**Original Article**

**Pharmacogenetic Contribution of Leptin Gene Polymorphism in Cutaneous T-Cell Lymphoma**

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**Abstract:** Leptin has recently attracted more attention due to its specific effects in the pathogenesis of malignancies. The aim of this study was to investigate the possible association between variants of -2548 G/A polymorphism in leptin (LEP) gene and cutaneous T-cell lymphomas (CTCL), with respect to the treatment responsiveness. A sample of 91 patients with CTCL was compared to 198 control individuals. The CTCL men with AG and/or GG genotype were more likely to receive the topical steroids treatment (odds ratio 7.88, 95% confidential interval 1.51-41.04) when compared to AA patients. Our data supports the possible involvement of LEP-2548G/A polymorphism in CTCL treatment responsiveness and thus might provide important information for individual therapy tailoring.

**Key Words:** Leptin, gene polymorphism, pharmacogenetics, cutaneous T-cell lymphoma

**Introduction**

Mycosis fungoides and its leukemic variant, Sezary syndrome, represent the most common forms of cutaneous T cell lymphoma (CTCL). These disorders are characterized by the progressive accumulation of cells that resemble activated/memory CD4+ T cells. Unlike their normal counterparts, these malignant lymphocytes have prolonged life spans and are resistant to dying following treatment with most chemotherapeutic agents [1]. According to other authors, the usually protracted and indolent course of CTCL is consistent with an accumulation of lymphocytes rather than being a true proliferative disorder, perhaps as the result of defective lymphocyte apoptosis [2].

Leptin is an adipocyte-derived hormone that acts as a major regulator for food intake and energy homeostasis. Leptin deficiency or resistance can result in profound obesity, diabetes, and infertility in humans. Broad effects on reproduction, hematopoiesis, angiogenesis, blood pressure, bone mass, lymphoid organ homeostasis, and T lymphocyte systems have been reported [3]. Leptin has direct effects on T cell-mediated immunity. This adipokine modulates T-cell proliferation, increasing the proliferation of naive T cells while reducing the proliferation of memory T cells, and protects T lymphocytes from apoptosis. Leptin modulates T cell-derived cytokine production and increases expression of the activation markers CD25 and CD71 in CD4+ and CD8+ cells [4]. It favours Th1 responses while inhibiting the secretion of Th2 cytokines [5].

Exogenous leptin was observed to restore in vitro T cell proliferation and cytokine synthesis in patients with common variable immunodeficiency syndrome [6]. In three morbidly obese children with congenital deficiency in leptin (the frame shift mutation $\Delta$G133 in LEP gene), their leptin deficiency was observed to be associated with a reduced number of circulating CD4+ T cells and impaired T cell proliferation and cytokine release, all of which were reversed by recombinant human leptin administration [7].

Leptin also promotes lymphocyte survival in vitro by suppressing apoptosis in experimental animal models. CD4+ T cells isolated from
leptin receptor (ObR)-deficient mice displayed a reduced proliferative response, compared with normal controls [8]. The expression of ObR was found to be up-regulated following T-cell activation in mice.

In humans, the -2548G/A polymorphism in leptin (LEP) gene was observed to influence leptin gene expression, possibly at the transcriptional level: AA subjects had higher levels of serum leptin than GA/GG subjects did. Adipose tissue leptin secretion rate in AA subjects was observed to be twice as high as in GA/GG subjects [9].

Based on these facts, aim of the study was to analyse possible association of LEP-2548G/A polymorphism and CTCL and, to analyse possible pharmacogenetic association of LEP-2548G/A polymorphism in CTCL patients.

Material and Methods

Subjects

91 patients with CTCL, diagnosed and treated at the 1st Department of Dermatology of St Ann’s Faculty Hospital Brno (55 men and 36 women, median age 63, range 26-101 years) were compared to 198 controls (133 men and 65 women, median age 58, age range 26-80) without personal or family history of skin diseases and without personal history of malignancy. The patients with CTCL were classified according to Tumor Node Metastasis (TNM) Classification for Cutaneous T-Cell Lymphoma according to Kashani-Sabet et al., 2001 [10].

In CTCL men, 53% (N=29) were treated by topical steroids, 22% (N=12) in combination with phototherapy, 3% (N=1) together with retinoids, 2% (N=1) together with retinoids and phototherapy. The CTCL women were treated by topical steroids in 61% (N=22), 25% (N=9) in combination with phototherapy, 6% (N=2) together with retinoids. Phototherapy was indicated in 53% (N=29) of CTCL men and in 50% (N=18) of CTCL women. No information about genotyping results was given to dermatologists in the period of therapy decision.

Genotyping for LEP-2548G/A

The common non-coding polymorphism LEP-2548G/A within the promoter of leptin gene (dbSNP ID rs7799039) was investigated as previously described [11]. Genotype distributions and allele frequencies in LEP-2548G/A polymorphisms were detected by PCR method with restriction analysis and compared between these two groups. Forward primer 5’-TTTCTGTAATTTCCCGTGAG-3’ and reverse 5’-AAAGCAAAGACAGGATAAAAA-3’ primer were used. Restriction analysis was performed with Hin6 I (G↓CGC) at 37°C for 4 hours. The products were separated on 2% agarose gel Serva and stained with ethidium bromide.

This study was approved by the Committee for Ethics of Medical Experiments on Human Subjects, Faculty of Medicine, Masaryk University, Brno (no. 64/93, 1993) and was performed in adherence to the Declaration of Helsinki Guidelines. Participants gave their written informed consent which has been archived.

Statistical Analyses

In all groups of subjects, distributions of genotype and allelic frequencies and their differences were calculated using χ² tests. Consistency of genotype frequencies with the Hardy-Weinberg equilibrium was tested using a χ² test on a contingency table of observed versus predicted genotype frequencies.

Odds ratio (OR) and 95% confidence interval were calculated to estimate the risks related to detected polymorphisms. To calculate the significance of OR, Fisher’s exact test was used. The corrected P values for multiple comparisons (Pcorr) were calculated by Holm’s test when necessary. The program package Statistica v. 6.0 (Statsoft Inc., Tulsa, OK) was used.

Results

Three genotypes were detected: GG (181/181 bp), AA (242/242 bp) and GA (181/242 bp, Figure 1). No significant differences either in genotype distribution or in allelic frequency of LEP-2548G/A polymorphism were observed between CTCL patients and the control subjects. The allele G frequency was 0.555 in CTCL men, 0.442 in CTCL women, 0.538 in control men and 0.569 in control women. Similarly, no significant differences were
proved among patients with different clinical stage of the CTCL (Figure 2).

On the other hand, significant differences were observed both in genotype distribution (Pg=0.01) and allelic frequency (Pa=0.003) between CTCL men with and without topical therapy (steroids) (Figure 3). Odds ratio for AG+GG genotypes was calculated for CTCL men with topical therapy as 7.88, 95% confidential interval 1.51-41.04, Pcorr=0.02. Thus, CTCL men with GG and GA genotypes were significantly more frequently treated by topical steroids compared to CTCL men with genotype AA. All patients enrolled in the study were treated by one physician and they were in complete remission when DNA sampling was performed. Because the study had a retrospective schedule, it was not possible to compare possible different responsiveness of the genotypes to the different treatment.

Finally, a marginally significant difference in allelic frequency of the polymorphism was proved between CTCL women with and without phototherapy (PUVA or SUP, Pa=0.09). Again, more CTCL women with GG and/or GA genotypes were treated by phototherapy compared to AA carriers. Although the frequency of G allele was higher in CTCL women treated with phototherapy compared to those which were treated alternatively (0.694 to 0.555), the difference was not significant at 5% level. But, so far, only 36 women with CTCL had been evaluated (Figure 4).

Discussion

We have not found comparable studies in the literature focusing on possible leptin gene variability in CTCL and/or the pharmacogenetic aspects of leptin gene in relation to CTCL treatment responsiveness. As described
Figure 3 Difference in genotype distribution of LEP-2485G/A polymorphisms and topical treatment in CTCL patients-men. Pg= probability of differences in genotype distribution; Pa= probability of difference in allelic frequencies.

previously by Willet et al in 2005 [12], A allele carriers of LEP-2548 were more likely to develop nonHodgkin lymphomas than G allele carriers, suggesting an important role of the LEP-2548 G/A polymorphism in the development of malignant lymphomas. Furthermore, the AA genotype of LEP-2548G/A polymorphism was associated with lung

Figure 4 Difference in genotype distribution of LEP-2485G/A polymorphisms and phototherapy treatment in CTCL patients-women
administration of exogenous glucocorticoids had been observed after systemic leptin, although increased circulating leptin does not seem to act as a susceptibility gene for CTCL. However, in our highly homogeneous Czech Caucasian population studied, variability in this polymorphism was associated with significantly impaired responsiveness to different treatment modalities. The CTCL patients with expected lower plasma leptin levels (i.e. GA and/or GG genotype carriers) were more likely to be treated by topical steroids (CTCL men) or by phototherapy (CTCL women).

The local steroid does not influence circulating leptin, although increased circulating leptin levels had been observed after systemic administration of exogenous glucocorticoids [16]. A very complicated intracranial interplay of skin leptin, cortisol and testosterone can be expected.

The probable basis for T-cell disease like CTCL treatment produced by PUVA is through selective cytotoxic effects on clonal T-lymphocyte populations that are concentrated in diseased skin [17]. The differential cytotoxic effects of ultraviolet A light (UVA) on an established T cell line treated with female and male sex hormones were investigated. CD4+ Jurkat T cells were treated with 17beta-estradiol (EST) or testosterone (TE). EST alone, without UVA, was found to enhance Jurkat T cell survival. However, EST exhibited a dose-related cytotoxicity in the presence of UVA which was not observed for TE. Thus, EST and TE have differential effects on UVA-induced cytotoxicity in Jurkat T-lymphocyte [18]. This suggests that women may be more susceptible to the harmful effects of PUVA than men.

To conclude, our results support the hypothesis that LEP-2548G/A polymorphism might be involved in the pharmacogenetic aspects of CTCL. The clinical importance of this finding has to be further evaluated in sufficiently long follow-up studies. Nevertheless, our data might provide important information for individual therapeutic tailoring.

References


