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# Possible Genetic Dysregulation in Pediatric CFS*

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## ABSTRACT

*Hypocortisolism is a frequent finding in individuals with chronic fatigue syndrome* (*CFS*) *and could play an explanatory role in the development of illness symptomatology. The etiologic mechanism behind this finding could be genetic variance in glucocorticoid receptor expression* (*GR*) *or increased resistance to the effects of glucocorticoids. Several investigators believe that allelic variance in a GR* (*NR*3*C*1) *mediates the expression of chronic fatigue possibly through influence on hypothalamic-pituitary-adrenal* (*HPA*) *axis function* [1]. *In addition, several immunologic variables are associated with CFS. The nuclear factor kappa beta* (*NFkB*) *pathway is heavily involved in cellular transcription and regulation and has been shown to be associated with the development of CFS. The NFkB pathway is directly regulated by and influences the presence of GR* [2]. *Our study focused on assessing whether such inflammatory transcription is occurring during adolescent years. Findings indicated decreased expression of NFKB*1, *NFKB*2, *and NR*3*C*1. *A decrease in the expression of these genes may have effects on immune cell function and cytokine production that could explain immunologic findings seen in individuals with CFS.*

***Keywords***: *Pediatric, Chronic Fatigue Syndrome, Hypocortisolism, Glucocorticoid Receptor Expression*

## 1. Introduction

In adult populations, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has been associated with CFS (Johnson & DeLuca, 2005) [3]. Adults with CFS tend to display lower levels of cortisol, the main signaling hormone of the HPA axis [4,5]. The literature is replete with findings implicating HPA axis dysregulation with CFS either through lower baseline cortisol [6], a lack of responsiveness on the part of the HPA axis [7], a pattern of glucocorticoid resistance [8], or disruption or dysregulation of the expected diurnal cortisol pattern [9].

A gene for defective cortisol binding protein has been associated with CFS [10]. Defective cortisol binding protein can lower the ability to respond to cortisol. Smith *et al*. [11] found that increased expression of genes influencing the HPA axis and changing cortisol production predicted the prevalence of unexplained chronic fatigue. A gene study by Rajeevan *et al*. [1] also implicated cortisol in CFS, finding single nucleotide polymorphisms in the glucocorticoid receptor (GR) gene (several such alleles were associated with increased risk for CFS). Cortisol binding is influenced by elements of the neuroendocrine pathways.

Complex interactions of neurological, immune, and endocrine systems, operating from the individual's genetic substrate, work in conjunction with environmental factors to influence onset of CFS and its clinical course. In particular, altered functioning of the HPA axis is implicated in the reduced capability to regulate responses to stress as seen in disorders such as CFS [12]. Variance in the expression of genes associated with HPA axis function has been associated with CFS across several studies with adult populations [13]. Previous work has found variance in the expression of GR (NR3C1) in individuals with CFS compared to controls [11]. Those with CFS may have a decreased sensitivity to the effects of cortisol due to a down-regulation of GR [8].

It is apparent that a degree of HPA axis dysfunction is involved in the pathogenic process of adult CFS, and it is possible that similar variables may predict the existence of HPA axis dysfunction among pediatric CFS cases involving children, adolescents, and young adults. For example, Mathew *et al*. [14] found the presence of HPA axis dysregulation in adolescence may serve as a predictor of this illness. Miike *et al*. [15] found that cortisol secretion was reduced in a pediatric population with CFS

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in comparison to controls. Segal, Hindmarsh, and Viner [16] found among adolescents with CFS a subtle alteration in adrenal functioning suggesting a reduction in central stimulation of adrenal glands, with females exhibiting a more attenuated response to ACTH than males. There is a consistent body of literature that has found dysregulation of glucocorticoid function to be associated with CFS in both adult and adolescent populations.

The present study focused on steroid receptor expression in a pediatric population with CFS, and we examined the presence of several specific genes associated with the development of CFS in adult populations. One of the primary hypotheses underlying the proposed work is that a pattern of hypocortisolism seen in adults with CFS [9] would be manifested in a pediatric population with the same disease. In addition, NR3C1 is one of the main transcriptional regulators of the GR, and polymorphisms of the NR3C1 gene have been associated with CFS in adults [1]. We also measured NFKB1 and NFKB2**,** which have been associated with inflammatory responses known to be associated with the development of CFS in adult populations [17]. The initiation of inflammatory changes might precede the development of fatigue symptomatology by several years. Therefore, we wanted to determine whether such inflammatory transcription was occurring during adolescent years.

## 2. Method

Data were collected in the mornings from a sample of adolescents with CFS (n = 6) which was obtained from the Chicago metropolitan area. Five were Caucasian and one Asian-American. Three were female and three were male. The average age was 17.8 years (range from 16 to 21). All were diagnosed with CFS by a physician who was familiar with this illness.

Serum cortisol served as an indicator of hypothalamic-pituitary-adrenal (HPA) axis function. Serum was obtained through centrifugation for a period of 20 minutes at 1000 X gravity. The collected samples were labeled with a unique identifier and preserved in a –80 degree centigrade freezer until time of assay. A commercially available enzyme linked immunoabsorbent assay (sensitivity: 0.030-3111 ng/ml) was used to determine cortisol concentration (R&D Systems, Minneapolis, MN).

For the children, one tube of peripheral blood (10ml) was preserved in lithium heparin. From this sample, peripheral blood mononuclear cells were obtained and preserved in accordance to a protocol provided by Panomics Inc (Fremont, CA). Preserved cells were then shipped to Panomics Inc. for analysis of the desired mRNA transcripts using a QuantiGene Plex System. This system employed blood lysate and branched DNA in creating a sandwich nucleic acid hybridization, which was then bound to a biotinalyted probe. Results were obtained through the use of laser excited fluorescent signal. Sample mean fluorescent intensity was calculated in relation to the mean signal of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Sample plates were read through the use of a BioPlex System (BioRad Laboratories, Hercules, CA).

Rationale for Selection of Cortisol and mRNA transcripts:

We examined for the presence of messenger RNA transcripts affecting receptor expression. The rationale for the selection of the examined transcripts (NR3C1, NFKB1, NFKB2) was based upon previous evidence demonstrating a possible relationship between these transcripts and CFS, along with an evaluation of their known biologic activity. Based on previous findings with adult populations, we also elected to examine cortisol level in this population.

The NR3C1 gene encodes a protein receptor for glucocorticoids. The encoded protein can bind to DNA and other proteins influencing transcriptional regulation. Mutations in the structure of this protein can influence glucocorticoid binding resulting in a degree of resistance to the actions of glucocorticoid.

The nuclear factor kappa beta (NFkB) pathway is one of the main regulators of inflammatory response across multiple cell populations. This pathway influences and is influenced by the release of cytokines and other inflammatory mediators. NFKB1 is involved in cell differentiation and pro-inflammatory immune response. NFKB2 is also involved in cell differentiation and pro-inflammatory immune response but has a stronger role on B cell linages and on apoptosis (programmed cell death). The expression of the nuclear receptor family NFKB1 (nuclear factor of kappa light polypeptide, p105) and NFKB2 (nuclear factor of kappa light polypeptide gene enhancer p 49/100) are also directly regulated by and influence the presence of GR [18]. Determining the levels of NFKB1 and NFKB2 provides a means of determining relative efficacy of GR function and an insight into regulation of this receptor in an adolescent population with fatigue. In this exploratory study, we chose to focus on those that have been demonstrated in adult populations to be associated with CFS. Thus, we examined for variance in the expression of factors involved in the regulation and expression of discrete components of immune function in a population of adolescents with CFS.

## 3. Results

The obtained samples demonstrated low mean cortisol values (M = 56.73 ng/ml, SD = 24.73). These pediatric CFS values are considerably lower than those found in control pediatric samples (6-16 years of age, M = 91.0

 

ng/ml, SD = 19) [19] and provide support for the presence of hypocortisolism in pediatric samples with CFS.

Additionally, the six samples were sent to the assay service of Panomics (Fremont, CA) for determination of three discrete genes conceptualized as associated with development of CFS; NRC31, NFKB1 and NFKB2. Results were then normalized to GAPDH and expression ratios derived. Mean expression ratios and ranges are provided in **Table 1**.

With gene expression ratios, values less than one indicate a down-regulation of function in relationship to the housekeeping gene (GAPDH) whereas values greater than one would indicate increased expression. These data demonstrate a pattern of down-regulation of gene expression in the pediatric sample with CFS, concomitant with reduced cortisol levels.

## 4. Discussion

The study's main findings were hypocortisolism and the down-regulated expression of NR3C1 (the encoding gene for the GR), NFKB1, and NFKB2. The reduced expression of the gene for the GR provides evidence for dysfunction of the HPA axis in those with CFS. The expression of NFKB is associated with proinflammatory immunologic responses and is induced by stimuli such as reactive oxygen species, mitogens, cytokines TNFα, and IL-1. In our pediatric sample, there was a marked down-regulation of NFKB1 and NFKB2. However, in response to down regulated endogenous glucocorticoid levels, transcription of inflammatory genes by NFKB might be expected to be up regulated. Glucocorticoid has significant suppressive effects on the expression of NFkB, an action that occurs through ligand binding of the glucocorticoid receptor. In the presence of down-regulation of the glucocorticoid receptor, it is possible that suppression of NFkB is inactive. Alternatively, there may be a disruption of an associated co-receptor or molecule that inhibits adequate GR binding, leading to a state in which NFkb and NR3C1 levels are both reduced.

NFKB is a proinflammatory transcriptional factor and prevents apoptosis and immune suppression which are typically evidenced by up regulated CD8+. In a study of girls with CFS, Ter Wolbeek *et al*., [20] also found decreased levels of the pro-inflammatory cytokines IL-6 and TNF-α, but found increased levels of the anti-inflammatory cytokines IL-10 and interferon (IF)-gamma. However, evidence for a process of anti inflammatory transcription or immune suppression would be supported by a predominance of TH2 type immune response and elevated cortisol levels [2].

One possible explanation for this down regulation of all three genes is a bidirectional competitive inhibition or trans repression exerted upon NFKB1/NFKB2 and NR3C1 by one another. This process may be the end result of a prolonged activation of the NFKB pathway, such that expressions of NFKB1/NFKB2 are depleted via the production of their own inhibitor IKBα upon translocation to the nucleus. IKBα (a member of the IKB family of inhibitory proteins) creates a feedback control loop which prevents NFKB levels from becoming too high and creating excessive inflammation. IKBα will inhibit proinflammatory transcription by NFKB via nega- tive feedback, which could possibly explain the down regulation of the NFKB1and NFKB2 genes. The initiation of the triggering of the NFKB pathway is likely dir- ected by the presence of several factors: mitogens, bacte- ria, UV exposure, viruses, cytokines IL-1 and TNF α along with other proinflammatory, and reactive oxygen species (all of which have the potential to trigger inflammation and suppress cortisol levels) [18,21]. It is possible that a prolonged activation of this inflammatory pathway has led to the down regulation of the production of NFKB1 and NFKB2 by its inhibitor IKBα. This inhibition would then leave the cell in an anti inflammatory state, possibly leading to the down regulation of the NR3C1 gene coding for the GCR, as its expression would not be necessary when the cell is in an anti inflammatory or suppressed state.

A second possible explanation for why all three genes are down regulated is competition between NFKB and the GCR for limited amounts of the following co activators: steroid receptor coactivator-1 (SRC-1) and CREB-Binding protein (CBP). Both of these co activators bind to NR3C1 and NFKB and are necessary for the transactivation (transport into the nucleus where transcription is carried out) of both NFKB and the GCR [22]. Inadequate supplies or insufficient levels of these co activators could lead to the down regulated expression of each of the three genes that code for transcriptional factors. It is also possible that NFKB1 and NFKB2 are involved in affecting the degree to which the glucocorticoid receptor (GCR) displays sensitivity to its ligand (cortisol). Both the expression and sensitivity of the GCR to its ligand may be affected by any number of factors including but not

**Table 1. Mean normalized expression ratios and ranges for genes of interest.**

| Variable | NFkB1 | NFkB2 | NR3C1 |
|---|---|---|---|
| Pediatric | 0.09 (0.05-0.13) | 0.10 (0.04-0.14) | 0.04 (0.02-0.10) |
| Universal RNA | 0.23 | 0.41 | 0.65 |

 

limited to: posttranslational modifications, the effects of signaling cascades, reduced or altered expression of heat shock proteins, DNA bending, variations in the receptor protein, dimerization of an alternative receptor, recaptor chaperone defect [18,21].

These data demonstrate that in a pediatric population with CFS, there is decreased expression of the gene encoding for the GR. In an adolescent population, there may be an increased level of vulnerability to disruptions of the HPA axis. In a vulnerable population, such disruptions may have end effects on cognitive pathways and lifelong neuroendocrine responsiveness [23]. While much of the speculation of the effects of cortisol on development has tended to investigate the effects of hypercortisolism, the lack of adequate hormone may be as equally disruptive. These data highlight the importance of examining this pathway in an adolescent population. These findings should be considered as preliminary, given the small sample size, and there is a need for replication with a larger data set.

## REFERENCES


[1] M. S. Rajeevan, A. K. Smith, I. Dimulescu, E. R. Unger, S. D. Vernon, C. Heim and W. C. Reeves, "Glucocorticoid Receptor Polymorphisms and Haplotypes Associated with Chronic Fatigue Syndrome," *Genes*, *Brain*, & *Behavior*, Vol. 6, No. 2, 2007, pp. 167-176.

[2] A. Amsterdam, K. Tajima and R. Sasson, "Cell-Specific Regulation of Apoptosis by Glucocorticoids: Implication to their Anti-Inflammatory Action," *Biochemical Pharmacology*, Vol. 64, No. 5-6, 2002, pp. 843-850.

[3] S. K. Johnson and J. DeLuca, "Chronic Fatigue Syndrome and the Brain," In: J. DeLuca, Ed., *Fatigue as a Window to the Brain*, MIT Press, Cambridge, 2005, pp. 137-156.

[4] A. J. Cleare, "The Neuroendocrinology of Chronic Fatigue Syndrome," *Endocrine Reviews*, Vol. 24, No. 2, 2003, pp. 236-252.

[5] J. Gaab, D. Huster, R. Peisen, V. Engert, V. Heitz, T. Schad, T. H. Schurmeyer, and U. Ehlert, "Hypothalamic-Pituitary-Adrenal Axis Reactivity in Chronic Fatigue Syndrome and Health under Psychological, Physiological, and Pharmacological Stimulation," *Psychosomatic Medicine*, Vol. 64, No. 6, 2001, pp. 951-962.

[6] W. K. Jerjes, A. J. Cleare, S. Wessel, P. J. Wood, and N. F. Taylor, "Diurnal Patterns of Salivary Cortisol and Cortisone Output in Chronic Fatigue Syndrome," *Journal of Affective Disorders*, Vol. 87, No. 2-3, 2005, pp. 299-304.

[7] T. G. Dinan, T. Majeed, E. Lavelle, L. V. Scott, C. Berti, and P. Behan, "Blunted Serotonin-Mediated Activation of the Hypothalamic-Pituitary-Adrenal Axis in Chronic Fatigue Syndrome," *Psychoneuroendocrinology*, Vol. 22, No. 4, 1997, pp. 261-267.

[8] A. Kavelaar, W. Kuis, L. Knook, G. Sinnema and C. J. Heijnen, "Disturbed Neuroendocrine-Immune Interactions in Chronic Fatigue Syndrome," *Journal of Clinical Endocrinology & Metabolism*, Vol. 85, No. 2, 2000, pp. 692- 696.

[9] S. R. Torres-Harding, M. Sorenson, L. Jason, N. Reynolds, M. Brown, K. Maher and M. A. Fletcher, "The Associations between Basal Salivary Cortisol and Illness Symptomatology in Chronic Fatigue Syndrome," *Journal of Applied Biobehavioral Research*, Vol. 13, No. 3, 2008, pp. 157- 180.

[10] D. J. Torpy, and J. T. Ho, "Corticosteroid-Binding Globulin Gene Polymorphisms: Clinical Implications and Links to Idiopathic Chronic Fatigue Disorders," *Clinical Endocrinology*, Vol. 67, No. 2, 2007, pp. 161-167.

[11] A. K. Smith, P. D. White, E. Aslakson, U. Vollmer-Conna and M. S. Rajeevan, "Polymorphisms in Genes Regulating the HPA Axis Associated with Empirically Delineated Classes of Unexplained Chronic Fatigue," *Pharmacogenomics*, Vol. 7, No. 3, 2006, pp. 387-394.

[12] F. Tanriverdi, Z. Karaca, K. Unluhizarci and F. Kelestimur, "The Hypothalamo Pituitary-Adrenal Axis in Chronic Fatigue Syndrome and Fibromyalgia Syndrome," *Stress*, Vol. 10, No. 1, 2007, pp. 13-25.

[13] J. R. Kerr, R. Petty, B. Burke, J. Gough, D. Fear, L. I. Sinclair, D. L. Mattey, S. C. Richards, J. Montgomery, D. A. Baldwin, P. Kellam, T. J. Harrison, G. E. Griffin, J. Main, D. Enlander, D. J. Nutt and S. T. Holgate, "Gene Expression Subtypes in Patients with Chronic Fatigue Syndrome/Myalgic Encephalomyelitis," *Journal of Infectious Diseases*, Vol. 197, No. 8, 2008, pp. 1171-1184.

[14] S. J. Mathew, J. D. Coplan, R. R. Goetz, A. Feder, S. Greenwald, R. E. Dahl, N. D. Ryan, J. J. Mann and M. M. Weissman, "Differentiating Depressed Adolescent 24 h Cortisol Secretion in Light of their Adult Clinical Outcome," *Neuropsychopharmacology*, Vol. 28, No. 7, 2003, pp. 1336-1343.

[15] T. Miike, A. Tomoda, T. Jhodoi, N. Iwatani and H. Mabe, "Learning and Memorization Impairment in Childhood Chronic Fatigue Syndrome Manifesting as School Phobia in Japan," *Brain and Development*, Vol. 26, 2004, pp. 442-447.

[16] T. Y. Segal, P. C. Hindmarsh and R. M. Viner, "Disturbed Adrenal Function in Adolescents with Chronic Fatigue Syndrome," *Journal of Pediatric Endocrinology & Metabolism*, Vol. 18, No. 3, 2005, pp. 295-301.

[17] M. Maes, I. Mihaylova, M. Kubera and E. Bosmans, "Not in the Mind but in the Cell: Increased Production of Cyclo-Oxygenase-2 and Inducible NO Synthase in Chronic Fatigue Syndrome," *Neuro Endocrinology Letters*, Vol. 28, No. 4, 2007, pp. 463-469.

[18] K. de Brosscher, W. V. Berghe and G. Haegeman, "The Interplay between the Glucocorticoid Receptor and Nuclear Factor-kb or Activator Protein-1: Molecular Mechanisms for Gene Repression," *Endocrine Reviews*, Vol. 24, No. 4, 2003, pp. 488-522.

[19] M. Phillip, M. Aviram, E. Leiberman, Z. Zadik, Y. Giat, J.


 

Levy and A. Tal, "Integrated Plasma Cortisol Conc-Entration in Children with Asthma Receiving Long-Term Inhaled Corticosteroids," *Pediatric Pulmonology*, Vol. 12, No. 2, 1992, pp. 84-89.

[20] M. Ter Wolbeek, L. J. P. van Doornen, A. Kavelaars, E. M. van de Putte, M. Schedlowski and C. J. Heijnen, "Longitudinal Analysis of Pro- and Anti-Inflammatory Cytokine Production in Severely Fatigued Adolescents," *Brain, Behavior, and Immunity*, Vol. 21, No. 8, 2007, pp. 1063- 1074.

[21] D. Kovalovsky, D. Refojo, F. Holsboer and E. Arzt, "Molecular Mechanisms and Th1/Th2 Pathways in Corticosteroid Regulation of Cytokine Production," *Journal of Neuroimmunology*, Vol. 109, No. 1, 2000, pp. 23-29.

[22] K. de Bosscher, M. L. Schmitz, W. Vanden Berghe, S. Plaisance, W. Fiers and G. Haegeman, "Glucocorticoid-Mediated Repression of Nuclear Factor-Kappab-Dependent Transcription Involves Direct Interference with Transactivation," *Proceedings of the National Academy of Science USA*, Vol. 94, No. 25, 1997, pp. 13504-13509.

[23] C. M. McCormick and I. Z. Mathews, "Adolescent Development, Hypothalamic-Pituitary-Adrenal Function, and Programming of Adult Learning and Memory," *Progress in Neuropsychopharmacology and Biological Psychiatry*, Vol. 34, No. 5, 2009, pp. 756-765.

 