

# A pilot study exploring the role of glucocorticoid receptor variants in primary biliary cirrhosis and primary sclerosing cholangitis

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## ABSTRACT

**Background:** In primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) significant therapeutic effects of glucocorticoids have not been documented. The most important clinical problem in patients with these diseases is fatigue, which is occasionally invalidating. Abnormalities in the hypothalamo-pituitary-adrenal axis have been suggested as a cause of fatigue. Most effects of glucocorticoids are mediated by the glucocorticoid receptor (hGR  $\alpha$ ). Recently a causative role for a splicing variant of the glucocorticoid receptor (hGR  $\beta$ ) has been proposed in glucocorticoid resistance in asthma and ulcerative colitis, whereas another splicing variant (hGR P) might be associated with glucocorticoid-resistant haematological malignancies. The aims of the present pilot study were to assess abnormalities in glucocorticoid receptor expression and to relate these abnormalities to the development of fatigue and to disease activity and severity in autoimmune cholestatic liver disease.

**Methods:** Five fatigued and five nonfatigued patients with PBC or PSC were included, and the results were compared with healthy controls.

**Results:** The expression of hGR P was not different from controls, but hGR  $\beta$  mRNA was significantly increased ( $p=0.02$ ) and hGR  $\alpha$  mRNA decreased ( $p=0.015$ ). There were no significant differences between fatigued and nonfatigued patients. A significant negative correlation between the serum activity of alkaline phosphatase and hGR  $\alpha$  and hGR P mRNA was found.

**Conclusion:** Although there was no relation with fatigue, abnormalities in hGR expression appear to occur in patients with these diseases, and may play a role in its pathophysiology and the poor response to glucocorticoid treatment.

## INTRODUCTION

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are chronic cholestatic liver diseases with a relatively favourable prognosis for most patients.<sup>1,2</sup> Clinically, the most frequent and occasionally invalidating symptom is fatigue. In PBC, the prevalence of fatigue of any severity is around 85%. Although fatigue has not been studied as extensively in PSC, it appears to occur with comparable frequency in patients with this disease.<sup>3,5</sup> There is no correlation with the biochemical or histological severity of the disease.<sup>6</sup> Although several studies have attempted to elucidate the pathophysiological mechanisms causing fatigue in cholestatic liver diseases, these have so far remained unknown.<sup>7</sup> In addition, there have been no reports of drugs or other treatment modalities with a beneficial effect on fatigue. Although the widely used drug ursodeoxycholic acid improves the biochemical abnormalities in these diseases, it usually has no effect on fatigue.<sup>6,8</sup>

Since fatigue is the most important and still an untreatable problem in many patients with these diseases, attempting to elucidate the mechanisms leading to fatigue may be an important step in finding an effective treatment. One of the possible mechanisms is dysfunction of the hypothalamo-pituitary-adrenal axis, a role of which has been implicated in the pathophysiology of chronic fatigue.<sup>9</sup> Since the actions of glucocorticoids are mediated by the intracellular glucocorticoid receptor, this receptor has been studied for defects associated with abnormalities in glucocorticoid function.<sup>10</sup> Previously, three splicing variants of the glucocorticoid receptor have been described. The hGR  $\alpha$  is the active form of the hGR, while hGR  $\beta$  and hGR P are derived by alternative splicing of the original transcript.<sup>11,12</sup>

The hGR P has been reported to increase the activity of hGR  $\alpha$  in several cell lines, and it has been suggested that it may be related to glucocorticoid resistance in haematological malignancies.<sup>12,13</sup> Increased expression of the  $\beta$ -variant of the glucocorticoid receptor (hGR), which is formed by alternative splicing of the hGR gene-transcript and is present in normal human tissues, was associated with glucocorticoid resistance in asthma and ulcerative colitis.<sup>14-19</sup> Although the mechanism causing the increased expression of hGR  $\beta$  is partially unclear, it has been repeatedly found that induction by proinflammatory cytokines may be involved.<sup>20-22</sup> This finding led to the hypothesis that glucocorticoid resistance might be the result of an abnormal inflammatory response.<sup>23</sup> Since an increased production of inflammatory cytokines has also been observed in cholestatic liver diseases, the expression of hGR  $\beta$  might be increased in these diseases.<sup>24-27</sup> In addition, glucocorticoid treatment is not recommended in these diseases since studies assessing the efficacy of glucocorticoids found only modest effects, suggesting that relative glucocorticoid resistance might exist.<sup>28,29</sup> No studies attempting to find a relation between expression of hGR  $\beta$  in chronic inflammatory diseases and fatigue have been reported. We hypothesised that increased expression of hGR  $\beta$  might not only be present in these diseases, but that it might also be associated with fatigue. The present study was performed to determine whether levels of the variants of the hGR in peripheral blood mononuclear leukocytes are different from controls in these cholestatic liver diseases, as well as to assess the relation between hGR expression and fatigue.

## PATIENTS AND METHODS

In the present pilot study five patients with a diagnosis of PBC or PSC without fatigue and five patients with chronic and significant fatigue were included. Sex, age and dose of ursodeoxycholic acid were recorded. Serum activity of aspartate aminotransferase, alkaline phosphatase, total serum bilirubin and total immunoglobulin M were measured as markers of disease severity and activity. Fatigue severity was quantified using a visual analogue scale (VAS) and the Fisk fatigue severity scale (FFSS).<sup>30</sup> The FFSS includes social, cognitive and physical domains, in which these aspects of fatigue are quantified. It has been validated for use in primary biliary cirrhosis.<sup>31</sup> A visual analogue scale was used in order to quantify pruritus. Informed consent was obtained from each patient and the study was approved by the institutional review committee.

### Laboratory techniques

Blood samples were obtained from a group of 12 healthy controls and the 10 patients. To isolate peripheral blood

mononuclear leukocytes, the samples were diluted twofold with saline and layered over Ficoll-Hypaque (Pharmacia, Uppsala, Sweden).

Density gradient centrifugation was performed at 1410 rpm for 30 minutes at room temperature. The peripheral blood mononuclear leukocytes enriched interphase was isolated and washed twice with saline and the final pellet was suspended with saline. RNA was immediately isolated using a high-resolution RNA isolation kit (Roche Diagnostics GmbH, Mannheim, Germany). After RNA elution in 55  $\mu$ l elution buffer the concentration of isolated RNA was measured using a Ribo-Green RNA Quantitation Reagent and Kit (Brunschwig Chemie, Amsterdam, the Netherlands). Using 5  $\mu$ M Random Hexamers and 200 nM Oligo-dt-primers in a first-strand cDNA synthesis kit (Applied Biosystems, Foster City, USA), 800 ng of total RNA was used for reverse transcription reaction of 50  $\mu$ l. Reactions lacking reverse transcriptase were also run to generate controls for assessment of genomic DNA contamination. For the different hGR splice variants 2  $\mu$ l of the resulting cDNA were amplified in real-time PCR assays on the ABI Prism 7700 (ABS, Nieuwerkerk a/d IJssel, the Netherlands) in a total volume of 25  $\mu$ l containing 300 pmol of each primer and 200 pmol probe in a qPCR-core kit (Eurogentec, Liege, Belgium). After an initial denaturation at 95  $^{\circ}$ C for ten minutes, PCR was performed for 42 cycles of denaturation for 15 seconds and annealing for one minute at 60  $^{\circ}$ C. To detect the expression of the hGR splice variants we used the same upstream primer: 5'-TGT TTT GCT CCT GAT CTG A-3', encoding part of exon 6, as well as the same taqman probe: 5'-FAM-TGA CTC TAC CCT GCA TGT ACG AC-TAMRA-3', encoding part of exon 7, for all isoforms. To discriminate hGR  $\alpha$ ,  $\beta$  and P from each other we used specific downstream primers. The sequences of these reverse primers are as follows: rev- $\alpha$ : 5'-TCG GGG AAT TCA ATA CTC A-3', encoding part of exon 9 $\alpha$ , rev- $\beta$ : 5'-TGA GCG CCA AGA TTG T-3', encoding part of exon 9 $\beta$ , and rev-P: 5'-GTT TCT GCC ATA CCT ATT TG-3', encoding part of intron 7. The expression levels were determined relatively by using the expression of the HPRT housekeeping gene (hyoxantine phosphoribosyltransferase with the forward primer (500 pmol): 5'-CAC TGG CAA AAC AAT GCA GAC T-3', the reverse primer (500 pmol): 5'-GTC TGG CTT ATA TCC AAC ACT TCG T-3', and the probe (200 pmol): 5'-FAM-CAA GCT TGC GAC CTT GAC CAT CTT TGG A-TAMRA-3'.

Because of the supposed interactions between the hGR  $\beta$  and hGR P variants with the active hGR  $\alpha$  variant, we calculated the hGR  $\alpha$ /hGR  $\beta$  and the hGR  $\alpha$ /hGR P ratios.

### Statistical analysis

Differences in the expression of variants of hGR mRNA between patients and controls, and differences between fatigued and nonfatigued patients were tested using

Mann-Whitney's nonparametric test for independent samples. Correlations between the severity of fatigue and laboratory values and the hGR expression were tested using Pearson's correlation method. Logarithmic transformations of laboratory values were used. All statistical tests were performed using SPSS version 9.0.

## RESULTS

Ten patients with cholestatic liver disease were included in the study, five of whom complained about fatigue. Seven patients had a diagnosis of PBC and three had been diagnosed with PSC. A summary of patient characteristics is shown in *table 1*.

To assess differences in hGR mRNA levels related to the presence of the disease, we compared the levels of the three variants of the hGR, as well as the hGR  $\alpha$ /hGR P ratio and the hGR  $\alpha$ /hGR  $\beta$  ratio with the levels in a group of healthy controls. These tests resulted in non-significant p values for hGR P and p values of 0.015 for the hGR  $\alpha$  variant and 0.02 for the hGR  $\beta$  variant, with decreased numbers of hGR  $\alpha$  and increased numbers of hGR  $\beta$  mRNA in patients vs controls. In addition, the hGR  $\alpha$ /hGR  $\beta$  ratio was significantly decreased in patients (*table 2*).

Tests for differences between fatigued and nonfatigued patients were performed, and resulted in p values of 0.99 for hGR  $\alpha$ , 0.65 for hGR  $\beta$  and 0.28 for hGR P. No significant correlations were found between GR mRNA levels and quantified fatigue (*table 3*). Finally, correlation testing was performed to find associations between the GR variants and the markers of disease

**Table 2**

*Glucocorticoid receptor mRNA levels, number of copies in 2  $\mu$ l cDNA obtained from a 50  $\mu$ l reverse transcriptase reaction of 800 ng RNA*

RELATIVE NUMBER OF COPIES	MEAN (PATIENTS)	MEAN (CONTROLS)	P VALUE
hGR $\alpha$	41,887	56,025	0.02
hGR $\beta$	69	42	0.02
hGR P	8,889	11,922	0.09
hGR $\alpha$ / hGR P	4.7	5.1	0.19
hGR $\alpha$ / hGR $\beta$	679	1,523	0.001
Total	50,844	67,752	0.02

*hGR = human glucocorticoid receptor.*

severity and activity. A significant, negative correlation was found between the serum alkaline phosphatase activity and hGR  $\alpha$  and hGR P, as well as total hGR mRNA. For the levels of aspartate aminotransferase, bilirubin and immunoglobulin M, no significant correlations were found (*table 4*). *Figure 1* illustrates this relation between alkaline phosphatase and total hGR mRNA.

## DISCUSSION

In the present study we found increased levels of hGR  $\beta$  mRNA and decreased levels of hGR  $\alpha$  mRNA in patients with cholestatic liver disease compared with healthy controls. As a result, the hGR  $\alpha$ /hGR  $\beta$  ratio was significantly decreased. In addition, there was a significant inverse

**Table 1**  
*Patient characteristics*

	ALL PATIENTS	FATIGUE +	FATIGUE -
Male/female	4 / 6	3 / 2	1 / 4
PSC/PBC	3 / 7	3 / 2	0 / 5
Age	58 (40-76)	53 (40-62)	64 (55-76)
UDCA dose (mg/day)	900 (0-1200)	900 (0-1200)	900 (600-1200)
Bilirubin ( $\mu$ mol/l)	15.5 (7-89)	15 (10-89)	16 (7-43)
Alkaline phosphatase (U/l)	171 (72-441)	188 (122-441)	154 (72-427)
Aspartate aminotransferase (U/l)	40 (27-241)	39 (27-241)	40 (30-65)
Immunoglobulin M (g/l)	1.8 (1.1-3.9)	2.8 (1.2-3.9)	1.6 (1.1-2.4)
Visual analogue score for pruritus (cm)	0.6 (0-3.3)	0.9 (0-2.5)	0 (0-3.3)
Visual analogue score for fatigue (cm)	3.15 (0-9.3)	6.2 (4.3-9.3)	0 (0-2.0)
FFSS physical domain	9 (1-32)	24 (10-32)	1 (1-8)
FFSS cognitive domain	4.5 (0-21)	7 (4-21)	2 (0-5)
FFSS social domain	7.5 (0-42)	31 (9-42)	3 (0-6)

*Values are medium (range). PSC = primary sclerosing cholangitis, PBC = primary biliary cirrhosis, FFSS = Fisk fatigue severity scale.*

**Table 3**  
Correlation between hGR mRNA and fatigue

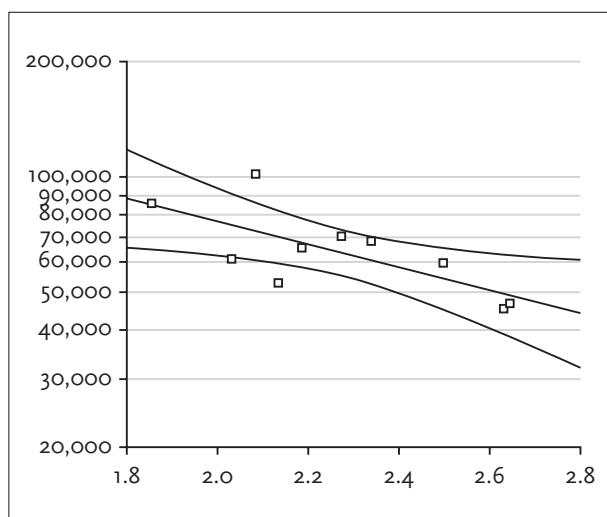
	HGR $\alpha$		HGR $\beta$		HGR P		TOTAL	
	coeff.	P	coeff.	P	coeff.	P	coeff.	p
VAS	-0.089	0.81	-0.29	0.43	0.12	0.75	-0.02	0.96
FFSS physical domain	-0.26	0.47	-0.30	0.40	-0.07	0.86	-0.20	0.58
FFSS cognitive domain	-0.93	0.80	0.21	0.55	0.14	0.71	-0.01	0.97
FFSS social domain	-0.26	0.47	-0.10	0.79	0.15	0.69	-0.12	0.74

Coeff. = coefficient, hGR = human glucocorticoid receptor, VAS = visual analogue scale, FFSS = Fisk fatigue severity scale.

**Table 4**  
Correlation between hGR mRNA and biochemical markers of disease activity and severity

	HGR $\alpha$		HGR $\beta$		HGR P		TOTAL	
	coeff.	P	coeff.	P	coeff.	P	coeff.	p
Total serum bilirubin	-0.38	0.28	0.11	0.76	-0.063	0.86	-0.28	0.43
Alkaline phosphatase	-0.65	0.041	-0.28	0.44	-0.64	0.049	-0.68	0.03
Aspartate aminotransferase	-0.39	0.27	-0.20	0.58	-0.61	0.063	-0.48	0.16
Immunoglobulin M	-0.10	0.78	0.11	0.76	-0.31	0.39	-0.18	0.62

hGR = human glucocorticoid receptor, coeff. = coefficient.



**Figure 1**  
Relation between total GR mRNA and serum activity of alkaline phosphatase

<sup>10</sup>Log(alkaline phosphatase) is shown on the X-axis and total hGR mRNA is shown on the Y-axis.

relation between the hGR  $\alpha$  and hGR P variants and the serum activity of alkaline phosphatase, a routinely used marker of disease activity in PBC and PSC. A correlation between the receptor variants and fatigue was not found. An association between increased levels of hGR  $\beta$  mRNA

and glucocorticoid resistance in asthma and ulcerative colitis has been reported previously.<sup>14-17,20,21</sup> In addition, increased expression of this variant has been observed in patients with hormone resistant nephrotic syndrome, chronic lymphatic leukaemia and nasal polyps.<sup>32-35</sup> Several *in vitro* studies have shown that expression of hGR  $\beta$  can be induced by the inflammatory cytokines Il-2, Il-4, Il-7, Il-8 and TNF- $\alpha$ .<sup>20-22</sup> The increase of hGR  $\beta$  as a result of cytokine exposure correlated with a decrease in glucocorticoid sensitivity in one of these studies.<sup>22</sup> Further, the frequency of a polymorphism associated with increased stability of hGR  $\beta$  mRNA was increased in patients with rheumatoid arthritis. The authors suggested that this could be a cause of glucocorticoid resistance, which is a common problem in this condition.<sup>36</sup> However, despite a significant number of studies reporting it, controversy regarding the negative effects of this variant does still exist, since several other *in vivo* and *in vitro* studies found no effects of the hGR  $\beta$  variant, and the mechanisms responsible for the dominant negative effect are largely unknown.<sup>13,19,37-39</sup> In the present study, levels of hGR  $\beta$  mRNA were much lower than those of the other variants. This does not exclude a role for this variant in causing glucocorticoid resistance, since similar results have been obtained in previous studies reporting quantitative hGR mRNA levels, and it might have several explanations.<sup>14,35</sup> First, mRNA does not necessarily correspond with protein levels, and hGR  $\beta$  protein levels could better reflect the

mechanism leading to glucocorticoid resistance, although a previous paper reported very low or undetectable hGR  $\beta$  protein levels in the presence of similar mRNA levels as in the present study.<sup>19</sup> Second, in the present study blood samples were studied, whereas the disease occurs primarily in the liver. Thus, studying blood samples may have diluted the hypothetically higher intrahepatic hGR  $\beta$  levels. Third, significantly lower levels of hGR  $\beta$  compared with total hGR levels might be needed to induce glucocorticoid resistance, although the mechanism responsible for this presumed dominant negative effect of the hGR  $\beta$  variant is unclear.<sup>23</sup>

Thus, the increased levels of hGR  $\beta$  mRNA in patients in the present study compared with healthy controls may have been caused by the inflammatory nature of these liver diseases, and it can be hypothesised that the modest efficacy of glucocorticoid treatment in these diseases could be caused by an increased expression of hGR  $\beta$ .<sup>28,29</sup> Another explanation for the increased hGR  $\beta$  mRNA levels, in parallel to the hypothesis by Derijk *et al.* in rheumatoid arthritis, is that patients with increased hGR  $\beta$  expression are at increased risk of developing autoimmune diseases due to resistance to endogenous glucocorticoids.<sup>36</sup>

An inverse correlation between the levels of hGR  $\alpha$  and hGR P, and therefore total hGR mRNA, and the serum activity of alkaline phosphatase was found, whereas we found no correlation with the other markers of disease activity or severity. Such a relation with disease activity has been reported previously in patients with systemic lupus erythematosus, where glucocorticoid sensitivity correlated with total hGR levels.<sup>40</sup> In patients with rheumatoid arthritis, hGR levels were decreased in patients compared with controls.<sup>41,42</sup> These studies suggest that, in addition to hGR  $\beta$  expression, hGR  $\alpha$  and hGR P levels might also play a role in determining disease activity and glucocorticoid resistance.

The most important limitation of the present study is its small sample size, and therefore confirmation of the results of the present study in a subsequent larger study would be valuable. In addition, the present study design does not allow conclusions with regard to the cause of abnormalities in hGR expression.

In conclusion, we found increased expression of hGR  $\beta$  mRNA in patients with cholestatic liver diseases as compared with controls and an inverse relation between the hGR  $\alpha$  and hGR P mRNA and the serum activity of alkaline phosphatase. This suggests that the glucocorticoid receptor might be involved in the pathogenesis of these diseases as well as in their relative glucocorticoid resistance. Since we found no correlation with fatigue, it seems unlikely that differential expression of hGR variants plays a major role in the aetiology of this distressing symptom of PBC and PSC.

## REFERENCES

1. Hoogstraten HJF van, Hansen BE, Buuren HR van, Kate FJW ten, Berge Henegouwen GP van, et al. Prognostic factors and long-term effects of ursodeoxycholic acid on liver biochemical parameters in patients with primary biliary cirrhosis. *J Hepatol* 1999;31:256-62.
2. Broome U, Olsson R, Loof L, Bodemar G, Hultcrantz R, Danielsson A, et al. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. *Gut* 1996;38(4):610-5.
3. Huet PM, Deslauriers J, Tran A, Faucher C, Charbonneau J. Impact of fatigue on the quality of life of patients with primary biliary cirrhosis. *Am J Gastroenterol* 2000;95(3):760-7.
4. Wiesner RH, Grambsch PM, Dickson ER, Ludwig J, MacCarty RL, Hunter EB, et al. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. *Hepatology* 1989;10(4):430-6.
5. Wiesner RH, LaRusso NF, Ludwig J, Dickson ER. Comparison of the clinicopathologic features of primary sclerosing cholangitis and primary biliary cirrhosis. *Gastroenterology* 1985;88(1 Pt1):108-14.
6. Cauch-Dudek K, Abbey S, Stewart DE, Heathcote EJ. Fatigue in primary biliary cirrhosis. *Gut* 1998;43(5):705-10.
7. Bergasa NV, Mehlman JK, Jones EA. Pruritus and fatigue in primary biliary cirrhosis. *Baillieres Best Pract Res Clin Gastroenterol* 2000;14(4):643-55.
8. Gluud C, Christensen E. Ursodeoxycholic acid for primary biliary cirrhosis. *Cochrane Database Syst Rev* 2002(1):CD000551.
9. Kavelaars A, Kuis W, Knook L, Sinnema G, Heijnen CJ. Disturbed neuro-endocrine-immune interactions in chronic fatigue syndrome. *J Clin Endocrinol Metab* 2000;85(2):692-6.
10. Beato M, Truss M, Chavez S. Control of transcription by steroid hormones. *Ann NY Acad Sci* 1996;784:93-123.
11. Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, et al. Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* 1985;318(6047):635-41.
12. Krett NL, Pillay S, Moalli PA, Greipp PR, Rosen ST. A variant glucocorticoid receptor messenger RNA is expressed in multiple myeloma patients. *Cancer Res* 1995;55(13):2727-9.
13. Lange P de, Segeren CM, Koper JW, Wiemer E, Sonneveld P, Brinkmann AO, et al. Expression in hematological malignancies of a glucocorticoid receptor splice variant that augments glucocorticoid receptor-mediated effects in transfected cells. *Cancer Res* 2001;61(10):3937-41.
14. Honda M, Orii F, Ayabe T, Imai S, Ashida T, Obara T, et al. Expression of glucocorticoid receptor beta in lymphocytes of patients with glucocorticoid-resistant ulcerative colitis. *Gastroenterology* 2000;118(5):859-66.
15. Christodoulouopoulos P, Leung DY, Elliott MW, Hogg JC, Muro S, Toda M, et al. Increased number of glucocorticoid receptor-beta-expressing cells in the airways in fatal asthma [In Process Citation]. *J Allergy Clin Immunol* 2000;106(3):479-84.
16. Hamid QA, Wenzel SE, Hauk PJ, Tsigopoulos A, Wallaert B, Lafitte JJ, et al. Increased glucocorticoid receptor beta in airway cells of glucocorticoid-insensitive asthma. *Am J Respir Crit Care Med* 1999;159(5 Pt1):1600-4.
17. Sousa AR, Lane SJ, Cidlowski JA, Staynov DZ, Lee TH. Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform. *J Allergy Clin Immunol* 2000;105(5):943-50.

18. Oakley RH, Webster JC, Sar M, Parker CR Jr, Cidlowski JA. Expression and subcellular distribution of the beta-isoform of the human glucocorticoid receptor. *Endocrinology* 1997;138(11):5028-38.
19. Pujols L, Mullol J, Roca-Ferrer J, Torrego A, Xaubet A, Cidlowski JA, et al. Expression of glucocorticoid receptor alpha- and beta-isoforms in human cells and tissues. *Am J Physiol Cell Physiol* 2002;283(4):C1324-31.
20. Leung DYM, Hamid Q, Vottero A, Szeffler SJ, Surs W, Minshall E, et al. Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. *J Exp Med* 1997;186(9):1567-74.
21. Orii F, Ashida T, Nomura M, Maemoto A, Fujiki T, Ayabe T, et al. Quantitative analysis for human glucocorticoid receptor alpha/beta mRNA in IBD. *Biochem Biophys Res Commun* 2002;296(5):1286-94.
22. Webster JC, Oakley RH, Jewell CM, Cidlowski JA. Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: a mechanism for the generation of glucocorticoid resistance. *Proc Natl Acad Sci U S A* 2001;98(12):6865-70.
23. Vottero A, Chrousos GP. Glucocorticoid Receptor beta: View I. *Trends Endocrinol Metab* 1999;10(8):333-8.
24. Harada K, Water J van de, Leung PS, Coppel RL, Ansari A, Nakanuma Y, et al. In situ nucleic acid hybridization of cytokines in primary biliary cirrhosis: predominance of the Th1 subset. *Hepatology* 1997;25(4):791-6.
25. Yamashiki M, Kosaka Y, Nishimura A, Watanabe S, Nomoto M, Ichida F. Analysis of serum cytokine levels in primary biliary cirrhosis patients and healthy adults. *J Clin Lab Anal* 1998;12(2):77-82.
26. Lohr HF, Schlaak JF, Gerken G, Fleischer B, Dienes HP, Meyer zum Buschenfelde KH. Phenotypical analysis and cytokine release of liver-infiltrating and peripheral blood T lymphocytes from patients with chronic hepatitis of different etiology. *Liver* 1994;14(3):161-6.
27. Miller LC, Kaplan MM. Serum interleukin-2 and tumor necrosis factor-alpha in primary biliary cirrhosis: decrease by colchicine and relationship to HLA-DR4. *Am J Gastroenterol* 1992;87(4):465-70.
28. Leuschner M, Maier KP, Schlichting J, Strahl S, Herrmann G, Dahm HH, et al. Oral budesonide and ursodeoxycholic acid for treatment of primary biliary cirrhosis: results of a prospective double-blind trial. *Gastroenterology* 1999;117(4):918-25.
29. Hoogstraten HJ van, Vleggaar FP, Boland GJ, Steenberg W van, Griffioen P, Hop WC, et al. Budesonide or prednisone in combination with ursodeoxycholic acid in primary sclerosing cholangitis: a randomized double-blind pilot study. Belgian-Dutch PSC Study Group [see comments]. *Am J Gastroenterol* 2000;95(8):2015-22.
30. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. *Clin Infect Dis* 1994;18(Suppl 1):S79-83.
31. Prince MI, James OF, Holland NP, Jones DE. Validation of a fatigue impact score in primary biliary cirrhosis: towards a standard for clinical and trial use. *J Hepatol* 2000;32(3):368-73.
32. Liu Y, Song L, Li B. The expression of glucocorticoid receptor beta messenger RNA in peripheral white blood cells of hormone-resistant nephrotic syndrome patients. *Zhonghua Nei Ke Za Zhi* 2001;40(11):725-8.
33. Shahidi H, Vottero A, Stratakis CA, Taymans SE, Karl M, Longui CA, et al. Imbalanced expression of the glucocorticoid receptor isoforms in cultured lymphocytes from a patient with systemic glucocorticoid resistance and chronic lymphocytic leukemia. *Biochem Biophys Res Commun* 1999;254(3):559-65.
34. Hamilos DL, Leung DY, Muro S, Kahn AM, Hamilos SS, Thawley SE, et al. GRbeta expression in nasal polyp inflammatory cells and its relationship to the anti-inflammatory effects of intranasal fluticasone. *J Allergy Clin Immunol* 2001;108(1):59-68.
35. Pujols L, Mullol J, Benitez P, Torrego A, Xaubet A, Haro J de, et al. Expression of the glucocorticoid receptor alpha and beta isoforms in human nasal mucosa and polyp epithelial cells. *Respir Med* 2003;97(1):90-6.
36. Derijk RH, Schaaf MJ, Turner G, Datson NA, Vreugdenhil E, Cidlowski J, et al. A human glucocorticoid receptor gene variant that increases the stability of the glucocorticoid receptor beta-isoform mRNA is associated with rheumatoid arthritis. *J Rheumatol* 2001;28(11):2383-8.
37. Lange P de, Koper JW, Brinkmann AO, Jong FH de, Lamberts SW. Natural variants of the beta isoform of the human glucocorticoid receptor do not alter sensitivity to glucocorticoids. *Mol Cell Endocrinol* 1999;153(1-2):163-8.
38. Hecht K, Carlstedt-Duke J, Stierna P, Gustafsson J, Bronnegard M, Wikstrom AC. Evidence that the beta-isoform of the human glucocorticoid receptor does not act as a physiologically significant repressor. *J Biol Chem* 1997;272(42):26659-64.
39. Carlstedt-Duke J. Glucocorticoid Receptor beta: View II. *Trends Endocrinol Metab* 1999;10(8):339-42.
40. Tanaka H, Akama H, Ichikawa Y, Makino I, Homma M. Glucocorticoid receptor in patients with lupus nephritis: relationship between receptor levels in mononuclear leukocytes and effect of glucocorticoid therapy. *J Rheumatol* 1992;19(6):878-83.
41. Schlaghecke R, Kornely E, Wollenhaupt J, Specker C. Glucocorticoid receptors in rheumatoid arthritis. *Arthritis Rheum* 1992;35(7):740-4.
42. Huisman AM, Everdingen AA van, Wenting MJ, Siewertsz Van Reesema DR, Lafeber FP, Jacobs JW, et al. Glucocorticoid receptor downregulation in early diagnosed rheumatoid arthritis. *Ann NY Acad Sci* 2002;966:64-7.