Potential Association of Estrogen Receptor 2 Variant with Chronic Periodontitis

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Abstract

Objective: Sex steroid hormones significantly contribute to the homeostasis of periodontium. Estrogens can modulate inflammation and healing process in periodontal tissue by affecting blood vessels and immune system. However, the association between estrogen receptor 1 gene (ESR1) and estrogen receptor 2 gene (ESR2) polymorphisms in chronic periodontitis remains unclear.

Material and Methods: The current case-control study contained 43 chronic periodontitis patients and 40 healthy control individuals from Polish population. Two single-nucleotide polymorphisms of ESR1 and three SNP of ESR2 were studied using polymerase chain reaction-restriction fragments length polymorphism technique with the use of restriction enzymes.

Results and Conclusion: No significant differences were found, neither in allele nor genotype frequency between chronic periodontitis patients and healthy controls for studied ESR1 (neither) and ESR2 (rs4986938, rs1256049) polymorphisms. The frequency of the rs1256120 genotype was 72.1, 23.3 and 4.6% (TT, TC and CC, respectively) in the chronic periodontitis patients, versus 47.5, 42.5 and 10.0% (respectively) in the control group. Interestingly, significant differences were found in allele distribution (p=0.023) and genotypes distribution in a recessive model (TT vs. TC and CC; p=0.039) between the chronic periodontitis and controls. A higher frequency of the T allele and TT genotype in periodontitis patients might suggest an association between the rs1256120 polymorphism in ESR2 and an increased risk of chronic periodontitis. These results might contribute to understanding the pathogenesis of periodontitis and facilitate development of prevention strategies based on genotyping.

Keywords: Chronic Periodontitis; Estrogen Receptor 1; Estrogen Receptor 2; Single-Nucleotide Polymorphisms

Introduction

The homeostasis of the periodontium involves complex multifactorial relationships, in which sex steroid hormones play an important role. Estrogens modulate all periodontal tissues during periods of hormone fluctuation [1-3] both in men and women. These effects are primarily seen as gingival manifestation [4]. Despite the lack of influence of steroid hormones on gingival sulcus microbiota [5], they can modulate inflammation and healing processes in periodontal tissue by an impact on blood vessels [6] and immune system [7]. Currently there is no doubt that the prevalence of gingival diseases increases with sex steroid hormone levels, even when oral hygiene remained unchanged during puberty [1], pregnancy [2] and menopause [3]. It is also confirmed that periodontology does not exhibit clinically obvious changes during the menstrual cycle [5] and oral contraceptives [8]. The pleiotropic and tissue-specific effects of estrogen are determined by different expression of specific estrogen receptor (ER) subtypes (ESR1 and ESR2) and their co-regulators. Moreover, there is a complex interaction between both receptors in which ESR2 may function as an antagonist of ESR1 [9]. This relationship was detected especially in bone metabolism examination.

Both estrogen receptors subtypes are expressed in osteoblasts, osteoblast-like cells and bones in male and female [10], but in an animal model it was shown that in male the ESR1 alone decreased bone turnover and increased trabecular bone volume, whereas in female both receptors influenced this process [11]. Expression of both subtypes of estrogen receptors, ESR1 and ESR2, was evaluated in periodontal ligament (PDL) cells [10]. However only ESR2 protein expression was detected with immunocytochemical and western blotting methods. The immunoreactivity staining of ESR2 was shown predominantly in the nuclei of PDL cells. It was also shown that during estrogens differentiation the levels of ESR2 mRNA protein were significantly higher compared with control.
group. That may suggests a role of ESR2 in estrogenic differentiation process [10]. Estrogen receptors expression was also detected in gingival tissue obtained from patients with diagnosed chronic desquamative gingivitis [12], oral mucosa and salivary glands [13]. In salivary epithelium both types of estrogen receptors are expressed [13], but ESR2 is suggested as a predominant type in salivary glands [14].

The human ESR1 gene (ESR1) is located on chromosome 6q25 and comprises eight exons separated by seven intronic regions [15]. The ER2 gene (ESR2) is located on chromosome 14q23-24.1 and comprises also eight exons and two additional on 5' and one on 3', which do not take a part in translation process [16].

Several single nucleotide polymorphisms (SNP) of the ESR1 gene have been identified, of which the MspI C>T SNP (rs1784705) in the first exon and Haell I C>T (rs922331), PoulI C>T (rs2234693) and Xbol A>G (rs9340799) SNP in the first intron have been studied most frequently. These polymorphisms have been associated with several diseases such as endometrial cancer [17], breast cancer [18], ovarian cancer [19], endometriosis [20], osteoporosis [21], and cardiovascular disease [22]. There are only few papers concerning the correlation of ESR1 polymorphisms with chronic periodontitis [23,24]. The studies were done on Chinese population. So far there is no research on ESR2 gene polymorphism in chronic periodontitis (CP), despite the fact that genetic association studies on ESR2 gene in some pathologic conditions were associated with periodontitis, bone mineral density - BMD [25], obesity [26], and Alzheimer’s disease [27]. The aim of this study was to investigate the frequency of ESR1 and ESR2 polymorphisms in Polish population in order to verify an association between polymorphic variants of ESR1/2 and chronic periodontitis.

Material and Methods

Ethics Statement
The study was approved by the Institutional Review Board of the Poznan University of Medical Sciences (No 87/09). The informed consent was obtained from all the patients and all controls.

Patients

Forty-three Caucasian patients, 35 women and 8 men (aged 48.42±3.24) with clinically diagnosed severe generalized chronic periodontitis, according to American Academy of Periodontology classification criteria [28] were qualified for the research. The qualification criteria included the following: no periodontal disease treatment [27]. The aim of this study was to investigate the frequency of ESR1 and ESR2 polymorphisms in Polish population in order to verify an association between polymorphic variants of ESR1/2 and chronic periodontitis.

Genotype Analysis

Sample Collection and DNA Extraction: A volume of 2 mL of peripheral blood samples were collected in 5-monovette tubes from all patients and controls and collected with EDTA as anticoagulant (SARSTEDT AG & Co., Numbrecht, Germany). Afterwards, the genomic DNA from each sample was isolated using AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Scientific, Inc., Union City, CA, USA) according to manufacturer protocol. DNA concentration and purity was determined spectrophotometrically. For each PCR reaction 50 to 200 ng of gDNA was used.

DNA Amplification by PCR: 1374 bp fragment of ESR1 gene and 620 bp, 646 bp and 582 bp fragments of ESR2 gene were amplified using specific primers as previously described [30-32]. PCR was carried out in a total volume of 25 µL. The reaction mixtures contained: 50 to 200 ng of gDNA, 1x KAPA HiFi HotStart ReadyMix PCR Kit (containing KAPA HiFi HotStart DNA Polymerase, 2.5 mM MgCl2, reaction buffer and dNTP Mix; all from Kapa Biosystems, Wilmington, MA, USA), 300 nM of each primer (Genomed, Warsaw, Poland) and PCR grade water. 2 µL of the PCR products were separated in 2% agarose gel stained with ethidium bromide to confirm PCR product identity relative to molecular mass marker Nova100 (Novazym, Poznan, Poland) was used.

Restriction Fragment Length Polymorphism (RFLP) Analysis: The reaction was conducted in a total volume of 15 µL containing: 15 µL of each PCR product, 1 µL of FastDigest Green Buffer, 1 FDU of the respective FastDigest Enzyme (Bioline GmbH, Germany) and DNeasy free water. The restriction reaction was incubated for one hour at 37°C and followed by thermal inactivation of the restriction enzyme at 85°C. Genotypes were determined by examining cleaved DNA fragments in 2% agarose gel electrophoresis in the presence of ethidium bromide followed by visualization. The results were described as AA or GG or AG for rrs9340799, TT or
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Statistical Analysis

The differences in allele or genotype distribution for the CP patients versus controls were evaluated using the Chi$^2$ test or Chi$^2$ test with Yate correction. The Hardy-Weinberg equilibrium (HWE) was computed with goodness-of fit Chi$^2$ test. The strength of the association between the CP and the ESR1 and ESR2 polymorphisms was estimated using odds ratios (ORs), with the corresponding 95% confidence intervals (CIs). P<0.05 was considered statistically significant. All analyses were performed using Statistica version 12.0 (Stat Soft.).

Results

Distribution of the ESR1 and ESR2 Genotypes

Genotype distribution and allele frequency of ESR1 and ESR2 SNPs were successfully performed in all subjects. The genotype distribution for the control group did not deviate for HWE equilibrium. For all samples, the frequency of the AA, AG and GG genotypes for the rs9340799 polymorphism was 18.1, 65.0 and 16.9%, respectively, and the frequency of the TT, TC and CC for the rs2234693 was 19.3, 54.2 and 26.5%, respectively. The frequency of the AA, AG and GG genotypes for the rs4986938 polymorphism was 7.2, 49.4 and 43.4%, respectively, the frequency of the AA, AG and GG for the rs1256049 was 0, 12.1 and 87.9%, respectively, and the frequency of the TT, TC and CC for the rs1256120 was 60.3, 32.5 and 7.2%, respectively.

Association of ESR1 Gene SNPs with Chronic Periodontitis

The frequency of the AA, AG and GG genotypes for the rs9340799 polymorphism was 13.9, 67.4 and 18.6%, respectively in the chronic periodontitis patients, versus 22.5, 62.5 and 15.0% in the control group. No significant differences were found, either in allele (p=0.53) or genotype (p=0.58) frequency between chronic periodontitis patients and healthy controls (Table 2). The frequency of the TT, TC and CC genotypes for the rs2234693 polymorphism was 13.9, 53.5 and 32.5%, respectively in the chronic periodontitis patients, versus 25.0, 55.0 and 20.0%, respectively, in the control group. No significant differences were found, either in allele (p=0.17) or genotype (p=0.28) frequency between chronic periodontitis patients and healthy controls (Table 2).

Table 2: PAallele and genotype frequency (rs9340799 and rs2234693) for chronic periodontitis patients and healthy controls.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Geno-type</th>
<th>CP patientsN (%)</th>
<th>Healthy controls N (%)</th>
<th>P value$^a$</th>
<th>P value$^b$</th>
<th>Odds ratio$^e$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XbaI</td>
<td>AA</td>
<td>6 (13.9)</td>
<td>9</td>
<td>0.88</td>
<td>0.46</td>
<td>0.77 (0.24-2.46)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>29 (67.4)</td>
<td>25 (62.5)</td>
<td></td>
<td></td>
<td>0.59 (0.18-1.74)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>8 (18.6)</td>
<td>6 (15.0)</td>
<td></td>
<td></td>
<td>0.46 (0.16-1.49)</td>
</tr>
<tr>
<td>Allele</td>
<td>A</td>
<td>41 (47.7)</td>
<td>43 (53.8)</td>
<td></td>
<td>0.53</td>
<td>0.79 (0.43-1.44)</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>45 (52.3)</td>
<td>37 (46.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PvuII</td>
<td>TT</td>
<td>6 (13.9)</td>
<td>10 (25.0)</td>
<td>0.29</td>
<td>0.31</td>
<td>0.52 (0.19-1.41)</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>23 (53.5)</td>
<td>22 (55.0)</td>
<td></td>
<td></td>
<td>0.46 (0.16-1.49)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>14 (32.5)</td>
<td>8 (20.0)</td>
<td></td>
<td></td>
<td>0.62 (0.34-1.15)</td>
</tr>
<tr>
<td>Allele</td>
<td>T</td>
<td>35 (40.7)</td>
<td>42 (52.5)</td>
<td></td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>51 (59.3)</td>
<td>38 (47.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: $^a$Chi2 test; $^b$multiplicative P value for genotypes and P value for allele
$^c$dominant model contrast: for XbaI AA and AG vs GG, for PvuII TT and TC vs CC
$^d$recessive model contrast: for XbaI AA vs AG and GG, for PvuII TT vs TC and CC
$^e$Odds ratio (95% CI) for dominant model contrast, recessive model contrast and for allele
CP- chronic periodontitis; SNP – single nucleotide polymorphism;
Association of ESR2 Gene SNPs with Chronic Periodontitis

The frequency of the AA, AG and GG genotypes for the rs4986938 polymorphism was 6.9, 55.8 and 37.2%, respectively, in the chronic periodontitis patients, versus 7.5, 42.5 and 50.0%, respectively, in the control group. No significant differences were found, either in allele (p=0.49) or genotype (p=0.46) frequency between chronic periodontitis patients and healthy controls (Table 3). The frequency of the AA, AG and GG genotypes for the rs1256049 polymorphism was 0.0, 11.6 and 88.4%, respectively, in the chronic periodontitis patients, versus 0.0, 12.5 and 87.5%, respectively, in the control group. No significant differences were found, either in allele (p=0.9) or genotype (p=0.83) frequency between chronic periodontitis patients and healthy controls (Table 3). The frequency of the TT, TC and CC genotypes for the rs1256120 polymorphism was 72.1, 23.3 and 4.6%, respectively, in the chronic periodontitis patients, versus 47.5, 42.5 and 10.0%, respectively, in the control group. Significant differences were not found in the genotype distribution (p=0.07) between the CP patients and healthy controls however, the results were close to the significance indicating a potential trend (Table 3) and (Figure 1A). Significant differences were found in allele distribution (p=0.023) between the CP patients and healthy controls (Table 3) and (Figure 1B). An increased frequency of the T allele in periodontitis patients was detected: OR=2.35; IC95% (1.11-4.91). A significant difference for genotypes distribution in the recessive models (TT vs. TC and CC) between the CP and controls was noted (p=0.039) (Table 3) and (Figure 1C). We clearly observed an increment of TT genotype in periodontitis patients: OR=2.86; IC95% (1.15-7.10).

Table 3: Allele and genotype frequency (AluI, RsaI and AlwNI) for chronic periodontitis (CP) patients and healthy controls.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Geno-type</th>
<th>CP patients N (%)</th>
<th>Healthy controls N (%)</th>
<th>P value Dominant</th>
<th>P value Recessive</th>
<th>P multiplicative and P value allele dependent</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AluI Geno-type</td>
<td>AA</td>
<td>3 (6.9)</td>
<td>3 (7.5)</td>
<td>0.34</td>
<td>0.73</td>
<td>0.46</td>
<td>1.60 (0.7-4.05)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>24 (55.8)</td>
<td>17 (42.5)</td>
<td></td>
<td></td>
<td></td>
<td>0.93 (0.18-4.87)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>16 (37.2)</td>
<td>20 (50.0)</td>
<td></td>
<td></td>
<td></td>
<td>0.92 (0.25-3.45)</td>
</tr>
<tr>
<td></td>
<td>Allele</td>
<td>A</td>
<td>30 (34.9)</td>
<td>23 (28.7)</td>
<td></td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>56 (65.1)</td>
<td>57 (71.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RsaI Geno-type</td>
<td>AA</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.82</td>
<td>-</td>
<td>0.90</td>
<td>0.92 (0.25-3.45)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>5 (11.6)</td>
<td>5 (12.5)</td>
<td></td>
<td></td>
<td></td>
<td>0.46 (0.16-1.49)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>38 (88.4)</td>
<td>35 (87.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Allele</td>
<td>A</td>
<td>5 (5.8)</td>
<td>5 (6.3)</td>
<td></td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>81 (94.2)</td>
<td>75 (93.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AlwNI Geno-type</td>
<td>TT</td>
<td>31 (72.1)</td>
<td>19 (47.5)</td>
<td>0.60</td>
<td>0.039</td>
<td>0.07</td>
<td>2.28 (0.39-13.18)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>10 (23.3)</td>
<td>17 (42.5)</td>
<td></td>
<td></td>
<td></td>
<td>2.86 (1.15-7.10)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>2 (4.6)</td>
<td>4 (10.0)</td>
<td></td>
<td></td>
<td></td>
<td>2.35 (1.11-4.91)</td>
</tr>
<tr>
<td></td>
<td>Allele</td>
<td>T</td>
<td>72 (83.7)</td>
<td>55 (68.8)</td>
<td></td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>14 (16.3)</td>
<td>25 (31.2)</td>
<td></td>
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</tbody>
</table>
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Figure 1: The genotype and alleles frequencies of rs1256120 polymorphism in control (C) and chronic periodontitis (CP) groups.

a. Bordering difference was found for genotype distribution
b. Significant differences were found in allele distribution and
c. For genotypes distribution in the recessive models.

Discussion

Periodontitis as a complex disease and by definition is recognized as relatively common, mostly adult onset, slowly progressive and chronic in nature. The pathogenesis of chronic periodontitis is multifactorial. While imbalance between microbial factors and host immune response is believed to initiate periodontal disease, the genetic and environmental risk factors play a role in the susceptibility and progression of destruction process of tooth surrounding tissues [33]. The well-supported data exist and characterize the periodontitis as disease-modifying genes, which is polygenic and probably different in different ethnic populations. Evidence for the role of a genetic component in chronic periodontitis has been conducted from twin and family studies, that reduced the risk of population stratification, and revealed that chronic periodontitis had approximately 50% heritability [34]. Most of the studies, using single nucleotide polymorphism identification, were focused on the genetic modification of the host response. The meta-analysis of Nicolopoulos et al. [35] revealed a association between chronic periodontitis and IL1A-889 and IL1B+3953R-allele carriage as well separately and in composite genotype in Caucasians and compared the possibility of IL1B+3953 genotype as a genetic risk factor for chronic periodontitis patients from this ethnic group. On the other hand the IL1 gene cluster polymorphisms cannot be consider as a risk factor for the worldwide chronic periodontitis population [36]. Similarly the IL6-174 polymorphism may be associated with chronic periodontitis susceptibility. The carriage rates of the IL6-174 R-allele varied in Caucasian population from 44-54% and in Brazilian population from 37-67% [36].

Well documented, as a genetic marker for CP, is polymorphism of IL10-592 [36] and vitamin D receptor (VDR) gene. The frequency of the VDR Taq1 R-allele range between 42-78% across Caucasian, Brazilian and Turkish populations, but in Chinese and Japanese population substantially lower rates between 5-23% were indicated. In Asians the high range of VDR Fok1 polymorphisms was detected [36]. ESR1 is one of the candidate genes studied in relation to susceptibility to chronic periodontitis. Our results did not corroborate any association of the rs9340799 SNPs with the CP previously described in Chinese population [23,24]. Zhang et al. [23] investigated the relationship between the ESR1 gene polymorphisms in 90 patients with aggressive periodontitis and 34 patients with chronic periodontitis in Han Chinese patients. Their observations indicated that the XX genotype might be a risk indicator for chronic periodontitis in Han Chinese population. The association was gender-dependent and was significant for chronic periodontitis female but not for male patients. Weng et al. [37] performed a meta-analysis concerning the association between ESR1 gene rs9340799 and rs2234693 polymorphism and periodontitis susceptibility in Chinese population. They concluded that the homozygote (rs9340799) genotype might increase the risk of CP in the Han Chinese population but not in the Hui population.

This underlines the probable population dependent variety and implies further studies in other populations. The association between ESR1 gen polymorphism and risk of periodontitis was also analyzed according to bone metabolism. In an animal model a complete deletion of ESR1 led to a decrease in bone turnover and an increase in trabecular bone volume in both genders. Deletion
of ESR2 led to different responses in males, where bone was unaffected, and in females, where bone resorption was decreased and trabecular bone volume increased. Deletion of both ESRs led to a profound decrease in trabecular bone volume in females, which was associated with a decrease in bone turnover. It is suggested that only ESR1 regulate bone remodeling in males, whereas in females both receptor subtypes influenced this process [38]. Mutation in the exon of the ESR1 gene was indicated to result in severe osteoporosis in both female and male mice [39]. In humans it was indicated by Payne et al. [40] that estrogen deficiency in osteoporotic or osteopenic women is associated with increasing frequency of alveolar bone height loss, crestal and subcrestal density loss comparing to women with normal BMD. Zhang et al. [24] examining Chinese population revealed that there were no significant differences between ESR1 rs9340799, ESR1 R-α rs2234693 genotypes and BMD in pre- and post-menopausal women with chronic periodontitis. It was demonstrated that rs2234693 and rs9340799 polymorphisms of the ESR1 gene were associated with osteoporosis and altered bone mineral density [41,42].

For the ESR2 gene polymorphism we found a significant association of the rs1256120 polymorphism with chronic periodontitis in Caucasian population. The presence of T allele and TT homozygote was significantly higher in CP patients comparing with healthy controls. To our knowledge, this is the first report on ESR2 gene polymorphism in chronic periodontitis patients. ESR2 receptor is predominant subtype expressed in human periodontal ligament [10], which attach the tooth to the bone tissue and play a dominant role in guided tissue regeneration. ESR2 is expressed in bone, as well as ESR1, and it is suggested to function as an inhibitor of ESR1 transcriptional activity [9]. It was also indicated that production of reactive oxygen species (ROS), which play a complex role in periodontal tissue breakdown [43], is estrogen hormone dependent and it is dependent on both receptors, but rather on ESR2 [44]. Perhaps the ESR2 gene polymorphism may be one of the risk factors in severe periodontitis, but further studies are needed to confirm this hypothesis. Although we used rigorous clinical criteria, it is still possible that patients, who were evaluated at a single time point with severe chronic periodontitis had experienced periods of aggressive periodontitis. A bigger group of patients is also needed. Taking into consideration lack of published data in this field and the dominant position of the ESR2 receptor in periodontium, further studies are needed to better understand the role of genetic background of chronic periodontitis susceptibility.

Acknowledgement

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