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Clinical risk factors and the role of VDR gene polymorphisms in diabetic retinopathy in Polish type 2 diabetes patients

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Abstract Evidence exists that some clinical, metabolic and genetic risk factors are associated with the development of diabetic retinopathy (DR). The aim of the study was: (1) to define the prevalence of DR in the examined group of 267 patients with type 2 diabetes mellitus (T2DM) from a Polish population; (2) to identify in cross-sectional analysis, the clinical features associated with DR in the study group; and (3) to search for the association of 4 markers of vitamin D receptor (VDR), a candidate gene for vascular complications in diabetes, with DR. The examined group consisted of 146 female and 121 male T2DM patients (mean age at examination: 61.3±9.4 years; age at T2DM diagnosis: 50.0±9.2; T2DM duration: 11.3±7.8 years; body mass index (BMI): 30.5±5.5 kg/m^2; HbA1c: 7.8±1.5%). In all patients, the clinical and metabolic profile was determined. Diagnosis of DR was determined by a trained ophthalmologist by ophthalmoscopy after pupillary dilatation. Colour photographic documentation was made. The examined T2DM patients were genotyped for FokI, ApaI, BsmI and TaqI frequent VDR polymorphisms based on the restriction fragment length polymorphism method. The statistical analysis was performed using univariate and multivariate logistic regression (SAS) and haplotype analysis (Haplostat). DR was detected in 85 (31.8%) patients with T2DM. The multivariate analysis revealed that significant predictors of this complication were: never-smoking status (odds ratio 2.2, 95% confidence interval 1.2–4), urea serum level (1.3, 1.1–1.5), HbA1c level (1.4, 1.1–1.8) and insulin treatment (2.7, 1.4–5.1). Other features such as age of T2DM diagnosis, T2DM duration prior to ophthalmic exam, obesity (BMI>30), serum creatinine level, albumin/creatinine ratio and arterial hypertension were univariate predictors of DR, however they lost significance as independent predictors in multivariate analysis. Similarly, the alleles, genotypes, haplotype and haplotype combination of VDR were not associated with the examined complication. However, there was a suggestion of a possible slight association between the fbaT haplotype and DR (p=0.11). In conclusion, our study showed that DR in T2DM patients remains a frequent complication in Polish T2DM patients. We were able to confirm the role of some clinical risk factors, surprisingly including not-smoking status, as was previously shown in the UK Prospective Diabetes Study (UKPDS). VDR gene polymorphisms did not constitute a risk factor for this size of study group.

Key words Diabetes • Retinopathy • Gene • Risk

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Introduction

One of the most important contemporary medical problems is an epidemic of type 2 diabetes mellitus (T2DM). It is estimated that the number of people with diabetes worldwide exceeds 200 million, most of them being

patients with T2DM [1, 2]. In the societies of the industrialised world, the prevalence of this disease has reached a few percent of entire populations and is still growing [1, 2]. For many decades T2DM, earlier called non-insulin-dependent diabetes, has been regarded as a less dangerous type of disease by both patients and their doctors. Scientists, physicians, patients and entire societies must now realise that T2DM is a leading cause of premature death, mainly from cardiovascular causes and occurrence of complications [3]. Diabetic eye disease, first of all retinopathy, remains a major cause of blindness in the world [4].

A few clinical risk factors of retinopathy in T2DM have been defined so far in some populations. For example, in the milestone UK Prospective Diabetes Study (UKPDS) project, the development of diabetic retinopathy (DR) in T2DM patients was associated with baseline glycaemia, glycaemic exposure over several years, higher blood pressure and not smoking [5]. In other studies, some of those clinical and metabolic risk factors were confirmed [6, 7]. In addition, T2DM complications, such as DR, seem to be also influenced by genetic factors [8, 9]. A number of loci have been studied so far in order to explain the genetic susceptibility to this complication. An example is genes associated with endothelial physiology, vasoconstriction, coagulation, oxidative processes and growth factors [10–12]. Some of those studies produced promising results, but few have been replicated in other populations. One should also mention aldose reductase, a gene from the polyol pathway, associated with DR in T2DM in several studies [13, 14]. Among interesting candidates are genes associated with vitamin D metabolism. Vitamin D not only affects calcium metabolism, but also has anti-proliferative and anti-angiogenic effect. It also regulates the apoptosis process [15, 16]. The active form of this steroid acts through a specific vitamin D receptor (VDR) [17]. This protein is widely expressed in human tissues and organs, including retina [18, 19]. Thus, a hypothesis that variants in the VDR gene may influence susceptibility to DR seems justified. Moreover, two polymorphisms of VDR have been associated with DR in type 1 diabetes in a French population [20, 21].

The aim of the study was: (1) to define the prevalence of DR in a group of patients with T2DM from a Polish population; (2) to identify, in a cross-sectional study, the clinical features associated with this complication; and (3) to search for the association of 4 markers of the VDR gene with DR.

Subjects and methods

We recently reported the results of a T2DM association study with four polymorphisms of the VDR gene that included 308 T2DM patients from a Polish population [22]. All of those patients were contacted and invited for an eye examination. We included in this project 267 unrelated T2DM individuals who agreed to an ophthalmological evaluation. All study individuals were Caucasians and residents of South-Eastern Poland. During the ascertainment, as previously described [22, 23], the current WHO definitions and criteria were used. Briefly, the patients received a standard questionnaire that contained questions regarding the age of T2DM diagnosis, family history, the treatment method, smoking status and other medical issues. Only patients with clinical diagnosis of T2DM and no insulin therapy for at least two years soon after diagnosis were recruited. The study individuals underwent a basic physical examination, which included measurements of height, weight and blood pressure. The individual was classified as having arterial hypertension if he/she met one of the following criteria: (1) a diagnosis of hypertension in previous medical history; (2) antihypertensive treatment prior to the entry of the study; and (3) systolic or diastolic blood pressure ≥140 mmHg or ≥90 mmHg, respectively, during the examination at the study entry. A history of coronary artery disease was established using past medical history and examination of previous and current ECGs. HbA1c was measured by HLPC method (Biorad). Concentrations of cholesterol and triglycerides were measured using enzymatic methods, with HDL cholesterol measured after precipitation of VLDL. The concentration of LDL cholesterol was calculated using the Friedewald formula. Urea serum levels were also determined using the urease method. DNA for genetic analysis was isolated using the standard method (DNAzol, GIBCO). This study was performed according to the Helsinki Declaration and it was accepted by the Ethical Committee of the Jagiellonian University, Medical College. All patients signed informed consent forms.

The presence of retinopathy was determined by a trained ophthalmologist by ophthalmoscopy after pupillary dilatation using 0.5% Tropicamid. The colour photographic documentation was made by a photo camera (Retinal Camera GENESIS); the same procedure as previously described for the EURODIAB study was used [24]. Briefly, two photographs of each eye were taken for every patient. In some cases, an additional picture was made. The final diagnosis regarding the presence of retinopathy was based on both ophthalmoscopy and photographic documentation. The eye evaluation and the analysis of the photographs for the entire group were performed by the same ophthalmologist. Based on this examination, the patients were assigned to one of two groups: (a) no evidence of DR; or (b) presence of DR of any stage.

The study group was genotyped for four VDR sequence differences located in exon 2, intron 8 (two) and exon 9 that can be detected using specific restriction enzyme FokI, BsmI, ApaI and TaqI, respectively. The details of the genotyping procedure have been described previously [22]. Three locus haplotypes formed by BsmI, ApaI and TaqI polymorphisms and carried by each study individual were inferred by the Haplostat program.

Data on quantitative characteristics are expressed as means±SD. Data on qualitative characteristics are expressed as percent values or absolute numbers as indicated. Comparisons between groups were made with the χ^2 test (nominal data) or Student's t-test (interval data). A value of $p<0.05$ was considered statistically significant. Hardy–Weinberg equilibrium was tested by the χ^2 method. Univariate and multivariable logistic regression for the association with DR was performed using the SAS program. We used a two-stage strategy to build a multivariable

regression model. The number of clinical and genetic variables available for analysis was too high, compared with the number of the patients with DR, to build a stable model. Therefore, we used an initial univariate screen of association of variables with retinopathy. Variables that were associated with retinopathy at p value <0.25 were entered into a multivariable model and then standard computer-assisted selection methods were used. Forward, backward and stepwise selection methods led to the same final multivariable model described below.

Results

Overall, we included 267 T2DM patients in the study (female:male 146:121). The mean age at examination was 61.2±12.4 years, age at T2DM diagnosis 50.0±9.2 years, T2DM duration 11.6±7.8 years, body mass index (BMI) 30.5±5.5 kg/m^2 and HbA1c 7.8±1.5%. DR of different stages was detected in 85 (31.8%) patients with T2DM; only 11 (4.1%) were diagnosed with proliferative retinopathy. A relatively high percentage of T2DM patients were on insulin (54.3%), which should be attributed to long disease duration in the study group. Comparison of the clinical parameters between the groups with and without DR is shown in Table 1. T2DM with retinopathy differed from those without DR regarding some examined features. They were diagnosed with T2DM at earlier age, had longer duration of T2DM and had higher HbA1c. In addition, they were more frequently on insulin therapy and diagnosed with arterial hypertension (Table 1).

The distribution of genotypes of the examined VDR gene polymorphisms is shown in Table 2. Genotypes of each VDR marker were in Hardy–Weinberg equilibrium in each group separately, and in the total cohort of 267 T2DM patients. No difference in the distribution of either VDR genotypes or alleles was seen between patients with and without DR. Similarly, no positive finding was recorded for haplotypes and their combinations created by three markers being in linkage disequilibrium (BsmI, ApaI and TaqI) (data not shown).

In order to identify clinical and gene risk factor interactions for DR, the multivariate analysis revealed that significant predictors of this microvascular complication were: never-smoking status (odds ratio 2.2, 95% confidence interval 1.2–4), urea serum level (1.3, 1.1–1.5), HbA1c level (1.4, 1.1–1.8) and insulin treatment (2.7, 1.4–5.1). The other features such as age of T2DM diagnosis, T2DM duration prior to ophthalmic exam, obesity (BMI>30), serum creatinine level, albumin/creatinine ratio and arterial hypertension were univariate predictors of DR, however they lost significance as independent predictors in multivariate analysis. Similarly, the alleles, genotypes, haplotype and haplotype combination of VDR were not associated with the examined complication. However, there was a suggestion of a possible slight association between fbaT haplotype and DR (p=0.11).

Lack of association between the genetic markers and DR could be attributed to heterogeneity in the duration of diabetes, so that control subjects with short duration of diabetes could in the future switch into the retinopathy group after sufficient observation time. To remedy this possible heterogeneity, we performed a stratified analysis comparing susceptible *vs*. resistant phenotype. In order to do that, we compared all the patients with any degree of retinopathy (85 diabetic individuals) to those with no retinal changes in spite of disease duration above the median

Table 1 Clinical characteristic of the patients with and without retinopathy

Clinical parameters	Patients	
	Without retinopathy, n=182	With retinopathy, n=85
Gender (female/male)	97/85	49/36
Age of T2DM diagnosis (years)[a]	51.3 (±9.0)	47.0 (±9.0)*
T2DM duration (years)[a]	9.7 (±7.2)	14.6 (±6.2)*
BMI (kg/m^2)[a]	30.2 (±5.6)	31.3 (±5.3)
HbA1c (%)[a]	7.5 (±1.3)	8.4 (±1.6)*
Total cholesterol (mmol/l)[a]	5.49 (±1)	5.22 (±1.1)
Urea serum level (mmol/ml)[a]	6.0 (±1.6)	8.1 (±4.2)
Insulin therapy (%)	44.2%	76.2*
Hypertension (%)	83.2	93.9*
Coronary artery disease (%)	37.5	45.7
Never smoking (%)	45.7	63.3*

The high proportion of insulin-treated (either alone or in combination with oral medications) T2DM patients is related to the relatively long mean duration of disease

[a]Data are as mean±SD

*p<0.05 for comparison of T2DM patients with and without DR

Table 2 VDR genotypes in patients with and without DR

VDR genotype	T2DM patients with DR (n=85)	T2DM patients without DR (n=182)	p value for the additive model
Marker FokI			
F/F	(25%)	(27.8%)	0.47
F/f	(50%)	(51.1%)	
f/f	(25%)	(21.1%)	
Marker BsmI			
B/B	(11.9%)	(11.6%)	0.79
B/b	(42.9%)	(45.9%)	
b/b	(45.2%)	(42.5%)	
Marker ApaI			
A/A	(20.5%)	(21.6%)	0.23
A/a	(45.8%)	(54.7%)	
a/a	(33.7%)	(23.8%)	
Marker TaqI			
T/T	(46.4%)	(44.8%)	0.66
T/t	(45.2%)	(44.8%)	
t/t	(8.3%)	(10.5%)	

(8 years) for the sub-group without retinopathy (93 individuals). In this additional multivariate analysis, we found that significant predictors of DR were HbA1c (odds ratio 1.3, 95% confidence interval 1.1–1.7) and BMI (1.1, 1.0–1.2). No evidence for the association was found for other examined variables, including the alleles, genotypes and haplotypes of the VDR gene.

Discussion

We report here the prevalence of DR in a group of 267 people with T2DM from a Polish population. DR of different stages was detected in 85 (31.8%) patients from the study group. It should be emphasised that the average duration of the disease for the subjects included in our study was more than 11 years. This suggests that the prevalence of DR in Polish T2DM patients is currently lower than that reported from the British population of the UKPDS. In the group of 1919 patients described by Stratton et al., already at T2DM diagnosis 37% showed evidence of DR and over the next 6 years 22% of those initially free of disease developed the retinal complication [5]. The difference between this and the British study with respect to the prevalence of DR could probably be explained by the stricter criteria of diabetes and more aggressive treatment that followed two milestone trials in the field of type 1 and type 2 diabetes: DCCT and UKPDS, respectively [25, 26]. On the other hand, in the study from the Polish population published in 1990, the prevalence of DR was similar to our data [27]. A possible limitation of our project is possible selection bias, as the response from the patients with severe DR could have been lower than from those free of the examined complication or with earlier stage of its development.

In general, this study confirmed the importance of previously reported clinical risk factors. As expected, the DR diagnosis was associated with the measure of metabolic compensation, which in our study was HbA1c level. The presence of serum urea level among the risk factors for DR in our study probably reflects the previously described frequent co-existence of two microvascular complications: diabetic nephropathy and retinopathy [28]. The association of insulin therapy with the examined complication most likely reflects the fact that this treatment is sometimes introduced after the occurrence of microvascular complications in diabetic subjects and that insulin therapy is commonly reserved for the most severely affected patients. The most unexpected finding of our study is the presence of non-smoking status among the list of risk factors for DR. This finding, however, is in agreement with previously reported UKPDS data [5]. The potential explanations of the possible association of DR with non-smoking status were widely discussed in Stratton et al.'s paper [5]. This list includes lower blood pressure among smokers and pharmacological effect of nicotine or other compounds present in tobacco smoke as well as anti-angiogenic properties of tobacco smoke. The potential bias due to lower response rate of smokers in the scientific studies should also be considered, however taking into account the magnitude of the identified association with never-smoking status, this seems to be an unlikely explanation. No matter what the true explanation is, doctors around the world should strongly advise diabetic patients against smoking due to very well documented deleterious effects regarding macrovascular complications and lung diseases [29, 30]. Unlike UKPDS, we did not identify arterial

hypertension as a risk factor for DR in multivariate analysis. As a majority of patients in both the DR and non-DR groups had arterial hypertension, the statistical multivariate models that include hypertension could not reach convergence. Additionally, in our study, this feature was not used as a continuous variable. Finally, it should be emphasised that our project was a smaller analysis than the prospective, multicentre UKPDS and it was designed as a cross-sectional study.

Additionally, the genetic part of our results should be discussed. Contrary to both French studies, we did not find any association with the examined VDR variants [20, 21]. We included in our project TaqI and FokI markers that were found to be associated with DR in type 1 diabetes patients from the French population. There are a few possible reasons for the discrepancy between our study and those previously published projects. First, this may be associated with the distinct genetic background of type 1 and type 2 diabetes, or different genetic susceptibility to DR in the French and Polish populations. Second, our study may be lacking power to detect the putative association. Third, the French researchers included only cases of severe DR in their analysis. Finally, our study may be limited by the fact that no minimal diabetes duration was used as a formal criterion for inclusion in the study. On the other hand, the fact that a substantial portion of T2DM subjects has retinopathy at presentation suggests that there is considerable time between onset of the disease and its clinical presentation [29].

Our study should be perhaps criticised for not using ‘gold standard’ 7-field 30° stereo photography, as defined by the Early Treatment Diabetic Retinopathy Study [30]. In the current study, however, we employed exactly the same methodology as used earlier in the EURODIAB IDDM complications study. It was previously reported that this system compared well with the standard procedure and was acceptably accurate, repeatable and relatively simple to apply [24]. The Department of Metabolic Diseases in Krakow was one of the centres participating in EURODIAB [31] and the medical team that carried out the photographic documentation for our current project participated in that previous study.

In conclusion, our study showed that DR in T2DM patients remains a frequent complication in Polish patients. We were able to confirm the role of some clinical risk factors, surprisingly including not-smoking status, as was previously shown in the UKPDS study. VDR gene polymorphisms did not constitute a risk factor at this size of study group.

References

1. Zimmet P, Alberti KG, Shaw J (2001) Global and societal implications of the diabetes epidemic. Nature 414:782–787
2. King H, Aubert RE, Herman WH (1998) Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. Diabetes Care 21:1414–1431
3. Selby JV, Ray GT, Zhang D, Colby CJ (1997) Excess costs of medical care for patients with diabetes in a managed care population. Diabetes Care 20:1396–1402
4. Stefansson E, Bek T, Porta M, Larsen N, Kristinsson JK, Agardh E (2000) Screening and prevention of diabetic blindness. Acta Ophthalmol Scand 78:374–385
5. Stratton IM, Kohner EM, Aldington SJ, Turner RC, Holman RR, Manley SE, Matthews DR (2001) UKPDS 50: risk factors for incidence and progression of retinopathy in Type II diabetes over 6 years from diagnosis. Diabetologia 44:156–163
6. Henricsson M, Nilsson A, Janzon L, Groop L (1997) The effect of glycaemic control and the introduction of insulin therapy on retinopathy in non-insulin-dependent diabetes mellitus. Diabet Med 14:123–131
7. Henricsson M, Gottsater A, Jeppsson JO, Fernlund P, Sundkvist G (1998) The frequency and severity of retinopathy are related to HbA1c values after, but not at, the diagnosis of NIDDM. J Intern Med 244:149–154
8. Rema M, Saravanan G, Deepa R, Mohan V (2002) Familial clustering of diabetic retinopathy in South Indian Type 2 diabetic patients. Diabet Med 19:910–916
9. Imperatore G, Hanson RL, Pettitt DJ, Kobes S, Bennett PH, Knowler WC (1998) Sib-pair linkage analysis for susceptibility genes for microvascular complications among Pima Indians with type 2 diabetes. Pima Diabetes Genes Group. Diabetes 47:821–830
10. Globocnik-Petrovic M, Hawlina M, Peterlin B, Petrovic D (2003) Insertion/deletion plasminogen activator inhibitor 1 and insertion/deletion angiotensin-converting enzyme gene polymorphisms in diabetic retinopathy in type 2 diabetes. Ophthalmologica 217:219–224
11. Nagi DK, McCormack LJ, Mohamed-Ali V, Yudkin JS, Knowler WC, Grant PJ (1997) Diabetic retinopathy, promoter (4G/5G) polymorphism of PAI-1 gene, and PAI-1 activity in Pima Indians with type 2 diabetes. Diabetes Care 20:1304–1309
12. Maeda M, Yamamoto I, Fukuda M, Nishida M, Fujitsu J, Nonen S, Igarashi T, Motomura T, Inaba M, Fujio Y, Azuma J (2003) MTHFR gene polymorphism as a risk factor for diabetic retinopathy in type 2 diabetic patients without serum creatinine elevation. Diabetes Care 26:547–548
13. Ichikawa F, Yamada K, Ishiyama-Shigemoto S, Yuan X, Nonaka K (1999) Association of an (A-C)n dinucleotide repeat polymorphic marker at the 5'-region of the aldose reductase gene with retinopathy but not with nephropathy or neuropathy in Japanese patients with Type 2 diabetes mellitus. Diabet Med 16:744–748
14. Wang Y, Ng MC, Lee SC, So WY, Tong PC, Cockram CS, Critchley JA, Chan JC (2003) Phenotypic heterogeneity and associations of two aldose reductase gene polymorphisms with nephropathy and retinopathy in type 2 diabetes. Diabetes Care 26:2410–2415
15. Reichel H, Koeffler HP, Norman AW (1989) The role of the vitamin D endocrine system in health and disease. N Engl J Med 320:980–991
16. Norman AW (1994) The vitamin D endocrine system: identification of another piece of the puzzle. Endocrinology 134:1601A–1601C

17. Feldman D (1999) Vitamin D, parathyroid hormone, and calcium: a complex regulatory network. Am J Med 107:637–639
18. Schreiner DS, Jande SS, Lawson DE (1985) Target cells of vitamin D in the vertebrate retina. Acta Anat (Basel) 121:153–162
19. Johnson JA, Grande JP, Roche PC, Campbell RJ, Kumar R (1995) Immuno-localization of the calcitriol receptor, calbindin-D28k and the plasma membrane calcium pump in the human eye. Curr Eye Res 14:101–108
20. Taverna MJ, Sola A, Guyot-Argenton C, Pacher N, Bruzzo F, Slama G, Reach G, Selam JL (2002) Taq I polymorphism of the vitamin D receptor and risk of severe diabetic retinopathy. Diabetologia 45:436–442
21. Taverna MJ, Selam JL, Slama G (2005) Association between a protein polymorphism in the start codon of the vitamin D receptor gene and severe diabetic retinopathy in C-peptide-negative type 1 diabetes. J Clin Endocrinol Metab 90:4803–4808
22. Malecki MT, Frey J, Moczulski D, Klupa T, Kozek E, Sieradzki J (2003) Vitamin D receptor gene polymorphisms and association with type 2 diabetes mellitus in a Polish population. Exp Clin Endocrinol Diabetes 111:505–509
23. Malecki MT, Moczulski DK, Klupa T, Wanic K, Cyganek K, Frey J, Sieradzki J (2002) Homozygous combination of calpain 10 gene haplotypes is associated with type 2 diabetes mellitus in a Polish population. Eur J Endocrinol 146:695–699
24. Aldington SJ, Kohner EM, Meuer S, Klein R, Sjolie AK (1995) Methodology for retinal photography and assessment of diabetic retinopathy: the EURODIAB IDDM complications study. Diabetologia 38:437–444
25. (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med 329:977–986
26. UK Prospective Diabetes Study (UKPDS) Group (1998) Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 352:837–853
27. Luzniak P, Czech A, Taton J (1997) Prospective studies of diabetic retinopathy in a cohort of patients with type II diabetes mellitus. Pol Merkuriusz Lek 2:14–17
28. Krolewski AS, Warram JH, Freire M (1996) Epidemiology of late diabetic complications. A basis for the development and evaluation of preventive programs. Endocrinol Metab Clin North Am 25:217–242
29. Harris MI, Klein R, Welborn TA, Knuiman MW (1992) Onset of NIDDM occurs at least 4–7 yr before clinical diagnosis. Diabetes Care 15:815–819
30. Early Treatment Diabetic Retinopathy Study Research Group (1991) Grading diabetic retinopathy from stereoscopic color fundus photographs – an extension of the modified Airlie House classification. ETDRS report number 10. Ophthalmology 98[Suppl 5]:786–806
31. – (1994) Microvascular and acute complications in IDDM patients: the EURODIAB IDDM Complications Study. Diabetologia 37:278–285