

NEW FACTORS OF DIABETIC RETINOPATHY PROGRESSION IN TYPE 2 DIABETES MELLITUS PATIENTS

Abstract. The objective of the study was to investigate the possible role of the rs759853 and rs9640883 polymorphisms of the AKR1B1 gene by comparing the distribution of genotypes and alleles in the control group and in patients with type 2 diabetes mellitus and diabetic retinopathy (DR). A total of 302 patients were involved in this study, where 98 patients were in the control group; Group 1 – 76 patients with type 2 diabetes mellitus showing no visible changes in the fundus; Group 2 – 64 patients with non-proliferative DR and Group 3 – 64 patients with proliferative DR. The analysis of polymorphic loci was performed using unified TaqMan Mutation Detection Assays Thermo Fisher Scientific test systems (USA). Statistical analysis of the results of studies was performed using SPSS 11.0, Medstat, MedCalc (MedCalc Software bvba, 1993-2013).

It was determined that there was a significant increase in the frequency of the G/A heterozygote of the rs759853 polymorphism in type 2 diabetes mellitus as compared to the control group, whereas the development of DR was associated with an increase in the frequency of the mutant A/A homozygote, especially in proliferative DR. In case of progressive DR, a significant decrease in the G allele frequency and an increase in the A allele frequency of the rs759853 polymorphism of the AKR1B1 gene were detected. In case of progressive DR, there were also found an increase in the G allele frequency and a decrease in the A allele frequency of the rs9640883 of the AKR1B1 gene. The homozygous genotype of the mutant A/A allele of the rs9640883 polymorphism was not detected in patients with DR at all, and this justified the idea of the protective effect of the minor A allele in the development of DR.

The study showed the role of the rs759853 and rs9640883 polymorphisms of the AKR1B1 gene. The main factors contributing to the progression of DR included the increased frequency of the A allele of rs759853 and G allele of rs9640883; the protective role was played by the A allele and A/A genotype of rs9640883.

Key words: type 2 diabetes mellitus, diabetic retinopathy, rs759853, rs9640883, AKR1B1.

INTRODUCTION. Genetic factors play a significant role in the development of diabetic retinopathy (DR) in type 2 diabetes mellitus (type 2 DM) [1-4]. According to latest data, genetic factors account for up to 50% of the risk of developing the DR. These factors can be considered as essential factors leading to complications of DM, in particular, DR [5, 6]. A number of studies have shown the influence of heredity on the development of DR in various populations, regardless of the level of hyperglycemia and associated environmental risk factors [7, 8].

The pathogenesis of DR involves many factors and, first of all, metabolic disorders: carbohydrate, lipid, protein and electrolyte metabolism disorders. Chronic hyperglycemia activates the polyol pathway of glucose metabolism thereby stimulating the accumulation of sorbitol and fructose in cells. In the absence of hyperglycemia, the conversion of glucose to sorbitol does not exceed 1%, while this indicator increases to 7-8% in type 2 DM. A key enzyme of the polyol pathway is aldose reductase converting glucose to sorbitol. Activation of this enzyme causes disorders in intracellular homeostasis, since the end products of glucose metabolism via the sorbitol pathway (sorbitol and fructose) do not penetrate the cell membrane and accumulate in cells, causing swelling and leading to cell death through osmotic lysis [8, 9]. Thus, the research into the influence of genetic polymorphisms (rs759853 and rs9640883 of the AKR1B1 gene) affecting the aldose reductase activity is relevant and up-to-date.

Objective of the study: to investigate the possible role of the rs759853 and rs9640883 polymorphisms of the AKR1B1 gene by comparing the distribution of

genotypes and alleles in the control group and in patients with type 2 DM and DR.

MATERIAL AND METHODS. The study was conducted at the Department of Ophthalmology of Danylo Halytsky Lviv National Medical University. The control group consisted of 98 patients having neither type 2 DM nor any other ophthalmic diseases. The first group included 76 patients with stage 1 DR (no visible changes in the fundus). The second group consisted of 64 patients diagnosed with non-proliferative DR (NPDR), and the third group included 64 patients diagnosed with proliferative DR (PDR). The ophthalmological examinations included general examination and biomicroscopy of the protective apparatus, anterior and posterior eye segments with a slit lamp (Haag-Streit BQ 900, Swiss) and biomicroscopy lense (Super Pupil XL, Volk Optical, USA).

Polymorphic DNA loci were analyzed using unified TaqMan Mutation Detection Assays Thermo Fisher Scientific test systems (USA). The rs759853 polymorphism of the AKR1B1 gene is located in Chr. 7:134143958 as per NCBI Build 37. This polymorphism is a G-to-A simple nucleotide substitution in an intron of the AKR1B1 gene (NM_001628.2: c.-144 C>T). Here G is an ancestral allele, A is a minor allele with minor allele frequency = 0.2768 according to MAF Source: 1000 Genomes (<http://www.1000genomes.org/>). The rs9640883 polymorphism of the AKR1B1 gene is located in Chr.7:134116633 as per NCBI Build 37. This polymorphism is a G-to-A simple nucleotide substitution in an intron of the AKR1B1 gene

(XR_928003.1: n.94+433 C>T). Here G is an ancestral allele, A is a minor allele with minor allele frequency = 0.2853 according to MAF Source: 1000 Genomes (<http://www.1000genomes.org/>). Statistical analysis of the results of studies was performed using SPSS 11.0, Medstat (Yu.Ye. Liakh. V.H. Guryanov), and MedCalc (MedCalc SoftWare bvba, 1993-2013). A P value of <0.05 was considered statistically significant in all analyses.

RESULTS AND DISCUSSION. The G/G genotype of the rs759853 polymorphism prevailed in patients of the control group (47.6%) (Fig. 1). Its frequency decreased in the presence of type 2 DM (13.1% – Group 1; 28.1% – Group 2 and 21.9% – Group 3; pFet <0.05 in all cases). Therefore, one might have argued that in type 2 DM there was a redistribution of the frequency of the ancestral genotype toward a significant decrease, which was maximally expressed in Group 1 (3.6 times).

Fig. 1. Distribution of genotypes and alleles of the rs759853 polymorphism of the AKR1B1 gene by groups of patients (as a percentage of the number of people in this group)

*Remark: * – significance of frequency differences between groups determined by Fisher's exact test (pFet <0.05).*

The frequency of heterozygous G/A genotype was higher in patient of Group 1 as compared to the control group: 81.6% versus 42.1% (pFet <0.05). The frequency of G/A genotype distribution in patients of Group 2 and 3 did not differ as compared to the control group (37.5% in both groups versus 42.1% in the control group). That is, the level of heterozygosity in type 2 DM without signs of retinopathy significantly increased (1.9 times), whereas there was no difference as compared to the control group in the presence of DR.

The distribution analysis of the minor and, on the basis of genetic events, mutant A/A genotype showed the following results. The frequency of the mutant genotype was significantly higher in the DR groups as compared to the control group: 34.4% in Group 2 and 40.6% in Group 3 versus 10.3% in control group. This means that the frequency of the mutant A/A homozygote increases significantly in the presence of

DR (3.3 times in NPDR and, to a greater extent, 3.9 times in PDR).

Thus, the increased frequency of heterozygotes was associated with the development of type 2 DM without retinopathy, whereas the increased frequency of mutant homozygote was associated with the development of DR and, to a greater extent, with its proliferative type.

The frequency of the ancestral G allele of the rs759853 polymorphism in patients of the control group was 68.7% (see Fig. 1). This allele showed a tendency to decrease in patients of the examined groups: 53.9% – Group 1; 46.9% – Group 2; 40.2% – Group 3 (pFet <0.05 in all cases). Therefore, one might have argued that the progression of DR decreased the frequency of the ancestral G allele with a minimum value in patients with PDR (1.7 lower as compared to the control group). On the contrary, the frequency of the mutant A allele increased significantly as compared to the control group (31.3%) and reached: 46.1% – Group 1; 53.1% – Group 2 and 59.8% – Group 3 (pFet <0.05 in all cases). The maximum value was also found in patients with PDR (1.9 times higher as compared to the control group).

In view of this, patients with increasing severity of the pathological process and progression of DR showed a clear pattern – a decrease in the frequency of the ancestral G allele and an increase in the frequency of the mutant A allele of the rs759853 polymorphism of the AKR1B1 gene.

Figure 2 presents data on another rs9640883 polymorphism of the AKR1B1 gene. The ancestral G/G homozygote of the rs9640883 polymorphism was found in almost half of patients of the control group (45.8%). Patients from all groups showed the increased frequency of this genotype, which was statistically significant. For instance, its frequency in Group 1 was 63.1% (1.4 times increased as compared to the control group), in Group 2 – 75.0% (1.6 times increased), in Group 3 – 68.8% (1.5 times increased; pFet <0.05 in all cases). That is, the frequency of the ancestral G/G genotype was significantly increased in type 2 DM (1.4–1.6 times).

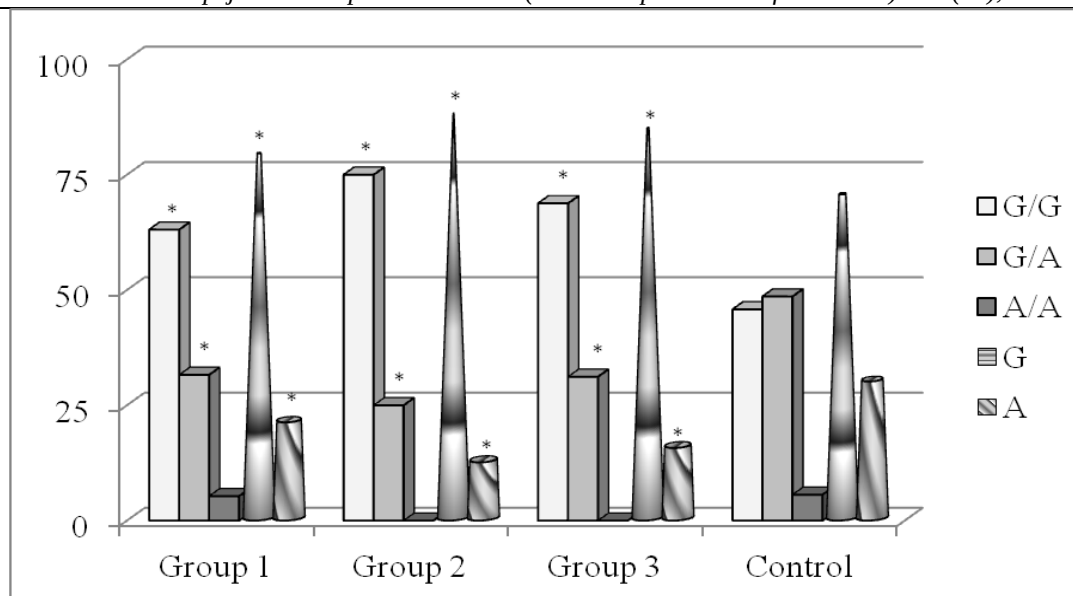


Fig. 2. Distribution of genotypes and alleles of the rs9640883 polymorphism of the AKR1B1 gene by groups of patients (as a percentage of the number of people in this group)

Remark: * – significance of frequency differences between groups determined by Fisher's exact test ($p_{Fet} < 0.05$)

The frequency of the heterozygous G/A genotype was statistically significantly lower in all patient groups as compared to the control group: 1.5–1.9 times ($p_{Fet} < 0.05$ in all cases). Therefore, the level of heterozygosity in type 2 DM was significantly decreased. The minor A/A genotype frequency of his polymorphism was quite low in the control group (5.6%); in Group 1, it was not statistically significantly different from those in the control group (5.6% and 5.3%, respectively), whereas no cases of homozygous genotype of minor allele were found in groups of patients with DR ($n=128$). That is, it can be assumed that there is a protective effect against the development of DR of A/A homozygous carrier state of this polymorphism.

The frequency of the ancestral G allele of the rs9640883 polymorphism was 70.1% in the control group. At the same time this allele showed a tendency

to increase in patients of the examined groups: 78.9% – Group 1; 87.5% – Group 2 and 84.4% – Group 3 ($p_{Fet} < 0.05$ in all cases). The mutant A allele frequency was statistically significantly decreased as compared to the control group (29.9%) and reached: 21.1% – Group 1; 12.5% – Group 2 and 15.6% – Group 3.

Thus, patients with increasing severity of the pathological process and progression of DR showed a clear pattern – an increase in the frequency of the ancestral G allele and a decrease in the frequency of the mutant A allele of the rs9640883 polymorphism of the AKR1B1 gene. Taking into account the fact that the homozygous genotype of the mutant A/A allele was not detected in patients with DR at all, one could justify the idea that the minor A allele has a protective effect in these cases.

Figure 3 presents the comparative analysis of the (A) minor alleles frequency for both polymorphisms.

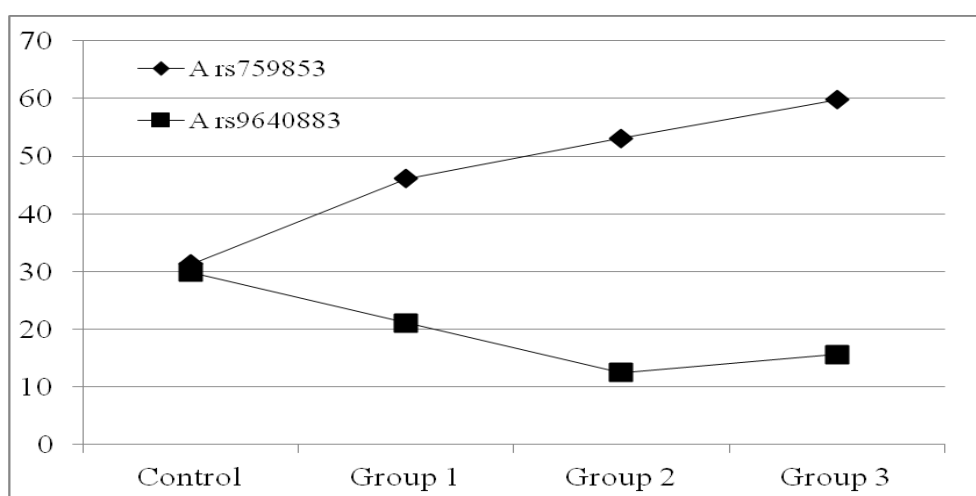


Fig. 3. Comparative dynamics of the (A) minor alleles frequency of the rs759853 and rs9640883 polymorphisms of the AKR1B1 gene

Remark: * – significance of frequency differences between the control group and the examined groups determined by Fisher's exact test ($p_{Fet} < 0.05$ for all observations)

Both alleles in the control group had approximately the same frequency: 31.3% for the rs759853 polymorphism and 29.9% for the rs9640883 polymorphism. With increasing severity of pathological processes in DM without retinopathy (o NPDR, o PDR), the frequency of the minor allele of the rs759853 polymorphism gradually increased (to a maximum of PDR–59.8%). This made it possible to consider it as a marker of the severity of the pathological process and, at the same time, highlighted its role as a causative factor in the development of eye lesions in type 2 DM.

The A allele frequency of the rs9640883 polymorphism, on the contrary, gradually decreased (to a minimum of 12.5% in NPDR and 15.6% in PDR). That means that this allele can be regarded as protective factor in the development of pathological processes. It is proved by the fact of absence of homozygotes of the minor A/A allele of the rs9640883 polymorphism in all patients with DR (in this study – 128 people).

CONCLUSIONS. The increased G/A heterozygote frequency of the rs759853 polymorphism was associated with the development of type 2 DM without retinopathy; the increased frequency of the mutant A/A homozygote was associated with the development of DR, and, to a greater extent, its proliferative type. In general, patients with increasing severity of the pathological process and progression of DR showed a clear pattern – a decrease in the frequency of the ancestral G allele and an increase in the frequency of the mutant A allele of the rs759853 polymorphism of the AKR1B1 gene, which could be considered a risk factor for the development of DR.

Patients with increasing severity of the pathological process and progression of DR showed a clear pattern – an increase in the frequency of the ancestral G allele and a decrease in the frequency of the mutant A allele of the rs9640883 polymorphism of the

AKR1B1 gene. The homozygous genotype of the mutant A/A allele was not detected in patients with DR at all, and this justified the idea of the protective effect of the minor A allele in the development of DR.

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CONNECTIVE TISSUE PATHOLOGY AS A RISK FACTOR FOR INTESTINAL FISTULA DEVELOPMENT

Summary. Retro- and prospective trial was based on data about 30 patients, suffering intestinal fistulas, who were treated in the Shalimov National Institute of Surgery and Transplantology during 2016-2019. There was revealed that the most informative phenotypical markers of undifferentiated dysplasia of the connective tissue (UDCT) in patients with intestinal fistulas are visceral (83,3%), vascular (70%), arrhythmic (70%) pathologies. It is established that a direct correlation between the level of biochemical markers of the collagen biodegradation and the UDCT degree may be applied for prognostication of development and course of complications in patients, suffering entero- colcutaneous fistulas. The presence of connective tissue dysplasia in patients with intestinal fistulas was proved to be the aggravating comorbidity factor, that is difficult to treat and is accompanied by high mortality rates.

Severe degree of UDCT in the patients, intestinal fistulas, constitutes unfavorable prognostic sign and enhances the mortality by 62.5%. The presence of UDCT in the patients with intestinal fistulas is an aggravating