Possible Role of Endoplasmatic Reticulum Stress in Psoriasis Vulgaris

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Abstract

Plaque psoriasis is a chronic immune-mediated inflammatory skin disease. The role of cytokines in the development of psoriasis is well known and has been studied for decades. Endoplasmic reticulum (ER) stress is the cellular response to the disturbed homeostasis in the ER. ER stress is involved in different human pathologies, including chronic inflammatory and degenerative conditions. The aim of our study was to explore whether the single nucleotide polymorphisms (SNPs) in ER stress related genes are associated with a higher risk for plaque psoriasis. We studied 29 SNPs in the following ER stress genes: ATF6 (chr1), HSPA5 (chr9), HSP90B1 (chr12), ERN1 (chr17), XBP1 (chr22). Single marker analysis resulted in significant associations with the HSP90B1 gene (rs17034977, p=0.0232) and with the ERN1 gene (rs9916168, p<0.0001). Haplotype analysis revealed that the AGCCCG block in the HSP90B1 gene differed statistically significantly between patients and controls (p=0.0197). Our study suggests that the variations in the ER stress related genes may contribute to the genetic susceptibility to psoriasis and the genes under investigation may be involved in the pathogenesis of this inflammatory disease.

Keywords: ER stress; Psoriasis; Unfolded protein response; Susceptibility

Introduction

Psoriasis is a chronic inflammatory disease affecting predominantly the skin with the involvement of autoimmune mediated mechanisms. It affects about 2 to 3% of the Caucasian population. Typical pathogenic features include an increased renewal of epidermal keratinocytes, the enlargement of the germinating compartment papillomatosis, altered epidermal differentiation, angiogenesis, lymphangiogenesis and inflammatory infiltration. Despite the progress in our understanding of underlying pathomechanisms, the ultimate cause of psoriasis remains elusive. The genetic contribution to disease is substantial and recent large-scale association studies have identified 37 psoriasis risk regions, with HLA-C*06 being the main risk gene [1-3].

The endoplasmic reticulum (ER) is a sophisticated luminal network in which protein synthesis, maturation, folding, and transport take place. Homeostasis in the ER is monitored and maintained via unfolded protein response (UPR). A number of biological, psychological or pathological stimuli can perturb protein folding in the ER, leading to accumulation of unfolded or misfolded proteins in the ER lumen – a condition referred to as “ER stress” [4,5]. In response to increased demands of producing secreted or membrane proteins, cells adapt themselves to the stress conditions via UPR; i.e., attenuation of general translation, induction of ER chaperones and foldases, and activation of ER-Associated Degradation (ERAD) to eliminate immature proteins [6,7].

Three major transducers for sensing ER stress have been identified on the membrane of the ER; i.e., RNA-dependent protein kinase-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring ER-to-nucleus signal kinase 1 (IRE1). These proteins bear domains protruding into the ER lumen, which sense ER stress, coupled to cytosolic effector domains. ATF6 is a sensor molecule on the ER membrane which is responsible for the sensing of unfolded proteins. Upon ER stress, the inactive form of ATF6 (p90ATF6) is transported to the Golgi apparatus and is activated by a two-step cleavage by Site-1 protease and Site-2 protease, to produce the active
form of ATF6 (p50ATF6). The free ATF6 fragment migrates to the nucleus to activate transcription [9,10]. IRE1 in humans is encoded by the ERN1 gene. IRE1 [11] excise an intron from XBP1 mRNA, generating a spliced version of mRNA coding for a more potent form of a UPR transcription factor. XBP1 is a potent inducer of a subset of UPR target genes [5]. It is required for ER expansion and the development and survival of a variety of secretory cells as previously noted, as well as the adaptation of cells to a variety of stressful tissue environments such as that associated with hypoxia and calcium and glucose deprivation, among others [12].

The ER stress response has been recognized in a wide range of diseases, including rheumatoid arthritis, cancer, hypoxia and neurodegenerative disorders [13-15]. The aim of this study was to investigate a potential genetic association of ER stress related genes for psoriasis vulgaris in Estonian psoriasis patients.

Materials and Methods

Study subjects

Unrelated plaque psoriasis patients of the Estonian population (n=566, age range 18-89 years, mean age of onset 28.1 years) were enrolled at the Department of Dermatology, University of Tartu, as described [16]. Psoriasis patients were also divided into two subgroups based on the age of onset of the disease. Those with the onset before 40 years of age were considered as early onset psoriasis patients (n=434, age range 18-84 years, mean age of onset 20.9 years) and those with the onset of disease at the age of 40 years and later were considered as late onset psoriasis patients (n=132, age range 41-89 years, mean age of onset 51.8 years).

The control cohort was comprised of healthy unrelated individuals (n=308) without a personal or family history of psoriasis. Control subjects were recruited from among medical students, health care personnel and patients presenting at the dermatological outpatient clinic with either facial teleangiecitsis or skin tags.

The clinical parameters of study participants are shown in Table 1. The Ethics Review Committee on Human Research of the University of Tartu approved the study and written informed consent was obtained from all participants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Plaque psoriasis (N=566) frequency (%)</th>
<th>Early onset (N=434) frequency (%)</th>
<th>Late onset (N=132) frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>304</td>
<td>234</td>
<td>69</td>
</tr>
<tr>
<td>Female</td>
<td>262</td>
<td>199</td>
<td>63</td>
</tr>
<tr>
<td>Family History</td>
<td>246 (43.46)</td>
<td>216 (49.77)</td>
<td>30 (22.73)</td>
</tr>
<tr>
<td>PASI score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 10</td>
<td>161 (28.45)</td>
<td>111 (25.58)</td>
<td>49 (37.12)</td>
</tr>
<tr>
<td>11-20</td>
<td>165 (29.15)</td>
<td>123 (28.34)</td>
<td>43 (32.58)</td>
</tr>
<tr>
<td>≥ 21</td>
<td>240 (42.4)</td>
<td>200 (46.08)</td>
<td>40 (30.3)</td>
</tr>
<tr>
<td>BSA &lt; 10%</td>
<td>121 (21.38)</td>
<td>85 (19.58)</td>
<td>36 (27.27)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PSA score</th>
<th>Frequency (%)</th>
<th>Frequency (%)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-30%</td>
<td>190 (33.57)</td>
<td>141 (32.49)</td>
<td>49 (37.12)</td>
</tr>
<tr>
<td>&gt;31%</td>
<td>255 (45.05)</td>
<td>208 (47.93)</td>
<td>47 (35.61)</td>
</tr>
<tr>
<td>Seasonality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring-Summer</td>
<td>42 (7.42)</td>
<td>336 (77.42)</td>
<td>70 (53.03)</td>
</tr>
<tr>
<td>Autumn-Winter</td>
<td>406 (71.73)</td>
<td>27 (6.22)</td>
<td>15 (11.36)</td>
</tr>
<tr>
<td>None</td>
<td>105 (18.55)</td>
<td>62 (14.29)</td>
<td>43 (32.58)</td>
</tr>
<tr>
<td>Do not Know</td>
<td>13 (2.3)</td>
<td>9 (2.07)</td>
<td>4 (3.03)</td>
</tr>
<tr>
<td>Nail Involvement</td>
<td>277 (48.94)</td>
<td>233 (51.38)</td>
<td>54 (40.91)</td>
</tr>
<tr>
<td>PSA</td>
<td>127 (22.44)</td>
<td>107 (24.65)</td>
<td>20 (15.15)</td>
</tr>
</tbody>
</table>

Table 1: Demographic data of psoriatic patients.

Genotyping

DNA was obtained from peripheral blood leucocytes by standard salting-out method. Single nucleotide polymorphisms (SNPs) were analyzed using the SNPlex Genotyping System [17]. SNPlex Genotyping System utilizes a suite of pre-optimized universal assay reagent kits and a set of SNP-specific ligation probes allowing the genotyping of up to 48 SNPs in a single reaction. This system is based on the oligonucleotide ligation/PCR assay (OLA/PCR) with a universal ZipChute probe detection for high-throughput SNP genotyping. Fluorescently labelled ZipChute probes are hybridized to complementary ZipCode sequences that are part of genotype-specific amplicons. These ZipChute probes are eluted and detected by electrophoretic separation on 3730 Genetic Analyzer. The GeneMapper 3.7 software was used for automated allele calling of all possible SNPs in each DNA sample.

SNPbrowser version 3.5 was used for the SNP selection and SNPlex assay pool design. Selection conditions were as follows – LD Map database was from Applied Biosystems, SNP selection was based on density with spacing criterion around 10 kb, minor allele frequency cut-off 5% and non-synonymous SNPs always included.

Statistical Analysis

For statistical analysis of single marker associations R program (http://www.r-project.org) was used. For haplotype analysis we used SHEsis program. A p-value<0.05 was considered statistically significant for all analyses. The significance level (p-value) was corrected by Bonferroni multiple comparisons analysis. Cases and controls were considered separately.

Results

We genotyped 29 SNPs in five ER stress related genes (namely ATF6, HSPA5, HSP90B1, ERN1 and XBP1) in the total of 566 psoriasis vulgaris patients and 308 healthy control individuals. Psoriasis patients were divided into two different groups- early and late onset, as described above. Genotype distributions of the analyzed polymorphisms of the studied genes were in Hardy-Weinberg equilibrium both in the group of patient with psoriasis and the control group. Comparison of genotypic frequencies between cases and controls in the psoriasis group resulted in two statistically significantly associated SNPs. Namely, HSP90B1 (rs17034977, p=0.0233) and ERN1 (rs9916168, p<0.0001) (Table 2).
Table 2: Associations of ER stress genes in Psoriasis Vulgaris patients (P-value<0.05 was considered statistically significant for all analyses).

Comparison of genotypic frequencies between cases and controls in the early and late onset psoriasis groups revealed that ERN1 (rs9916168) was statistically significant both the early and late onset psoriasis group (p=0.0005 and p=0.0314, respectively), whereas HSP90B1 (rs17034977) gave statistically significant association only in the late onset psoriasis group (p=0.0088) (Table 2).

These differences remained significant after the Bonferroni correction (giving p=0.0004 and p=0.031 for ERN1 gene and p=0.025 for HSP90B1 gene, respectively).

<table>
<thead>
<tr>
<th>Halophytes of the HSP90B1 gene</th>
<th>Psoriasis patients (%) (N=566)</th>
<th>Control samples (%) (N=208)</th>
<th>OR (95%CI)</th>
<th>p-value</th>
<th>PASI ≥ 21</th>
<th>BSA ≥ 31%</th>
<th>Seasonality (autumn-winter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AACCGG</td>
<td>5.0</td>
<td>4.3</td>
<td>1.138 (0.670-1.935)</td>
<td>0.6316</td>
<td>0.7512</td>
<td>0.8985</td>
<td>0.4472</td>
</tr>
<tr>
<td>AGCCG</td>
<td>12.0</td>
<td>11.7</td>
<td>1.010 (0.717-1.424)</td>
<td>0.9546</td>
<td>0.6466</td>
<td>0.7144</td>
<td>0.6104</td>
</tr>
<tr>
<td>AGCGG</td>
<td>3.9</td>
<td>6.6</td>
<td>0.581 (0.343-0.917)</td>
<td>0.0197</td>
<td>0.0013</td>
<td>0.0301</td>
<td>0.0100</td>
</tr>
<tr>
<td>AGTAAG</td>
<td>12.7</td>
<td>12.4</td>
<td>1.014 (0.726-1.417)</td>
<td>0.9338</td>
<td>0.9100</td>
<td>0.8852</td>
<td>0.9421</td>
</tr>
<tr>
<td>AGTCAAG</td>
<td>12.0</td>
<td>10.6</td>
<td>1.130 (0.794-1.609)</td>
<td>0.4967</td>
<td>0.7076</td>
<td>0.9624</td>
<td>0.6889</td>
</tr>
<tr>
<td>AGTCAG</td>
<td>15.1</td>
<td>13.4</td>
<td>1.134 (0.824-1.561)</td>
<td>0.4405</td>
<td>0.2708</td>
<td>0.4187</td>
<td>0.3281</td>
</tr>
<tr>
<td>GATAAG</td>
<td>35.5</td>
<td>35.7</td>
<td>0.986 (0.765-1.220)</td>
<td>0.084</td>
<td>0.5570</td>
<td>0.7285</td>
<td>0.6231</td>
</tr>
</tbody>
</table>

Table 3: Haplotype distribution of HSP90B1 gene polymorphisms in Psoriasis Vulgaris patients and controls (P-value<0.05 was considered statistically significant for all analyses).

We also performed stratified association analyses based on disease activity (Psoriasis Area and Severity Index - PASI score and Body Surface Area - BSA) and seasonality, age of onset and associated nail and joint involvement. The rs17034977 in the HSP90B1 gene showed statistically significant association only in the late onset psoriasis group (p=0.0088) (Table 2). These differences remained significant after the Bonferroni correction (giving p=0.0004 and p=0.031 for ERN1 gene and p=0.025 for HSP90B1 gene, respectively).
Haplotyp analysis for the genes under investigation was performed and analysis for the HSP90BI gene revealed seven haplotype blocks (Table 3). Haplotype block AGCCCG differed statistically significantly between patients and controls and emerged as a risk haplotype with OR, 95%CI, and P-value of 0.561, 0.343-0.917, 0.0197. The AGCCCG haplotype block was also statistically significantly associated with different clinical parameters of psoriasis, namely PASI (PASI ≥ 21) (p=0.0013), BSA (BSA ≥ 31%) (p=0.0031), and seasonality (p=0.0100).

Haplotype analysis for the ATP6, ERN1, HSPA5 and XBPI gene was performed, but no statistically significant associations in haplotype blocks was found (data not shown).

Discussion
The purpose of this study was to analyze ER stress related genes and to assess their impact on the risk of plaque psoriasis in the Estonian population. ER stress and the attendant UPR can lead to cell death and ER stress is related to chronic inflammatory diseases [18,19]. Moreover, the conditions that lead to an increase in protein misfolding or a decrease in the ability of the cell to handle these proteins in the ER can result in cellular dysfunction and cause different types of diseases [18,20-26]. ER stress pathways are also linked to the mechanisms involved in immunity and inflammation. ER stress may be both a trigger and a consequence of chronic inflammation. Chronic inflammation is often associated with diseases that arise because of primary misfolding mutations and ER stress. Similarly, ER stress and activation of the UPR is a feature of many chronic inflammatory diseases [8]. Psoriasis is a chronic inflammatory disease arising through the interplay between genetic risk variants and the environment. The number of psoriasis susceptibility variants has increased with the development of large-scale genetics and, so far, primarily the genes shared between psoriasis phenotypes have been captured [1].

We found no previous data about the possible associations between psoriasis vulgaris and ER stress genes. However, in this study, we showed that ER stress genes were associated with genetic susceptibility to plaque psoriasis. ERN1 (rs9916168) and HSP90BI (rs17034977) [27] were significantly associated within the group with plaque psoriasis compared to the healthy control individuals. Thus, we conclude that the ER stress associated genes may play a role in the development of plaque psoriasis.

ERN1 is endoplasmic reticulum to nucleus signaling 1 and it is a human homologue of the yeast Ire1 gene product. This gene is important in altering gene expression as a response to an endoplasmic reticulum-based stress signal [28]. ERN1 has quite diverse functions that are all related to the regulation of ER stress response. This gene is involved in the broader regulation of cell fate during unfolded protein response [29]. Therefore, it is involved in quite diverse cellular functions. ERN1 senses bacterial proteins invading ER and activate innate immune response [30]. ERN1 has also been shown to be involved in inflammation and in neurodegeneration [17,31,32]. For instance, the role of ER stress in the pathogenesis of rheumatoid arthritis is well established [14]. XBPI, CHOP and GRP78 have all been shown to be involved in the development of rheumatoid arthritis [33,34]. In several studies the role of ER stress in synovial damage has been shown. Therefore, ER stress can be involved in the psoriatic arthritis that is very common in psoriasis patients, however in our study group we were unable to show it. Moreover, ER stress and the ERN1 gene are involved in the Toll-like receptor-mediated signaling during RA. Macrophages from the synovial fluid of rheumatoid arthritis patients have significantly activated IRE1a. Myeloid–specific deletion of the IRE1a gene protected mice from inflammatory arthritis [35].

Another association was found with the HSP90BI gene, which is an ER chaperone and regulates the activity, stability and subcellular localization of a large number of client proteins to which it binds in a selective manner together with associated cofactors [7]. In our study, we found that the HSP90BI gene SNP rs17034977 was significantly associated with psoriasis vulgaris. This may support the fact that HSP90B1 is induced by the accumulation of misfolded proteins and it facilitates cell repair by stabilizing and refolding denatured proteins after stress [6].

There is a good reason to believe that ER-stress is involved in psoriasis as increased ER-stress is a feature of epidermal differentiation [36,7]. The increased epidermal proliferation typical for psoriatic epidermis will increase the burden of ER-stress and thus ER-stress signaling. ER-stress is also increased during UV-A and UV-B irradiation of mammalian epidermis and dermis [37,38]. As UV-B therapy improves psoriasis and thus over-activates UV-induced ER-stress, it is hard to believe that ER-stress is the underlying pathomechanism in psoriasis. Nevertheless, the role of ER-stress in psoriasis and genetic susceptibility to disease pathophysiology is interesting link to ER-stress-mediated inflammatory responses [39,40]. Our findings provide a new insights into the association between ER stress related genes and psoriasis vulgaris. However, the role of ER stress response in the pathogenesis of this disease remains to be defined.

Conflict of Interest
The authors have declared no conflicts of interest.

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