

Toll-like receptor 4 polymorphisms in patients with Stargardt disease: A family study

Polimorfismos de TLR4 en pacientes con enfermedad de Stargardt: un estudio familiar

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Abstract

Purpose: Retinal pigment epithelial cells exhibit a propensity for apoptosis in Stargardt macular dystrophy (STGD). Previously, single-nucleotide polymorphisms (SNPs) in the toll-like receptor 4 (TLR4) gene have been related to apoptosis and inflammatory response. Therefore, this study was undertaken to investigate whether TLR4 SNPs are associated with STGD in a family-based study. **Methods:** Four blood-related Mexican patients with a clinical diagnosis of STGD (4 women) and 12 of their unaffected relatives were included in the study. A total of 109 subjects (40 men and 69 women; age, 63.28 ± 7.93 years) without macular affections, family history, or inherited macular dystrophies were used as controls. SNPs rs4986790, rs1927911, rs12377632, rs2149356, and rs11536889 of the TLR4 gene were genotyped using a Taqman[®] Allelic Discrimination Assay. **Results:** The frequency of the minor allele of rs4986790 (G) was significantly higher in STGD patients compared to control subjects (25% vs. 1%, $p = 0.0012$). The genotype carrying the minor allele of rs4986790 (AG) was more frequent in STGD patients (50%) compared with their relatives and unrelated control subjects (8 and 2.75%, respectively), with statistical significance ($p = 0.0048$). The allele and genotype frequencies of the remaining SNPs were not significant between STGD patients and control subjects ($p > 0.5$). Unaffected relatives of STGD patients showed allele and genotype frequencies similar to those observed in control subjects. **Conclusion:** Minor alleles of the SNPs rs4986790 (G) and genotypes carrying it (AG) were related to clinical STGD in one family. Interestingly, rs4986790 has been described as a promoter of apoptosis. Therefore, this TLR4 gene polymorphism should be considered as a marker in future studies.

Key words: Stargardt disease. Toll-like receptor 4. Polymorphisms. Family-based genetic study.

Resumen

Objetivo: Las células del epitelio pigmentario retiniano tienden a la apoptosis en la distrofia macular de Stargardt (STGD). Anteriormente, los polimorfismos de un solo nucleótido (SNP) del gen TLR4 se habían relacionado con la apoptosis y la

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inflamación. Por tanto, este estudio se realizó para investigar si los SNP de TLR4 están asociados con la STGD en un estudio familiar. **Métodos:** Se incluyeron 4 pacientes mexicanos consanguíneos con diagnóstico clínico de STGD (4 mujeres) y 12 familiares no afectados. Se utilizaron como control 109 sujetos (40 hombres y 69 mujeres; edad, 63.28 ± 7.93 años) sin enfermedades maculares ni antecedentes familiares. Los SNP rs4986790, rs1927911, rs12377632, rs2149356 y rs11536889 del gen TLR4 fueron genotipados usando un ensayo de discriminación alélica Taqman®. **Resultados:** La frecuencia del alelo menor rs4986790 (G) fue significativamente mayor en pacientes con STGD en comparación con los controles (25 vs 1%; $p = 0.0012$). El genotipo portador del alelo menor rs4986790 (AG) fue más frecuente en los pacientes con STGD (50%) en comparación con sus familiares y controles (8 y 2.75%, respectivamente) de manera significativa ($p = 0.0048$). Las frecuencias alélicas y genotípicas de los SNP restantes no fueron significativas entre pacientes con STGD y controles ($p > 0.5$). Los familiares no afectados mostraron frecuencias alélicas y genotípicas similares a las encontradas en los controles. **Conclusión:** El alelo menor del SNP rs4986790 (G) y el genotipo portador (AG) se relacionaron con la enfermedad de Stargardt clínica. Curiosamente, el rs4986790 se ha descrito como un promotor de la apoptosis. Así, pues, este polimorfismo del gen TLR4 debe considerarse como un marcador en estudios futuros.

Palabras clave: Enfermedad de stargardt. Receptor tipo toll 4. Polimorfismos. Estudio genético familiar.

Introduction

Stargardt disease Stargardt macular dystrophy (STGD) (Online Mendelian Inheritance in Man [OMIM]: 248200) is an autosomal recessive form of juvenile-onset macular dystrophy first described in 1909 by German ophthalmologist Karl Stargardt¹. It is the most common type of hereditary recessive macular dystrophy with an estimated incidence of 1 in 10,000 live births, accounting approximately for 7% of all retinal diseases and typically presenting in children and young adults^{2,3}. The diagnosis of STGD is classically based on family history, visual acuity, fundus examination, fluorescein angiography, fundus autofluorescence, and electroretinography⁴.

The typical form of STGD (STGD1, OMIM #248200) is caused by mutations in the ATP-binding cassette transporter 4 (ABCA4) gene located in 1p22.1, with an autosomal recessive homozygous or compound heterozygous transmission. Mutations of the prominin-like protein 1 gene (PROM1) located in 4p15.32 cause an autosomal dominant form (STGD4, ONIM #603786). Dominant mutations of the fatty acid elongase 4 (ELOVL4) gene (6q14.1) cause Stargardt-like macular dystrophies (STGD3, OMIM #600110)⁵.

The pathogenesis of Stargardt disease associated with ABCA4 gene mutations has been well established. ABCA4 participates in the retinoid cycle as a "flippase" that transports the by-product N-retinylidene-phosphatidylethanolamine (N-retinylidene-PE) across the disc membranes. Through this mechanism, ABCA4 prevents retinoid accumulation in the subcellular space after rhodopsin photobleaching⁵. Fail to transport N-retinylidene-PE across the disc membrane leads to its entrapment and reaction with all-*trans*-retinal (atRAL) to form di-retinoid-pyridinium-phosphatidylethanolamine

(A2PE). When photoreceptors renew, A2PE converts into di-retinoid-pyridinium-ethanolamine (A2E), a Vitamin A dimer, which becomes permanently trapped in the retinal pigment epithelium (RPE) because it cannot be further hydrolyzed. A2E is a major component of lipofuscin, a hallmark of cellular degeneration⁵. Markedly accumulated atRAL may re-associate with opsin, and the resulting complex is less effective in activating the visual cascade than photoactivated rhodopsin; this may explain the prolonged dark adaptation found in STGD patients^{5,6}. Lipofuscin can accumulate up to 5 times above normal values, and A2E excess in the RPE affects its function and survival because it acts as a detergent compromising cell membrane and metabolic functions. Furthermore, in the presence of oxygen and blue-wavelength light, lipofuscin forms free epoxide radicals that induce RPE cell death⁶. Death of RPE cells leads to secondary photoreceptor degeneration⁷.

Microglial activation by endogenous retinal proteins arising from injured photoreceptors plays an important role in the pathogenesis of retinal degeneration. In a comprehensive study in a STGD mouse model (caused by delayed clearance of atRAL from the retina) demonstrates an important contribution of toll-like receptor 4 (TLR4)-mediated microglial activation in retinal inflammation that aggravates retinal cell death⁸.

The TLR4 gene has been associated in several reports with apoptosis of retinal cells. For example, TLR4 and its signaling pathway have been related to retinal ganglion cells (RGCs) death in the retina⁹⁻¹¹ and to apoptosis of RGCs and retinal endothelial cells in high-glucose models that imitate the microenvironment of diabetic retinopathy^{12,13}.

TLR4 is a member of the family of pattern recognition receptors that serve as a key part of the innate immune

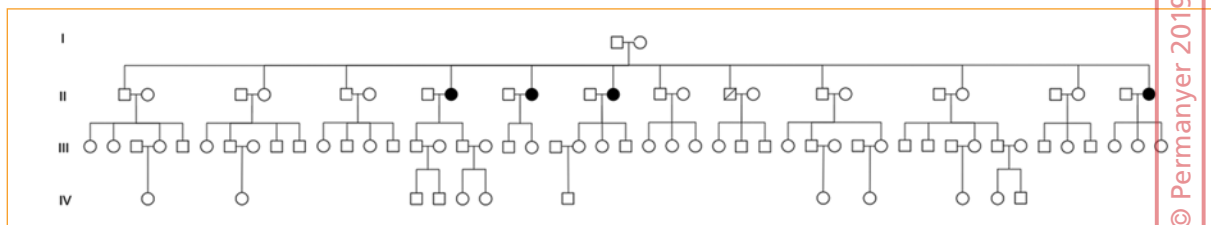


Figure 1. Pedigree of the family affected by Stargardt macular dystrophy (STGD). Affected individuals are shown in shaded and healthy subjects are shown as open figures. Circles represent females and squares represent males. Four siblings in the second generation (II: 8, II: 9, II: 10, and II: 22) are affected. This pattern supports the autosomal recessive transmission observed in STGD. STGD; Stargardt disease.

system. It has been identified in the central nervous system and in the retina⁹. The TLR4 gene is located in the locus 9q33.1 which spans a genomic region of ~13.3 kb with four exons (Assembly; GRCh38.p2, RefSeqGene; NG_011475.1, Gene ID; TLR4 7099)¹⁴ that encodes a transmembrane pathogen recognition receptor involved in the detection of lipopolysaccharides (LPS) of Gram-negative bacteria and other exogenous or endogenous ligands¹⁵. This gene displays several single-nucleotide polymorphisms (SNPs) that presumably influence the activity and function of its product¹⁶. It has been shown that TLR4 polymorphisms in humans may contribute to the severity of eye degenerative diseases such as glaucoma^{17,18} or age-related macular degeneration¹⁹.

Based on studies that suggest the participation of TLR4 in the pathogenesis of Stargardt disease, and the association of TLR polymorphisms with retinal degenerative disorders, we decided to evaluate the association of SNPs in coding and non-coding regions of the TLR4 gene (locus 9q32-q33) with macular Stargardt disease in a family with a clinical diagnosis of Stargardt disease. Remarkably, this genetic mark has been described as pro-apoptotic in other studies.

Patients and methods

Patients

DNA was obtained from four affected patients (4 women) and 12 unaffected relatives from a multigenerational Mexican family. The pedigree of the affected family is shown in [fig. 1](#). We included a total of 109 (40 men and 69 women; age 63.28 ± 7.93 years) control samples from a matched Mexican population. Patients diagnosed with STGD underwent a complete ocular examination, fluorescein angiography, and high-definition optical coherence tomography (HD-OCT). Furthermore,

electroretinography was performed to corroborate the diagnosis of STGD. Fundus photograph, red-free fundus photograph, and HD-OCT images of STGD patients are presented in [fig. 2](#). Control subjects had a full clinical history taken, none of them had a family history of macular disease, and they were carefully examined to rule out any ocular disease. Informed consent was obtained from all individuals participating in the study. The study was approved by the local ethics committee and complied with the guidelines of the declaration of Helsinki. All study details were explained to patients and control subjects before acquiring the informed consent for genetic screening.

Sample preparation and genotyping

Target SNPs are located in non-coding regions of the TLR4 gene (locus 9q32-q33); rs1927911 G/A, rs12377632 T/C, and rs2149356 G/T within introns, rs11536889 G/C within the 3' untranslated region, and rs4986790 A/G (Asp299Gly) located at the third exon (Transcript variant 1; RefSeq: NM_138554.3). Position of the polymorphisms is 8595, 11271, 12740, 16672, and 13843, respectively (Assembly; GRCh38.p2, RefSeqGene; NG_011475.1, Gene ID; TLR4 7099).

Genomic DNA was extracted from peripheral blood leukocytes and purified with the Qiagen QIAmp DNA Blood Kit (Qiagen, Valencia, CA, USA). Genotyping of the target SNPs was performed using validated TaqMan SNP genotyping assays (TaqMan: Applied Biosystems, Inc. [ABI], Foster City, CA). SNPs were amplified using real-time polymerase chain reaction (PCR) using 0.5 μ M primers and TaqMan Genotyping Master Mix (TaqMan: Applied Biosystems, Inc. [ABI], Foster City, CA) with 30 ng template DNA in the amplification mixture (25 μ L). The fluorescence signal of the probe was detected with the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Inc. [ABI], Carlsbad, CA).

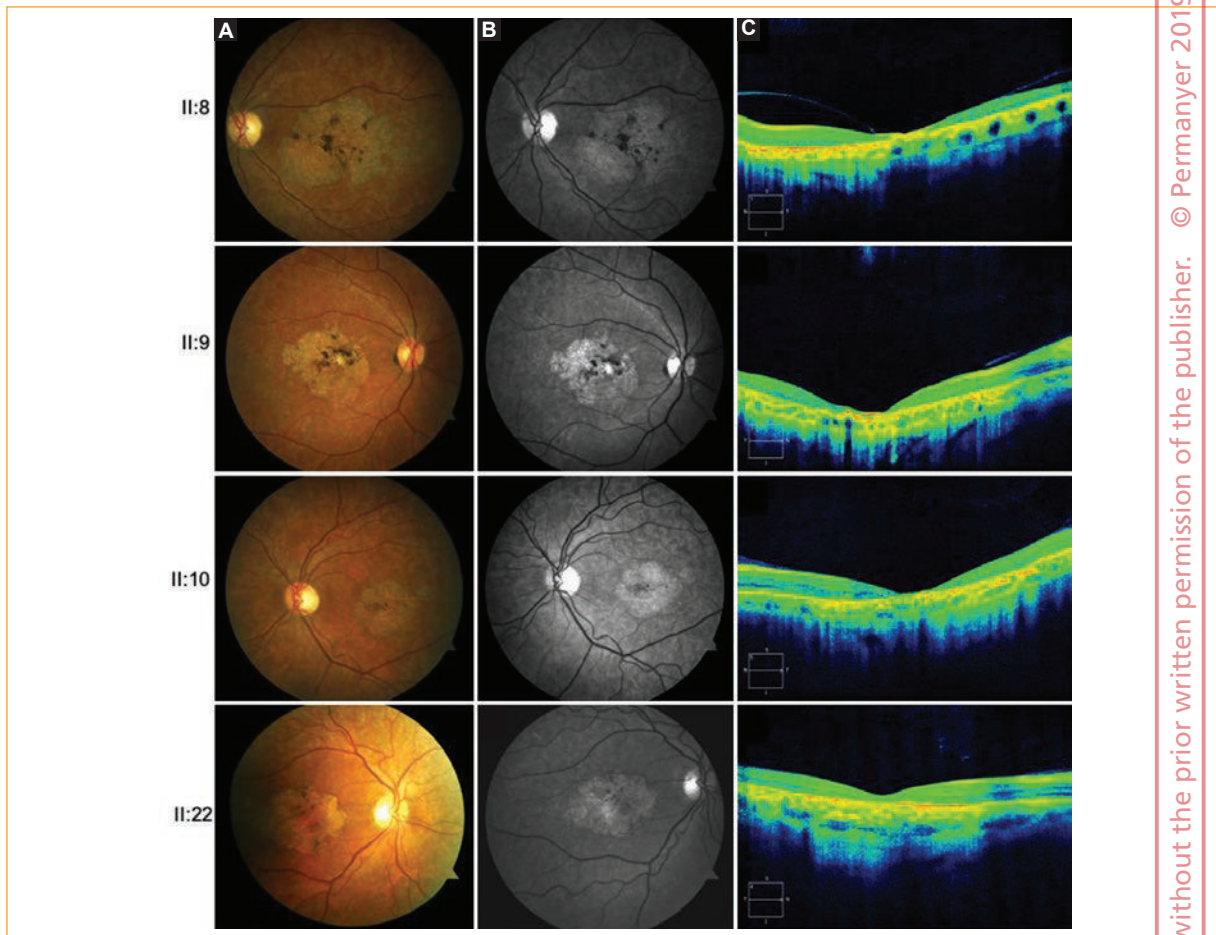


Figure 2. Representative images of the diagnostic studies of affected subjects. Fundus photograph (A), red-free fundus photograph (B), and macular high-definition optical coherence tomography images (C) of Stargardt disease (STGD) patients are presented. Rows 1, 2, 3, and 4 correspond to diagnostic images of II: 8, II: 9, II: 10, and II: 22 STGD patients.

Statistical analysis

IBM SPSS statistics software (version 21.0, SPSS, Inc., Chicago, IL) was used for statistical analyses. The Hardy-Weinberg equilibrium was analyzed using gene frequencies obtained by simple gene counting and the X² test with Yates' correction for comparing observed and expected values. Differences in the genotype frequencies among the cases and controls were tested by Fisher exact or X² depending on the cell counts. The inferred haplotypes and LD (linkage disequilibrium) quantified between the biallelic loci were estimated using the SNPStats online software (<http://bioinfo.iconologia.net/SNPStats>). The significance of an association was determined by contingency tables analysis using Chi-square or Fisher exact tests. Significance was defined as $p < 0.05$.

Results

Allele and genotype frequencies

SNPs allele frequencies in STGD patients, their relatives and control subjects are shown in [table 1](#). The SNPs rs1927911 G/A, rs12377632 T/C, rs2149356 G/T, rs11536889 G/C, and rs4986790 A/G were genotyped in all subjects and were in Hardy-Weinberg equilibrium. The frequency of the minor allele of rs4986790 A/G (G) was significantly higher in STGD patients compared to control subjects (25% vs. 1%, $p = 0.0012$).

The genotype frequencies of the analyzed SNPs are shown in [table 2](#). The genotype carrying the minor allele of rs4986790 (AG) was more frequent in the STGD patients (50%) compared to their relatives and unrelated control subjects (8 and 2.75%, respectively), with statistical significance ($p = 0.0048$).

Table 1. Frequency of SNPs alleles in STGD and control groups

SNP/Alleles		STGD	Relatives	Control	p	p
		n = 8*	n = 24	n = 218*	Case versus control	Overall
rs4986790	A/G	2 (0.25)	1 (0.04)	3 (0.01)	0.00120	0.00772
rs1927911	G/A	2 (0.25)	6 (0.25)	56 (0.26)	0.71229	0.99651
rs12377632	T/C	3 (0.375)	5 (0.21)	105 (0.48)	0.81624	0.06467
rs2149356	G/T	4 (0.5)	7 (0.29)	60 (0.28)	0.32393	0.38194
rs11536889	G/C	0 (0)	0 (0)	28 (0.13)	0.59151	0.26700

*Minor allele frequency followed by its proportion. SNPs: single-nucleotide polymorphisms; STGD: Stargardt disease.

Table 2. Frequency of genotypes in target TLR4 single-nucleotide polymorphisms

Gene position	SNP	*SNP position	SNP location	Genotypes	STGD n = 4	Relatives n = 12	Control n = 109	p
9q32-q33	rs4986790	13843	Exon	A/A	2 (0.5)	11 (0.92)	106 (0.9724)	0.0048
				A/G	2 (0.5)	1 (0.08)	3 (0.0275)	
				G/G	0 (0)	0 (0)	0 (0)	
	rs1927911	8595	Intron	G/G	2 (0.5)	6 (0.5)	64 (0.587)	0.55
				A/G	2 (0.5)	6 (0.5)	34 (0.312)	
				A/A	0	0 (0)	11 (0.101)	
	rs12377632	11271	Intron	T/T	2 (0.5)	7 (0.58)	31 (0.284)	0.29
				C/T	2 (0.5)	5 (0.42)	51 (0.468)	
				C/C	0	0 (0)	27 (0.248)	
rs2149356	12740	Intron	G/G	1 (0.25)	6 (0.5)	61 (0.56)	0.44	
			T/G	2 (0.5)	5 (0.42)	36 (0.33)		
			T/T	1 (0.25)	1 (0.08)	12 (0.11)		
rs11536889	16672	3 × UTR	G/G	4 (1)	12 (1)	84 (0.771)	0.36	
			C/G	0 (0)	0 (0)	22 (0.202)		
			C/C	0 (0)	0 (0)	3 (0.028)		

*RefSeqGene, NG_011475.1; Gene ID, TLR4 7099. SNP: single-nucleotide polymorphism; UTR: untranslated region; SNPs: single-nucleotide polymorphisms; STGD: Stargardt disease.

The allele and genotype frequencies of remaining SNPs were non-significant between cases and control subjects ($p > 0.05$). Unaffected relatives of STGD patients showed allele and genotype frequencies similar to those observed in control subjects.

Haplotype analysis

Tag SNPs were located in one haplotype block, and the magnitude of the LD between biallelic loci was very

high, with a pairwise $D' > 0.98$ for all cases when comparing STGD patients to control subjects. The haplotypes with a frequency higher than 1% are shown in table 3. We found a significant risk haplotype. As shown in table 3, the GGTTG haplotype reached the higher odds ratio (OR) ($p = 0.011$, OR = 33.84, 95% confidence interval = 2.38-481.17). Interestingly, minor allele of rs4986790 A/G (G) is present in the risk haplotype.

Table 3. Haplotype frequencies of Tag SNPs in the TLR4 gene between cases and control subjects

Tag SNPs					Control	STGD	Relatives	Haplotype association with Stargardt*	
rs4986790	rs1927911	rs2149352	rs12377632	rs11536889	n = 109	n = 4	n = 12	OR (95% CI)	
A	G	G	C	G	0.3532	0.25	0.2083	1	
A	A	T	T	G	0.2523	0.25	0.25	1.57 (0.21-11.71)	0.66
A	G	G	T	G	0.2385	0.25	0.5	1.32 (0.15-11.30)	0.8
G	G	T	T	G	0.0138	0.25	0.0417	33.84 (2.38-481.17)	0.011

*OR values were calculated using two-way frequency tables with control and Stargardt groups. CI: confidence interval; OR: odds ratio; SNPs: single-nucleotide polymorphisms.

Discussion

TLRs are transmembrane proteins capable of activating signal pathways of inflammatory and immune responses. They are expressed in the innate immune system as well as in the central nervous system, including the retina. Thirteen TLRs (TLR1-TLR13) have been identified in humans so far⁹. TLR4 is a transmembrane receptor involved in the detection of LPS of Gram-negative bacteria and many other exogenous or endogenous ligands. It has an extracellular (leucine-rich) domain responsible for the recognition, a transmembrane domain, and an intracellular domain (similar to the interleukin [IL]-1 domain)¹⁵. After activation, several proteins are involved in intracellular signaling: TIR-domain-containing adapter-inducing interferon (TRIF) and myeloid differentiation primary response gene 88 (MYD88) adapters²⁰. TLR4, through nuclear factor kappa B (NF- κ B), is capable of initiating the production of pro-inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor- α (TNF- α). Furthermore, by activating interferon (IFN) regulatory factor 3 (IRF3), it initiates the expression of IFN-inducible genes and IFN- β ²¹. All the aforementioned pathways control cell survival and apoptosis²².

The human TLR4 gene displays several SNPs that have been reported to be associated with a wide variety of both infectious and non-infectious diseases including rheumatoid arthritis^{23,24}, bronchial asthma²⁵, chronic periodontitis²², susceptibility to pulmonary aspergillosis and tuberculosis^{26,27}, lung cancer²⁸, and peptic ulcer risk²⁹.

SNPs of the TLR4 gene presumably influence the activity and function of its product¹⁶. For instance, the TLR4 gene variations rs4986790 and rs4986791 lead to missense variants (Asp299Gly and Thr399Ile, respectively) that alter the extracellular domain of the protein, affecting TLR4 function by decreasing its

responsiveness^{30,31}. The mechanism by which these SNPs induce hypo-responsiveness remains to be clarified, but currently, it is known that these mutations induce conformational changes that affect protein folding and stability, interactions with messenger proteins, and expression on the cell surface³². Besides, it has been demonstrated that Asp299Gly induces deficient recruitment of MYD88 and TRIF³³.

As previously mentioned, the TLR4 gene has been associated with retinal cells apoptosis, including RGCs and retinal endothelial cells⁹⁻¹³, but intriguingly, the TLR4 polymorphisms rs4986790 and rs4986791 can induce apoptosis of activated hepatic stellate cells by reducing phospho-ERK and Bcl-2³⁴.

Apoptosis of REP⁶ and photoreceptor degeneration⁷ is the hallmark phenomena in STGD. Although the pathophysiology of these processes has been established based on the ABCA4 gene mutations and their effects in STGD⁵⁻⁷, the contribution of other genes should be explored to discover other injury mechanisms in STGD.

Interestingly, rs4986790 has been described as an apoptosis promoter³⁴. The significance of this effect should be evaluated, but for STGD, it could be related to TLR4-mediated microglial activation, as it was suggested in previous studies.⁸

Conclusion

Minor allele of the SNPs rs4986790 and genotypes carrying it was related to clinical STGD in one family. We are aware that the main limitation of our study is the reduced number of patients included, but we consider that evidence on these and other reports support that the TLR4 gene and its polymorphisms should be considered in future studies to determine if they are useful molecular markers for the diagnosis, prognosis, or treatment of STGD.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

References

- Zahid S, Jayasundera T, Rhoades W, Branham K, Khan N, Niziol LM, et al. Clinical phenotypes and prognostic full-field electroretinographic findings in stargardt disease. *Am J Ophthalmol.* 2013;155:465-73000.
- Sohrab MA, Allikmets R, Guarnaccia MM, Smith RT. Preimplantation genetic diagnosis for stargardt disease. *Am J Ophthalmol.* 2010;149:651-500.
- Heathfield L, Lacerda M, Nossek C, Roberts L, Ramesar RS. Stargardt disease: towards developing a model to predict phenotype. *Eur J Hum Genet.* 2013;21:1173-6.
- Berisha F, Feke GT, Aliyeva S, Hirai K, Pfeiffer N, Hirose T, et al. Evaluation of macular abnormalities in stargardt's disease using optical coherence tomography and scanning laser ophthalmoscope microperimetry. *Graefes Arch Clin Exp Ophthalmol.* 2009;247:303-9.
- Tanna P, Strauss RW, Fujinami K, Michaelides M. Stargardt disease: clinical features, molecular genetics, animal models and therapeutic options. *Br J Ophthalmol.* 2017;101:25-30.
- Sparrow JR, Boulton M. RPE lipofuscin and its role in retinal pathobiology. *Exp Eye Res.* 2005;80:595-606.
- Ma H, Thapa A, Morris L, Redmond TM, Baehr W, Ding XQ, et al. Suppressing thyroid hormone signaling preserves cone photoreceptors in mouse models of retinal degeneration. *Proc Natl Acad Sci U S A.* 2014;111:3602-7.
- Kohn H, Chen Y, Kevany BM, Pearlman E, Miyagi M, Maeda T, et al. Photoreceptor proteins initiate microglial activation via toll-like receptor 4 in retinal degeneration mediated by all-trans-retinal. *J Biol Chem.* 2013;288:15326-41.
- Morzaev D, Nicholson JD, Caspi T, Weiss S, Hochhauser E, Goldenberg-Cohen N, et al. Toll-like receptor-4 knockout mice are more resistant to optic nerve crush damage than wild-type mice. *Clin Exp Ophthalmol.* 2015;43:655-65.
- Okamoto T, Ozawa Y, Kamoshita M, Osada H, Toda E, Kurihara T, et al. The neuroprotective effect of rapamycin as a modulator of the mTOR-NF- κ B axis during retinal inflammation. *PLoS One.* 2016;11:e0146517.
- Ulbrich F, Schallner N, Coburn M, Loop T, Lagrèze WA, Biermann J, et al. Argon inhalation attenuates retinal apoptosis after ischemia/reperfusion injury in a time- and dose-dependent manner in rats. *PLoS One.* 2014;9:e115984.
- Hu L, Yang H, Ai M, Jiang S. Inhibition of TLR4 alleviates the inflammation and apoptosis of retinal ganglion cells in high glucose. *Graefes Arch Clin Exp Ophthalmol.* 2017;255:2199-210.
- Zhang TH, Huang CM, Gao X, Wang JW, Hao LL, Ji Q, et al. Gastrodin inhibits high glucose-induced human retinal endothelial cell apoptosis by regulating the SIRT1/TLR4/NF- κ Bp65 signaling pathway. *Mol Med Rep.* 2018;17:7774-80.
- Schneider S, Koch W, Hoppmann P, Ubrich R, Kemmer S, Steinlechner E, et al. Association of toll-like receptor 4 polymorphism with age-dependent systolic blood pressure increase in patients with coronary artery disease. *Immun Ageing.* 2015;12:4.
- Rallabhandi P, Bell J, Boukhvalova MS, Medvedev A, Lorenz E, Arditi M, et al. Analysis of TLR4 polymorphic variants: new insights into TLR4/MD-2/CD14 stoichiometry, structure, and signaling. *J Immunol.* 2006;177:322-32.
- Ferwerda B, McCall MB, Verheijen K, Kullberg BJ, van der Ven AJ, Van der Meer JW, et al. Functional consequences of toll-like receptor 4 polymorphisms. *Mol Med.* 2008;14:346-52.
- Navarro-Partida J, Martinez-Rizo AB, Ramirez-Barrera P, Velazquez-Fernandez JB, Mondragon-Jaimes VA, Santos-Garcia A, et al. Association of toll-like receptor 4 single-nucleotide polymorphisms asp299Gly and thr399Ile with the risk of primary open angle glaucoma. *Graefes Arch Clin Exp Ophthalmol.* 2017;255:995-1001.
- Navarro-Partida J, Alvarado Castillo B, Martinez-Rizo AB, Rosales-Diaz R, Velazquez-Fernandez JB, Santos A, et al. Association of single-nucleotide polymorphisms in non-coding regions of the TLR4 gene with primary open angle glaucoma in a mexican population. *Ophthalmic Genet.* 2017;38:325-9.
- Sarli A, Skalidakis I, Velissari A, Koutsandrea C, Stefanotou M, Petersen MB, et al. Investigation of associations of *ARMS2*, *CD14*, and *TLR4* gene polymorphisms with wet age-related macular degeneration in a greek population. *Clin Ophthalmol.* 2017;11:1347-58.
- Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, et al. Role of adaptor TRIF in the myD88-independent toll-like receptor signaling pathway. *Science.* 2003;301:640-3.
- Kutikhin AG. Impact of toll-like receptor 4 polymorphisms on risk of cancer. *Hum Immunol.* 2011;72:193-206.
- Sellers RM, Payne JB, Yu F, LeVan TD, Walker C, Mikuls TR, et al. TLR4 asp299Gly polymorphism may be protective against chronic periodontitis. *J Periodontol Res.* 2016;51:203-11.
- Kuuliala K, Orpana A, Leirisalo-Repo M, Kautiainen H, Hurme M, Hanonon P, et al. Polymorphism at position +896 of the toll-like receptor 4 gene interferes with rapid response to treatment in rheumatoid arthritis. *Ann Rheum Dis.* 2006;65:1241-3.
- Radstake TR, Franke B, Hanssen S, Netea MG, Welsing P, Barrera P, et al. The toll-like receptor 4 asp299Gly functional variant is associated with decreased rheumatoid arthritis disease susceptibility but does not influence disease severity and/or outcome. *Arthritis Rheum.* 2004;50:999-1001.
- Werner M, Topp R, Wimmer K, Richter K, Bischof W, Wjst M, et al. TLR4 gene variants modify endotoxin effects on asthma. *J Allergy Clin Immunol.* 2003;112:323-30.
- Carvalho A, Pasqualotto AC, Pitzurra L, Romani L, Denning DW, Rodrigues F, et al. Polymorphisms in toll-like receptor genes and susceptibility to pulmonary aspergillosis. *J Infect Dis.* 2008;197:618-21.
- Schurz H, Daya M, Möller M, Hoal EG, Salie M. TLR1, 2, 4, 6 and 9 variants associated with tuberculosis susceptibility: a systematic review and meta-analysis. *PLoS One.* 2015;10:e0139711.
- Kurt H, Ozbayer C, Bayramoglu A, Gunes HV, Degirmenci B, Oner KS, et al. Determination of the relationship between rs4986790 and rs4986791 variants of TLR4 gene and lung cancer. *Inflammation.* 2016;39:166-71.
- Pohjanen VM, Koivurova OP, Huhta H, Helminen O, Mäkinen JM, Karhukorpi JM, et al. Toll-like receptor 4 wild type homozygosity of polymorphisms +896 and +1196 is associated with high gastrin serum levels and peptic ulcer risk. *PLoS One.* 2015;10:e0131553.
- Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet.* 2000;25:187-91.
- Long H, O'Connor BP, Zemans RL, Zhou X, Yang IV, Schwartz DA, et al. The toll-like receptor 4 polymorphism asp299Gly but not thr399Ile influences TLR4 signaling and function. *PLoS One.* 2014;9:e93550.
- Ohto U, Yamakawa N, Akashi-Takamura S, Miyake K, Shimizu T. Structural analyses of human toll-like receptor 4 polymorphisms D299C and T399I. *J Biol Chem.* 2012;287:40611-7.
- Figuerola L, Xiong Y, Song C, Piao W, Vogel SN, Medvedev AE, et al. The asp299Gly polymorphism alters TLR4 signaling by interfering with recruitment of myD88 and TRIF. *J Immunol.* 2012;188:4506-15.
- Guo J, Loke J, Zheng F, Hong F, Yea S, Fukata M, et al. Functional linkage of cirrhosis-predictive single nucleotide polymorphisms of toll-like receptor 4 to hepatic stellate cell responses. *Hepatology.* 2009;49:960-8.

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