Coll. Antropol. **36** (2012) 2: 451–457
Original scientific paper

Polymorphisms of Vitamin D Receptor Gene in the Population of Eastern Croatia with Psoriasis vulgaris and Diabetes mellitus

Melita Vukšić Polić[1,2], Ivana Ručević[3], Vladimira Barišić-Druško[3], Maja Miškulin[2,4], Ljubica Glavaš-Obrovac[5], Mario Štefanić[2,5], Ivan Karner[2,5], Jasna Lipozenčić[6], Tatjana Bačun[2,7] and Ivan Mihaljević[2,5]

[1] »J. J. Strossmayer« University, Osijek University Hospital Center, Department of Dermatology, Osijek, Croatia
[2] »J. J. Strossmayer« University, School of Medicine, Osijek, Croatia
[3] Private Practice for Dermatology and Venereology, Osijek, Croatia
[4] Institute of Public Health for the Osijek-Baranya County, Osijek, Croatia
[5] »J. J. Strossmayer« University, Osijek University Hospital Center, Clinical Institute of Nuclear Medicine and Radiation Prevention, Osijek, Croatia
[6] University of Zagreb, School of Medicine, Department of Dermatology and Venereology, Zagreb, Croatia
[7] »J. J. Strossmayer« University, Osijek University Hospital Center, Clinic for Internal Medicine, Osijek, Croatia

ABSTRACT

The aim of this study was to evaluate the possible association between polymorphisms in the Vitamin D receptor gene (VDR gene) and tendency for development of psoriasis vulgaris and diabetes mellitus in the population of Slavonia, which is a region in the Eastern Croatia. In order to conduct the mentioned evaluation the restriction fragment length polymorphisms (ApaI, BsmI and TaqI) in the Vitamin D receptor gene were researched in three groups of patients: patients suffering only from psoriasis vulgaris, patients suffering only from diabetes mellitus, and patients suffering at the same time from both diseases. Four most common genotypes were found in all standardized control patients: triple heterozygotes BbAaTt (in 29.3% of the studied patients), bbAaTT (in 18.6% of the studied patients), bbaaTT (in 12.9% of the studied patients) and BbAATt (in 8.6% of the studied patients). Three most common VDR 3'-RFLP haplotypes determined in this study were: three-component baT, Bat and bAT haplotype. Results of the Hardy-Weinberg equilibrium showed presence of BsmI *polymorphism genotype frequencies disequilibrium in the group of patients suffering from psoriasis and* ApaI *polymorphism in the group of patients suffering from both diseases. According to the same statistical test all conditions for* TaqI *polymorphism genotype frequency were fulfilled in all groups of studied patients. There was no significant difference in distribution of* BsmI, ApaI *or* TaqI *polymorphism genotype frequencies between control patients and any of the subgroup of studied patients. In studied population none of analysed polymorphisms individually was associated with the risk of development of psoriasis, diabetes or combined phenotype.*

Key words: *psoriasis vulgaris, diabetes mellitus, Vitamin D receptor gene polymorphisms*

Introduction

Psoriasis and diabetes represent serious public health problems. Psoriasis is a chronic immune-inflammatory-mediated skin disease that affects up to 3.0% population worldwide[1]. Diabetes mellitus is a chronic disease caused by the inability of pancreatic beta cells to maintain adequate insulin secretion to prevent hyperglycemia. According to the International Diabetes Federation global prevalence of disease is 6.6%[2]. They have negative influence on the quality of life of affected patients due to the fact that both diseases are chronic and because of later complications (psoriatic arthritis, diabetic neuropathy, nephropathy, retinopathy and micro-angiopathy)[3–5]. Association between psoriasis and diabetes was discovered several decades ago. In 1897, Strauss drew particular at-

Received for publication February 17, 2010

tention to development of diabetes mellitus in patients with psoriasis[6]. Since then, many authors studied pathological values of blood sugar (GUK) in patients with psoriasis vulgaris[7,8] as well as statistical correlation between psoriasis and diabetes[9–14]. The prevalence of psoriasis and diabetes in Eastern Croatia is significant and more and more often one patient is suffering from both diseases. The prevalence of psoriasis and diabetes mellitus in this part of Croatia is 1.6% and 6.7%, respectively[15,16]. There are data showing genetic background in etiology of psoriasis and diabetes. Some authors consider that one of the causes for development of both psoriasis and diabetes could be polymorphism in the vitamin D receptor gene[17,18].

Vitamin D receptor (VDR) belongs to the group of steroid and thyroid hormone receptors, the family of activation transcription factors and the super family of the core's hormone receptors that include thyroid, steroid and retinoid receptors[19–21]. It has been found in high concentration in T lymphocytes and in macrophages population. The maximal concentration was found in immature immune thymus cells and in mature CD-8 T lymphocytes[19,20]. In the skin VDR was found in keratinocytes[22], Langerhan's cells[23], monocytes[24], fibroblasts[25], endothelial cells[26] and in activated T lymphocytes[27]. Presence of VDR in most of the immune system cells, especially in dendritic cells, as well as in CD8+ lymphocytes, CD4+ lymphocytes[28] and in monocytes/macrophages[29], further emphasizes its immunoregulatory effect[20,30]. In respect to that, the role of Vitamin D in autoimmune diseases is being researched, especially in therapeutic sense[31]. The authors have been researching correlation between diabetes mellitus, as an autoimmune disease, and Vitamin D for years. De Luca and associates concluded that 1,25-Dihydroxiviatmin D3 can prevent and suppress experimental autoimmune encephalomyelitis, rheumatoid arthritis, systemic lupus, and type I diabetes. The authors explained it by claiming that Vitamin D has a suppressive effect on inflammatory T cells. Therewith Vitamin D could have important role in new therapeutic approaches to autoimmune diseases[31]. Casteels and associates suggest that 1,25(OH)2D3 could have protective role against onset of diabetes as a result of increased apoptosis of the Th1 autoimmune effector[32]. Also, Mathieu and associates confirmed in their study that long-term therapy with high dosage of 1,25(OH)2D3 can decrease insulinitis incidence in spontaneous autoimmune diabetes without significant side effects[33]. The gene encoding VDR was located on chromosome 12, region 12q13.11 and it is covering 62 359 base pairs (bp). Its length is 75 kb of the genomic DNA, it is composed of eight encoding exons (II-IX), six non-coding exons (Ia to If), several promoting regions and 3ž-UTR poki A tract. The difference between exons and introns is that exons are codes of proteins, while introns are not involved with the coding for proteins. The intron sequences were once considered to be junk DNA, however, people have recently realized that some of them may be functional. These DNAs may harbor a variety of elements that regulate transcription, e.g., untranslated RNAs and splicing control elements[34]. Exons II to IX form structural part of the VDR gene. hVDR promoters were found in exons If, Ia and Id. That region is responsible for different connections during transcription, resulting in occurrence of three types of hVDR mRNA, through which VDR protein is going to be translated[20,35]. Specificity of the VDR gene in comparison to other core receptor genes lies in the presence of exon 5. Several allelic versions of the VDR gene were identified. Polymorphisms in VDR gene were mostly researched by three restriction enzymes: Apal, BsmI and TaqI[19,20].

By several occasions scientists were examining certain variants of alleles and haplotypes as markers for possible development of osteoporosis and metabolic bone disorders with higher fracture risk. Special attention was given to the possible role of VDR gene variants in development of breast cancer, prostate gland cancer, osteoarthritis, artery arteriosclerosis, diabetes mellitus, hyperparathyroidism, certain infective diseases and psoriasis[19].

There are several polymorphisms in hVDR gene, whereat there are significant differences in studied VDR polymorphisms between different races or ethnic groups. The objective of many studies was to research associations between different VDR genotypes (AA, Aa, aa, BB, Bb, bb, TT, Tt, tt) and tendency for development of certain autoimmune diseases, such as Addison's disease, autoimmune hepatitis, Grave's disease[36–38].

The objective of this study was to research gene variants, that is, restriction fragment length polymorphisms (*Apa*I, *Bsm*I and *Taq*I) in the Vitamin D receptor gene in patients suffering only from psoriasis vulgaris, patients suffering only from diabetes mellitus and patients suffering at the same time from both diseases, as well as possible association between polymorphisms in VDR gene (VDR gene) and tendency for development of psoriasis vulgaris and diabetes mellitus in the population of Eastern Croatia.

Materials and Methods

Patients

The study was performed in 2007. It included 100 randomly selected patients in the region of Eastern Croatia: 35 patients suffering both from psoriasis and diabetes, 40 patients suffering from psoriasis but not from diabetes and 25 patients suffering from diabetes but not from psoriasis. All patients were diagnosed at the Clinic for Internal Medicine and at the Department for Dermatology at the Clinical Hospital Centre Osijek in Osijek, in accordance with existing criteria for diagnosing psoriasis and diabetes in Croatia and worldwide. The mean age of all patients included in the study was 54.6±16.1 (the range was from 10 to 80 years of age). 46 of them were women and 54 were men. Diagnosing criteria for psoriasis was pathohistological diagnostics. All patients were examined by dermatologists/venereologists and endocrinologists, blood samples were collected from the patients and blood glucose level was measured. Also, detailed psoriasis and diabetes anamnesis was obtained. All data

were entered into the questionnaires prepared for this study only. During study no difference was made between patients suffering from diabetes Type I or Type II and psoriasis – all patients were studied together. The group of healthy control patients included 40 volunteers, who had no family connections. They were also coming form the region of Eastern Croatia, the mean age was 39.8±13.8 (the range was from 22 to 70 years of age). 20 of them were female and 20 were male. None of them had clinical signs or family history of psoriasis and/or diabetes mellitus. The study was approved by the Ethic Committee of the Clinical Hospital Centre Osijek and it has been performed in accordance with its instructions. A blood sample (5 mL) was collected from each patient, both psoriatic and diabetic, as well as from healthy control patients. Genotype analysis was made in the Clinical Institute for Nuclear Medicine and Radiation Prevention at the Clinical Hospital Centre Osijek. The blood samples were encoded and after the study was finished they were destroyed.

Methods

High Pure Template Preparation kit, Light Cycler FastStart DNA Master SYBR Green I Kit, DNA Molecular Weight Marker VIII were purchased from Roche Diagnostics, Mannheim, Germany.

- Primer 1 (5'-GGGAGACGTAGCAAAGG-3'),
- Primer 2 (5'-AGAGGTCAAGGGTCACTG-3'),
- Primer 3 (5'-CAGAGCATGGACAGGGAGCAAG-3') and
- Primer 4 (5'-GCAACTCCTCATGGCTGAGGTCTCA-3') were purchased from Invitrogen (Paisley, UK)

Restriction enzymes *Bsm*I, *Taq*I and *Apa*I are products of Sigma (Taufkirchen, Germany).

Genotyping

The genome DNA was extracted from 200 μL EDTA blood using the isolation kit according to the producer's instructions.

Every DNA sample underwent 40 cycles of PCR on the LightCycler machine (Roche Diagnostics, Mannheim, Germany). Quality of PCR products was verified by melting curve analysis. Restriction fragment length polymorphisms were encoded as Bb (*Bsm*I), Aa (*Apa*I) and Tt (TaqI), where a capital letter means absence of the restriction site and a small letter means presence of the restriction site.

The *BsmI* restriction site (rs1544410) was detected by amplifying the region that was overcoming the site with one primer in exon 7 (Primer 1) and with second primer in intron 8 of the VDR gene (Primer 2). The amplification started with an initial denaturation step at 95°C, then 5 seconds at 51°C and 15 seconds at 72°C. The length of PCR products was 359 base pairs (bp). After the amplification, the PCR products were digested with 5 units of the *BsmI* restriction enzymes for 2 hours at 65°C. Then agarose gel (3%) electrophoresis with ethidium bromide was performed. The bands were visualized under UV-transilluminator (Pharmacia Biotech, Uppsala, Sweden). Presence of the *Bsm*I restriction site on both alleles (determined as bb) resulted in 182 and 177 bp fragments, whereas absence (BB) resulted in one non-digested 359 bp fragment.

VDR gene region containing *Apa*I (rs 7975232) and *Taq*I (rs 731236) restriction sites was obtained during PCR reaction by using Primer 3 located in intron 8 and primer 4 located in exon 9 of the VDR gene. The amplification started with an initial denaturation step at 95°C for 10 minutes, followed by 40 amplifying cycles at 95°C for 10 seconds, then 5 seconds at 69°C and 30 seconds at 72°C – the length of PCR products was 740 bp. In order to detect *Apa*I and *Taq*I restriction sites, every PCR product was digested with 3 units for 2 hours at 65°C and with 10 units of the *Apa*I enzyme for 2 hours at 65°C. After digestion with *Apa*I, genotypes were identified as AA (one non-digested PCR fragment of 740 bp), aa (two fragments of 515 and 225 bp) and heterozygote Aa, depending on the presence or absence of a restriction site. *Taq*I digestion revealed one obligatory restriction site, homozygote TT (absence of the specific *Taq*I restriction site) providing fragments of 490 bp and 245 bp. Homozygote tt provides fragments of 290, 245 and 205 bp and heterozygote Tt provides fragments of 490, 290, 245 and 205 bp.

Statistics

Departures from Hardy-Weinberg equilibrium were assessed by using a permutation version of the Guo-Thompson exact test (10000 replications)[39]. Pairwise linkage disequilibrium was assessed by two measures, Lewontin's |D'| and r2 coefficients[40] (Haploview 4.0RC2 package, http://www.broad.mit.edu/mpg/haploview)[41]. For continuous, unpaired data, Kruskall-Wallis and Mann-Whitney U-test were used.

For estimates of strength of association, odds ratios and 95% confidence intervals were calculated using exact logistic regression models from StatXact/LogXact-7 software (Cytel Inc, Cambridge, MA, USA)[42].

To correct for multiple testing, the Westfall-Young max(T) permutation procedure[43] (10000 randomizations), which accounts for the dependence structure between RFLPs, phenotypes and models. was applied (co-dominant model, PLINK version 0.99r, http://pngu.mgh.harvard.edu/purcell/plink)[44].

Haplotype patterns were estimated by Stephens-Donnelly coalescent-based Bayesian method[45,46] (PHASE 2.1, www.stat.washington.edu/stephens) and haplotype frequency distributions compared by permutation testing (10000 replicates). To maximize statistical power, only common observed haplotypes (>10% in pooled samples) were analyzed.

Permutation-corrected p-values <0.05 were taken as significant.

Power calculations were performed using the Genetic Power Calculator program (http://pngu.mgh.harvard.edu/~purcell/gpc)[47]. A multiplicative model, two-tailed type I error rate $\alpha=0.05$ and a population disease prevalence of 0.02 (PV) and 0.06 (DM) were assumed.

Results

Genotyping showed that four most common genotypes (61% of all detected combinations) in studied patients in the region of Eastern Croatia were as follows (by descending order): triple heterozygote genotype BbAaTt (29.3%, 41 out of 140 patients), genotype bbAaTT (18.6%, 26 out of 140 patients), genotype bbaaTT (12.9%, 18 out of 140 patients) and genotype BbAATt (8.6%, 12 out of all 140 standardized patients) (Table 1).

Tri-component baT, Bat and bAT haplotypes are three most common VDR 3'-RFLP haplotypes in the studied population (Table2).

Conditions of the Hardy-Weinberg equilibrium fulfil *Taq*I polymorphism genotype frequencies in all patient groups, as well as all polymorphisms in the combined sample of all patients and in the control patients sample (Table 3).

Hardy-Weinberg disequilibrium of *Bsm*I genotype frequencies (PV), i.e., *Apa*I polymorphism (DM+PV), were found in patients with PV (psoriasis vulgaris), as well as in the subgroup of patients with DM+PV (diabetes mellitus + psoriasis vulgaris).

Strong linkage disequilibria were observed for all markers based on the calculation of |D'| (*Bsm*I-*Apa*I $0.826 \leq |D'| \leq 1$ *Apa*I-*Taq*I) and r^2 coefficients (*Bsm*I-*Apa*I $0.372 \leq r2 \leq 0.441$ *Apa*I-*Taq*I, $p < 10^{-37}$, χ^2-test, df=1, n=100).

Under the presumption of permutation allele effects model, no significant differences in distribution of genotype frequencies, as well as of allele frequencies of the *Bsm*I, *Apa*I or *Taq*I polymorphisms were found between control patients and any of patient subgroups (PV, DM, PV+DM).

There were no significant differences in allele-specific risks, as well as no new significant associations between tested phenotypes.

We had >80% power to detect allelic ORs of ≤0.3 (≥3.33), 0.37 (≥2.65) and 0.41 (≥2.45) corresponding to the minor allele frequencies of 10, 20 and 30% (PV arm of the study), i.e., ≤0.29 (≥3.4), 0.36 (≥2.8) and 0.38 (≥2.65) for the DM arm of the study.

TABLE 1
DISTRIBUTION OF COMBINED ABSOLUTE GENOTYPE FREQUENCIES OF THE *BSM*I, *APA*I AND *TAQ*I POLYMORPHISMS IN DISEASE-AFFECTED PATIENS SAMPLE AND IN CONTROL PATIENTS SAMPLE

VDR genotype	DM-PV (N=35)	PV (N=40)	DM (N=25)	Control patients (N=40)
BBAATt	1	2	–	1
BBAAtt	–	1	5	7
BBAaTT	–	–	–	1
BBAaTt	1	–	–	1
BbAATT	–	–	–	1
BbAATt	2	6	3	1
BbAAtt	–	4	–	–
BbAaTT	–	5	2	2
BbAaTt	16	8	7	10
BbaaTT	–	4	–	1
bbAATT	1	–	–	–
bbAAtt	–	–	–	1
bbAaTT	8	5	5	8
bbAaTt	–	–	–	2
bbaaTT	6	5	3	4

Discussion and Conclusion

This study showed that there were no significant differences in allele-specific risks, as well as no new significant associations between tested phenotypes. 15 out of 27 theoretic possible combinations of *Bsm*I, *Apa*I and *Taq*I genotypes in block 3'-B1 of the VDR gene were found[48]. According to the available literature there were several researchers who studied VDR locus as candidate gene likely to cause development of diabetes mellitus Type I or Type II. McDermott and associates studied VDR gene and etiology of diabetes mellitus Type I in 93 families from South India. They analysed three VDR polymorphisms using restriction enzymes *Taq*I, *Apa*I

TABLE 2
RELATIVE *BSM*I-*APA*I-*TAQ*I HAPLOTYPE FREQUENCIES (%) IN CONTROL PATIENTS AND IN SUBGROUPS OF DISEASE-AFFECTED PATIENTS (STEPHENS-DONNELLY BAYES ALGORYTHM, 95% BOOTSTRAP CI*)

Haplotype	R^2_h	Control patients (N=40)	Disease-affected patients		
			DM (N=25)	PV (N=40)	DM-PV (N=35)
baT	0.980	37.1 (28.2–47.5)	39.1 (26–56)	37.5 (27–48.4)	51.2 (42.9–61.4)
BAt	0.989	32.9 (22–45.9)	39.5 (28–52)	26.1 (17.6–34.7)	29.7 (21.4–38.6)
bAT	0.922	13.2 (6.3–20.1)	16.4 (8–26)	14.9 (5.6–25)	17.1 (8.6–25.7)
BAT	0.781	5.8 (1.3–11.2)	3.6 (0–12)	8.1 (0–16.9)	1.4 (0–4.3)
bAt	0.762	5.3 (0–12.2)	–	6.9 (1.6–15.4)	–
BaT	0.776	4.5 (0–9.7)	–	6.3 (1.5–13.2)	–
Global haplotype effect**	0.544	0.976		0.064	

TABLE 3
HARDY-WEINBERG EQUILIBRIUM TEST RESULTS

RFLP (NCBI dbSNP ID)	Control patients (N=40)	DM (N=25)	PV (N=40)	DM+PV (N=35)	Combined sample (N=140)
	Exact P*	Exact P*	Exact P*	Exact P*	Exact P*
*Bsm*I (rs1544410)	0.193	1	0.021	0.444	0.478
*Apa*I (rs7975232)	0.213	0.682	0.537	0.046	0.092
*Taq*I (rs731236)	0.19	0.423	0.718	0.132	0.462

and *Bsm*I. According to the results of that study allele *b* and haplotypes *bT* and *bAT* could encourage development of diabetes mellitus Type I[49]. Malecki and associates performed case-control study, in which they analysed association between F*ok*I, *Bsm*I, *Ap*aI and *Taq*I polymorphisms of the VDR gene and tendency for development of diabetes mellitus Type II in the population of Poland. 308 patients suffering from diabetes mellitus and 240 healthy control patients were included in the study. Researches did not find any difference between allele frequency neither in control patients nor in diabetic patients. They found equal distribution of haplotypes and their combinations in both studied patient groups, whereupon VDR gene polymorphisms would not influence development of diabetes in Polish patients. Nevertheless, researchers suggested further study following the same path but performed on a bigger patient sample[50]. Pani and associates performed a study that included 152 German families. They analyzed 4 polymorphic restriction sites in the VDR gene (F*ok*I, *Bsm*I, *Ap*aI and *Taq*I) considered as potential causative agents for development of diabetes mellitus Type I in the German population. According to their finding, the restrictive site F*ok*I should not influence development of diabetes mellitus at all[51]. Škrabić and associates included in their study 134 patients suffering from diabetes mellitus Type I and 132 healthy control patients. The objective of their study was either to determine or to deny association between VDR gene polymorphisms and tendency for development of diabetes mellitus Type I in the population of Dalmatia in the Southern Croatia. The results of the study confirmed high association between BBAAtt genotype combination and development of the diabetes mellitus Type I. Also, authors of the study suggested further, extended research[52]. The study showed that under presumption of allele effective multiplicative model there are no significant differences in distribution of genotype frequencies of the *Bsm*I, *Ap*aI or *Taq*I polymorphisms between control patients and subgroup of diabetes patients. According to the co-dominant model, the most common genotypes found in both control patients and diabetes patients were Bb, Aa and TT. The analysis of allele frequencies of the *Taq*I, *Ap*aI and *Bsm*I polymorphisms showed that the most common alleles in diabetes patients are T, A and b. Haplotype analysis performed in this study showed that the haplotype Bat is the most common haplotype in diabetes patients. The study examined VDR gene and its polymorphisms also in psoriasis patients. Differences in distribution of genotype frequencies of the *Bsm*I, *Ap*aI and *Taq*I polymorphisms between control patients and patients suffering from psoriasis vulgaris were not found. In both groups the most common genotypes were Bb, Aa and TT. Single most common alleles found in both control and psoriasis patients were T, A and b. Haplotype analysis determined baT haplotype as the most common one in psoriasis patients. Saeki and associates compared VDR gene polymorphisms found in 115 patients suffering from psoriasis vulgaris and in 69 healthy control patients in the Japanese population. The results showed higher prevalence of bb and TT genotypes in affected patients. Also, there was no significant difference in distribution of genotype frequencies of the *Ap*aI polymorphisms in both examined patient groups. The study showed that B and t alleles had lower prevalence in affected patients group, whereas equal frequency of allele A was found in both examined groups. Taking into consideration the obtained results, in the conclusion the authors confirmed possible role of the VDR gene allele variants in predisposition to psoriasis vulgaris[53]. Park and associates studied the Korean population (104 psoriasis patients and 104 control patients were including). Opposite to the previously mentioned studies, results of their study showed prevalence of allele A in patients suffering from psoriasis vulgaris compared to the control group patients. At the same time, there was a significant correlation between homozygous AA and development of psoriasis vulgaris at the early age of patients[54]. In her study, Ručević examined possible association between psoriasis vulgaris and VDR gene polymorphisms for *Ap*aI, *Bsm*I and *Taq*I. None of analyzed polymorphisms individually, also after the adjustment according to the age of patients, did not show differential age, gender or phenotype specific effects on the risk of developing psoriasis vulgaris. Also, in general population there was no association between any analyzed polymorphism individually and a measurable risk for development of psoriasis vulgaris, psoriasis Type I or Type II. However, the allele Bt is associated with individual protective effect in psoriasis vulgaris Type I, but not in Type II. Ručević concluded that VDR polymorphism can be associated with psoriasis but with significant race differences[55].

In this study we examined VDR gene and its polymorphisms in patients affected with both diseases (psoriasis vulgaris and diabetes mellitus). There are no studies in the available literature about other researchers examining more detailed the VDR gene as potential causative agent for development of both psoriasis vulgaris and diabetes mellitus in one patient. Thereby it makes it diffi-

cult to discuss or compare possibly same or different results obtained by other authors. When comparing control patients and a subgroup of patients suffering from both psoriasis vulgaris and diabetes mellitus (PV+DM), we did not find any difference in distribution of genotype frequencies of the BsmI, ApaI polymorphisms, with exception of TaqI polymorphisms. The most common genotypes in both studied groups are Bb and Aa. Tt genotype is the most common genotype in patients suffering from both PV and DM and TT is the most common in control patients. T, a and b alleles are the most common in control patients and T, a and b in PV+DM patients. The results of the Haplotype analysis method showed that haplotype baT is the most common in both PV+DM patients and control patients group. Further to the above results, in all analyzed patient groups we detected 3 out of 9 haploid alleles with frequency ≥10%. In accordance with other published results[56] also this study showed that the most common VDR 3'-RFLP haplotypes are tri-component baT, Bat and bAT. For more precise assessment of population haplotype frequencies we need significantly bigger patients sample.

To conclude: within restrictions determined by the size and characteristics of the sample, by the limited statistical power and multiplicity of tests, by application of empiric statistics that is maintaining statistical testing validity in set conditions, in studied population there is no association between analyzed polymorphisms individually and risk of psoriasis vulgaris, risk of diabetes mellitus or combined phenotype. However, taking into consideration limited statistical power, one can not exclude with certainty a significant residual effect of individual polymorphisms, which was not possible to determine in a given sample. This conclusion should be basis for further studies with bigger samples.

Acknowledgements

This work was partially financially supported by the Ministry of Science, Education and Sports of the Republic of Croatia. (Project No. 219-2190372-2068).

REFERENCES

1. CROOM KF, MCCORMACK PL, Am J Clin Dermatol, 10 (2009) 43. DOI: 10.2165/0128071-200910010-00008 — 2. UNWIN N, WHITING D, GAN D, JACQMAIN O, GHYOOT G, IDF Diabetes Atlas, fourth edition, The International Diabetes Federation, accessed 05.03.2010. Available from: URL: http://www.diabetesatlas.org/ — 3. BRAUN-FALCO O, PLEWIG G, WOLFF HH, BURGDORFER WHC, Erythematosqumaous Diseases. In: BRAUN FALCO O, PLEWIG G, WOLFF HH, BURGDORFER WHC, (Eds) Dermatology 2nd edition (Springer, Berlin, 2000). DOI: 10.1007/978-3-642-97931-6 — 4. METELKO Ž, GRANIĆ M, ŠKRABALO Z, Diabetes mellitus. In: VRHOVAC B, BAKRAN I, GRANIĆ M, (Eds) Internal medicine 2nd edition In Croat. (Naprijed, Zagreb, 1997). — 5. PAŠIĆ A, Erythematosqumaous and Papulous Dermatosis. In: LIPOZENČIĆ J, (Ed) Dermatovenerology 3rd edition In Croat. (Medicinska naklada, Zagreb, 2008). — 6. STRAUSS H, Deutsche Med Wschr, 309 (1897) 23. — 7. ROST GA, Br J Dermat, 57 (1932) 44. DOI: 10.1111/j.1365-2133.1932.tb09571.x — 8. RAVAUT P, BITH D, Bull Soc Franc Derm Syph, 99 (1926) 33. — 9. LOMHOLT G, Diabetes mellitus and Psoriasis. In: LOMHOLT G (Ed) Psoriasis (G.E.C. GAD, Copenhagen, 1963). — 10. REEDS RE JR, FUSARO RM, FISHER I, Arch Derm, 205 (1964) 89. DOI:10.1001/archderm.1964.01590260043007 — 11. BINAZZI M, CALANDRA P, LISI P, Arch Derm, 254 (1975) 43. DOI: 10.1007/BF00561533 — 12. LINDEGARD B, Dermatologica, 72 (1986) 298. — 13. HENSELER T, CHRISTOPHERS E, J Am Acad Dermatol, 32 (1995) 982. DOI: 10.1016/0190-9622(95)91336-X — 14. SHAPIRO J, COHEN AD, DAVID M, HODAK E, CHODIK G, VINER A, KREMER, HEYMANN A, J Am Acad Dermatol, 56 (2007) 629. DOI: 10.1016/j.jaad.2006.09.017 — 15. BARIŠIĆ-DRUŠKO V, PALJAN D, KANSKY A, VUJASINOVIĆ S, Acta Derm Venerol Suppl, 146 (1989) 178. — 16. METELKO Z, PAVLIC-RENAR I, POLJICANIN T, SZIROVITZA L, TUREK S, Diabetes Res Clin Pract, 81 (2008) 263. DOI: 10.1016/j.diabres.2008.04.016 — 17. VALDIVIELSO JM, FERNANDEZ E, Clin Chim Acta, 371 (2006) 1. DOI: 10.1016/j.cca.2006.02.016 — 18. VAN ETTEN E, VERLINDEN L, GIULIETTI A, RAMOS-LOPEZ E, BRANISTEANU DD, FERREIRA GB, OVERBERGH L, VERSTUYF A, BOUILLON R, ROPE BO, BADENHOOP K, MATHIEU C, Europ J Immunol, 37 (2007) 395. DOI: 10.1002/eji.200636043 — 19. ZMUDA JM, CAULEY JA, FERRELL RE, Epidemiol Rev, 22 (2000) 203. DOI: 10.1093/oxfordjournals.epirev.a018033 — 20. HAUSSLER MR, WHITFIELD KG, J Bon Min Res, 13 (1998) 325. — 21. EVANS RM, Science, 240 (1988) 889. DOI: 10.1126/science.3283939 — 22. FELDMAN D, CHEN T, HIRST M, COLSTON K, KARASEK M, CONE C, J Clin Endocrinol Metab, 51 (1980) 1463. DOI: 10.1210/jcem-51-6-1463 — 23. DAM TN, MOLLER B, HINDKJAER, KRAGBALLE K, J Investig Dermatol Symp Proc, 1 (1996) 72. — 24. RANSON M, POSEN S, MASON RS, J Invest Dermatol, 91 (1988) 593. DOI: 10.1111/1523-1747.ep12477126 — 25. EIL C, MARX S, Proc Natl Acad Sci USA, 78 (1981) 2562. — 26. MERKE J, MILDE P, LEWICKA S, HÜGEL U, KLAUS G, MANGELSDORF DJ, HAUSSLER MR, RAUTERBERG EW, RITZ E, J Clin Invest, 83 (1989) 1903. DOI: 10.1172/JCI114097 — 27. BHALLA AK, AMENTO EP, CLEMENS TL, HOLICK MF, KRANE SM, J Clin Endocrinol Metab, 57 (1983) 1308. DOI: 10.1210/jcem-57-6-1308 — 28. VELDMAN CM, CANTORNA MC, DELUCA HF, Arch Biochem Biophys, 374 (2000) 334. DOI: 10.1006/abbi.1999.1605 — 29. PROVVEDINI DM, TSOUKAS CD, DEFTOS LJ, MANOLAGAS SC, Science, 221 (1983) 1181. DOI: 10.1126/science.6310748 — 30. HUNG-YI CHUANG, KUEI-TING YU, J Occup Health, 46 (2004) 316. DOI: 10.1539/joh.46.316 — 31. DELUCA H, CANTORNA M, FASEB J, 15 (2001) 2579. DOI: 10.1096/fj.01-0433rev — 32. CASTEELS KM, WAER M, BOUILLON E, DEPOVERE J, VALCKX D, LAUREYS JM, MATHIEU C, Clin Exp Immunol, 112 (1998) 181. DOI: 10.1046/j.1365-2249.1998.00568.x — 33. MATHIEU C, LAUREYS J, SOBIS H, VANDEPUTTE M, WAER M, BOUILLON R, Diabetes, 41 (1992) 1491. DOI: 10.2337/diabetes.41.11.1491 — 34. MAJEWSKI J, OTT J, Genome Res, 12 (2002) 1827. DOI: 10.1101/gr.606402 — 35. MIYAMOTO K, KESTERSON RA, YAMAMOTO H, TAKETANI Y, NISHIWAKI E, TATSUMI S, INOUE Y, MORITA K, TAKEDA E, PIKE JW, Mol Endocrinol, 11 (1997) 1165. — 36. PANI AM, SEISSLER J, USADEL KH, BADENHOOP K, Eur J Endocrinol, 147 (2002) 635. — 37. VOGEL A, STRASSBURG CP, MANNS MP, Hepatology, 35 (2002) 126. — 38. ŠTEFANIĆ M, KARNER I, GLAVAŠ-OBROVAC LJ, PAPIĆ S, VRDOLJAK D, LEVAK G, Croat Med J, 46 (2005) 639. — 39. GUO SW, THOMPSON EA, Biometrics, 48 (1992) 361. DOI: 10.2307/2532296 — 40. LEWONTIN RC, Genetics, 49 (1964) 49. — 41. BARRETT JC, FRY B, MALLER J, DALY MJ, Bioinformatics, 21 (2005) 263. DOI: 10.1093/bioinformatics/bth457 — 42. HIRJI KF, MEHTA CR, PATEL NR, JASA, 82 (1987) 1110. DOI: 10.2307/2289388 — 43. WESTFALL PH, YOUNG SS, Resampling-based multiple testing: examples and methods for P-value adjustment (John Wiley & Sons, New York, 1993). — 44. PURCELL S, NEALE B, TODD-BROWN K, THOMAS L, FERREIRA MA, BENDER D, MALLER J, SKLAR P, DE BAKKER PI, DALY MJ, SHAM PC, Am J Hum Genet, 81 (2007) 559. DOI: 10.1086/519795 — 45. STEPHENS M, SMITH NJ, DONNELLY P, Am J Hum Genet, 68 (2001) 978. — 46. STEPHENS M, DONNELLY P, Am J Hum Genet, 73 (2003) 1162. — 47. PURCELL S, CHERNY SS, SHAM PC, Bioinformatics, 19 (2003) 149. DOI: 10.1093/bioinformatics/19.1.149 — 48. NEJENTSEV S, GODFREY L, SNOOK H, RANCE H, NUTLAND S, WALKER NM, LAM AC, GUJA C, IONESCU-TIRGOVISTE C, UNDLIEN DE, RØNNINGEN KS, TUOMILEHTO-WOLF E, TUOMILEHTO J, NEWPORT MJ, CLAYTON DG, TODD JA, Hum Mol Genet, 13 (2004) 1633. — 49. MCDERMOTT MF, RAMACHANDRAN A, OGUNKOLADE BW, AGANNA E, CURTIS D, BOUCHER BJ, SNEHALATHA C, HITMAN GA, Diabetologia, 40 (1997)

971. DOI: 10.1007/s001250050776 — 50. MALECKI MT, FREY J, MOCZULSKI D, KLUPA T, KOZEK E, SIERADZKI J, Exp Clin Endocrin & Diab, 111 (2003) 505. — 51. PANI MA, KNAPP M, DONNER H, BRAUN J, BAUR MP, USADEL KH, BADENHOOP K, Diabetes, 49 (2000) 504. DOI: 10.2337/diabetes.49.3.504 — 52. ŠKRABIĆ V, ZEMUNIK T, ŠITUM M, TERZIĆ J, Diabetes Res Clin Prat, 59 (2002) 31. — 53. SAEKI H, ASANO N, TSUNEMI Y, TAKEKOSHI T. KISHIMOTO M, MITSUI H, TADA Y, TOEII H, KOMINE M, ASAHINA A, TAMAKI K, J Dermatol Sci, 30 (2002) 167. DOI: 10.1016/S0923-1811(02)00073-7 — 54. PARK BS, PARK JS, LEE DY, YOUN JI, KIM IG, J Invest Dermatol, 112 (1999) 113. — 55. RUČEVIĆ I, Vitmin D receptor polymorphism and psoriasis vulgaris. PhD Thesis. In Croat. »J. J. Strossmayer« University, Osijek, 2007). — 56. STRANGER BE, FORREST MS, DUNNING M, INGLE CE, Science, 315 (2007) 848 DOI: 10.1126/science.1136678.

M. Vukšić Polić

»J. J. Strossmayer« University, Osijek University Hospital Center, Department of Dermatology, Osijek, Josipa Huttlera 4, 31 000 Osijek, Croatia
e-mail: melyderma@gmail.com

POLIMORFIZMI VDR GENA U OBOLJELIH OD VULGARNE PSORIJAZE I ŠEĆERNE BOLESTI S PODRUČJA ISTOČNE HRVATSKE

S A Ž E T A K

Cilj ovoga rada bio je istražiti moguću udruženost polimorfizama u genu za vitamin D receptor (VDR gen) sa sklonošću obolijevanju od vulgarne psorijaze i šećerne bolesti u populaciji Slavonije, odnosno Istočne Hrvatske. S obzirom na to polimorfizmi u duljini restrikcijskih odsječaka (*ApaI*, *BsmI* i *TaqI)* u genu za receptor vitamina D su analizirani u bolesnika oboljelih samo od vulgarne psorijaze, u bolesnika oboljelih samo od šećerne bolesti i u bolesnika oboljelih istovremeno od vulgarne psorijaze i šećerne bolesti. U svih tipiziranih kontrolnih ispitanika nađena su četiri najčešće zastupljena genotipa: trostruki heterozigoti BbAaTt (kod 29,3% ispitanika), bbAaTT (kod 18,6% ispitanika), bbaaTT (kod 12,9% ispitanika) i BbAATt genotip (kod 8,6% ispitanika). U ovoj studiji tri najčešća VDR 3'-RFLP haplotipa su bili trokomponentni baT, BAt i bAT haplotip. Rezultati Hardy-Weinberg-ovog ekvilibrija uputili su na disekvilibrij genotipskih frekvencija BsmI polimorfizma u skupini oboljelih od vulgarne psorijaze i ApaI polimorfizma u oboljelih od obje bolesti. Prema istom statističkom testu ispunjeni su uvjeti u svim skupinama ispitanika za genotipske frekvencije *TaqI* polimorfizma. U distribuciji genotipskih frekvencija *BsmI*, *ApaI* ili *TaqI* polimorfizama između kontrolnih ispitanika i bilo koje podskupine nije bilo značajnih razlika. Niti jedan analizirani polimorfizam pojedinačno nije bio udružen s rizikom obolijevanja od vulgarne psorijaze, šećerne bolesti ili kombiniranog fenotipa u ispitivanoj populaciji.