

## The Association Between Polymorphism of Vitamin D Receptor *FokI* and Chronic Periodontitis in Sumatera Utara

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### Abstract

The vitamin D receptor (VDR) plays an important role in bone metabolism and immune response modulation, both of which are important for the development of chronic periodontitis (CP). Therefore the polymorphism of VDR could give significant effect on the CP susceptibility. This study investigated the association between the VDR single nucleotide polymorphism (SNP) *FokI* and chronic periodontitis. The genotypes of 43 CP patients and 40 periodontally healthy control subjects were analyzed. The DNA was extracted from the buccal epithelial cell and the VDR *FokI* polymorphism were determined by using the Polymerase Chain Reaction (PCR). This is followed by Restriction Fragment Length Polymorphism (RFLP) by using *FokI* smart cut enzyme digest and electrophoresis. The frequencies of genotypes and alleles were compared between patients and control subjects. The genotypes and alleles of vitamin D receptor *FokI* distribution was significantly different between two groups ( $P=0.016$  and  $P=0.018$ , respectively). The *F* allele had the potential to increase the susceptibility of chronic periodontitis (OR = 2.18, 95% confidence interval = 1.161-4.111) as well as the *FF* genotype (OR = 4.44, 95% confidence interval = 1.125-17.566). The current findings confirm that the polymorphism of VDR *FokI* were significantly associated with chronic periodontitis.

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### Introduction

Chronic periodontitis is an inflammatory disease characterized by slow progression of connective tissue destruction and alveolar bone resorption.<sup>1</sup> This disease contributed to the sixth highest cases over the global population, with the prevalence of 10.8% from around the world.<sup>2</sup> There is a tendency for the disease to increase in severity as people age.<sup>3</sup> About 96.2% population at  $\geq 30$  years of age in Indonesia has periodontal problem.<sup>4</sup> Almost 25% adults between age 35-54 years in Australia and 5% of 35-49 years old US population have either moderate or severe periodontitis.<sup>5</sup>

Periodontitis is a multifactorial disease

and it is now widely accepted that susceptibility to inflammation is also determined by intrinsic factors such as genetics.<sup>6</sup> The interactions of local, systemic, immunological and genetic predisposition determine the onset, progress and severity of the disease.<sup>7</sup> Variation in genetic such as single nucleotide polymorphisms (SNPs) may contribute to the innate and adaptive immune response or the periodontal tissue structure itself.<sup>8</sup>

As bacterial accumulation and alveolar bone destruction are major characteristics of periodontal disease, it is more likely a regulator to immune response and bone remodeling such as vitamin D receptor (VDR) and its polymorphism influenced the periodontitis susceptibility.<sup>9</sup> The function of activated VDR as a ligand-activated transcription factor, is responsible in calcium and phosphate homeostasis, immunomodulation, cellular growth, cell differentiation and apoptosis.<sup>10</sup> The binding of  $1,25(\text{OH})_2\text{D}_3$  and heterodimerization with other nuclear hormone receptor such as retinoid X receptors allowing VDR to recognize specific DNA sequence or vitamin D response element

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(VDRE) in the promoter region of genes, to regulate the transcription of various target gene that it regulates.<sup>11</sup>

Previous study suggested the role of vitamin D receptors in macrophages, dendritic cells(DC) and activated T and B cells lymphocytes in modulating the immune response.<sup>12</sup> These immune modulating role are also responsible in suppressing the pro inflammatory cytokine expression and inducing the antimicrobial peptide formation like cathelicidin-LL37 as innate immune response in the periodontal disease.<sup>13</sup> The activated VDR also gives a direct effects promoting bone resorption by increasing the number and activity of osteoclast.<sup>14</sup> A persistent microbial challenge in the periodontal tissue may disrupt the dynamic balance between bone-forming osteoblasts (OBLs) and bone-resorbing osteoclasts (OCLs) activity leading to the alveolar bone loss.<sup>15</sup>

The vitamin D receptor gene is located in chromosome 12(12q13-14).<sup>16</sup> Some of the polymorphisms found in the VDR gene related to the periodontitis susceptibility includes *Apal*, *Bsml*, *TaqI*, and *FokI*, but inconsistency result showed up.<sup>17</sup> Association of VDR *TaqI* polymorphism with periodontitis found in India, Italia, Brazil and China.<sup>18-21</sup> Studies in Jordan, some region in Asia and Brazil also found significant association of VDR *Apal* and *Bsml* polymorphism with periodontitis.<sup>17,20,22</sup> While no significant association found between all fourth VDR polymorphism with periodontitis in Columbia.<sup>23</sup>

Previous study identified *FokI* as the only polymorphism that gives significant impact to the structure and function of the encoded VDR protein.<sup>24</sup> Earlier studies suggested mixed relationship between *FokI* polymorphism and periodontitis. Previous studies on Chinese, Japanese, Libyan and Columbian population suggested negative relationship between VDR *FokI* polymorphism and periodontitis.<sup>23,25-27</sup>

Another studies on Japanese male, Chinese, South Korean and Thailand population on the other hand suggested positive relationship between *FokI* polymorphism and periodontitis.<sup>28-32</sup> This study focused on the association of VDR polymorphism *FokI* to the chronic periodontitis in the Sumatera Utara community.

## Materials and methods

This case-control study was comprised of 83 non-smoking subjects, 43 chronic periodontitis patients (13 males and 30 females) and 40 (11 males and 29 females) periodontally healthy control subjects with an age range between 20-65 years old that had been recruited from patients who visited the Department of Periodontics, Universitas Sumatera Utara. None of the CP patients and control subjects had systemic disease such as diabetes mellitus, hypertension, kidney disease, heart disease and human immunodeficiency virus infection determined via questionnaire and medical history review. Subjects who had received prophylaxis treatment for the last 6 months or had taken antibiotics, vitamin D, anti-inflammatory, anticonvulsant, or using orthodontic appliance or removable prosthetic appliance were also excluded from this study. All research works in this study had been granted an approval of the study protocol and by the health research ethical committee of Universitas Sumatera Utara.

Diagnosis and classification of study and control group were established based on the basis of clinical parameters. This includes the physical examination, medical and dental history, periodontal probing depth (PD) and assessment of clinical attachment level (CAL) using the periodontal probe (UNC-15). The CP group subjects were selected based on clinical examination on patients with at least 1 tooth with Probing Depth (PD)  $\geq$  5 mm and Clinical Attachment Loss (CAL)  $>$  0 mm. The dental radiographs show match alveolar bone destruction. None of the included healthy control participants has periodontal disease as determined by no clinical attachment loss and no probing depth  $>$  3 mm.

The collection of cells samples were carried out through buccal swab followed by DNA extraction using Geneaid Presto™ Buccal Swab gDNA Extraction Kit. VDR *FokI* gene were amplified by PCR by using 5'-AGC TGG CCC TGG CAC TGA-3' and 5' ATG GAA ACA CCT TGC TTC TTC TCC CTC-3' primers.<sup>30,33</sup> The amplification consisted of 94 °C hot start for 5 min, and 35 cycle of 94 °C for 30 s, 61 °C for 30 s, 72 °C for 1 min and final extension at 72 °C for 7 minutes.<sup>33</sup> The PCR products were then digested with the *FokI* cut smart restriction enzyme at 37 °C for 60 min. The visualization

was performed in 4% agarose gel electrophoresis and obtain 265 bp for *FF*, 169 and 96 bp for *ff* and all three bands for the heterozygote *Ff*.

The genotype distribution of VDR *FokI* were tested for Hardy-Weinberg equilibrium. Genotypes and alleles frequency distribution between study group and control were compared using chi squared test. Odd ratio (OR) with the 95% confidence intervals (95% CI) were used to calculate the risk associated with individual alleles and genotypes.

## Results

The demographic and clinical features of patients with chronic periodontitis and healthy control subjects are shown in Table 1.

The population of this study is comprised of non-smoker individuals both in case and control groups. Individuals with CP were likely older and less educated ( $P<0.001$ ). While no significant difference found in gender and scaling treatment experience between study groups.

The genotype distribution of VDR *FokI* polymorphism in CP and Control group were consistent with Hardy-Weinberg Equilibrium with  $P=0.38$  and  $P=1$  respectively. Allele *F* of the VDR *FokI* polymorphism was carried by 59.6% (99 out of 166) of the participants, of which, 34.9% (29 out of 83) were homozygous and 49.4% (41 out of 83) were heterozygous. The distribution of VDR *FokI* genotypes and alleles in CP patients and control subjects are shown in Table 2.

The proportion of *FF* genotype compare to *ff* genotype in CP patients were found to be higher than the control group ( $P=0.016$ ). The *F* allele frequency in CP patients were significantly higher than the control groups ( $P=0.018$ ). This study showed that individuals with *FF* genotype were significantly at higher risk to periodontitis ( $OR=6.33$ , 95% CI= 1.41-28.39). Individuals with *F* carrier (*FF+Ff*) also showed significantly higher risk to chronic periodontitis ( $OR=4.44$ , 95% CI= 1.12-17.56). The allele analysis hown similar result which *F* allele were at higher risk to periodontitis ( $OR=2.18$ , 95% CI = 1.161-4.111).

## Discussion

Vitamin D has been related to the periodontal disease progress due to its function in bone remodeling and immune response.<sup>34,35</sup>

The interaction of VDR with 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) activated the receptor to regulate target genes either activation or repression of transcription.<sup>11,36</sup> Therefore, VDR gene is an interesting gene candidate for its association with periodontitis.<sup>37</sup>

| Characteristic                         | CP Patients      | Control Subjects | p value* |
|--|------------------|------------------|----------|
| Number of individuals                  | n = 43           | n = 40           |          |
| Age (yr) ( $\pm$ SD)                   | 43.56 $\pm$ 10.6 | 31.95 $\pm$ 7.1  | <0.001   |
| Gender (%)                             |                  |                  | 0.813    |
| Female                                 | 30 (69.8)        | 29 (72.5)        |          |
| Male                                   | 13 (30.2)        | 11 (27.5)        |          |
| Educational (years) (%)                |                  |                  | <0.001   |
| ≤ 9                                    | 11(25.6)         | 2(5)             |          |
| 10-12                                  | 18(41.9)         | 8(20)            |          |
| ≥ 13                                   | 14(32.6)         | 30(75)           |          |
| Scaling treatment experience (time)    |                  |                  | 0.184    |
| < 1                                    | 22(51.2)         | 14(35)           |          |
| ≥ 1                                    | 21(48.8)         | 26(65)           |          |
| N teeth present ( $\pm$ SD)            | 25.95 $\pm$ 3.96 | 29 $\pm$ 2.20    | <0.001   |
| Average Probing Depth ( $\pm$ SD) (mm) | 3.90 $\pm$ 0.9   | 2.2 $\pm$ 0.30   | <0.001   |
| Average CAL ( $\pm$ SD) (mm)           | 5.4 $\pm$ 1.6    | 0                | <0.001   |

**Table 1.** Demographic and Clinical Characteristic of the Studied Population. \*Chi-square or t-test

|                                       | CP Patients           | Control Subjects  | P      | OR    | 95% CI     |
|---------------------------------------|-----------------------|-------------------|--------|-------|------------|
| Genotype                              | n=43                  | n=40              |        |       |            |
| <i>FF</i>                             | 19 (44.2)             | 10 (25)           | 0.016† | 6.33† | 1.41-28.39 |
| <i>Ff</i>                             | 21 (48.8)             | 20 (50)           | 0.086† | 3.50† | 0.84-14.60 |
| <i>ff</i>                             | 3 (7)                 | 10 (25)           | -      | -     | -          |
| ( <i>FF+Ff</i> ) / <i>ff</i>          | 40 (97) / 3 (7)       | 30 (75) / 10 (25) | 0.034* | 4.44* | 1.12-17.56 |
| <i>FF</i> / ( <i>Ff</i> + <i>ff</i> ) | 19 (44.2) / 24 (55.8) | 10 (25) / 30 (75) | 0.106* | 2.37* | 0.93-6.05  |
| Allele                                | n=86                  | n=80              |        |       |            |
| <i>F/f</i>                            | 59 (68.6)/27(31.4)    | 40(50)/40(50)     | 0.018* | 2.18* | 1.16-4.11  |

**Table 2.** Distribution of VDR *FokI* Genotypes and Alleles in CP Patients and Control Subjects. \*Chi-square † Regression Logistic.

There are various types of VDR polymorphisms that been associated to periodontitis such as *Apal*, *Bsml*, *TaqI* and *FokI*.<sup>23,37</sup> VDR *FokI* is the only VDR polymorphism which known for its VDR protein structural change and been recently explored for its functional effect differences.<sup>38</sup> The VDR *FokI* polymorphism has a missense on the first start codon (*ATG*→*ACG*) resulting in translational initiation at an in frame ATG 3 codons downstream and form 3 amino acids shorter VDR protein.<sup>38-40</sup> The presence of the *FokI* restriction endonuclease site produced 427 amino acids VDR protein (denoted *f*), whereas the absence of this site (denoted *F*) producing a VDR with 424 amino acids.<sup>41</sup>

Previous studies suggested that the genotype frequencies of VDR *FokI* showed significant racial differences. The *F* allele of the Columbian shows 87.5%, which of 36.3% were homozygous.<sup>23</sup> The frequency for *FF*, *Ff* and *ff* in South Korean study was about 35%, 48% and

17%, respectively.<sup>30</sup> In Japan, the frequency for *FF*, *Ff* and *ff* genotypes were about 35-40%, 45%, 15-20%.<sup>27,28</sup> The genotypes frequency of *FF*, *Ff* and *ff* among several study in china were about 27-29%, 48-49%, 22-25% respectively.<sup>26,29,31</sup> In present study, the *F* allele was 59.6% from all subjects and the proportion of *FF*, *Ff* and *ff* genotype were 34.9%, 49.4% and 15.7% respectively.

The present study suggested that the individuals with homozygote *FF* and Heterozygote *Ff* have significant risk of developing chronic periodontitis. A previous study suggested the significant difference of VDR *Fok I* risk genotype between no/mild periodontitis and severe periodontitis.<sup>32</sup> Individuals with allele *F* has 1.8 times risk to Aggressive Periodontitis.<sup>30</sup> The short VDR protein (*F* allele) were 1.5-2 times more active in gene transcription of the target gene than the long VDR protein.<sup>10,30</sup>

No association found in China, Japan, Libya and Columbia studies between VDR *FokI* polymorphism and periodontitis.<sup>17,25,27</sup> The racial and environmental differences among population may be a high significant factor.<sup>26</sup> Different characteristic of study subjects could alter the result. A previous study showed no relation of VDR *FokI* polymorphism periodontitis among pregnant women<sup>27</sup> whereas in other study by Naito, et al. in Japanese male subjects shows positive correlation to the risk of severe chronic periodontitis.<sup>27,28</sup>

Periodontitis is a multifactorial chronic disease which lead to periodontal connective and bone destruction.<sup>42</sup> During the exposure with microbial pathogens, the initial innate immune response of the periodontal tissue include the regulated expression of some host defense peptide such as human  $\beta$ -defensin and cathelicidin (LL37).<sup>43</sup> Apparently, gene that express human  $\beta$ -defensin 2 (hBD-2), human  $\beta$ -defensin 3 (hBD-3), and cathelicidin (LL-37) has Vitamin D Responsive Elements(VDRE) on its gene promoter.<sup>44</sup> The cathelicidin (LL-37) peptides produced by secondary granules of neutrophils and also found in many other cells including epithelial, gingival and macrophages.<sup>45</sup> This peptides are part of the host innate immune response that has antimicrobial, antifungal, and antiviral activities.<sup>46</sup>

During periodontitis, the synthesis and release of proinflammatory cytokines and prostanooids elevated.<sup>47</sup> A study using human

periodontal ligament fibroblasts (hPdLF) and primary human periodontal ligament cells (hPdLC) showed the role of vitamin D on the immune response. The presence of vitamin D activate VDR to suppress the cell to produce interleukin-6 (IL-6), interleukin-8 (IL-8), and monocyte chemotactic protein-1 (MCP-1) after induced by *Porphyromonas gingivalis* lipopolysaccharide (LPS) or heat-killed *P. gingivalis*.<sup>48</sup>

The interaction of genetic polymorphisms such as SNPs with environmental factors could influence disease susceptibility.<sup>26</sup> The CC+CT (*FF* and *Ff*) genotypes of *FokI* Polymorphism in non-smoker Chronic Periodontitis patients were 1.9 fold higher than the *TT*(*ff*) genotype, while the risk increased up to 9.6 fold when combine with the smoking habit.<sup>32</sup>

In the presence of vitamin D, there were suppression of IL-12 which known as the  $T_{h}1$  polarization factor. The IL-12 cytokine induced  $T_{h}17$  cells to differentiate to be  $T_{h}1$  cells which produce pro-inflammatory mediators.<sup>49</sup> A previous study on dendritic cells that induced by lipopolysaccharide showed that the homozygous *FF* genotype VDR were significantly higher in production of IL-12 than the *ff* genotype VDR.<sup>38</sup> The *FokI* polymorphism is located at the N-terminal end of the VDR molecule, while the ligand-binding domain localized in C-terminal.<sup>35,38</sup> Adding vitamin D showed no significant difference between these two structurally different VDR proteins. This finding indicated that the presence of the vitamin D as the ligand did not have influence in VDR function between two variance.<sup>35</sup>

There were approximately 11 genes of bone and mineral homeostasis that regulated by VDR and its ligand such as osteopontin, an ossification trigger, TRPV6, as calcium channel in the intestine, LRP5 for osteoblastogenesis, RANKL for bone resorption trough osteoclastogenesis and the repression of OPG as RANKL decoy receptor.<sup>50</sup> *FF* genotype and *F* allele has 2 times risk to discopathies and/osteochondrosis concomitant with disc herniation.<sup>51</sup>

As the resorption and formation of the bone are coupled, osteocalcin is considered as a valid marker of bone turnover, while the alkaline phosphatase is a part of the normal turnover of periodontal ligament, root cementum formation and maintenance, and bone homeostasis,

indicating their important role as biomarker in periodontal disease.<sup>52</sup> Higher level of alkaline phosphatase were found in Chronic periodontitis patients compared to those in gingivitis patients and healthy periodontal subjects.<sup>53</sup> Gingival crevicular fluid level of Osteocalcin was also found to be higher in chronic periodontitis patients compared to those in healthy periodontium.<sup>54</sup> An in-vitro study of gingival fibroblast and periodontal ligament cell culture showed that *FF* genotype were more active in induction of protein transcription such as osteocalcin and alkaline phosphatase. Therefore higher transcriptional activity were found in *FF*-VDR cell compared to *Ff*-VDR or *ff*-VDR.<sup>55</sup>

The overall result showed that *F* allele had potential to increase the risk of chronic periodontitis. Similar result was also found in Taiwan Han population with *F* allele(OR=2.02), which *f* allele were more protective to periodontitis.<sup>29</sup> Another study also found higher activity of the *F* human VDR demonstrated by the induction of 1,25(OH)<sub>2</sub>D<sub>3</sub>, relative to the *f* hVDR (4.2 fold compare to 2.6 fold respectively). The *F* hVDR interacted more efficiently with TFIIB than the *f* hVDR, thus providing a plausible mechanism for greater transactivation potency of the *F* hVDR.<sup>41</sup> Liganded VDR needed to interact directly or indirectly with basal transcription factor such as TFIIB to get the establishment of stable pre-initiation complex for the target gene.<sup>36</sup>

## Conclusions

The VDR *FokI* polymorphism genotype and allele showed significant difference between CP patients and control subjects. There were also an association between *FF* genotype and *F* allele to chronic periodontitis in our population. VDR genotype can be potentially used as a risk marker for determining the susceptibility of chronic periodontitis.

## Declaration of Interest

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