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Association of TLR4 rs1927911 polymorphism with diabetic retinopathy and diabetic macular edema in type 2 diabetic patients

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Background: *Genetic susceptibility is a factor in the development of ophthalmic complications in type 2 diabetes mellitus (T2DM). There are differences between polymorphisms of toll-like receptor-4 (TLR4) in terms of their association with microvascular complications of T2DM.*

Purpose: *To assess the association of TLR4 rs1927911 polymorphism with diabetic retinopathy (DR) and diabetic macular edema (DME) in T2DM.*

Material and Methods: *This study involved 81 type 2 diabetics (81 eyes) with both DR and DME and 50 type 2 diabetic controls (50 eyes) having normalized carbohydrate metabolism and neither DR nor DME. TLR4 rs1927911 genotypes were investigated by real time polymerase chain reaction (PCR) using Gene Amp® 7500 PCR System (Applied Biosystems, Foster City, CA) and TaqMan Mutation Detection Assays (Life Technologies, Carlsbad, CA).*

Results: *TLR4 rs1927911 polymorphism was associated with DR and DME ($p = 0.021$), and the risk of these complications was lower (OR = 0.42; 95% CI, 0.23–0.77) in allele A carriers than in ancestral allele G carriers. rs1927911 genotype distribution was significant by Pearson chi-square test under a dominant model (GG versus GA+AA; $\chi 2 = 6.38$; $p = 0.012$; OR = 0.37; 95% CI, 0.18-0.76). After patients were stratified by the stage of DR and the severity of DME, significant differences with regard to genotypes were observed only in proliferative DR and severe DME. Carriers of the heterozygous genotype and minor genotype AA showed less severe glycemia, lower HbA1c, and smaller central retinal thickness and total retinal volume than carriers of the ancestral genotype GG; this corresponded to less severe carbohydrate metabolism abnormalities and less severe retinal damage.*

Conclusion: *Pro-inflammatory pathways involve TLR4 under hyperglycemic conditions. Given the role of TLR4 in the mechanisms triggering the immune response, it may be supposed that the activity of these pathways is reduced in carriers of rs1927911 polymorphism, and it is this that causes reduced diabetic retinal damage.*

Keywords:
hyperglycemia, central retinal thickness, retinal volume, regression analysis

Introduction

The International Diabetes Federation (IDF) estimated the global population with diabetes mellitus (DM) to be 463 million in 2019 and 700 million in 2045. More than 95% of people with diabetes have type 2 DM (T2DM) [1]. Diabetic retinopathy (DR) and diabetic macular edema (DME) are the two most common ophthalmic complications of T2DM and leading causes of blindness in the working-age populations [2, 3].

DR is an inflammatory disease associated with hyperglycemia, and causes cell apoptosis, neurodegeneration, oxidative stress and neovascularization [4].

Toll-like receptors (TLR) are a family of pattern recognition receptors (PRR) responsible for the initiation of inflammatory and immune responses [5, 6]. TLR4 identifies both endogenous and exogenous ligands and is associated with various physiological and pathological pathways in the body. Due to hyperglycemia, TLR4 expression increases in diabetic retina, which activates various pathways leading to DR.

High mobility group box 1 (HMGB1) is a danger associated protein pattern receptor which can sense high glucose as a stressor [7, 8]. This results in increased inflammation through nuclear factor kappa beta (NFkB) with the involvement of the pathological pathway HMGB1/TLR4/NF-κB. The treatment of experimental diabetes with TLR4/NF-κB inhibitors has been reported to improve the morphology and biochemistry of retinal damage [9–11].

A genetic factor has been implicated in the development of ophthalmic complications of T2DM

[12–14]. Attention was given to the association of TLR4 polymorphisms and the risk of DR while considering the role of TLR4 in the development of DR [14–16]. Zhang and colleagues [15] and Buraczynska and colleagues [16] found TLR4 polymorphisms to be associated with increased risk of cardiovascular disorders and DR in T2DM, whereas Manolakis and colleagues [17] found that these polymorphisms provide protection against T2DM.

The purpose of the study was to assess the association of TLR4 rs1927911 polymorphism with DR and DME in T2DM.

Material and Methods

This was a prospective cohort case-control study. One hundred and thirty one patients with T2DM (131 eyes) were involved in this study. Of these, 81 had both DR and DME, and 50 (controls), neither DR nor DME. Patient age ranged from 53 to 85 years, and mean age was 65.7 ± 0.83 years. There was no significant difference between groups in terms of gender distribution: the percentages for women and men were 52.0% and 48%, respectively, for controls and 45.7% and 54.3%, respectively, for patients with both DR and DME ($p = 0.495$).

Approval for the study was obtained from the Bioethics Committee. The procedures followed were in accordance with the ethical standards of the Helsinki Declaration of the World Medical Association, European Convention on Human Rights and Biomedicine (1977), relevant provisions of WHO's Constitution, Council for International Organizations of Medical Science, International Code of Medical Ethics (1983), and Ministry of Health Order No. 690, dated 23 September, 2009. Informed consent was obtained from all participants.

Patients involved in the study had T2DM and had no previous history of attending a tertiary eye care institution. Carbohydrate metabolism was assessed by measuring fast blood glucose and glycosylated hemoglobin A1c (HbA1c) levels.

Patients underwent an eye examination which included visual acuity assessment, static Humphrey perimetry, refractometry, intraocular pressure (IOP) measurement, slit lamp biomicroscopy, ophthalmoscopy with Volk 90D lens and Goldmann three-mirror lens (Volk Optical, Mentor, OH) and fundus photography (the ETDRS seven standard fields) [18]. In addition, swept-source optical coherence tomography (OCT) (Topcon DRI OCT Triton plus, Tokyo, Japan) was used to take 7 x 7 mm 3D Macula Map scans in all patients.

DR severity was graded as per the 2002 guidelines of the American Academy of Ophthalmology. Patients with DR were divided into three groups based on examination results: group 1 of 10 patients with mild non-proliferative DR (NPDR); group 2 of 33 patients with moderate or severe non-proliferative NPDR; and group 3 of 38 patients with proliferative DR (PDR). The control group comprised 50 patients (50 eyes) with T2DM, a disease duration of no longer than 5 years, normalized carbohydrate metabolism, DR0 (i.e., no DR) and no DME.

The presence of DME was based on (a) an increased retinal macular thickness in the ETDRS subfields compared to the upper limit of normal for patient's age and gender and (b) the presence of intraretinal fluid on OCT scans. The retinal thickness deviation map (a graph comparing patient's deviation with normative age-matched database) was generated, with orange and pink pixels on the map indicating increased retinal thickness reported in percentiles. OCT images of good quality (with OCT image quality score > 40 were included in the analysis).

DME severity was graded as per the 2003 guidelines of the American Academy of Ophthalmology. DME0 (no DME) conformed to retinal images with normal macular thickness in all nine ETDRS subfields (green coded) in the absence of intraretinal fluid on OCT scans. DME1 (mild DME) conformed to increased macular thickness (orange or pink coded) in ETDRS subfields 7, 6, 8 and 9, and normal macular thickness (green coded) in ETDRS subfields 1-5, in the absence of intraretinal fluid in ETDRS subfields 1-5. DME2 (moderate DME) conformed to increased macular thickness (orange or pink coded) in ETDRS subfields 2-5, and normal macular thickness (green coded) in ETDRS subfield 1. DME3 (severe DME) conformed to increased macular thickness (orange coded) in ETDRS subfield 1.

In addition, central retinal thickness (CRT, μm) and total retinal volume (TRV, mm^3) were assessed.

TLR4 rs1927911 genotypes were investigated by real time polymerase chain reaction (PCR) using Gene Amp® 7500 PCR System (Applied Biosystems, Foster City, CA). Genomic DNA was extracted from venous blood samples using PureLink Genomic DNA Kit for Purification of Genomic DNA (Invitrogen, Carlsbad, CA). Mutation detection was performed using TaqMan Mutation Detection Assays (Life Technologies, Carlsbad, CA). Genetic studies were conducted at the Research Institute of Experimental and Clinical Medicine, Bogomolets National Medical University (director, Cand Sc (Med) Iu.G. Klys).

Statistical analyses were performed using MedStat and MedCalc v.15.1 (MedCalc Software bvba). In the course of genetic data analysis, we analyzed the general table for cases and genotype and allele frequencies. Thereafter, we analyzed the frequency differences indicating the effects of genotypes and alleles on the development of the disease [19]. Odds ratio (OR) and 95% confidence interval (CI) values were considered for the statistically significant difference related to the association with T2DM. Logistic regression models were built. The presence of DR and DME of any stage (predicted dependent variable Y=1, 81 cases) or the absence of DR and DME (predicted dependent variable Y = 0, 50 cases) was used the output variable.

Results

The genotype distribution among patients included in the current study was similar to that reported for 1000 Genomes Project Phase 3 (http://www.internationalgenome.org/) ($\chi^2 = 2.96$; $p = 0.228$). The project determined genotype frequencies of TLR4 rs1927911 in individuals of the European population of the European population,

and found the frequencies of the ancestral GG genotype, heterozygous GA genotype, and minor homozygous AA genotype to be of 0.547, 0.394 and 0.060, respectively, versus 0.626, 0.313 and 0.061, respectively, in our studies. In addition, the allele distribution among patients included in the current study was similar to that reported for 1000 Genomes Project Phase 3 ($\chi^2 = 1.48$; $p = 0.224$). The project found the frequencies of ancestral allele G and minor allele A to be of 0.744 and 0.256, respectively, versus 0.782 and 0.218, respectively, in our studies. Therefore, the results for the control group conformed to the results of 1000 Genomes Project Phase 3 for the European population in terms of the genotype and allele distributions for TLR4 rs1927911.

The ancestral GG genotype was significantly more frequent, whereas the heterozygous GA genotype and minor homozygous AA genotype were significantly less frequent in type 2 diabetics with DR and DME compared to type 2 diabetic controls ($p = 0.021$; Fig. 1). Correspondingly, the minor A allele was significantly less frequent in type 2 diabetics with DR and DME compared to controls ($p = 0.005$).

Therefore, it may be hypothesized that the rs1927911 polymorphism is associated with ophthalmic complications of T2DM. In this connection, we determined the effects of rs1927911 allele and genotype frequency distributions on T2DM and their associations with the disease (Table 1). The Hardy-Weinberg equilibrium was met for rs1927911 in the controls and cases (χ^2=0.52; df=1; p=0.471 and χ^2=1.30; df=1; p=0.254, respectively).

rs1927911 polymorphism was found to be associated with the disease ($\chi^2 = 7.72$; $p = 0.021$). In addition, the minor homozygous AA genotype was associated with a decreased risk (OR = 0.39; 95% CI, 0.18-0.86) of the disease. Correspondingly, the minor A allele was associated with a decreased risk (OR = 0.42; 95% CI, 0.23-0.77) of the disease, too. Therefore, the presence of the A allele could be considered as a protective factor against ophthalmic complications in T2DM.

Type 2 diabetic patients with the minor A allele had a 2.4-times decreased risk of developing ophthalmic complications compared to type 2 diabetic patients with the G allele.

Moreover, dominant and recessive models were compared (Table 2).

rs1927911 genotype distribution was significant by Pearson chi-square test under a dominant model (GG versus GA+AA), but not under a recessive model ($\chi^2 = 6.38$; $p = 0.012$ and $\chi^2 = 1.18$; $p = 0.278$, respectively). This finding confirmed an association between the presence of the minor allele A in type 2 diabetics and diabetic ophthalmic complications. Therefore, in type 2 diabetics, rs1927911 was a factor associated with the protection against such ophthalmic complications as DR and DME.

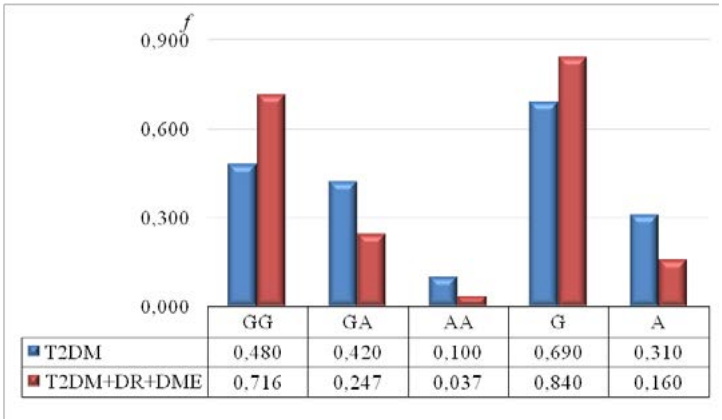

Fig. 1. Genotype and allele frequencies for TLR4 rs1927911 in type 2 diabetic controls and type 2 diabetics with both diabetic retinopathy (DR) and diabetic macular edema (DME). Differences between groups in terms of genotype and allele frequencies were significant (Pearson Chi-square test; p = 0.021 and p = 0.05, respectively). The ordinate displays frequencies (f), and the abscess displays genotypes and alleles. Note: GG, GA, and AA, genotypes of TLR4 rs1927911; G and A, alleles of TLR4 rs1927911; T2DM, a group of type 2 diabetic controls; T2DM+DR+DME, a group of type 2 diabetics with both DR and DME

Table 1. Effects of rs1927911 genotype and allele frequency distributions on the development of diabetic ophthalmic complications and the association of rs1927911 genotypes with the disease

Genotypes and alleles	T2DM+DR+DME, n (f)	T2DM, n (f)	χ^2	p	OR	95% CI
G/G	58 (0.72)	24 (0.48)	7.72	0.021	Reference	
G/A	20 (0.25)	21 (0.42)			0.25	0.05–1.12
A/A	3 (0.04)	5 (0.10)			0.39	0.18–0.86
G	136 (0.84)	69 (0.69)	7.26	0.007	Reference	
A	26 (0.16)	31 (0.31)			0.42	0.23–0.77

Notes: T2DM+DR+DME, a group of type 2 diabetics with both DR and DME (cases); T2DM, a group of type 2 diabetic controls; n (f), number and frequency of carriers of a certain genotype or allele; χ^2, Pearson Chi-square with adjustment for continuity; p, significance of difference between groups; OR, odds ratio; 95% CI, ninety-five percent confidence interval for odds ratio

Table 2. Effects of rs1927911 genotype frequency distributions on the development of diabetic ophthalmic complications (dominant and recessive models of inheritance)

Genotypes		T2DM+DR+ DME, n (f)	T2DM, n (f)	χ^2	p	OR	95% CI
Dominant	G/G	58 (0.72)	24 (0.48)	6.38	0.012	Reference	
	G/A+A/A	23 (0.28)	26 (0.52)			0.37	0.18–0.76
Recessive	G/G+G/A	78 (0.96)	45 (0.90)	1.18	0.278	Reference	
	A/A	3 (0.04)	5 (0.10)			–	–

Notes: T2DM+DR+DME, a group of type 2 diabetics with both DR and DME (cases); T2DM, a group of type 2 diabetic controls; n (f), number and frequency of carriers of a certain genotype or allele; χ^2, Pearson Chi-square with adjustment for continuity; p, significance of difference between groups; OR, odds ratio; 95% CI, ninety-five percent confidence interval for odds ratio

Table 3. RLR4 rs1927911 genotype and allele frequency distributions in patients stratified by the stage of diabetic retinopathy (DR)

Genotypes and alleles	Групи хворих за стадіями ДР, n (f)			
	Controls (DR0)	Group 1 (mild NPDR)	Group 2 (moderate or severe NPDR)	Group 3 (PDR)
G/G	24 (0.48)	5 (0.50)	23 (0.70)	30 (0.79)
G/A	21 (0.42)	3 (0.30)	10 (0.30)	7 (0.18)
A/A	5 (0.10)	0 (0.20)	0	1 (0.03)
p	-	>0.999	0.174	0.036
G	69 (0.69)	13 (0.65)	56 (0.85)	67 (0.88)
A	31 (0.31)	7 (0.35)	10 (0.15)	9 (0.12)
p	-	>0.0999	0.087	0.010

Note: n (f), number and frequency of carriers of a certain genotype or allele; p, significance of difference (Chi square with Bonferroni correction); NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy

Thereafter, this association was checked with patients stratified by the stage of DR (Table 3) and the severity of DME (Table 4). Significant differences ($p < 0.001$) with regard to both genotypes and alleles were observed only in PDR (Table 3) and severe DME (Table 4).

We found relationships of rs1927911 genotypes with carbohydrate metabolism characteristics and retinal OCT characteristics (Table 5).

In the presence of DR and DME, blood glucose and HbA1c levels were 1.8 times and 1.4 times, respectively, increased in diabetic carriers of rs1927911 GG and GA genotypes, and these differences were significant ($p < 0.001$).

Of note that both blood glucose and HbA1c levels were higher in homozygous compared to heterozygous carriers of these variants, which could reflect a greater effect of the ancestral G allele in the former carriers than in the latter carriers. Correspondingly, the presence of the minor allele corresponded to less severe carbohydrate metabolism abnormalities.

A Kruskal-Wallis one-way analysis of variance found no effect of rs1927911 genotypes on blood glucose and HbA1c levels in the absence and in the presence of DR and DME ($p > 0.1$).

CRT and TRV were 1.8 times and 1.4 times, respectively, larger in the presence of DR and DME than in the absence of DR and DME in carriers of any genotype, and these differences were significant ($p < 0.001$). Of note were substantially (1.7-1.8 times) and significantly ($p < 0.001$) larger CRT and TRV in wild homozygous carriers, whereas in heterozygous and minor homozygous carriers with ophthalmic complications, the CRT was the same or even smaller than in those without these complications.

Table 4. RLR4 rs1927911 genotype and allele frequency distributions in patients stratified by the severity of diabetic macular edema (DME)

Genotypes	Groups of patients stratified by the severity of DME, n (f), n (f)			
	No DME	Mild DME	Moderate DME	Severe DME
G/G	24 (0.48)	6 (0.40)	12 (0.54)	40 (0.91)
G/A	21 (0.42)	6 (0.40)	10 (0.45)	4 (0.09)
A/A	5 (0.10)	3 (0.20)	0	0)
p	-	>0.999	0.915	<0.001
G	69 (0.69)	18 (0.60)	34 (0.77)	84 (0.95)
A	31 (0.31)	12 (0.40)	10 (0.23)	4 (0.05)
p	-	>0.999	0.915	<0.001

Note: n (f), number and frequency of carriers of a certain genotype or allele; p, significance of difference (Chi square with Bonferroni correction)

In the presence of ophthalmic complications, TRV was less substantial in heterozygous and minor homozygous carriers than in wild homozygous carriers.

A Kruskal-Wallis one-way analysis of variance found effects of rs1927911 genotypes on CRT and TRV in the presence of DR and DME ($p < 0.001$). Both CRT and TRV were lower in heterozygous carriers and especially in minor homozygous carriers. Therefore, rs1927911 polymorphism contributed to a reduction in diabetic retinal damage.

No significant relationship ($p > 0.4$) was found between rs1927911 polymorphism and IOP for this sample of patients.

Univariate regression analysis found DR and DME to be associated with rs1927911 genotypes (Table 6).

A reduction ($p = 0.019$) in the risk of developing DR was found in rs1927911 AG genotype carriers (OR = 0.39; 95% CI, 0.18–0.86) compared with GG genotype carriers. Fig. 2 shows a receiver operating characteristic (ROC) curve for this test.

An area under curve (AUC) of 0.62 (95% CI, 0.53–0.71) indicated a mild association between rs1927911 and the risk of DR. The test sensitivity and specificity were 71.6% (95% CI, 60.5%–81.1%) and 52.0% (95% CI, 37.4%–66.3%) for the optimal cut-off point (rs1927911 GG).

Therefore, TLR4 rs1927911 polymorphism was found to be associated with such ophthalmic complications of T2DM as DR and DME ($p = 0.021$), and the risk of these complications was lower (OR = 0.42; 95% CI, 0.23–0.77) in allele A carriers than in ancestral allele G carriers.

Discussion

Our findings are in agreement with those by Xu and colleagues [20], who found an association between TLR4 four polymorphisms (including rs1927911) and DR in Chinese patients with T2DM. DR is shown to be regulated by inadequate activation of members of the immune system [21]. TLR4 is a key mediator of innate immunity, and genetic changes in TLR4 support inflammation under hyperglycemic conditions.

It is possible that carriers of the minor allele A at rs1927911 are less susceptible to the development of pro-inflammatory retinal changes implemented through HMGB1/TLR4/NF-κB, a pathological signaling circuit [7, 8]. Hyperactivity of this component of innate immunity activation is likely to be a protective factor against ophthalmic diabetic complications.

This hypothesis was confirmed by the findings of the present study, with carbohydrate metabolism abnormalities and retinal damage being less severe in carriers of the heterozygous genotype and especially carriers of the minor AA genotype. In addition, the risk of DR and DME was lower in carriers of these genotypes compared to carriers of the ancestral GG genotype (OR = 0.37; 95% CI, 0.18–0.76).

This explanation of the role of rs1729911 polymorphism is in agreement with findings of a study by Seidel and colleagues [22] who generated diabetic endothelial cell specific and Müller cell specific TLR4 knockout mice to determine cell specific actions of TLR4 in the retina. Diabetic Cdh5-Cre TLR4 mice, PDGFRα-Cre TLR4 mice, and TLR4 floxed mice were evaluated

Table 5. Effects of rs1927911 genotypes on carbohydrate metabolism characteristics, CRT, TRV and IOP

Characteristic	Group	Genotypes			H	p
		G/G	G/A	A/A		
Glucose, mmol/l	T2DM	4.80±0.77	4.81±0.59	4.79±0.68	0.38	0.826
	T2DM+DR+DME	8.48±3.38	7.52±1.97	5.87±1.08	2.43	0.297
p*		<0.001	<0.001	0.099		
HbA1c,%	T2DM	5.42±0.36	5.3±0.45	5.38±0.43	1.16	0.558
	T2DM+DR+DME	7.40±0.78	6.96±0.66	6.70±0.72	4.53	0.103
p*		<0.001	<0.001	0.653		
CRT, μm	T2DM	239.6±13.4	235.7±14.0	224.2±19.1	3.49	0.174
	T2DM+DR+DME	438.9±111.6	245.6±52.9	178.3±20.6	44.1	<0.001
p*		<0.001	0.254	0.036		
TRV, mm^3	T2DM	5.91±0.57	5.69±0.60	5.56±0.59	2.88	0.236
	T2DM+DR+DME	10.41±2.16	8.60±1.99	7.18±0.37	16.9	<0.001
p*		<0.001	<0.001	<0.001		
IOP, mmHg	T2DM	14.92±2.59	14.71±2.65	17.0±2.92	2.96	0.221
	T2DM+DR+DME	15.29±3.70	14.05±3.22	18.67±3.51	4.74	0.091
p*		0.523	0.609	0.445		

Note: H, Kruskal-Wallis statistic; p, statistical significance of intragroup differences; p*, statistical significance of differences between groups (Mann-Whitney test); T2DM+DR+DME, a group of type 2 diabetics with both DR and DME (cases); T2DM, a group of type 2 diabetic controls

Table 6. Univariate logistic regression analysis of the risk of diabetic retinopathy (DR) and diabetic macular edema (DME) in carriers of different rs1927911 genotypes

Independent variable		Model coefficient, b±m	Significance of the difference of the OR from 1, p	OR (95% CI)
rs1927911	G/G	Reference		
	G/A	-0,93±0,40	0,019	0,39 (0,18–0,86)
	A/A	-1,39±0,77	0,070	0,25 (0,05–1,12)

Note: CI, confidence interval; DME, diabetic macular edema; DR, diabetic retinopathy; OR, odds ratio

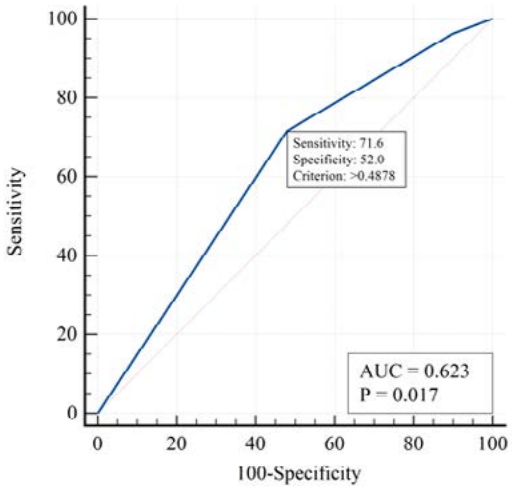

Fig. 2. Receiver operating characteristic (ROC) curve for predicting the risk of diabetic retinopathy on the basis of TLR4 rs1927911 genotypes

for retinal permeability, neuronal damage, and numbers of degenerative capillaries, all changes commonly observed in diabetic retina. Global loss of TLR4 reduced diabetic retinal inflammation, protein level of key inflammatory mediators and vascular endothelial growth factor levels. In addition, global loss of TLR4 reduced retinal capillary permeability and retinal neuronal and vascular damage in diabetic endothelial cell specific TLR4 knockout mice.

Fu and Liu [23] injected streptozotocin (STZ) into wild-type and TLR4 knockout mice to induce diabetes and investigate the role of TLR4 in DR. Deletion of TLR4 in diabetic mice significantly improved DR compared to wild-type mice, as judged by the enhanced thickness of retinal tissue. Furthermore, TLR4-dependent NF-kB pathway, inflammatory cytokine release and the expressions of VEGF and glial fibrillary protein (GFAP), which were all remarkably stimulated in STZ-injected wild-type mice, were inhibited in STZ-injected knockout mice.

In a study by Wang and colleagues [24], TLR4 was detected in CD31-labeled human retinal vasculae endothelia and its expression was markedly increased in fibrovascular membranes from DR patients and in retinal vascular endothelial cells of mice with STZ-induced diabetes. The expression of TLR4 and interleukin (IL)-1β was enhanced by high glucose in cultured human microvascular endothelial cells and was inhibited by TLR4 siRNA knock-down and TLR antagonist.

TLR4 plays a crucial role in the inflammation and apoptosis of retinal ganglion cells (RGCs) cultured in high glucose in rats with STZ-induced diabetes [25]. The administration of TAK-242, an inhibitor of TLR4, inhibited inflammation (via four TLR4 downstream signaling molecules MyD88, NF-κB, TRAF6, NLRP3), pro-inflammatory cytokines (IL-1β, IL-18) and apoptosis of RGCs.

Pro-inflammatory pathways involve TLR4 under hyperglycemic conditions; the above supposes that the activity of these pathways is reduced in carriers of rs1927911 polymorphism, and it is this that causes reduced diabetic retinal damage. The hypothesis requires experimental confirmation in future studies e.g., on cultured retinal endothelial cells and RGCs in carriers of different genotypes of rs1927911.

The findings of these future studies will have an impact on the research of the efficacy of TLR4 inhibitors [9–11, 25].

Conclusion

First, TLR4 rs1927911 polymorphism was associated with DR and DME (p = 0.021): the minor A allele was associated with a decreased risk (OR = 0.42; 95% CI, 0.23-0.77) of the disease.

Second, rs1927911 genotype distribution was significant by Pearson chi-square test under a dominant model (GG versus GA+AA) ($\chi 2$ = 6.38; p = 0.012; OR = 0.37; 95% CI, 0.18-0.76). After patients were stratified by the stage of DR and the severity of DME, significant differences with regard to genotypes were observed only in PDR and severe DME.

Third, carriers of the heterozygous genotype and minor genotype AA showed less severe glycemia and lower HbA1c, CRT and TRV than carriers of the ancestral genotype GG; this corresponded to less severe carbohydrate metabolism abnormalities and less severe diabetic retinal damage.

Finally, a reduction in the risk of developing DR (p = 0.019) was found in rs1927911 GA genotype carriers (OR = 0.39; 95% CI, 0.18–0.86) compared with GG genotype carriers.

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