Prevalence of vitamin D receptor gene *Fok I* polymorphism in patients with systemic lupus erythematosus – a preliminary report

Jarosław Bogaczewicz[1], Anna Sysa-Jędrzejowska[1], Jacek Łukaszkiewicz[2], Beata Kaleta[2], Anna Woźniacka[1]

[1]I Department of Dermatology and Venereology, Medical University of Lodz, Poland
Head: Prof. Anna Sysa-Jędrzejowska MD, PhD
[2]Department of Biochemistry and Clinical Chemistry, Medical Univeristy of Warsaw, Poland
Head: Prof. Dariusz Sitkiewicz PhD

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Abstract

Introduction: Recognition of the allele responsible for the phenotype of osseous tissue in patients with systemic lupus erythematosus (SLE) would be an important step forward in diagnostic and preventive management in osteoporosis.

Aim: To assess the frequency of the nuclear receptor of vitamin D (VDR) gene polymorphism *Fok I* in SLE patients in comparison to the control group.

Material and methods: The study included 56 patients with SLE. The control group comprised 65 samples of blood samples received from healthy blood donors. DNA isolation was performed using Boom's technology on silicone magnetic particles in NucliSens® miniMAG™, followed by real-time polymerase chain reaction with the Simple Probe for *Fok I*.

Results: In SLE patients, the distribution of ff homozygotes was 14.29% patients; that of the Ff heterozygotes was 60.71%, and of the FF homozygotes was 25%. In the control group, the distribution was 15.38% for ff homozygotes, 61.54% for Ff heterozygotes, and 23.08% for FF homozygotes. No significant difference in the frequency of *Fok I* between SLE patients and the control group was found.

Conclusions: Our preliminary report indicates that the distribution of *Fok I* VDR gene polymorphism in patients with SLE is not significantly different from that of the general population.

Key words: vitamin D, polymorphism, systemic lupus erythematosus.

Introduction

Patients with systemic lupus erythematosus (SLE) are at high risk of osteoporosis [1, 2]. The frequency of osteoporosis in SLE is estimated at 18% and increases up to 68% in patients undergoing systemic glucocorticoid therapy [1, 2]. Reasons for bone loss include limitation of locomotor activity, impaired function of kidneys, chronic inflammation, actions of cytokines and other mediators of inflammation, insufficient vitamin D status, earlier menopause, and also undertaken therapy, especially with glucocorticoids [3, 4]. Van Staa *et al.* showed that the risk of fractures was increased proportionally to the dose of oral glucocorticoids [5]. In patients treated with a daily dose of prednisolone < 2.5 mg, the risk of fractures was 1.55 times higher than in the control group of untreated persons. The daily dose of prednisolone in the range of 2.5-7.5 mg was associated with two times higher risk, and at the dose > 7.5 mg over five times higher. This means that in patients taking the equivalent of prednisolone > 7.5 mg a day within a period of 3 months, the probability of a fracture of the spine is over five times higher than in the control group [5]. The risk of osteoporotic fracture in patients treated with glucocorticoids through the period of 5-10 years affects over 30% of them, and one needs to take into account that in older patients the femoral neck fracture is connected with approximately 35% risk of death within a period of 1 year [6]. On the other hand, one ought to remember that the average daily consumption of calcium by an average Polish man is evaluated at 400 mg, whereas the recommended intake is about

Address for correspondence: Jarosław Bogaczewicz MD, PhD, I Department of Dermatology and Venereology, Medical University of Lodz, 5 Krzemieniecka, 94-017 Lodz, Poland, tel.: +48 42 686 79 81, fax: 042 688 45 65, e-mail: jaroslaw.bogaczewicz@umed.lodz.pl

1000-1500 mg [7]. That is why the chronic course of SLE along with systemic glucocorticoid therapy significantly increases the risk of osteoporosis. Undoubtedly, genetic background in the development of osteoporosis is also essential. Searching for genetic factors is facilitated by estimation of frequencies of given alleles in individuals with a given phenotype. Any involvement of a selected gene with bone metabolism is a starting point to research its relationship with osteoporosis [8]. The discovery of a gene coding the receptor of vitamin D (vitamin D receptor – VDR) shed new light on the connections between genetic background and bone mineral density. Several lines of evidence indicate the existence of VDR polymorphism [9]. The term polymorphism defines a simultaneous occurrence of different allelic forms of a given genotype in the population.

Aim

To assess frequency of the nuclear receptor of vitamin D (VDR) gene polymorphism *Fok I* in SLE patients in comparison to the control group.

Material and methods

The study covered 56 patients with SLE, including 50 women and 6 men, at the age of 44.18 ±11.71 years, treated at the Department of Dermatology and Venereology of the Medical University of Lodz. The control group comprised 65 blood samples received from healthy blood donors.

The DNA isolation from the full blood was performed using Boom's technology on silicone magnetic particles in NucliSens® miniMAG™, followed by real-time polymerase chain reaction (RT-PCR) with the Simple Probe for *Fok I*. The probe specifically hybridizes with the sequence of DNA containing the selected polymorphism. This procedure enables one to identify a single change of mononucleotide in a sequence of DNA of the VDR gene *Fok I* (single-nucleotide polymorphism – SNP). The genotype TT represents the polymorphism ff, TC represents Ff, and CC represents FF. The following reagents were used: lysis buffer from bioMerieux (catalogue No. #200292), a set of extraction reagents from bioMerieux (catalogue No. #200293), LightCycler® 480 Probes Master from Roche (catalogue No. #04-707-494-001), and the probe Light SNiP rs2228570 (*Fok I*) Hu VDR from TIB MOLBIOL. The study was approved by the local Ethics Committee (No. RNN/67/08/KE).

Table 1. Polymorphism *Fok I* of VDR gene polymorphism in patients with SLE and control group

	Polymorphism [%]		
	ff	Ff	FF
SLE (n = 56)	14.29	60.71	25
Control group (n = 65)	15.38	61.54	23.08

Results

The frequencies of *Fok I* genotypes in SLE patients and in the control group are shown in Table I. 14.29% of SLE patients were ff homozygotes, 60.71% were Ff heterozygotes, and 25% were FF homozygotes. In the control group, ff homozygotes accounted for 15.38% of persons, Ff heterozygotes for 61.54%, and FF homozygotes for 23.08%. The comparison of the frequency of *Fok I* based on the analysis with the χ^2 test did not reveal any significant difference between SLE patients and the control group (Fig. 1).

Discussion

The product of gene expression of VDR is a transcription factor that binds with calcitriol. This enables the expression of approximately 200 genes to be launched. Molecular characteristics and cloning of the human VDR was undertaken by Baker *et al.* in 1988 [10]. The VDR contains a sequence of 427 amino acids [10, 11]. Up to now there is only one study addressing the *Fok I* VDR polymorphism in patients with SLE [12]. Huang *et al.* did not find significant differences in the frequency of *Fok I* between 52 patients with SLE and with the control group [12]. It needs to be underlined that the patients and the control group of the aforementioned study were recruited among a Chinese population [12]. 21.2% of SLE patients were FF homozygotes, 65.4% Ff heterozygotes,

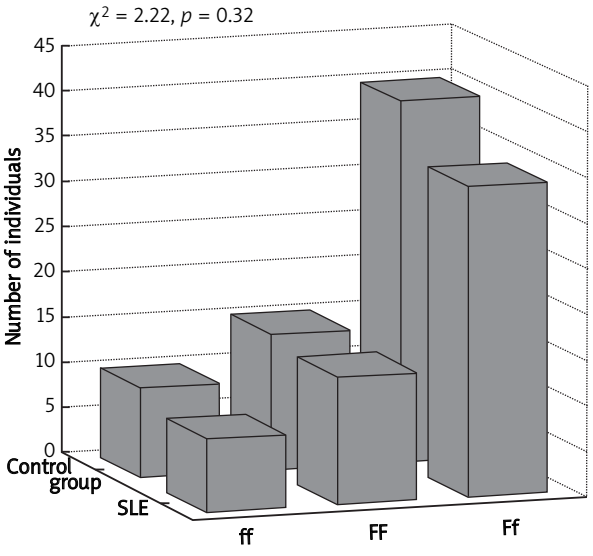

Fig. 1. Analysis of statistical significance of differences in prevalence of VDR gene *Fok I* polymorphism in SLE patients and control group

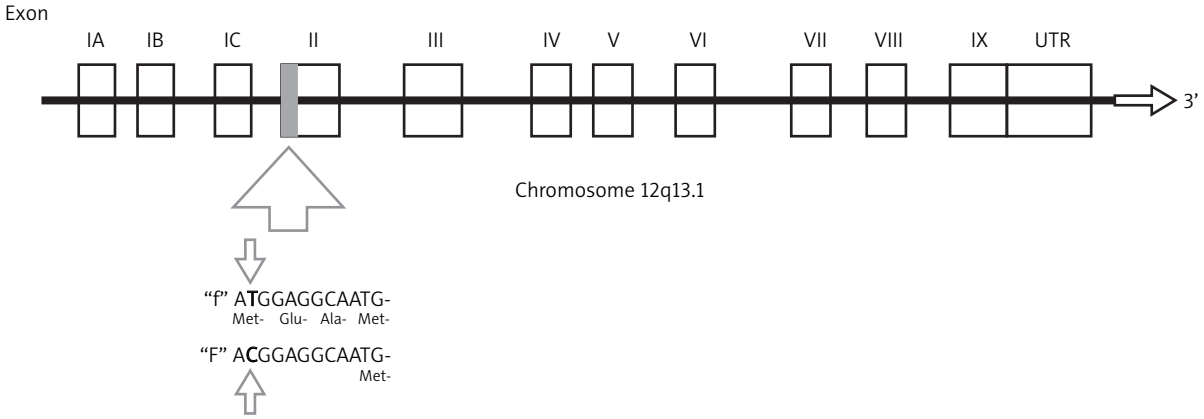

Fig. 2. Diagram of VDR gene and location of *Fok I* polymorphism

and 13.4% ff homozygotes. In the control group, 23.3% of persons were FF homozygotes, 47.8% Ff heterozygotes, and 28.9% ff homozygotes [12]. In our study, the analysis of *Fok I* was undertaken in the Polish population. In accordance with the results of the Chinese authors, we did not reveal significant differences in the frequency of *Fok I* between SLE patients and healthy individuals. Ascertained frequencies undoubtedly result from the polymorphism, as the criterion that differentiates a polymorphism from a mutation is precisely the frequency. If in the population a change of a sequence of nucleotides occurs more often than in 1%, it is a polymorphism. The study of Ozaki *et al.* based on the polymerase chain reaction method (PCR) and restriction fragment length polymorphism (RFLP) investigated the frequency of another VDR gene polymorphism *BsmI* in 58 patients with SLE [13]. Allele B in BsmI polymorphism represents in the RFLP method longer fragments of DNA (with greater molecular mass), whereas allele b represents shorter fragments. Ozaki *et al.* found that the genotype BB occurred more often in SLE patients in comparison to the control group [13]. These results were in accordance with those of Huang *et al.*, that revealed higher frequency of the genotype BB in patients with SLE than in the control group [14]. Genotype bb is profitable for bone parameters, while the occurrence of allele B in VDR is connected with decreased bone mass [8]. The density of bone mass in the lumbar spine in individuals with genotype bb was significantly higher than in the case of BB, and the difference appeared to represent approximately 10 years of life. The genotype bb occurs in the Japanese population more than twice as often as in Caucasians (respectively 77% and 33%) [8]. On the other hand, there are reports indicating a lack of correlation between the VDR genotype and bone mineral density as well as showing populations in which the bb genotype was connected with lower bone mass. Reasons for the discrepancies are suspected to involve linkage disequilibrium between the VDR gene and another gene located in proximity, that may also influence the bone mass [8]. *Fok I* polymorphism is due to exchange of nucleotides, i.e. transition T to C in exon 2, defined as letter F, that results in elimination of the origination site of translation, and it in turn leads to elimination of 3 amino acids in the VDR molecule (Fig. 2). Laboratory studies show that shortened VDR is characterized by greater transcriptional activity as a consequence of enhanced binding to transcription factor IIB [15]. Unquestionably, the detection of one or several genes determining a broad spectrum of phenotypic features of osseous tissue, including both parameters of endurance, bone turnover, and susceptibility to fractures, would constitute a step forward in diagnosis and prophylaxis of osteoporosis in patients with systemic lupus erythematosus. However, much more probable is the involvement of many different genes, from which each may separately influence the expression of a given feature. Therefore, it seems that a group of genetic factors influence bone metabolism. Moreover, expression of a given allele may be connected with other genes and alleles [8]. Despite the great diversity of variants of the VDR gene, determining whether the occurrence of a given polymorphism may dispose to the development of SLE or given symptoms encounters difficulties. This results from the fact that up to now the exact role played by VDR polymorphism is not fully understood. For instance, Oakley-Girvan *et al.* ascertained that in FF homozygotes of Afro-American origin, the risk of prostate cancer is 1.9 times increased [16]. On the other hand, Chen *et al.* found 1.34 times higher risk of breast cancer in ff homozygotes in comparison to the FF genotype [17], and in the study of a Chinese population ff homozygotes were found to be at 2.3 times increased risk of tuberculosis [18]. All these results indicate the importance of the role of VDR polymorphism and the necessity of further investigations on large groups of patients [19-21].

Conclusions

Our preliminary report indicates that the distribution of *Fok I* VDR gene polymorphism in patients with SLE is

not significantly different from that of the general population.

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