with serine protease inhibitors. *Proc Natl Acad Sci U S A.* Aug 1987;84(15):5153-5157.

[12] Kojima N, Sakata S, Komaki T, Matsuda M, Miura K. [Preparation of an immunoadsorbent interacting specifically with cortisol-binding globulin (CBG) and its application to the interaction between CBG and cortisol--evaluation of the association constant (Ka) between CBG and cortisol in normal subjects]. *Nippon Naibunpi Gakkai Zasshi.* May 20 1987;63(5):695-701.

[13] Hammond GL, Smith CL, Underhill DA. Molecular studies of corticosteroid binding globulin structure, biosynthesis and function. *J Steroid Biochem Mol Biol.* 1991;40(4-6):755-762.

[14] Avvakumov GV, Warmels-Rodenhiser S, Hammond GL. Glycosylation of human corticosteroid-binding globulin at aspargine 238 is necessary for steroid binding. *J Biol Chem.* Jan 15 1993;268(2):862-866.

[15] Gagliardi L, Ho JT, Torpy DJ. Corticosteroid-binding globulin: the clinical significance of altered levels and heritable mutations. *Mol Cell Endocrinol.* Mar 5 2010;316(1):24-34.

[16] Klieber MA, Underhill C, Hammond GL, Muller YA. Corticosteroid-binding globulin, a structural basis for steroid transport and proteinase-triggered release. *J Biol Chem.* Oct 5 2007;282(40):29594-29603.

[17] Seralini GE, Berube D, Gagne R, Hammond GL. The human corticosteroid binding globulin gene is located on chromosome 14q31-q32.1 near two other serine protease inhibitor genes. *Hum Genet.* Nov 1990;86(1):73-75.

[18] Gettins PG. Serpin structure, mechanism, and function. *Chem Rev.* Dec 2002;102(12):4751-4804.

[19] Qi X, Loiseau F, Chan WL, et al. Allosteric modulation of hormone release from thyroxine and corticosteroid-binding globulins. *J Biol Chem.* May 6 2011;286(18):16163-16173.

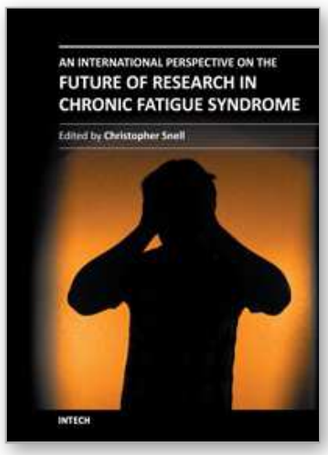
[20] Pemberton PA, Stein PE, Pepys MB, Potter JM, Carrell RW. Hormone binding globulins undergo serpin conformational change in inflammation. *Nature.* Nov 17 1988;336(6196):257-258.

[21] Zhou A, Wei Z, Stanley PL, Read RJ, Stein PE, Carrell RW. The S-to-R transition of corticosteroid-binding globulin and the mechanism of hormone release. *J Mol Biol.* Jun 27 2008;380(1):244-251.
[22] Pugeat M, Bonneton A, Perrot D, et al. Decreased immunoreactivity and binding activity of corticosteroid-binding globulin in serum in septic shock. *Clin Chem.* Aug 1989;35(8):1675-1679.
[23] Bernier J, Jobin N, Emptoz-Bonneton A, Pugeat MM, Garrel DR. Decreased corticosteroid-binding globulin in burn patients: relationship with interleukin-6 and fat in nutritional support. *Crit Care Med.* Mar 1998;26(3):452-460.
[24] Zouaghi H, Savu L, Guerot C, Gryman R, Coulon A, Nunez EA. Total and unbound cortisol-, progesterone-, oestrone- and transcortin-binding activities in sera from patients with myocardial infarction: evidence for differential responses of good and bad prognostic cases. *Eur J Clin Invest.* Dec 1985;15(6):365-370.
[25] Ho JT, Al-Musalhi H, Chapman MJ, et al. Septic shock and sepsis: a comparison of total and free plasma cortisol levels. *J Clin Endocrinol Metab.* Jan 2006;91(1):105-114.
[26] Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. *Ann Intern Med.* Jan 15 1998;128(2):127-137.
[27] Stewart PM, Clark PM. The low-dose corticotropin-stimulation test revisited: the less, the better? *Nat Clin Pract Endocrinol Metab.* Feb 2009;5(2):68-69.
[28] Demettre E, Bastide L, D'Haese A, et al. Ribonuclease L proteolysis in peripheral blood mononuclear cells of chronic fatigue syndrome patients. *J Biol Chem.* Sep 20 2002;277(38):35746-35751.
[29] Dunn JF, Nisula BC, Rodbard D. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J Clin Endocrinol Metab.* Jul 1981;53(1):58-68.
[30] Torpy DJ, Ho JT. Corticosteroid-binding globulin gene polymorphisms: clinical implications and links to idiopathic chronic fatigue disorders. *Clin Endocrinol (Oxf).* Aug 2007;67(2):161-167.
[31] Mendel CM. The free hormone hypothesis: a physiologically based mathematical model. *Endocr Rev.* Aug 1989;10(3):232-274.
[32] Adams JS. "Bound" to work: the free hormone hypothesis revisited. *Cell.* Sep 9 2005;122(5):647-649.
[33] Nakhla AM, Khan MS, Rosner W. Induction of adenylate cyclase in a mammary carcinoma cell line by human corticosteroid-binding globulin. *Biochem Biophys Res Commun.* Jun 30 1988;153(3):1012-1018.
[34] Rosner W, Hryb DJ, Khan MS, Singer CJ, Nakhla AM. Are corticosteroid-binding globulin and sex hormone-binding globulin hormones? *Ann N Y Acad Sci.* 1988; 538:137-145.
[35] Sumer-Bayraktar Z, Kolarich D, Campbell MP, Ali S, Packer NH, Thaysen-Andersen M. N-glycans modulate the function of human corticosteroid-binding globulin. *Mol Cell Proteomics.* May 10 2011.
[36] Perogamvros I, Kayahara M, Trainer PJ, Ray DW. Serum regulates cortisol bioactivity by corticosteroid-binding globulin dependent and independent mechanisms, as revealed by combined bioassay and physicochemical assay approaches. *Clin Endocrinol (Oxf).* Feb 21 2011.
[37] Hammes A, Andreassen TK, Spoelgen R, et al. Role of endocytosis in cellular uptake of sex steroids. *Cell.* Sep 9 2005;122(5):751-762.
[38] Croft P, Rigby AS, Boswell R, Schollum J, Silman A. The prevalence of chronic widespread pain in the general population. *J Rheumatol.* Apr 1993;20(4):710-713.

[39] Skapinakis P, Lewis G, Meltzer H. Clarifying the relationship between unexplained chronic fatigue and psychiatric morbidity: results from a community survey in Great Britain. *Am J Psychiatry.* Sep 2000;157(9):1492-1498.
[40] Darbishire L, Ridsdale L, Seed PT. Distinguishing patients with chronic fatigue from those with chronic fatigue syndrome: a diagnostic study in UK primary care. *Br J Gen Pract.* Jun 2003;53(491):441-445.
[41] Ridsdale L, Godfrey E, Chalder T, et al. Chronic fatigue in general practice: is counselling as good as cognitive behaviour therapy? A UK randomised trial. *Br J Gen Pract.* Jan 2001;51(462):19-24.
[42] Clauw DJ, Chrousos GP. Chronic pain and fatigue syndromes: overlapping clinical and neuroendocrine features and potential pathogenic mechanisms. *Neuro--immunomodulation.* May-Jun 1997;4(3):134-153.
[43] Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med.* Dec 15 1994;121(12):953-959.
[44] Holmes GP, Kaplan JE, Gantz NM, et al. Chronic fatigue syndrome: a working case definition. *Ann Intern Med.* Mar 1988;108(3):387-389.
[45] Cleare AJ. The neuroendocrinology of chronic fatigue syndrome. *Endocr Rev.* Apr 2003;24(2):236-252.
[46] Byrne E. Idiopathic chronic fatigue and myalgia syndrome (myalgic encephalomyelitis): some thoughts on nomenclature and aetiology. *Med J Aust.* Jan 18 1988;148(2):80-82.
[47] Chrousos GP, Kino T. Glucocorticoid signaling in the cell. Expanding clinical implications to complex human behavioral and somatic disorders. *Ann N Y Acad Sci.* Oct 2009;1179:153-166.
[48] Lorusso L, Mikhaylova SV, Capelli E, Ferrari D, Ngonga GK, Ricevuti G. Immunological aspects of chronic fatigue syndrome. *Autoimmun Rev.* Feb 2009;8(4):287-291.
[49] Arnett SV, Alleva LM, Korossy-Horwood R, Clark IA. Chronic fatigue syndrome - A neuroimmunological model. *Med Hypotheses.* Jul 2011;77(1):77-83.
[50] Roitman A, Bruchis S, Bauman B, Kaufman H, Laron Z. Total deficiency of corticosteroid-binding globulin. *Clin Endocrinol (Oxf).* Nov 1984;21(5):541-548.
[51] Schweitzer R, Kelly B, Foran A, Terry D, Whiting J. Quality of life in chronic fatigue syndrome. *Soc Sci Med.* Nov 1995;41(10):1367-1372.
[52] Anderson JS, Ferrans CE. The quality of life of persons with chronic fatigue syndrome. *J Nerv Ment Dis.* Jun 1997;185(6):359-367.
[53] Solomon L, Nisenbaum R, Reyes M, Papanicolaou DA, Reeves WC. Functional status of persons with chronic fatigue syndrome in the Wichita, Kansas, population. *Health Qual Life Outcomes.* 2003;1:48.
[54] Prins JB, van der Meer JW, Bleijenberg G. Chronic fatigue syndrome. *Lancet.* Jan 28 2006;367(9507):346-355.
[55] Wessely S. The epidemiology of chronic fatigue syndrome. *Epidemiol Psichiatr Soc.* Jan-Apr 1998;7(1):10-24.
[56] Reynolds KJ, Vernon SD, Bouchery E, Reeves WC. The economic impact of chronic fatigue syndrome. *Cost Eff Resour Alloc.* Jun 21 2004;2(1):4.
[57] Cairns R, Hotopf M. A systematic review describing the prognosis of chronic fatigue syndrome. *Occup Med (Lond).* Jan 2005;55(1):20-31.
[58] Kaiser J. Biomedicine. Genes and chronic fatigue: how strong is the evidence? *Science.* May 5 2006;312(5774):669-671.

[59] Walsh CM, Zainal NZ, Middleton SJ, Paykel ES. A family history study of chronic fatigue syndrome. *Psychiatr Genet.* Sep 2001;11(3):123-128.
[60] Buchwald D, Herrell R, Ashton S, et al. A twin study of chronic fatigue. *Psychosom Med.* Nov-Dec 2001;63(6):936-943.
[61] Albright F, Light K, Light A, Bateman L, Cannon-Albright LA. Evidence for a heritable predisposition to Chronic Fatigue Syndrome. *BMC Neurol.* 2011;11:62.
[62] Chrousos GP. Stress and disorders of the stress system. *Nat Rev Endocrinol.* Jul 2009;5(7):374-381.
[63] Demitrack MA, Dale JK, Straus SE, et al. Evidence for impaired activation of the hypothalamic-pituitary-adrenal axis in patients with chronic fatigue syndrome. *J Clin Endocrinol Metab.* Dec 1991;73(6):1224-1234.
[64] Poteliakhoff A. Adrenocortical activity and some clinical findings in acute and chronic fatigue. *J Psychosom Res.* 1981;25(2):91-95.
[65] Heim C, Ehlert U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology.* Jan 2000;25(1):1-35.
[66] Altemus M, Dale JK, Michelson D, Demitrack MA, Gold PW, Straus SE. Abnormalities in response to vasopressin infusion in chronic fatigue syndrome. *Psychoneuroendocrinology.* Feb 2001;26(2):175-188.
[67] Cleare AJ, Miell J, Heap E, et al. Hypothalamo-pituitary-adrenal axis dysfunction in chronic fatigue syndrome, and the effects of low-dose hydrocortisone therapy. *J Clin Endocrinol Metab.* Aug 2001;86(8):3545-3554.
[68] Hamilos DL, Nutter D, Gershtenson J, et al. Core body temperature is normal in chronic fatigue syndrome. *Biol Psychiatry.* Feb 15 1998;43(4):293-302.
[69] Scott LV, Dinan TG. Urinary free cortisol excretion in chronic fatigue syndrome, major depression and in healthy volunteers. *J Affect Disord.* Jan 1998;47(1-3):49-54.
[70] Young AH, Sharpe M, Clements A, Dowling B, Hawton KE, Cowen PJ. Basal activity of the hypothalamic-pituitary-adrenal axis in patients with the chronic fatigue syndrome (neurasthenia). *Biol Psychiatry.* Feb 1 1998;43(3):236-237.
[71] Gaab J, Huster D, Peisen R, et al. Low-dose dexamethasone suppression test in chronic fatigue syndrome and health. *Psychosom Med.* Mar-Apr 2002;64(2):311-318.
[72] Strickland P, Morriss R, Wearden A, Deakin B. A comparison of salivary cortisol in chronic fatigue syndrome, community depression and healthy controls. *J Affect Disord.* Jan 1998;47(1-3):191-194.
[73] Wood B, Wessely S, Papadopoulos A, Poon L, Checkley S. Salivary cortisol profiles in chronic fatigue syndrome. *Neuropsychobiology.* 1998;37(1):1-4.
[74] Crofford LJ, Pillemer SR, Kalogeras KT, et al. Hypothalamic-pituitary-adrenal axis perturbations in patients with fibromyalgia. *Arthritis Rheum.* Nov 1994;37(11):1583-1592.
[75] Griep EN, Boersma JW, Lentjes EG, Prins AP, van der Korst JK, de Kloet ER. Function of the hypothalamic-pituitary-adrenal axis in patients with fibromyalgia and low back pain. *J Rheumatol.* Jul 1998;25(7):1374-1381.
[76] McCain GA, Tilbe KS. Diurnal hormone variation in fibromyalgia syndrome: a comparison with rheumatoid arthritis. *J Rheumatol Suppl.* Nov 1989;19:154-157.
[77] Elwan O, Abdella M, el Bayad AB, Hamdy S. Hormonal changes in headache patients. *J Neurol Sci.* Nov 1991;106(1):75-81.
[78] Johansson F. Differences in serum cortisol concentrations in organic and psychogenic chronic pain syndromes. *J Psychosom Res.* 1982;26(3):351-358.

[79] Rohleder N, Schommer NC, Hellhammer DH, Engel R, Kirschbaum C. Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Psychosom Med.* Nov-Dec 2001;63(6):966-972.
[80] Van Baelen H, Brepoels R, De Moor P. Transcortin Leuven: a variant of human corticosteroid-binding globulin with decreased cortisol-binding affinity. *J Biol Chem.* Apr 10 1982;257(7):3397-3400.
[81] Emptoz-Bonneton A, Cousin P, Seguchi K, et al. Novel human corticosteroid-binding globulin variant with low cortisol-binding affinity. *J Clin Endocrinol Metab.* Jan 2000;85(1):361-367.
[82] Torpy DJ, Bachmann AW, Grice JE, et al. Familial corticosteroid-binding globulin deficiency due to a novel null mutation: association with fatigue and relative hypotension. *J Clin Endocrinol Metab.* Aug 2001;86(8):3692-3700.
[83] Perogamvros I, Underhill C, Henley DE, et al. Novel corticosteroid-binding globulin variant that lacks steroid binding activity. *J Clin Endocrinol Metab.* Oct 2010;95(10): E142-150.
[84] Hammond GL. Molecular properties of corticosteroid binding globulin and the sex-steroid binding proteins. *Endocr Rev.* Feb 1990;11(1):65-79.
[85] Brunner E, Baima J, Vieira TC, Vieira JG, Abucham J. Hereditary corticosteroid-binding globulin deficiency due to a missense mutation (Asp367Asn, CBG Lyon) in a Brazilian kindred. *Clin Endocrinol (Oxf).* Jun 2003;58(6):756-762.
[86] Torpy DJ, Bachmann AW, Gartside M, et al. Association between chronic fatigue syndrome and the corticosteroid-binding globulin gene ALA SER224 polymorphism. *Endocr Res.* Aug 2004;30(3):417-429.
[87] Cizza G BL, Smirne N, Maletta R, Tomaino C, Costanzo A, Gallo M, Lewis JG, Geracitano S, Grassod MB, Potenza G, Monteleone C, Brancati G, Ho JT, Torpy DJ, Bruni AC. Clinical manifestations of highly prevalent corticosteroid binding globulin mutations in a village in southern italy. *J Clin Endocrinol Metab.* 2011.
[88] Holliday KL, Nicholl BI, Macfarlane GJ, Thomson W, Davies KA, McBeth J. Genetic variation in the hypothalamic-pituitary-adrenal stress axis influences susceptibility to musculoskeletal pain: results from the EPIFUND study. *Ann Rheum Dis.* Mar 2010;69(3):556-560.
[89] Kato K, Sullivan PF, Evengard B, Pedersen NL. Importance of genetic influences on chronic widespread pain. *Arthritis Rheum.* May 2006;54(5):1682-1686.
[90] Capecchi MR. The new mouse genetics: altering the genome by gene targeting. *Trends Genet.* Mar 1989;5(3):70-76.
[91] Richard EM, Helbling JC, Tridon C, et al. Plasma transcortin influences endocrine and behavioral stress responses in mice. *Endocrinology.* Feb 2010;151(2):649-659.
[92] Overmier JB, Seligman ME. Effects of inescapable shock upon subsequent escape and avoidance responding. *J Comp Physiol Psychol.* Feb 1967;63(1):28-33.
[93] Henn FA, Vollmayr B. Stress models of depression: forming genetically vulnerable strains. *Neurosci Biobehav Rev.* 2005;29(4-5):799-804.
[94] Papolos DF. Switching, cycling, and antidepressant-induced effects on cycle frequency and course of illness in adult bipolar disorder: a brief review and commentary. *J Child Adolesc Psychopharmacol.* Summer 2003;13(2):165-171.
[95] Petersen HH, Andreassen TK, Breiderhoff T, et al. Hyporesponsiveness to glucocorticoids in mice genetically deficient for the corticosteroid binding globulin. *Mol Cell Biol.* Oct 2006;26(19):7236-7245.



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Edited by Dr. Christopher R. Snell

ISBN 978-953-51-0072-0
Hard cover, 104 pages
Publisher InTech
Published online 15, February, 2012
Published in print edition February, 2012

While the chapters in this book are a long way from solving the enigma that is CFS, they do represent important attempts to understand this complex and perplexing disease. A common theme in them all is CFS as a multisystem disease with the possibility of more than one cause and influenced by a variety of interacting factors. Further, they acknowledge the reality of CFS for persons with this disease and the importance of finding causes, treatments and ultimately a cure. As advanced biomedical research techniques are increasingly applied to the study of CFS, it is surely only a matter of time before biomarkers are identified, etiologies understood, and remedies devised.

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