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Association between SNPs of Metalloproteinases and Prostaglandin F2α Receptor Genes and Latanoprost Response in Open-Angle Glaucoma

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Purpose: To determine whether single nucleotide polymorphisms (SNPs) of genes coding for matrix metalloproteinases (MMPs) and the prostaglandin F2α receptor gene (*PTGFR*) are related to a response to latanoprost in a white Spanish population of glaucomatous patients.

Design: Case–control study.

Participants: One hundred twenty-four patients with open-angle glaucoma.

Methods: Genotyping was performed in 117 patients with primary open-angle glaucoma with a minimum treatment duration of 4 weeks. Candidate genes and individual polymorphisms were selected according to the effect on the mechanism of action of latanoprost. Multi-SNP haplotype analyses for associations also were tested.

Main Outcome Measures: Diurnal intraocular pressure reduction and genotyping of the SNPs in the MMPs and *PTGFR*.

Results: The *PTGFR* SNPs were associated with positive (rs6686438, rs10786455) and negative (rs3753380, rs6672484, rs11578155) responses to latanoprost. Multiple testing found 2 genes, *PTGFR* and *MMP-1*, were related to refractoriness to latanoprost.

Conclusions: The SNPs of the *PTGFR* and *MMP-1* genes may determine the latanoprost response in a white European Spanish population. This study identified 5 SNPs related to the latanoprost response; 1 SNP, rs3753380, already has been associated with a poor response to latanoprost in a healthy Japanese population. Latanoprost is a commonly used antiglaucomatous drug, and increased knowledge of its mechanism of action will lead to advances in pharmacogenetics. *Ophthalmology 2015;■:1–9 © 2015 by the American Academy of Ophthalmology.*

Latanoprost (Xalatan; Pfizer Laboratories, New York, NY) is one of the most powerful agents for reducing intraocular pressure (IOP). The mechanism of action is increased uveoscleral outflow facility via matrix metalloproteinase (MMP)-1, -2, -3, -9, and -17 in the ciliary muscle and sclera,[1,2] which reflects the direct response of the prostaglandin F receptor in the sclera and ciliary body.[3–5]

The response to a pharmacologic agent may be affected by genetic factors, which is the study of pharmacogenetics.[6] In glaucoma, β-blockers and prostaglandin analogs have been studied. Schwartz et al[7] reported a relationship between a single nucleotide polymorphism (SNP) at codon 389 in the β-adrenergic receptor gene and a greater response to betaxolol 0.25% (Betoptic; Alcon Laboratories, Fort Worth, TX); this response is related to a SNP at nucleotide 1165 where a substitution G→C results in an arginine→glycine (Arg→Gly) substitution at codon 389, changing the G protein structure near the binding domain, in normal healthy volunteers. Sakurai et al[8,9] reported an association between SNPs and a positive or negative response to latanoprost in healthy Japanese volunteers; SNPs rs3753380 and rs3766355 in the promoter and intron 1 regions of the prostaglandin F2α receptor (*PTGFR*) gene downregulated expression of the gene after a short course of latanoprost treatment (1 week), resulting in a diminished therapeutic effect.

The Latanoprost Study Group (LSG) determined a nonresponse rate of 18%, which was defined arbitrarily as an IOP reduction of less than 15% of the basal IOP after 2 weeks of treatment with latanoprost.[10] The rates of response to latanoprost vary among populations, from 4.1% in an Italian population[11] to 13.5% in American populations.[12] In this study, we evaluated possible associations between the SNPs of the genes coding for MMP-1, -2, -3, -9, and -17 and the

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Table 1. Eligibility Criteria

- Caucasian Spanish origin
- Male or female, older than 18 yrs
- Diagnosis of primary open-angle glaucoma according to the American Academy of Ophthalmology preferred practice pattern guidelines
  - Optic disc or retinal nerve fiber layer abnormalities
  - Reproducible visual field abnormality
  - Open anterior chamber angles
- Monotherapy treatment with latanoprost exceeding 4 weeks
- Documented response or nonresponse to latanoprost
- No history of ocular surgery, including laser

PTGFR gene in a white Spanish population of glaucomatous patients treated with latanoprost monotherapy.

Methods

We conducted a multicenter case–control study after the local ethics committees of 5 participating centers approved the study. The study protocol adhered to the tenets of the Declaration of Helsinki and all criteria stipulated by Spanish law 14/2007 about clinical studies. All patients provided written informed consent. The eligibility criteria are shown in Table 1.

Definition of Responders and Nonresponders to Latanoprost

After a minimum of 4 weeks of treatment with latanoprost, we followed the LSG criteria in terms of IOP reduction to define responders and nonresponders to latanoprost. Nonresponders were defined as those with an IOP reduction of less than 15% of the basal IOP, and responders were defined as those with an IOP reduction exceeding 15% of the basal IOP; hyperresponders, with an IOP reduction exceeding 30% of the basal IOP, were included among the responders.

Gene and Single Nucleotide Polymorphism Selection

We performed a candidate gene study. A bibliographic search of the public databases of the National Center for Biotechnology Information PubMed (available at: http://www.ncbi.nlm.nih.gov/PubMed/) and Online Mendelian Inheritance in Man (available at: http://www.ncbi.nlm.nih.gov/omim/) was conducted according to the mechanism of action of latanoprost in the extracellular matrix and trabecular or uveoscleral aqueous humor pathways. A study of the main agents involved in the mechanism of action of latanoprost: MMP-1, -2, -3, -9, and -17 and the *PTGFR* gene was performed.

To select SNPs, we searched the public databases HapMap (http://www.hapmap.ncbi.nlm.nih.gov) and dbSNP (http://www.ncbi.nlm.nih.org/SNP/) using the following criteria: location in the gene, with priority given to the SNPs in the promoter and exonic regions; SNPs known to be related to a latanoprost response, that is, SNPs rs3753380 (promoter region) and rs3766355 (intronic region) of the *PTGFR* gene; linkage disequilibrium, with priority given to tag SNPs; allelic frequency; and minor allelic frequency of 10% or more. The 71 nonsynonymous SNPs chosen were in chromosomes coding for the following genes: 1 (*PTGFR*), 11 (*MMP-1*, *-3*), 12 (*MMP-17*), 16 (*MMP-2*), and 20 (*MMP-9*; Table 2).

Validated SNPs were selected from the dbSNP database. Tag SNPs were selected automatically using the webserver SYSNP (http://www.sysnps.org). The SNPs were selected only if linkage disequilibrium was evaluated by having allelic frequencies of more than 0.05, genotype percentages of more than 75%, and an r^2 threshold of 0.8. For the *PTGFR* gene, we selected SNPs within 10 kb from the gene.

Intraocular Pressure Reduction and DNA Samples

Patients (67 women, 57 men; mean age ± standard deviation, 63.3±12.3 years) with a documented history of a response or nonresponse to latanoprost were included in the study at 5 Spanish reference centers. All patients instilled latanoprost at night. Intraocular pressure was measured using a Goldmann applanation tonometer (Carl Zeiss, Inc, Jena, Germany) mounted on a slit-lamp biomicroscope. An expert ophthalmologist

Table 2. Single Nucleotide Polymorphisms Evaluated and Chromosomal Location

Single Nucleotide Polymorphism	Chromosomal Location	Single Nucleotide Polymorphism	Chromosomal Location	Single Nucleotide Polymorphism	Chromosomal Location	Single Nucleotide Polymorphism	Chromosomal Location
rs1328441	1	rs3787268	20	rs10751701	12	rs17293823	11
rs1328449	1	rs3918249	20	rs10782665	1	rs243845	16
rs1417103	1	rs3918256	20	rs10873978	1	rs470215	11
rs1555541	1	rs4650581	1	rs10902456	12	rs470358	11
rs1581918	1	rs4964926	12	rs11162463	1	rs473027	1
rs1581920	1	rs4964927	12	rs11162488	1	rs475007	11
rs1861320	16	rs5031036	11	rs11162494	1	rs514921	11
rs1999012	1	rs6672484	1	rs11225426	11	rs518341	1
rs2071232	11	rs6686438	1	rs11246851	12	rs520171	1
rs2241145	16	rs6692239	1	rs11541998	16	rs520540	11
rs2274755	20	rs7125062	11	rs11578155	1	rs569444	11
rs2352039	1	rs7125320	11	rs11613757	12	rs650108	11
rs3025066	11	rs7484577	12	rs11835665	12	rs674345	1
rs3087864	12	rs7545762	1	rs12099648	12	rs724159	1
rs3753380	1	rs7945189	11	rs12568630	1	rs866770	16
rs3766332	1	rs10489950	1	rs12731181	1	rs1034186	1
rs3766354	1	rs10751699	12	rs12748050	1	rs1053605	16
rs3766355	1	rs10751700	12	rs12923011	16		

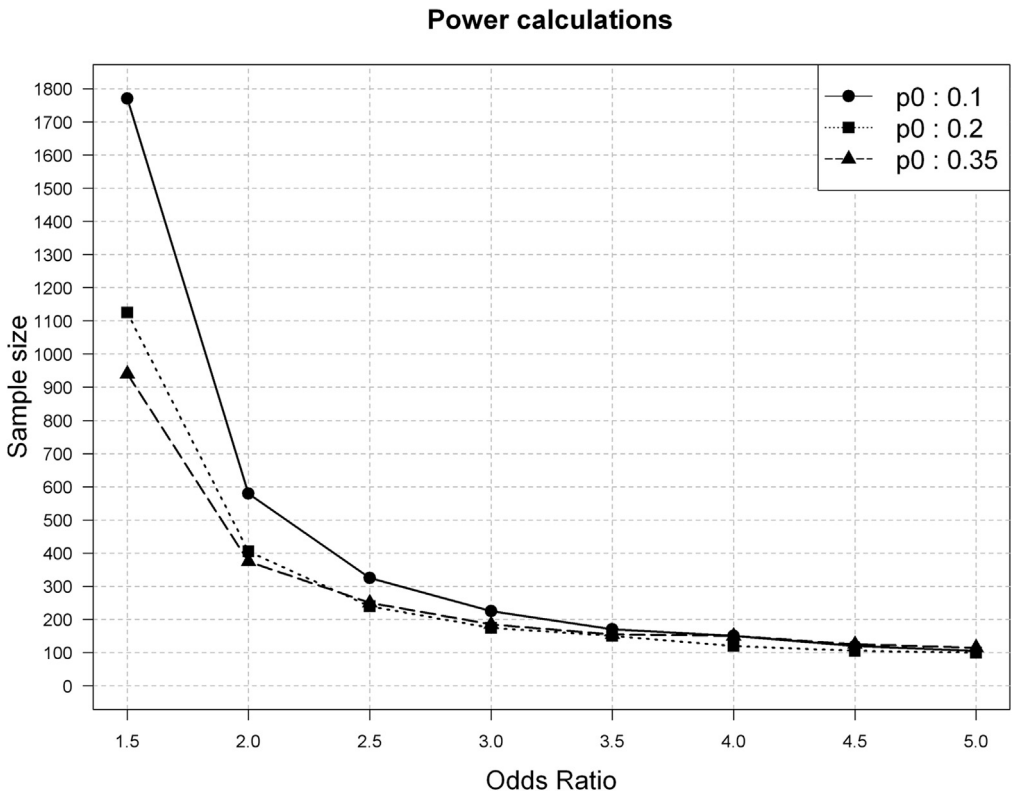

Figure 1. Sample size of case–control study to detect associations with different effect sizes and with type 1 error rate of 5%. p0 = frequency of the predisposing allele.

performed 2 measurements in each eye at 9 AM. The first tonometry measurement was performed before starting the treatment; after a minimum of 4 weeks, a second tonometry measurement was performed to evaluate the latanoprost response. After identifying the type of response, we obtained genomic DNA from a peripheral venous blood sample (6 ml) that was stored at 4°C until DNA was extracted. After being extracted, DNA was stored in Eppendorf tubes at −20° C, and DNA quantification was measured by spectrophotometry using the BioPhotometer (Eppendorf, Inc., Hamburg, Germany) starting with a 1.6 (260 nm/280 nm) absorbance quotient. DNA was sent to study in a 96-well plate (reference PN 4306737; Applied Biosystems–Thermo Fisher Scientific Corp., Foster City, CA). All DNA samples had a concentration of at least 75 ng/µl (range, 75.4–422 ng/µl). Genotyping was performed at the Santiago Node of the Spanish National Genotyping Center (CeGen-ISCIII) using the I-PLEX MassARRAY (increased plexing efficiency and flexibility for mass array) in the SEQUENOM platform (SEQUENOM, San Diego, CA).[13]

Statistical Analyses

Analyses were conducted using R software[14] (R Foundation for Statistical Computing, Vienna, Austria) including allelic[15] (Forner K, R-package version 0.1), genetics[16] (Warnes G, R-package version 1.3.7), and haplo.stats[17] (Sinnwell JP, Schaid DJ, R-package version 1.5.2) packages.

Statistical Power Analysis and Sample Size Calculation. Power for case–control association design was evaluated depending on several factors: the frequency of the predisposing allele, genotype, or haplotype; the accepted false-positive or type I error rate ($\alpha = 0.05$); the described prevalence of nonresponse to latanoprost in the studied population (5%); and the odds ratio (OR) or effect size. A sample size of 120 subjects would have 80% power to detect effect sizes of 4 with a frequency of the predisposing allele set at 20% and a 5% type 1 error rate (Fig 1). In the same scenario, setting the significance level at 10%, a detectable OR would be close to 3.

Preliminary Analysis. Hardy-Weinberg equilibrium was evaluated using the Pearson goodness-of-fit test or Fisher exact test when there was a low genotype count. Differential missing genotype data between cases and controls were investigated by testing the association between case–control status and a new variable, that is, 1 for all observed genotypes and 0 for missing genotypes. Allelic and genotyping frequencies and their 95% confidence intervals (CIs) were estimated and compared with allelic and genotyping frequencies for a European population. These frequencies were obtained using the package snpMatrix[18] (Clayton D, Leung HT, R-package version 1.14.6) according to the HapMap AFD_EUR_PANEL (48 samples).

Individual Single Nucleotide Polymorphism Analysis. The null hypothesis of no association between SNP genotypes and case–control status was checked using the Pearson chi-square and Fisher exact tests. In addition, 5 inheritance models were defined: the codominant model, in which every genotype gives a diverse and nonadditive risk; the dominant model, in which a single copy of a variant allele is sufficient to alter the risk; the recessive model, in which risk modification requires 2 copies of a variant allele; the overdominant model, in which the heterozygous alleles are compared with a pool of both homozygous alleles; and the additive

Table 3. Clinical Features and Differences between Groups

	Total Sample			Responders			Nonresponders			
	No.	% (95% Confidence Interval)	Mean ± Standard Deviation	No.	% (95% Confidence Interval)	Mean ± Standard Deviation	No.	% (95% Confidence Interval)	Mean ± Standard Deviation	P Value*
Age	117	—	66.44 ± 12.83	98	—	66.71 ± 12.92	19	—	65 ± 12.63	0.5941
Gender										0.8284
Male	55	47.01 (37.8–56.42)	—	47	85.45 (76.14–94.77)	—	8	14.55 (5.77–23.32)	—	
Female	62	52.99 (43.58–62.21)	—	51	82.26 (72.16–92.35)	—	11	17.74 (5.23–27.25)	—	
Diabetes	21	17.95 (11.7–26.36)	—	16	76.19 (67.67–84.71)	—	5	23.81 (5.59–42.03)	—	0.3304
Hypertension	44	37.61 (28.96–47.08)	—	34	77.27 (67.66–86.89)	—	10	22.73 (10.34–35.11)	—	0.223
Migraine	1	0.86 (0.1–5.37)	—	1	100	—	0	0	—	—
Basal IOP†	117	—	25.57 ± 4.15	98	—	26.19 ± 4.05	19	—	22.37 ± 3.14	**0.0001**
IOP after treatment?†	117	—	18 ± 3.38	98	—	17.12 ± 2.78	19	—	22.5 ± 2.58	**<0.0001**
Treatment time (mos)	117	—	17.14 ± 24.55	98	—	19.3 ± 26.08	19	—	6.1 ± 7.82	**0.0001**

IOP = intraocular pressure.
Boldface values indicate statistical significance.
*Associated with chi-square test for qualitative data and with *t* test for quantitative variables.
†Mean between both eyes.

model, in which each copy of a variant allele alters the risk in an additive form. Model selection was based on the Akaike information criterion. Allele-based association tests also were performed. Allele frequencies were compared using the unbiased and exact test proposed by Guedj et al.[19]

Multiple Single Nucleotide Polymorphism Analysis. Haplotypic frequencies were estimated using the expectation-maximization algorithm.[20] To evaluate the association between haplotypes and latanoprost response, the score test[21] was used and also was applied to all subhaplotypes from each gene (contiguous subsets of alleles, from 2 to the number of SNPs genotyped in this gene).

Multiple Testing. To solve the multiple testing problem, we used the false discovery rate (FDR) approach.[22] A global *P* value for each gene was calculated, considering single and multiple analyses. The method proposed by Rosenberg et al[23] for genetic case–control association studies was used. This is a 2-stage method. For the first stage, most significant single SNP and multiple SNP association tests within 1 gene are considered and adjusted for multiple comparisons. Then, an omnibus test is constructed in which distribution is computed from permutations, shuffling case and control labels. In the second stage, summary gene *P* values are adjusted for multiplicity using *Q* values.[24]

Results

Over 13 months, we selected 124 glaucomatous patients treated with latanoprost monotherapy. Seven DNA samples were discarded because 2 patients were close relatives and the other 5 patients had processing defects during genotyping. A total of 117 DNA samples were used for the study: 98 (83.8%) were responders, among whom 8 (7.7%) were hyperresponders, and 19 (16.2%) were nonresponders. In the responder group, the basal IOP was 25.6±4.1 mmHg (mean ± standard deviation), which decreased to 18.0±3.3 mmHg. The nonresponders had a basal IOP of 22.3±3.1 mmHg, which was 22.5±2.5 mmHg after treatment. All patients were treated with latanoprost for at least 4 weeks. Nonresponders received treatment for 6.1±7.8 months. Age, gender, and systemic diseases such as hypertension, diabetes, and migraine were not related to the response to latanoprost. Table 3 shows the distribution of clinical characteristics.

All 71 SNPs were verified to be in Hardy-Weinberg equilibrium using the FDR method, and only SNP rs7545762 (*PTGFR* gene) showed an inconsistent distribution in the nonresponder group; however, it had an adjusted *P* value near 1.0 for the multiple comparison test using the FDR method. In the allelic association studies, only SNPs within the *PTGFR* gene were significant; the percentages for responders and nonresponders with the frequency in the Caucasian European (CEU) population of the allele studied are shown in Table 4 (available at www.aaojournal.org).

Seven single SNP significant associations were observed in the *PTGFR* gene (Table 4, available at www.aaojournal.org). In the allelic association analysis, depicted in Table 5 (available at www.aaojournal.org), 5 SNPs also were significant: rs6686438 and rs1328441, in an additive inheritance model in which the minor allele increases the possibility of a positive response to latanoprost (OR, 0.2163; 95% CI, 0.0487–0.6363; and OR, 0.3199; 95% CI, 0.14–0.6779; respectively); rs10782665, under a dominant inheritance model for frequent variant increases 3 times the possibility of a positive response (OR, 0.3032; 95% CI, 0.1085–0.7161); rs6672484, under a dominant inheritance model, C→T increases the risk of a nonresponse to latanoprost (OR, 2.4479; 95% CI, 1.1891–5.0247); and rs11578155, in an

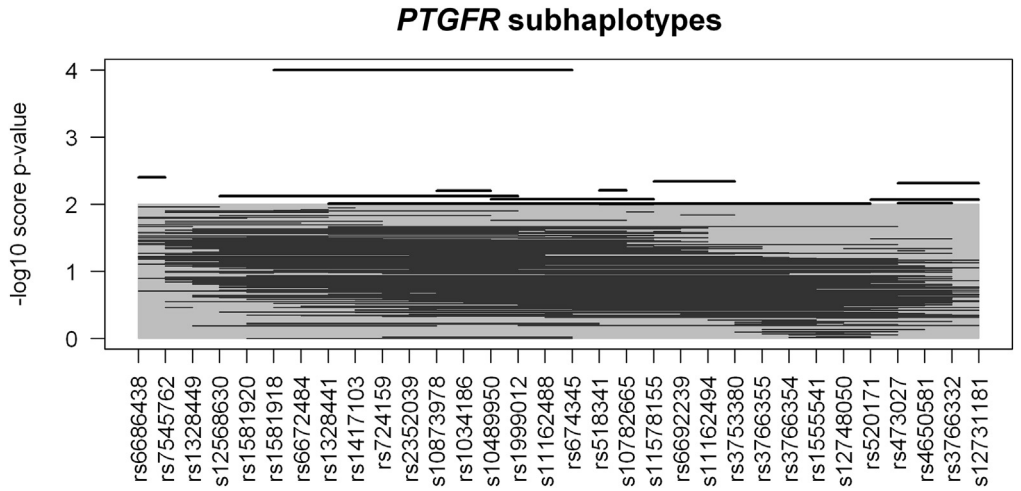

Figure 2. Association of subhaplotype blocks within the *PTGFR* gene. Each line represents 1 haplotypic block made up of the markers indicated on the *x*-axis. The $-\log_{10}$ (*P* value) for each association is indicated on the *y*-axis. Associations above the grey area are statistically significant at 0.01 significance level.

overdominant model where the possibility of a nonresponse to latanoprost is increased 3 times (OR, 2.9119; 95% CI, 1.0173−7.6915). Meanwhile, rs10489950 and rs3753380, under overdominant and dominant models, respectively, showed an allelic association close to the margin of statistical significance ($P = 0.0534$ and $P = 0.1505$, respectively). No other gene showed single SNP significant associations with response status.

After multiple comparison testing, subhaplotype analysis was positive only for the *PTGFR* and *MMP-1* genes; the other genes coding MMP-2, -3, -9, and -17 did not affect a response or lack of response. The *PTGFR* gene has 11 subhaplotypes related to a response or nonresponse (Fig 2); those in the uppermost portion of the graph were most significantly involved in the response to latanoprost. The *MMP-1* gene has 6 subhaplotypes associated, at a 0.01 significance level, with no response to latanoprost (Fig 3) and were located above the shaded areas. Tables 6 and 7 show estimated haplotypes statistically significant for *PTGFR* and *MMP-1* genes, respectively.

The 6 candidate genes studied were analyzed using a multiple testing strategy. With the FDR at the 5% level, we found only the *PTGFR* gene was associated significantly ($P_{OMNI} < 0.0001$) with a latanoprost response. When the FDR was at the 10% level, the *PTGFR* and *MMP-1* genes were associated significantly ($P_{OMNI} < 0.041$, corrected Q value of 0.093) with the response. The results are shown in Table 8.

Discussion

The first hurdle in this study was the lack of a standard definition of a nonresponder to latanoprost therapy. Some authors have defined no response as a reduction of the basal IOP by less than 10%[8,25−27]; others have defined no response as an IOP decrease of less than 15%.[10,11,28−30] There is also disparity in the rates of nonresponders,

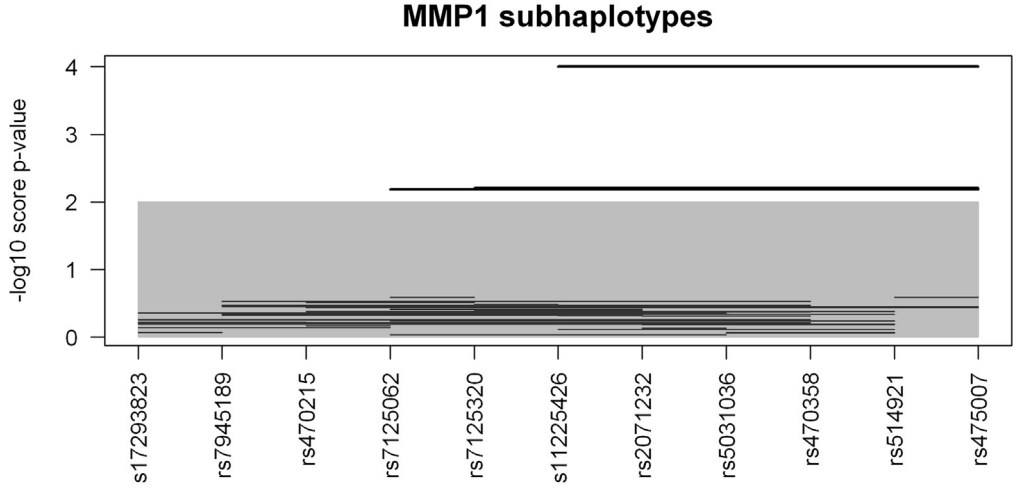

Figure 3. Association of subhaplotype blocks within the *MMP-1* gene. Each line represents 1 haplotypic block made up of the markers indicated on the *x*-axis. The $-\log_{10}$ (*P* value) for each association is showed on the *y*-axis. Subhaplotypes in the uppermost part of the graph show a stronger correlation with nonresponse.

Table 6. Frequencies of Haplotype Blocks within the *PTGFR* Gene Significantly Associated with Response or Nonresponse

Single Nucleotide Polymorphisms																															Haplotypic Frequencies (%)		Score Test P Value
rs6686438	*rs7545762*	*rs1328449*	*rs12568630*	*rs1581920*	*rs1581918*	*rs6672484*	*rs1328441*	*rs1417103*	*rs724159*	*rs2352039*	*rs10873978*	*rs1034186*	*rs10489950*	*rs1999012*	*rs11162488*	*rs674345*	*rs518341*	*rs10782665*	*rs11578155*	*rs6692239*	*rs11162494*	*rs3753380*	*rs3766355*	*rs3766354*	*rs1555541*	*rs12748050*	*rs520171*	*rs473027*	*rs4650581*	*rs3766332*	*rs12731181*	*Responders*	*Nonresponders*	
G	A																														32.68	60.22	0.0029
T	A																														29.57	5.57	0.0057
																	T	G													53.96	81.58	0.0015
																	T	T													38.78	15.79	0.0083
											C	T	C																		54.54	71.05	0.0411
											T	T	C																		37.76	18.42	0.0171
											T	T	T																		1.53	7.89	0.0213
																											G	T	T		8.81	34.03	0.001
																			G	C	T	G									7.22	18.42	0.0281
																											G	T	T	A	7.67	26.71	0.0027
																											G	T	T	G	0.64	5.48	0.0354
																										A	G	T	T	A	1.49	7.3	0.0277
																										C	G	T	T	A	6.2	19.61	0.0036
																										C	G	T	T	G	0.64	5.46	0.0356
													C	C	A	A	T	G	G												7.27	18.42	0.0293
													C	C	C	G	T	T	A												26.53	10.53	0.0399
													T	C	A	A	T	G	A												1.35	7.89	0.0213
						A	C	A	T	T	T	C	T	C	C	A	G															1.86	8.25	0.0357
			A	C	A	C	A	T	T	T	C	T	C	C																		1.79	10.53	0.0068
							A	T	T	G	C	T	C	C	A	A	T	G	G	C	T	G	G	C	A	C	C				6.12	18.42	0.0122
							A	T	T	G	C	T	C	G	A	G	T	G	A	T	A	G	G	C	G	C	C				1.02	7.89	0.0005
G	A	T	T	C	G	T	A	T	T	G	C	T	C	C	A	G	T	G	A	T	A	G	G	C	G	C	C	G	T	T	A	1.53	15.79	0.0244

which can vary according to the population studied. Rossetti et al[11] reported a low rate of nonresponders (4.1%) in a CEU Italian population; in a Spanish CEU population, Martinez Garcia and Pérez Garcia[30] identified an 11% rate of nonresponders. In Asiatic populations, Aung et al[28] reported a 5.4% rate of nonresponders in a Southeast Asian population after 4 weeks of treatment. Ikeda et al[26] found 31.8% of nonresponders in a Japanese population after 12 months of treatment with latanoprost. In other studies that compared latanoprost with other prostaglandin analogs, in studies of CEU-descendent American populations, the LSG[10] described a 20% rate of nonresponders and Scherer[31] found a 25% rate of nonresponders. In similar populations, the Travoprost Study Group[12] and the Bimatoprost/Latanoprost[29] Study Group found 13.5% and 51.5% of patients, respectively, to be nonresponders to latanoprost. In the current study, we used the same criteria as the LSG to define a nonresponder; we faced difficulties finding this type of patient because of the low percentage, with only 19 subjects among the large population of glaucomatous patients visiting the 5 referral centers involved in this study, which agrees with the data reported by Rossetti et al.[11] However, our final sample size was similar to that of the other studies. Another limitation is the relatively short follow-up period for assessment of latanoprost response, because there may be late nonresponders and late responders, as described by the LSG.[10] Moreover, the definition of 15% response rate is also exactly 50% of the average 30% basal IOP reduction and therefore is somewhat arbitrary, and so may have its weakness. Gender, age, and presence of systemic diseases such as diabetes and hypertension were unrelated to the latanoprost response and agreed with the results of other studies.[8,26]

Intraocular pressure fluctuations occur during the day,[32] and we recognize that this may be a limitation of every study of IOP reduction, including the current study. Ideally, a tension curve may have reduced this study bias, but it was unfeasible because of the timetables of the centers where the population was studied. However, experienced ophthalmologists measured the IOP a minimum of twice before and after treatment.

Genotypic association showed the SNPs rs6672484 (OR, 4.91), rs10489950 (OR, 5.94), and rs11578155 (OR, 4.13) had an increased likelihood of being related to a nonresponse to latanoprost therapy. We found that rs10782665 (OR, 0.22), rs6686438 (OR, 0.2), and rs1328441(OR, 0.33) protected against being a nonresponder and increased the likelihood of a positive response to latanoprost. rs3753380 Was within the limit of statistical significance when related to no response, although in a study performed in a healthy Japanese (JPN) population, it was associated with no response.[8]

Subhaplotype analysis of the *PTGFR* gene showed that 1 subhaplotype, rs11578155−rs3753380 (comprising 4 SNPs), was correlated strongly with haplotype GCTG in 18% of latanoprost nonresponders. This subhaplotype includes SNP rs3753380, which was described previously by Sakurai et al[8] as a risk factor for a poor response to latanoprost in a Japanese population. Subhaplotype

Table 7. Frequencies of Haplotype Blocks within the *MMP-1* Gene Significantly Associated with Response or Nonresponse

Single Nucleotide Polymorphisms											Haplotypic Frequencies (%)		Score Test
rs17293823	*rs7945189*	*rs470215*	*rs7125062*	*rs7125320*	*rs11225426*	*rs2071232*	*rs5031036*	*rs470358*	*rs514921*	*rs475007*	*Responders*	*Nonresponders*	**P Value**
						C	A	T	A	A	1.12	18.42	0.0015
					C	C	A	T	A	A	1.34	18.42	0.0022
				T	C	C	A	T	A	A	1.9	18.42	0.0031
			C	T	C	C	A	T	A	A	1.91	18.42	0.0031
G	C	A	C	T	C	C	A	T	A	A	1.91	18.42	0.0082

Table 8. Multiple Testing Using the Method Proposed by Rosenberg et al[23]

Gene	Single Nucleotide Polymorphism (n)	Individual Single Nucleotide Polymorphism–Associated Tests		Multiple Single Nucleotide Polymorphism–Association Tests		Omnibus Test		Q Value
		Minimum P *Value**	*Adjusted* P *Value*	*Minimum* P *Value**	*Adjusted* P *Value*	*Minimum* P *Value**	*Adjusted* P *Value*	
PTGFR	**32**	**0.0029**	**0.023**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
MMP-1	11	*0.0614*	0.711	**<0.0001**	**0.036**	**0.036**	**0.041**	*0.093*
MMP-3	4	0.2290	0.830	0.3897	0.616	0.616	0.776	0.952
MMP-17	10	*0.0944*	0.828	0.341	0.944	0.828	0.937	0.952
MMP-2	7	0.1359	0.796	0.1627	0.564	0.564	0.677	0.952
MMP-9	4	0.3366	0.928	0.6662	0.869	0.869	0.952	0.952

Boldface values indicate statistical significance at 0.05. Italicized values indicate *P* values less than 0.1.
*Minimum *P* value obtained.

rs11225426–rs4750507 of the *MMP-1* gene (comprising 6 SNPs) associated haplotype CCATAA with 18% of latanoprost nonresponders in the current sample.

McCarty et al[33] could not replicate in their study the findings of Sakurai et al[8] and reported no association between SNPs rs3753380 and rs3766355 of the *PTGFR* gene and prostaglandin analogs in an American population of European descendent, although that study did not describe which of the available prostaglandin analogs were studied.

According to the multiple comparisons analysis, we found a strong correlation between the *PTGFR* gene and a latanoprost response (FDR, 5%). To our knowledge, we are describing for the first time a relationship between the *MMP-1* gene and a latanoprost response, although this relationship is of borderline significance because of the relatively small sample size in our study.

This study had some limitations such as the small sample size and the assumption of proper treatment compliance by all patients; however, it has been reported that up to 30% of glaucomatous patients are noncompliant with treatment[34,35] and that 64% do not show proper adherence.[36] Electronic devices to assess compliance, such as the Travalert[37] dosing aid (Alcon Research, Fort Worth, TX), are unavailable for latanoprost. Some patients were classified as responders or nonresponders based on retrospective data collection, but in all cases, the drug was administered as initial monotherapy and IOP measurements were stated in the study protocol. The fact that the study was carried out at multiple sites increases the possibility of phenotyping errors when diagnosing glaucomatous damage and IOP measurements; however, because only experienced ophthalmologists participated, this tended to reduce this possibility. Wide fluctuations in IOP are recognized,[32] and the impossibility of observing all patients with a tension curve may have limited the current results. In addition, the central corneal thickness may affect the accuracy of the IOP measurement. However, the response to latanoprost was assessed by an absolute IOP reduction. Furthermore, this study included a uniform CEU glaucomatous population, with a minimal treatment time of 4 weeks according to the same LSG definitions of nonresponders to latanoprost, and all patients received latanoprost and not generic formulations to eliminate doubt about no response resulting from an improper concentration of the active pharmacologic agent.

Polymorphisms of the *PTGFR* and *MMP-1* genes may affect the response to latanoprost treatment in a CEU Spanish population. A better understanding of the mechanisms involved in the pharmacologic response to latanoprost may help to develop diagnostic tests to detect individuals carrying SNPs that may affect the response to latanoprost and facilitate personalized therapy. The nonresponder rates and SNP associations for the other prostaglandin analogs are not known, but it is possible that similar trends may be observed. Future studies with larger populations should include all prostaglandin analogs available on the market.

In conclusion, we identified 3 SNPs of the *PTGFR* gene that are related to refractoriness to latanoprost (rs3753380, rs6672484, and rs11578155) and 2 SNPs of the same gene associated with a positive response (rs6686438 and rs10786455). However, as with any genetic association, these results must be replicated independently in a study with the same design. Our results further indicated that SNPs of the *MMP-1* gene also may affect the response to latanoprost, although because of the current sample size, this association remains within the limits of significance, and further studies must be carried out to confirm this finding.

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References

1. Lindsey JD, Kashiwagi K, Kashiwagi F, Weinreb RN. Prostaglandins alter extracellular matrix adjacent to human ciliary muscle cells in vitro. Invest Ophthalmol Vis Sci 1997;38:2214–23.
2. Weinreb RN, Kashiwagi K, Kashiwagi F, et al. Prostaglandins increase matrix metalloproteinase release from human ciliary smooth muscle cells. Invest Ophthalmol Vis Sci 1997;38:2772–80.
3. Anthony TL, Lindsey JD, Aihara M, Weinreb RN. Detection of prostaglandin EP1, EP2 y FP receptor in human sclera. Invest Ophthalmol Vis Sci 2001;42:3182–6.
4. Gaton DD, Sagara T, Lindsey JD, et al. Increased matrix metalloproteinases 1, 2, and 3 in the monkey uveoscleral outflow pathway after topical prostaglandin F(2 alpha)-isopropyl ester treatment. Arch Ophthalmol 2001;119:1165–70.
5. Weinreb RN, Lindsey JD. Metalloproteinase gene transcription in human ciliary muscle cells with latanoprost. Invest Ophthalmol Vis Sci 2002;43:716–22.
6. Zhou SF, Di YM, Chan E. Clinical pharmacogenetics and potential application in personalized medicine. Curr Drug Metab 2008;9:738–84.
7. Schwartz SG, Puckett BJ, Allen RC, et al. Beta1-adrenergic receptor polymorphisms and clinical efficacy of betaxolol hydrochloride in normal volunteers. Ophthalmology 2005;112:2131–6.
8. Sakurai M, Higashide T, Takahashi M, Sugiyama K. Association between genetic polymorphisms of the prostaglandin F2alpha receptor gene and response to latanoprost. Ophthalmology 2007;114:1039–45.
9. Sakurai M, Higashide T, Ohkubo S, et al. Association between genetic polymorphisms of the prostaglandin F2α receptor gene, and response to latanoprost in patients with glaucoma and ocular hypertension. Br J Ophthalmol 2014;98:469–73.
10. Camras CB, Hedman K, US Latanoprost Study Group. Rate of response to latanoprost or timolol in patients with ocular hypertension or glaucoma. J Glaucoma 2003;12:466–9.
11. Rossetti L, Gandolfi S, Traverso C, et al. An evaluation of the rate of nonresponders to latanoprost therapy. J Glaucoma 2006;15:238–43.
12. Netland PA, Landry T, Sullivan EK, et al; Travoprost Study Group. Travoprost compared with latanoprost and timolol in patients with open-angle glaucoma or ocular hypertension. Am J Ophthalmol 2001;132:472–84.
13. Oeth P, Beaulieu M, Park C, et al. iPLEX™ Assay: increased plexing efficiency and flexibility for MassARRAY system through single base primer extension with mass-modified terminators. Application note 2005. Available at: http://www.sequenom.com/Assets/pdfs/appnotes. Accessed December 2012.

14. R Core Team. R: a language and environment for statistical computing 2012. Vienna, Austria: R Foundation for Statistical Computing, 2012. Available at: http://www.R-project.org. Accessed December 2012.
15. Forner K. Allelic: a fast, unbiased and exact allelic exact test. R package version 0.1. 2006. Available at: http://CRAN.R-project.org/package=allelic. Accessed December 2012.
16. Warnes G, with Gregor G, Leisch F, Man M. Genetics 2012: Population Genetics. R-package version 1.3.7. Available at: http://CRAN.R-project.org/package=genetics. Accessed December 2012.
17. Sinnwell JP, Schaid DJ. haplo.stats: Statistical analysis of haplotypes with traits and covariates when linkage phase is ambiguous. R package version 1.5.2., 2012. Available at: http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm. Accessed December 2012.
18. Clayton D, Leung HT. snpMatrix: the snp.matrix and X.snp.matrix classes. R-package version 1.14.6. 2011. Available at: http://wwwgene.cimr.cam.ac.uk/clayton/software/. Accessed: December 2012.
19. Guedj M, Wojcik J, Della-Chiesa E, et al. A fast, unbiased and exact allelic test for case-control association studies. Hum Hered 2006;61:210–21.
20. Fallin D, Schork NJ. Accuracy of haplotype frequency estimation for biallelic loci via the expectation-maximization algorithm for unphased diploid genotype data. Am J Hum Genet 2000;67:947–59.
21. Schaid DJ, Rowland CM, Tines DE, et al. Score test for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002;70:425–34.
22. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B Methodol 1995;57:289–300.
23. Rosenberg PS, Che A, Chen BE. Multiple hypothesis testing strategies for genetic case-control association studies. Statist Med 2006;25:3134–49.
24. Storey JD. The positive false discovery rate: a Bayesian interpretation and the q value. Ann Statist 2003;31:2013–35.
25. Susanna R Jr, Giampani J Jr, Borges AS, et al. A double-masked, randomized clinical trial comparing latanoprost with unoprostone in patients with open-angle glaucoma or ocular hypertension. Ophthalmology 2001;108:259–63.
26. Ikeda Y, Mori K, Ishibashi T, et al. Latanoprost nonresponders with open-angle glaucoma in the Japanese population. Jpn J Ophthalmol 2006;50:153–7.
27. Gandolfi SA, Cimino L. Effect of bimatoprost on patients with primary open-angle glaucoma or ocular hypertension who are nonresponders to latanoprost. Ophthalmology 2003;110:609–14.
28. Aung T, Chew PT, Yip CC, et al. A randomized double-masked crossover study comparing latanoprost 0.005% with unoprostone 0.12% in patients with primary open-angle glaucoma and ocular hypertension. Am J Ophthalmol 2001;131:636–42.
29. Choplin N, Bernstein P, Batoosingh AL, Whitcup SM, Bimatoprost/Latanoprost Study Group. A randomized, investigator-masked comparison of diurnal responder rates with bimatoprost and latanoprost in the lowering of intraocular pressure. Surv Ophthalmol 2004;49(Suppl 1):S19–25.
30. Martínez García A, Pérez García R. Efficacy of latanoprost monotherapy treatment in primary open-angle glaucoma. Arch Soc Esp Oftalmol 2002;77:269–74.
31. Scherer WJ. A retrospective review of non-responders to latanoprost. J Ocul Pharmacol Ther 2002;18:287–91.
32. Jonas JB, Budde WM, Stroux A, et al. Circadian intraocular pressure profiles in chronic open angle glaucomas. J Ophthalmic Vis Res 2010;5:92–100.
33. McCarty CA, Berg R, Patchett R, et al. Lack of association between polymorphisms in the prostaglandin F2α receptor and solute carrier organic anion transporter family 2A1 genes and intraocular pressure response to prostaglandin analogs. Ophthalmic Genet 2012;33:74–6.
34. Kholdebarin R, Campbell RJ, Jin YP, Buys YM. Multicenter study of compliance and drop administration in glaucoma. Can J Ophthalmol 2008;43:454–61.
35. Schwartz GF, Quigley HA. Adherence and persistence with glaucoma therapy. Surv Ophthalmol 2008;53(Suppl 1):S57–68.
36. Dreer L, Girkin C, Mansberger SL. Determinants of medication adherence to topical glaucoma therapy. J Glaucoma 2012;21:234–40.
37. Sánchez-Pulgarín M, Martínez-de-la-Casa JM, Escalada A, et al. The influence of the Travalert® dosing aid on medical treatment compliance and the quality of life of glaucoma patients. Arch Soc Esp Oftalmol 2011;86:282–6.

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Abbreviations and Acronyms:
CEU = Caucasian European; **CI** = confidence interval; **FDR** = false discovery rate; **IOP** = intraocular pressure; **LSG** = Latanoprost Study Group; **MMP** = metalloproteinase; **OR** = odds ratio; ***PTGFR*** = prostaglandin F2α receptor gene; **SNP** = single nucleotide polymorphism.

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Table 4. Genotype Frequencies and Associations for the *PTGFR* Gene

			Genotype Frequencies				Inheritance Model*				
			Responders		*Nonresponders*				95% CI		
SNP	**Genotype**	**CEU (%)**	*n*	*% (95% CI)*	*n*	*% (95% CI)*	*Selected model*	*OR*	*Lower*	*Upper*	*P-value*
rs6686438	GG	54.44	48	49.48 (39.53–59.43)	16	84.21 (67.81–100)	**Additive**	**0.2**	**0.06**	**0.7**	**0.0195**
G → T	GT	36.67	41	42.27 (32.44–52.1)	3	15.79 (0–32.19)					
	TT	8.89	8	8.25 (2.77–13.72)	0	0					
rs7545762	AA	37.80	38	38.78 (29.13–48.42)	6	31.58 (10.68–52.48)	Additive	0.85	0.4	1.81	0.1112
A → T	AT	46.95	46	46.94 (37.06–56.82)	13	68.42 (47.52–89.32)					
	TT	15.24	14	14.29 (7.36–21.21)	0	0					
rs1328449	CC	1.27	0	0	0	0	Overdominant	0.26	0.03	2.12	0.1378
T → C	CT	12.66	17	17.35 (9.85–24.84)	1	5.26 (0–15.3)					
	TT	86.08	81	82.65 (75.16–90.15)	18	94.74 (84.7–100)					
rs12568630	AA	0	0	0	0	0	*Overdominant*	*3.11*	*0.92*	*10.45*	*0.0801*
T → A	AT	10.11	10	10.31 (4.26–16.36)	5	26.32 (6.52–46.12)					
	TT	89.89	87	89.69 (83.64–95.74)	14	73.68 (53.88–93.48)					
rs1581920	CC	61.11	62	63.92 (54.36–73.47)	16	84.21 (67.81–100)	Overdominant	0.42	0.11	1.55	0.1606
C → T	CT	36.67	30	30.93 (21.73–40.13)	3	15.79 (0–32.19)					
	TT	2.22	5	5.15 (0.75–9.55)	0	0					
rs1581918	AA	3.33	5	5.1 (0.75–9.46)	1	5.26 (0–15.3)	Codominant	1.26	0.13	11.93	0.6170
G → A	AG	27.78	30	30.61 (21.49–39.74)	8	42.11 (19.9–64.31)		1.68	0.6	4.69	
	GG	68.89	63	64.29 (54.8–73.77)	10	52.63 (30.18–75.08)		1			
rs6672484	CC	54.44	55	56.7 (46.84–66.56)	4	21.05 (2.72–39.38)	**Dominant**	**4.91**	**1.52**	**15.88**	**0.0036**
C → T	CT	34.44	32	32.99 (23.63–42.35)	12	63.16 (41.47–84.85)					
	TT	11.11	10	10.31 (4.26–16.36)	3	15.79 (0–32.19)					
rs1328441	AA	32.95	23	23.71 (15.25–32.18)	10	52.63 (30.18–75.08)	**Additive**	**0.33**	**0.15**	**0.73**	**0.0029**
G → A	AG	45.45	45	46.39 (36.47–56.32)	8	42.11 (19.9–64.31)					
	GG	21.59	29	29.9 (20.79–39.01)	1	5.26 (0–15.3)					
rs1417103	CC	0	1	1.02 (0–3.01)	0	0	-	-	-	-	-
T → C	CT	12.73	12	12.24 (5.75–18.74)	0	0					
	TT	87.27	85	86.73 (80.02–93.45)	19	100					
rs724159	CC	10.91	10	10.31 (4.26–16.36)	0	0	Overdominant	0.73	0.27	2.02	0.5443
T → C	CT	40.61	43	44.33 (34.44–54.22)	7	36.84 (15.15–58.53)					
	TT	48.48	44	45.36 (35.45–55.27)	12	63.16 (41.47–84.85)					
rs2352039	GG	74.39	65	66.33 (56.97–75.68)	12	63.16 (41.47–84.85)	Overdominant	1.18	0.43	3.3	0.7247
G → T	GT	24.39	32	32.65 (23.37–41.94)	7	36.84 (15.15–58.53)					
	TT	1.22	1	1.02 (0–3.01)	0	0					
rs10873978	CC	40.00	34	34.69 (25.27–44.12)	11	57.89 (35.69–80.1)	*Dominant*	*0.39*	*0.14*	*1.05*	*0.0606*
C → T	CT	48.48	51	52.04 (42.15–61.93)	6	31.58 (10.68–52.48)					
	TT	11.52	13	13.27 (6.55–19.98)	2	10.53 (0–24.33)					
rs1034186	AA	0	0	0	0	0	Additive	0.39	0.05	3.22	0.3299
T → A	AT	12.36	12	12.37 (5.82–18.92)	1	5.26 (0–15.3)					
	TT	87.64	85	87.63 (81.08–94.18)	18	94.74 (84.7–100)					
rs10489950	CC	86.67	95	96.94 (93.53–100)	16	84.21 (67.81–100)	**Overdominant**	**5.94**	**1.1**	**32.04**	**0.0474**
C → T	CT	13.33	3	3.06 (0–6.47)	3	15.79 (0–32.19)					
	TT	0	0	0	0	0					
rs1999012	CC	73.03	60	61.86 (52.19–71.52)	14	73.68 (53.88–93.48)	Additive	0.85	0.36	2.03	0.7098
C → G	CG	26.97	33	34.02 (24.59–43.45)	3	15.79 (0–32.19)					
	GG	0.00	4	4.12 (0.17–8.08)	2	10.53 (0–24.33)					
rs11162488	AA	48.89	54	55.1 (45.25–64.95)	14	73.68 (53.88–93.48)	Overdominant	0.62	0.2	1.8	0.3747
A → C	AC	40.00	36	36.73 (27.19–46.28)	5	26.32 (6.52–46.12)					
	CC	11.11	8	8.16 (2.74–13.58)	0	0					
rs674345	AA	10.91	14	14.43 (7.44–21.43)	4	21.05 (2.72–39.38)	Codominant	1.67	0.43	6.59	0.7656
G → A	AG	47.88	42	43.3 (33.44–53.16)	8	42.11 (19.9–64.31)		1.12	0.37	3.36	
	GG	41.21	41	42.27 (32.44–52.1)	7	36.84 (15.15–58.53)		1			
rs518341	CC	1.11	0	.	0	0	Additive	0.33	0.04	2.67	0.2319
T → C	CT	20.00	14	14.43 (7.44–21.43)	1	5.26 (0–15.3)					
	TT	78.89	83	85.57 (78.57–92.56)	18	94.74 (84.7–100)					
rs10782665	GG	37.20	37	37.76 (28.16–47.35)	14	73.68 (53.88–93.48)	**Dominant**	**0.22**	**0.07**	**0.65**	**0.0037**
G → T	GT	46.34	46	46.94 (37.06–56.82)	4	21.05 (2.72–39.38)					
	TT	16.46	15	15.31 (8.18–22.43)	1	5.26 (0–15.3)					
rs11578155	AA	86.67	84	86.6 (79.82–93.38)	12	63.16 (41.47–84.85)	**Overdominant**	**4.13**	**1.36**	**12.55**	**0.0157**
A → G	AG	13.33	12	12.37 (5.82–18.92)	7	36.84 (15.15–58.53)					
	GG	0	1	1.03 (0–3.04)	0	0					

(*Continued*)

Table 4. (Continued.)

			Genotype Frequencies				Inheritance Model*				
			Responders		*Nonresponders*				95% CI		
SNP	**Genotype**	**CEU (%)**	*n*	*% (95% CI)*	*n*	*% (95% CI)*	*Selected model*	*OR*	*Lower*	*Upper*	*P-value*
rs6692239	CC	38.18	35	36.08 (26.53–45.64)	4	21.05 (2.72–39.38)	Codominant	1			0.4138
C → T	CT	44.24	48	49.48 (39.53–59.43)	12	63.16 (41.47–84.85)		2.19	0.65	7.35	
	TT	17.58	14	14.43 (7.44–21.43)	3	15.79 (0–32.19)		1.87	0.37	9.48	
rs11162494	AA	55.15	56	57.73 (47.9–67.56)	8	42.11 (19.9–64.31)	Codominant	1			0.3945
A → T	AT	40.61	33	34.02 (24.59–43.45)	8	42.11 (19.9–64.31)		1.7	0.58	4.95	
	TT	4.24	8	8.25 (2.77–13.72)	3	15.79 (0–32.19)		2.62	0.57	12	
rs3753380	AA	11.59	12	12.37 (5.82–18.92)	2	10.53 (0–24.33)	**Dominant**	**0.34**	**0.11**	**1**	**0.0399**
G → A	AG	41.46	38	39.18 (29.46–48.89)	3	15.79 (0–32.19)					
	GG	46.95	47	48.45 (38.51–58.4)	14	73.68 (53.88–93.48)					
rs3766355	GG	79.17	69	70.41 (61.37–79.45)	14	73.68 (53.88–93.48)	Codominant	1			1
G → T	GT	20.83	26	26.53 (17.79–35.27)	5	26.32 (6.52–46.12)		0.95	0.31	2.89	
	TT	0	3	3.06 (0–6.47)	0	0		0	0	0	
rs3766354	CC	80.61	76	78.35 (70.15–86.55)	15	78.95 (60.62–97.28)	Additive	0.97	0.29	3.22	0.9538
C → T	CT	18.18	21	21.65 (13.45–29.85)	4	21.05 (2.72–39.38)					
	TT	1.21	0	0	0	0					
rs1555541	AA	42.94	41	42.27 (32.44–52.1)	6	31.58 (10.68–52.48)	Codominant	1			0.6262
A → G	AG	42.33	40	41.24 (31.44–51.03)	10	52.63 (30.18–75.08)		1.71	0.57	5.14	
	GG	14.72	16	16.49 (9.11–23.88)	3	15.79 (0–32.19)		1.28	0.29	5.75	
rs12748050	CC	66.67	72	73.47 (64.73–82.21)	13	68.42 (47.52–89.32)	Codominant	1			0.7423
C → T	CT	27.27	23	23.47 (15.08–31.86)	6	31.58 (10.68–52.48)		1.44	0.49	4.2	
	TT	6.06	3	3.06 (0–6.47)	0	0		0	0	0	
rs520171	AA	1.82	5	5.1 (0.75–9.46)	0	0	Additive	0.93	0.38	2.30	0.6444
C → A	AC	30.30	23	23.47 (15.08–31.86)	6	31.58 (10.68–52.48)					
	CC	67.88	70	71.43 (62.48–80.37)	13	68.42 (47.52–89.32)					
rs473027	AA	47.27	50	52.08 (42.09–62.08)	6	31.58 (10.68–52.48)	Reccesive	1.81	0.44	7.43	0.42681
A → G	AG	43.64	37	38.54 (28.81–48.28)	10	52.63 (30.18–75.08)					
	GG	9.09	9	9.38 (3.54–15.21)	3	15.79 (0–32.19)					
rs4650581	AA	8.99	5	5.43 (0.8–10.07)	0	0	Additive	1.10	0.47	2.6	0.4027
T → A	AT	40.45	21	22.83 (14.25–31.4)	7	36.84 (15.15–58.53)					
	TT	50.56	66	71.74 (62.54–80.94)	12	63.16 (41.47–84.85)					
rs3766332	AA	0.61	0	0	0	0	Additive	0.33	0.04	2.67	0.2319
T → A	AT	11.59	14	14.43 (7.44–21.43)	1	5.26 (0–15.3)					
	TT	87.80	83	85.57 (78.57–92.56)	18	94.74 (84.7–100)					
rs12731181	AA	67.88	73	74.49 (65.86–83.12)	11	57.89 (35.69–80.1)	Additive	1.41	0.62	3.21	0.127
A → G	AG	27.27	20	20.41 (12.43–28.39)	8	42.11 (19.9–64.31)					
	GG	4.85	5	5.1 (0.75–9.46)	0	0					

CEU = Caucasian European; CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism.
Bolded values are statistically significant. Italicized values are *P* values less than 0.1.
*Inheritance models: Additive, each copy of the rare variant modify the risk; dominant, a single copy of the frequent variant is enough to modify the risk; recessive, two copies of the variant allele are necessary to change the risk; overdominant, heterozygosity modifies the risk; codominant, every genotype gives a diverse and nonadditive risk; -, invalid model.

Table 5. Allelic Frequencies and Associations for the *PTGFR* Gene

			Allelic Frequencies				Associations			
			Responders		*Nonresponders*			95% CI		
SNP	**Allele**	**CEU (%)**	*n*	*% (95% CI)*	*n*	*% (95% CI)*	*OR*	*Lower*	*Upper*	*P-value*
rs6686438	G	73.1	137	70.62 (64.21–77.03)	35	92.11 (83.53–100)	**0.2163**	**0.0487**	**0.6363**	**0.0076**
G → T	T	26.9	57	29.38 (22.97–35.79)	3	7.89 (0–16.47)				
rs7545762	A	59.2	122	62.24 (55.46–69.03)	25	65.79 (50.71–80.87)	0.8621	0.4028	1.7711	0.7079
A → T	T	40.8	74	37.76 (30.97–44.54)	13	34.21 (19.13–49.29)				
rs1328449	C	7.3	17	8.67 (4.73–12.61)	1	2.63 (0–7.72)	3.0978	0.6038	76.2042	0.2993
T → C	T	92.7	179	91.33 (87.39–95.27)	37	97.37 (92.28–100)				
rs12568630	A	4.7	10	5.15 (2.04–8.27)	5	13.16 (2.41–23.91)	*0.3559*	*0.1163*	*1.2352*	*0.0702*
T → A	T	95.3	184	94.85 (91.73–97.96)	33	86.84 (76.09–97.59)				
rs1581920	C	82.3	154	79.38 (73.69–85.07)	35	92.11 (83.53–100)	*0.3457*	*0.0773*	*1.0325*	*0.0792*
C → T	T	17.7	40	20.62 (14.93–26.31)	3	7.89 (0–16.47)				
rs1581918	A	16.9	40	20.41 (14.77–26.05)	10	26.32 (12.31–40.32)	0.7134	0.3263	1.6674	0.5254
G → A	G	83.1	156	79.59 (73.95–85.23)	28	73.68 (59.68–87.69)				
rs6672484	C	73.8	142	73.2 (66.96–79.43)	20	52.63 (36.76–68.51)	**2.4479**	**1.1891**	**5.0247**	**0.0182**
C → T	T	26.2	52	26.8 (20.57–33.04)	18	47.37 (31.49–63.24)				
rs1328441	A	41.3	91	46.91 (39.88–53.93)	28	73.68 (59.68–87.69)	**0.3199**	**0.1400**	**0.6779**	**0.0037**
G → A	G	58.7	103	53.09 (46.07–60.12)	10	26.32 (12.31–40.32)				
rs1417103	C	6.5	14	7.14 (3.54–10.75)	0	0	—	—	—	0.1537
T → C	T	93.5	182	92.86 (89.25–96.46)	38	100				
rs724159	C	33.3	63	32.47 (25.88–39.06)	7	18.42 (6.1–30.75)	2.0911	0.9134	5.4654	0.1175
T → C	T	66.7	131	67.53 (60.94–74.12)	31	81.58 (69.25–93.9)				
rs2352039	G	81.8	162	82.65 (77.35–87.95)	31	81.58 (69.25–93.9)	1.0907	0.4083	2.5801	1
G → T	T	18.2	34	17.35 (12.05–22.65)	7	18.42 (6.1–30.75)				
rs10873978	C	63.3	119	60.71 (53.88–67.55)	28	73.68 (59.68–87.69)	0.5582	0.2439	1.1857	0.1381
C → T	T	36.7	77	39.29 (32.45–46.12)	10	26.32 (12.31–40.32)				
rs1034186	A	4.7	12	6.19 (2.8–9.58)	1	2.63 (0–7.72)	2.1602	0.4018	54.0386	0.4694
T → A	T	95.3	182	93.81 (90.42–97.2)	37	97.37 (92.28–100)				
rs10489950	C	95.3	193	98.47 (96.75–100)	35	92.11 (83.53–100)	*5.4526*	*0.9047*	*32.8834*	*0.0534*
C → T	T	4.7	3	1.53 (0–3.25)	3	7.89 (0–16.47)				
rs1999012	C	86.7	153	78.87 (73.12–84.61)	31	81.58 (69.25–93.9)	0.8556	0.3230	1.9986	0.8341
C → G	G	13.3	41	21.13 (15.39–26.88)	7	18.42 (6.1–30.75)				
rs11162488	A	66.2	144	73.47 (67.29–79.65)	33	86.84 (76.09–97.59)	0.4311	0.1391	1.0815	0.1078
A → C	C	33.8	52	26.53 (20.35–32.71)	5	13.16 (2.41–23.91)				
rs674345	A	38.8	70	36.08 (29.32–42.84)	16	42.11 (26.41–57.8)	0.7754	0.3820	1.6022	0.5979
G → A	G	61.2	124	63.92 (57.16–70.68)	22	57.89 (42.2–73.59)				
rs518341	C	10	14	7.22 (3.58–10.86)	1	2.63 (0–7.72)	2.5428	0.4839	63.0934	0.4595
T → C	T	90	180	92.78 (89.14–96.42)	37	97.37 (92.28–100)				
rs10782665	G	59.7	120	61.22 (54.4–68.05)	32	84.21 (72.62–95.8)	**0.3032**	**0.1085**	**0.7161**	**0.0106**
G → T	T	40.3	76	38.78 (31.95–45.6)	6	15.79 (4.2–27.38)				
rs11578155	A	94.6	180	92.78 (89.14–96.42)	31	81.58 (69.25–93.9)	**2.9119**	**1.0173**	**7.6915**	**0.0375**
A → G	G	5.4	14	7.22 (3.58–10.86)	7	18.42 (6.1–30.75)				
rs6692239	C	61.4	118	60.82 (53.96–67.69)	20	52.63 (36.76–68.51)	1.3962	0.6861	2.8267	0.3543
C → T	T	38.6	76	39.18 (32.31–46.04)	18	47.37 (31.49–63.24)				
rs11162494	A	74.3	145	74.74 (68.63–80.86)	24	63.16 (47.82–78.5)	1.7278	0.8086	3.5858	0.1867
A → T	T	25.7	49	25.26 (19.14–31.37)	14	36.84 (21.5–52.18)				
rs3753380	A	33.5	62	31.96 (25.4–38.52)	7	18.42 (6.1–30.75)	2.0425	0.8917	5.3410	0.1505
G → A	G	66.5	132	68.04 (61.48–74.6)	31	81.58 (69.25–93.9)				
rs3766355	G	89.6	164	83.67 (78.5–88.85)	33	86.84 (76.09–97.59)	0.7950	0.2522	2.0515	0.6486
G → T	T	10.4	32	16.33 (11.15–21.5)	5	13.16 (2.41–23.91)				
rs3766354	C	90	173	89.18 (84.8–93.55)	34	89.47 (79.72–99.23)	0.9966	0.2697	2.8517	1
C → T	T	10	21	10.82 (6.45–15.2)	4	10.53 (0.77–20.28)				
rs1555541	A	68.5	122	62.89 (56.09–69.68)	22	57.89 (42.2–73.59)	1.2338	0.5976	2.5026	0.6033
A → G	G	31.5	72	37.11 (30.32–43.91)	16	42.11 (26.41–57.8)				
rs12748050	C	84	167	85.2 (80.23–90.17)	32	84.21 (72.62–95.8)	1.0979	0.3807	2.7257	1
C → T	T	16	29	14.8 (9.83–19.77)	6	15.79 (4.2–27.38)				
rs520171	A	18.2	33	16.84 (11.6–22.08)	6	15.79 (4.2–27.38)	1.0606	0.4318	3.0390	1
C → A	C	81.8	163	83.16 (77.92–88.4)	32	84.21 (72.62–95.8)				
rs473027	A	74.8	137	71.35 (64.96–77.75)	22	57.89 (42.2–73.59)	1.8100	0.8694	3.7084	0.1331
A → G	G	25.2	55	28.65 (22.25–35.04)	16	42.11 (26.41–57.8)				
rs4650581	A	27.3	31	16.85 (11.44–22.26)	7	18.42 (6.1–30.75)	0.8855	0.3712	2.3794	1
T → A	T	72.7	153	83.15 (77.74–88.56)	31	81.58 (69.25–93.9)				

(*Continued*)

Table 5. (Continued.)

			Allelic Frequencies				Associations			
			Responders		*Nonresponders*			95% *CI*		
SNP	**Allele**	**CEU (%)**	*n*	*% (95% CI)*	*n*	*% (95% CI)*	*OR*	*Lower*	*Upper*	*P-value*
rs3766332	A	5.4	14	7.22 (3.58—10.86)	1	2.63 (0—7.72)	2.5428	0.4839	63.0934	0.4595
T → A	T	94.6	180	92.78 (89.14—96.42)	37	97.37 (92.28—100)				
rs12731181	A	83.6	166	84.69 (79.65—89.73)	30	78.95 (65.98—91.91)	1.4890	0.5830	3.4620	0.4976
A → G	G	16.4	30	15.31 (10.27—20.35)	8	21.05 (8.09—34.02)				

CEU = Caucasian European; CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism.
Bolded values are statistically significant. Italicized values are *P* values less than 0.1.