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ORIGINAL PAPER

Analysis of a functional serotonin transporter promoter polymorphism in psoriasis vulgaris

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Abstract Serotonin is a monoamine acting as a neuromediator in the central and peripheral nervous system. Recently, serotonin has also been shown to influence T- and B-cell function. The serotonin transporter is central in the regulation of the serotonergic system and widely expressed on cells of the immune system. A functional length polymorphism in the promoter of the serotonin transporter gene (5-HTTLPR) has been implicated in the genetic background of depression. Psoriasis is a complex disease with a polygenetic inheritance. In light of the role of T-cell mediated inflammation in psoriasis and the increased prevalence of depression in psoriatic patients, we analyzed the 5-HTTLPR polymorphism in 309 patients with psoriasis vulgaris and 315 healthy control individuals. No significant differences in genotype distribution and allele frequencies were found. There was also no difference in the score of the Hamilton Rating Scale for Depression in patients with psoriasis ($n = 137$) characterized by carriage of different 5-HTTLPR genotypes. These findings argue against a major contribution of the 5-HTTLPR polymorphism to psoriasis susceptibility and the occurrence of depressive symptoms among psoriatic patients.

Keywords Psoriasis · Genetics · Depression · Serotonin · 5-HTTLPR

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Introduction

Psoriasis vulgaris is a complex disease with multifactorial inheritance affecting approximately 2–3% of the population. It is believed that cells of the innate and acquired immune system, among them dendritic cells and T-cells, collaborate to produce a pattern of cutaneous angiogenesis, inflammation and epidermal changes that underlie the formation of sharply demarcated erythematosquamous plaques, the typical clinical presentation of psoriasis [3]. Because psoriasis is a chronic, often widespread and stigmatizing condition that may extensively reduce the quality of life of affected patients, the observed increased prevalence of depressive symptoms among patients compared with healthy individuals [1] can possibly be regarded as secondary to the manifestation of a significant medical condition. It is also not excluded that the psoriatic inflammatory process plays a direct role via the constantly increased production of mediators that may also influence mental functions or that psoriasis and depression share common risk factors at the genetic level. On the other hand, it has been suggested that stress can aggravate skin inflammation in patients with psoriasis, indicating another level of possible interactions between psychological symptoms and skin inflammation.

The serotonergic system is a promising candidate for establishing a neuroimmunological link between psoriatic skin disease and psychological symptoms. In the central

nervous system, serotonergic neurons project to almost all regions of the brain and influence many physiological functions such as pain, sleep, motor functions, neuroendocrine circuits and the mood. The main producers of 5-HT are enterochromaffin cells of the gut and the serotonergic neurons of the brain, but 5-HT is stored in many cell types outside the central nervous system including platelets, lymphocytes, monocytes/macrophages and dendritic cells [6]. Recently, it has been shown that lymphocytes also synthesize and excrete 5-HT [17]. The production of 5-HT is increased at sites of inflammation and several functional activities of 5-HT have been revealed that play an important role in chronic inflammatory diseases such as psoriasis including the activation of mast cell migration and adhesion [9], the stimulation of cytokine release from dendritic cells [8] and the mediation of dendritic cell–T-cell interactions [17].

The expression and function of the serotonin transporter (5-HTT), which is responsible for the uptake of 5-HT into cells thereby removing 5-HT from the extracellular space, is a key parameter in the regulation of 5-HT-mediated effects in the central nervous system and the immune system [15]. The expression of 5-HTT is controlled by a repeat length polymorphism in the 5-HTT linked polymorphic region (5-HTTLPR). The long (high activity) allele of this polymorphism is associated with a higher number of 5-HTT molecules on lymphocytes [10]. The biological relevance of the 5-HTTLPR polymorphism is underscored by the recent finding of an association with primary pulmonary hypertension [5], and the observed influence on the development of depressive symptoms in individuals afflicted by a negative life event as well as on suicidal behavior [11].

In this study, we used a case–control design to obtain first evidence whether the 5-HTTLPR polymorphism is associated with psoriasis. We also compared depressive symptoms in psoriatic patients characterized by carriage of different 5-HTTLPR genotypes.

Subjects and methods

Study participants

Unrelated German Caucasian patients with an established diagnosis of chronic plaque-type psoriasis were enrolled from the Department of Dermatology, University Hospital Göttingen (309 individuals, 127 female, 182 male, age 46.0 ± 14.3 years (mean ± SD), positive family history in 140 cases). The control group consisted of healthy unrelated German Caucasians without a personal or family history of psoriasis (315 individuals, 146 female, 169 male, age 35.6 ± 12.1 years (mean ± SD)) recruited from the blood donor registry of the Department of Transfusion Medicine, University Hospital Göttingen and from local health-care personnel as described previously [20].

To assess the presence of depressive symptoms, the Hamilton Rating Scale for Depression (HAM-D) was performed in the subgroup of 137 patients of patients aged between 18 and 60 years who were recruited between May 2004 and November 2005. The HAM-D is a validated scale that is routinely used for the assessment of depression and rates the severity of 21 symptoms typical of depression, from which a sum score is calculated. The HAM-D can take on values between 0 and 67, and the score of >8, >14 and >19 is considered to reflect a mild, moderate and severe depression, respectively.

The study was approved by the Institutional Review Board of the Medical Faculty of the University of Göttingen, and informed consent was obtained from all participants.

Genotyping of the 5-HTT promoter polymorphism (5-HTTLPR)

Genomic DNA was prepared from peripheral blood mononuclear cells according to the published protocols [13].

The 5-HTT linked polymorphic region (5-HTTLPR) of the gene encoding SERT (*SCL6A4*) located on chromosome 17q11.1–12 was amplified by the flanking oligonucleotide primers 5′-GAG GGA CTG AGC TGG ACA AC-3′ and 5′-GCA GCA GAC AAC TGT GTT CAT C-3′. The short (s) allele is 44 bp shorter than the long (l) allele due to a 44 bp deletion [7]. PCR was performed in a final solution of 20 µl containing between 200 and 600 ng genomic DNA, 0.2 mM dNTP, 0.2 mM deaza-GTP, 0.5 µM of each primer, 2% dimethylsulfoxide, 2.5 U *Taq* polymerase in PCR buffer (Promega).

After an initial denaturation for 3 min at 95°C, 43 cycles of denaturing at 95°C for 30 s, annealing for 30 s and extension at 72°C for 1 min were performed, followed by a final extension at 72°C for 10 min. Annealing was performed at 65°C for the first four cycles, 64°C for the subsequent four cycles and 63°C during the final 35 cycles.

PCR products were visualized on an agarose gel containing ethidium bromide. The variants were detected according to their size relative to a 100 bp DNA ladder (Fig. 1).

Statistical analysis

To determine whether the genotype frequencies confirmed with Hardy–Weinberg equilibrium, the equivalence test proposed by Wellek was used (5% test level) with $\varepsilon = 0.1$ [24]. Odds ratios (ORs) and exact 95% confidence intervals (CIs) were calculated to compare the genotype frequencies. Differences in genotype frequencies were investigated using an exact Cochrane–Armitage trend test. Differences

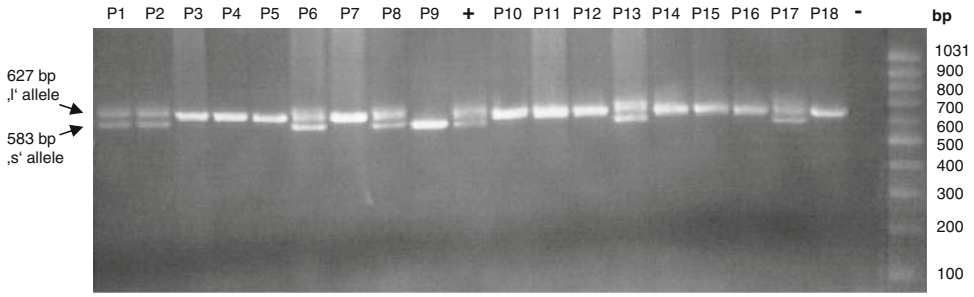

Fig. 1 Example for genotyping of 5-HTTLPR in 18 probands. + Positive control (genotype *ls*), − negative control, *lanes* P1, P2, P6, P8, P13, P17 genotype *ls*, *lane* P9 genotype *ss*, remaining probands genotype *ll*

Table 1 5-HTTLPR genotype distribution

	Psoriasis patients	Control group		
	n = 309	*n* = 315		
Genotype	*n* (%)	*n* (%)	OR	p^a
ll	104 (33.7)	108 (34.3)		
ls	144 (46.6)	155 (49.2)	1.08 (0.86–1.36)	
ss	61 (19.7)	52 (16.5)	1.17 (0.74–1.85)	0.496
	Type 1 psoriasis [b]	Type 2 psoriasis [c]		
	n = 224	*n* = 85		
Genotype	*n* (%)	*n* (%)		
ll	78 (34.8)	26 (30.6)		
ls	107 (47.8)	37 (43.5)	1.28 (0.89–1.84)	
ss	39 (17.4)	22 (25.9)	1.63 (0.79–3.39)	0.184

[a] Two-sided exact *p* from Cochrane-Armitage trend-test

[b] Type 1 psoriasis: age at onset <40 years

[c] Type 2 psoriasis: age at onset ≥40 years

in the HAM-D among different genotypes were compared using the non-parametric Mann–Whitney *U* test. The overall significance level was set to $p = 0.05$.

Results

The absolute and relative genotype frequencies of the 5-HTTLPR polymorphism are given in Table 1. The genotype frequencies in cases and controls were in Hardy–Weinberg equilibrium. The genotype frequencies were similar among patients with psoriasis vulgaris and healthy controls. In addition, there was no difference in genotype frequencies between patients with early onset- (type I; age at onset <40 years) and late onset psoriasis (type II; age at onset ≥40 years).

HAM-D was 5.1 ± 5.3 in psoriasis patients (mean ± SD), with 20% of patients suffering from an at least mild depression (HAM-D >8). HAM-D was significantly higher in female psoriasis patients compared with male patients (7.2 ± 5.9 vs. 3.7 ± 4.5, $p = 0.0001$).

To assess whether allelic variants of 5-HTTLPR polymorphism may be associated with depressive symptoms in psoriatic patients, the HAM-D was compared between patients with psoriasis ($n = 137$) characterized by carriage of 0, 1 or 2 s alleles, corresponding to the ll, ls and ss genotypes, respectively. Mean and SD as well as median and 25 and 75% percentiles of the HAM-D were similar in these subgroups (Table 2). As there was a significant difference in the HAM-D between male and female patients, HAM-D values were also compared separately in male and female carriers of 5-HTTLPR genotypes. No significant differences were observed in these subgroups (data not shown).

Discussion

To the best of our knowledge, this is the first study to investigate a possible relationship between a functionally relevant polymorphism in the promoter of the gene encoding the serotonin transporter and an inflammatory skin disorder. The control group showed genotype frequencies of the 5-HTTLPR polymorphism similar to those reported in an

Table 2 HAM-D in psoriasis patients according to 5-HTTLPR genotype

HAM-D	5-HTTLPR genotype		
	ll (*n* = 48)	ls (*n* = 67)	ss (*n* = 22)
Mean (SD)	5.4 (5.7)	5.3 (5.3)	4.7 (5.4)
Median (range)	4 (0;25)	4 (0;21)	4 (0;26)
25. percentile	1	1	2
75. percentile	8	7.5	7

earlier study with healthy German individuals [12]. We found no association of the 5-HTTLPR polymorphism with psoriasis vulgaris, and the obtained 95% CIs indicate that major effects are unlikely.

Depressive symptoms have been reported to be more prevalent among psoriatic patients than among healthy individuals [1]. However, it has not been established whether depression is exclusively a consequence of the impaired quality of life and stigmatization of the disease or whether neuroimmunological mechanisms may also contribute to depressive symptoms. The observation of elevated levels of proinflammatory cytokines in depressive patients and the relatively frequent induction of depressive symptoms during the therapeutic application of interferon-α point to a possible role of proinflammatory cytokines in the development of depressive disorders [19]. The reduction of depressive symptoms in patients with psoriasis treated with the TNF-antagonist etanercept parallel to the improvement of skin symptoms [23] could, therefore, be interpreted not only as an indirect effect, but also as a direct effect related to the modulation of peripheral or central neurological functions.

Both depression and psoriasis are complex diseases. Carriage of the 's' allele of the 5-HTTLPR polymorphism has been shown to influence susceptibility to develop depressive symptoms after stressful life events but also in association with other diseases such as Parkinson's disease [4, 14, 25]. In our study, we could not find a significant difference in the HAM-D among carriers of different 5-HTTLPR genotypes. However, there are significant limitations to this finding. Low overall HAM-D values among patients with psoriasis in our patient sample may indicate an under-representation of patients with more pronounced depressive symptoms. This is possibly related to the fact that the majority of patients was recruited from a specialized outpatient clinic with optimized patient care. From previous studies, it is evident that different factors are likely to confound the presence and severity of depressive symptoms in psoriasis including gender, severity of skin symptoms and impairment of quality of life [21]. In accordance with the earlier results [18], more pronounced depressive symptoms were observed among female compared with male patients in this study, possibly as a result of gender-specific differences in the prevalence of depression and in the impact of skin diseases on overall well being and self-perception. In light of these findings, it is likely that much larger studies are necessary to establish a relationship between depressive symptoms and genetic variations such as the 5-HTTLPR polymorphism in psoriasis, and that the results obtained with a subgroup of psoriatic patients in this study can only be regarded as preliminary.

The serotonin/serotonin transporter system is a possible link between neuropsychological and immunological functions that may particularly be relevant in diseases such as psoriasis that are characterized by stress-induced chronic inflammation and an increased prevalence of depression. In this pilot study, we failed to demonstrate an association between psoriasis and the functionally relevant 5-HTTLPR polymorphisms of the serotonin transporter. There was also no association between depressive symptoms as measured by the HAM-D and carriage of different 5-HTTLRP genotypes in a subgroup of patients with psoriasis. This does not exclude a small contribution of the 5-HTTLPR polymorphism to psoriasis susceptibility or a modulatory effect on the development of depressive symptoms in patients affected by the disease. The specific limitation of the study related to the small number of individuals enrolled is underlined by recent studies indicating that analysis of several thousand individuals may be necessary to detect associations between SNPs of genes involved in psoriasis pathophysiology and the disease [16]. However, smaller studies with hundreds of patients have, for example, been able to identify association between the 5-HTTLPR polymorphism and anxiety-related personality traits in line with the conclusion that the findings from the present investigation at least argue against a major role of the 5-HTTLPR polymorphism in psoriasis [22]. In addition, it may be necessary to analyse genetic influences on depression separately in male and female patients with psoriasis, as depressive symptoms tend to be more severe in woman—as in our study—and genetic effects of the 5-HTTLPR may be superimposed by gender effects. Finally, other functionally relevant genetic variations of the serotonergic system have been identified including a variable number of tandem repeat polymorphism in intron 2 of the 5-HTT gene and polymorphism of serotonin receptors that could play a role in the pathogenesis of psoriasis and depressive symptoms in psoriasis patients [2]. Larger prospective trials are needed to evaluate the genetic basis of the complex interaction of psoriasis, environmental stressors, gender and development of depressive symptoms.

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