4

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].

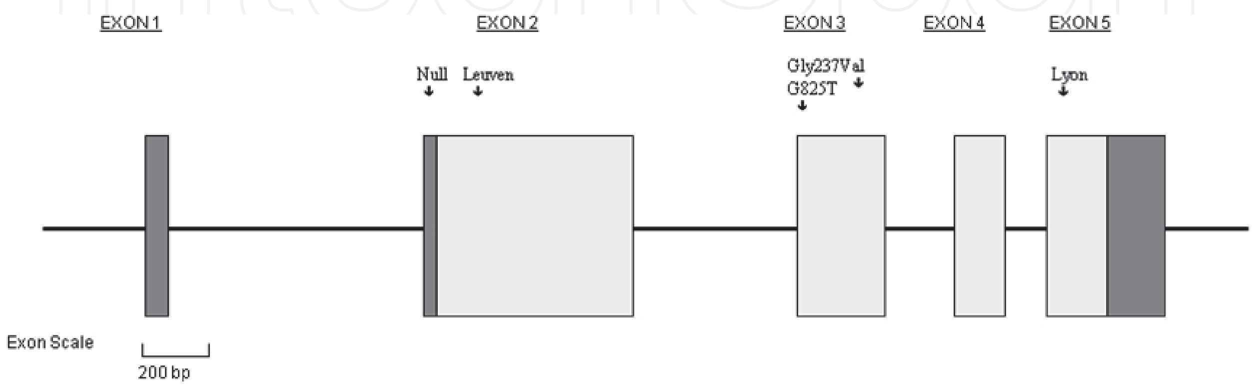

Fig. 1. Schematic diagram of the CBG gene and the location of CBG mutations. Darker shaded portions indicate regions of the exons that are untranslated. The mutations include CBG null (G121A; Trp-12X), transcortin Leuven (T433A; Leu93His), CBG Lyon (G1254A; Asp367Asn) and Ser/Ala224 polymorphism (G825T) 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 ($Asp^{367} \rightarrow Asn$) 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.

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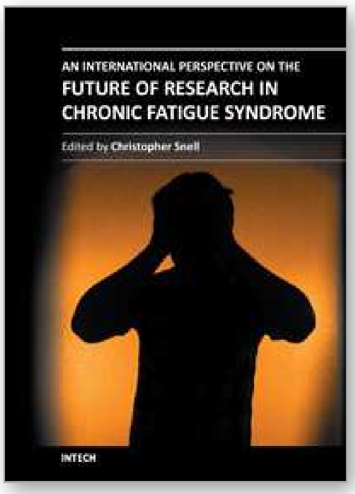

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While the chapters in this book are a long way from solving the enigma that is CFS, they do represent important attempts to understand this complex and perplexing disease. A common theme in them all is CFS as a multisystem disease with the possibility 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.