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Association between sorbitol dehydrogenase gene polymorphisms and type 2 diabetic retinopathy

Jacek P. Szaflik [a], Ireneusz Majsterek [b], Michal Kowalski [a], Pawel Rusin [b], Anna Sobczuk [c], Anna I. Borucka [a], Jerzy Szaflik [a], Janusz Blasiak [b,*]

[a] *Department of Ophthalmology, Medical University of Warsaw, Warsaw, Poland*
[b] *Department of Molecular Genetics, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland*
[c] *Polish Mother's Memorial Hospital, Lodz, Poland*

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Abstract

Diabetic retinopathy (DR) may affect 98% of diabetic patients, but its aetiology is poorly understood. Besides glycaemic exposure, genetic factors likely contribute to the onset of DR. The polyol pathway, including aldose reductase and sorbitol dehydrogenase (SDH), can be activated under hyperglycaemic conditions. In our work we searched for an association between the C−1214G and G−888C polymorphisms of the *SDH* gene promoter and the occurrence and progression of type 2 DR. Two hundred and fifteen unrelated individuals with type 2 diabetes mellitus (T2DM) were divided into three groups: without DR, with non-proliferative diabetic retinopathy (NPDR) and with proliferative diabetic retinopathy (PDR). Genotypes of the C−1214G (rs2055858) and G−888C (rs3759890) polymorphisms of the *SDH* gene were determined with DNA from the peripheral blood lymphocytes of patients by restriction fragment length polymorphism and allele-specific PCR, respectively. The genotype distributions were contrasted by the χ^2 test and the significance of the polymorphism was assessed by multiple logistic regression producing odds ratios (ORs) and 95% confidence intervals (CIs). We found an association (OR 1.73, 95% CI 1.06−2.83) between NPDR and the G allele of the G−888C polymorphism. There was no association between NPDR and the other polymorphisms of the *SDH* gene. No differences were found in the distributions of these polymorphisms between patients with PDR and those with NPDR. A weak association (OR 2.0, 95% CI 1.29−3.07) was found between DR and the G allele of the G−888C polymorphism. Analysis of the combined genotypes (haplotypes) of both polymorphisms revealed associations between the C/G−C/G genotype and NPDR (OR 2.95, 95% CI 1.07−8.13) as well as DR in general (OR 2.91, 95% CI 1.15−7.36). The G−888C polymorphism of the *SDH* gene may be associated with the onset of DR rather than with its progression, and its effect may be strengthened by the interaction with the C−1214G polymorphism, but this association is rather weak and requires further study.

Keywords: diabetic retinopathy; genetic polymorphism; sorbitol dehydrogenase; restriction fragment length polymorphism

1. Introduction

Diabetic retinopathy (DR) is the leading cause of blindness in the working-age group in the Western world (King et al., 1998). It is one of the most serious long-term complications of type 2 diabetes mellitus (T2DM). The prevalence of DR varies from 17% to 98%, depending on the duration of the diabetes (Klein and Klein, 1995). Ischaemia, macular oedema, and rapid progression of DR may cause vision loss (Lee et al., 1998). The earliest manifestations of DR are venous tortuosity and dot and blot haemorrhages with microaneurysm formation. Soft exudates reflect microinfarcts and hard exudates indicate lipid deposition in the retina. Haemorrhages in DR can be easily distinguished from those in hypertension by their depth and ability to diffuse. Visual loss in DR has two primary causes: progression from non-proliferative DR (NPDR) to the stage of microvascular proliferation called proliferative DR (PDR), in which new, pathological vessels are formed in the

* Corresponding author. Tel.: +48 42 6354334; fax: +48 42 6354484.
E-mail address: januszb@biol.uni.lodz.pl (J. Blasiak).

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retina and fibrotic scarring of the retina (Vinik, 2005), which is the culmination of PDR.

The aetiology of DR is still poorly understood. A large cohort study showed that 28.8% of diabetic patients develop retinopathy in the early stages of the disease, but 22.2% are not affected by DR (Klein et al., 1984). This suggests that high glycaemic exposure is not the only factor determining the onset of retinopathy in diabetic patients. Apparently, genetic factors play a role. Therefore, these factors need to be determined to select a subgroup of diabetic patients, who should be under special ophthalmologic care, and to identify a group in the general population, who should be aware of the serious consequences of developing diabetes.

The categorizations of NPDR and PDR can be used to assess the progression of DR. The most precise is the Early Treatment Diabetic Retinopathy Study Research Group scale, according to which retinopathy can be scored as: no retinopathy, non-proliferative, pre-proliferative, proliferative, and advanced proliferative retinopathy (Early Treatment Diabetic Retinopathy Study Research Group, 1991, http://clinicaltrials.gov/ct/show/NCT00000151). Again, the progression of DR seems to be unaffected by factors such as age, duration of diabetes, glycaemic haemoglobin level, and diabetic treatment. This suggests that genetic factors may contribute to the severity of DR, which can be expressed as its progression rate reflected in the classifying DR as NPDR or PDR. Thus, two issues need to be resolved: (1) which genetic factors determine the chance of developing DR in diabetic individuals and (2) the progression, expressed either as NPDR or PDR, of PDR in diabetic patients.

Mutations can contribute to the disease phenotype, and are commonly accepted genetic factors that indicate occurrence and progression of a disease, especially if it occurs frequently and takes the form of gene polymorphisms. An association between the polymorphisms in the 5′-untranslated region of the vascular endothelial growth factor (*VEGF*) gene and DR has been reported (Suganthalakshmi et al., 2006). This confirmed earlier suggestions about such an association (Awata et al., 2002). Polymorphisms of the insulin-like growth factor-1 (Rietveld et al., 2006), intercellular adhesion molecule 1 (ICAM-1) (Liu et al., 2006), aldose reductase (Awata et al., 2002; Rietveld et al., 2006), receptor for advanced glycation end products (RAGE) (Ramprasad et al., 2007), plasminogen activator inhibitor-1 (PAI-1) genes have also been reported as risk modifiers of DR (Funk et al., 2005; Nagi et al., 1997).

Several papers reported an association between DR and polymorphisms in the aldose reductase gene (Olmos et al., 2006; dos Santos et al., 2006; Kao et al., 1999). The enzyme coded by this gene, aldose reductase (AR2, EC 1.1.1.21), converts glucose to sorbitol and represents one of two enzymes of the polyol pathway that can be activated under hyperglycaemic conditions and may therefore be considered as an important biochemical factor in the development of long-term complications of diabetes, including retinopathy (Kinoshita and Nishimura, 1988). AR2 is the first and rate-limiting enzyme of the polyol pathway. The other component of the pathway, sorbitol dehydrogenase (SDH), which converts sorbitol to fructose, was investigated to a much lesser extent. This is probably a consequence of an apparently more direct role of AR2 than SDH in glucose metabolism, which is a hallmark of diabetes and its complications. However, Tilton et al. (1995) and Amano et al. (2003) suggested that SDH activity might make a greater contribution to the aetiology of DR than did the first step with AR2. Moreover, Amano et al. (2003) suggested that the polymorphisms of the promoter of the *SDH* gene could be correlated with its expression level in retinal cells in diabetes. Therefore, these polymorphisms may play a role in the pathogenesis of DR. This study was performed on a small Japanese population (97 cases and 46 controls) and a need for further research was stressed.

Searching for the meaning of genetic variability in the *SDH* gene seems to be important in light of recent work of Hellgren et al. (2007), who showed that the Tyr110Phe mutation in the gene abolished the enzymatic activity of its product and destabilized the protein into tetramers, dimers and monomers.

In our present work we investigated two polymorphisms of the sorbitol dehydrogenase gene (*SDH*) promoter: a C → G transversion located at −1214 position (the C−1214G polymorphism) and a G → C transversion at −888 (the G−888C polymorphism). These polymorphisms, located in the promoter region, may affect the level of transcription and, consequently, the activity of the SDH enzyme. We searched for an association between genotypes of these polymorphisms and the occurrence and progression of DR in a Polish population.

2. Methods

2.1. Clinical subjects

Peripheral blood samples were obtained from 215 individuals with T2DM diagnosed at the Clinical Hospital of Department of Ophthalmology, University of Warsaw, Warsaw, Poland in 2005. Of these, 82 had PDR, 72 had NPDR, and 61 individuals without DR served as controls. All subjects underwent ophthalmic examination, including best corrected visual acuity, slit lamp examination, and fundus examination using non-contact and contact fundus lenses with a slit lamp. The diagnosis or lack of DR was confirmed by fluorescein angiography (FA) in all but not 20 cases. The reasons for not performing FA were the general health status of the 16 patients and four patients' unwillingness to have FA performed. In this group the diagnosis or lack of DR was established based on fundus examination only. The FA examinations were performed with a Topcon TRC-50I IX fundus camera with digital Image Net ver. 2.14 image system (Topcon Co., Tokyo, Japan). The clinical characteristics of patients are listed in Table 1. The study was approved by the Bioethics Committee of Medical University of Warsaw and each patient gave written informed consent.

2.2. DNA preparation

Peripheral blood lymphocytes (PBLs) were isolated by centrifugation in a density gradient of Histopaque-1077 (15 min,