ORIGINAL ARTICLE

Two variants in vascular endothelial growth factor gene affect response to treatment for age-related macular degeneration in the Israeli population

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Abstract

Purpose: The aim of the study was to investigate the potential association between gene variation and the response of Israeli patients with age-related macular degeneration (AMD) to treatment.

Materials and Methods: The study sample included 100 patients with AMD. One eye per patient was analyzed. The medical files were reviewed for demographic, systemic and ocular data, and measurements of best corrected visual acuity (BCVA), and central retinal thickness (CRT) at each visit were recorded. Treatment consisted of intravitreal bevacizumab alone (1.25 mg) or followed by ranibizumab (0.5 mg) in the event of no initial response to bevacizumab, at 3 months intervals. Blood was collected from each patient, and genotyping for eight single-nucleotide polymorphisms (vascular endothelial growth factor A [VEGFA] rs3025039, rs2010963, rs699947; complement factor H rs800292; age-related maculopathy susceptibility 2 rs10490924; high-temperature requirement A-1 rs11200638; and pigment epithelium-derived factor rs9913583, rs1136287) was performed by allele-specific polymerase chain reaction. A favorable treatment response was defined as an improvement or no change in logMAR BCVA and a reduction in CRT by optical coherent tomography.

Results: The study group consisted of 54 male and 46 female patients mean 77.6, mean follow-up 40.43 months. Vision improved in 70 patients. A correlation with BCVA was noted for two of the polymorphisms studied, vascular endothelial growth factor A rs2010963 and rs699947. The correlation with BCVA was stronger than the correlation with CRT. There was no difference in mean follow-up time between responders and non-responders, and no association between age at disease onset and the genetic variants identified.

Conclusion: Identification of the gene variants VEGFA rs2010963 and rs69994 in Israeli patients with AMD may help to predict their response to treatment. Furthermore, such investigations are important to aid clinicians in selection of the preferred treatment.

Introduction

Age-related macular degeneration (AMD) is the leading cause of visual loss in the elderly population in developed countries.[1] There are two forms of the disease, exudative and atrophic. Choroidal neovascularization (CNV) secondary to exudative AMD is responsible for most cases of severe deterioration of vision.[1] Although environmental and behavioral factors play important roles in AMD, the disease is primarily due to genetic factors.[2] Multiple studies have implicated several genes in the alternative complement pathway, namely, complement factor (CF) H (CFH), CFB, CFI, and complements 2 and 3 (C2, C3).[3-5] This pathway and its related mechanisms have been extensively studied in AMD and are considered major susceptibility factors.[3] The region of the age-related maculopathy susceptibility 2/high temperature requirement A-1

genes (ARMS2/HTRA1) is also strongly associated with AMD, but its function has not been confirmed. Using genome-wide scanning and candidate-gene approaches, one study of AMD risk identified two major genetic loci: The well-characterized CFH gene at 1q31 and the still-debated ARMS2/LOC387715 gene at 10q26.[3] More recent genome-wide association studies found that the high-density lipoprotein cholesterol pathway (LIPC, ABCA1, and CETP) and extracellular matrix pathway (TIMP3, COL10A1, and COL8A1) are also apparently associated with AMD.[6,7] This is also true for the vascular endothelial growth factor A (VEGFA) gene in the angiogenesis pathway.[6,7] Pigment epithelium derived factor (PEDF) is also a potent antiangiogenic agent.[8] Imbalance between VEGF and PEDF contributes to the development of CNV in AMD.[9-11]

It is generally agreed that VEGF is an important trigger for the progression of exudative AMD.[12] Accordingly, intravitreal injection of bevacizumab, an anti-VEGF agent, has been found to be effective in the treatment of CNV due to AMD, improving vision for at least 6 months and reducing the risk of visual loss.[13] Early identification of CNV, before penetration of the subretinal space, would enable clinicians to start treatment earlier, before vision is compromised. Thus, following the establishment of correlations between genetic risk factors and phenotypes of exudative AMD, researchers are investigating ways to predict response to treatment. Several studies have suggested that single-nucleotide polymorphisms (SNPs) in genes associated with AMD may be involved in disease progression and response to treatment, although their specific role is not clearly understood.[14] The first meta-analytically identified SNP associated with response to treatment was CFH rs1061170 (T1277C, Y4202H).[15] The presence of the c allele tended to predict poor response. In a study from Korea CFH Y402H, LOC387715, and HTRA1 genotypes, yielded significant differences in response to treatment and need for additional injections were found between homozygote and heterozygote carriers of the risk allele of CFH Y402H.[16]

The aim of the present study was to extend the investigation of potential markers of treatment response in AMD to Israeli patients. Variations in the following genes were examined: VEGFA, CFH, ARMS2/LOC387715, HTRA1, and PEDF (also termed SERPINF1).

Materials and Methods

Patients

The study was approved by the Institutional and National Review Boards. Included were 100 patients (54 males and 46 females) clinically diagnosed with exudative AMD who were being followed in a tertiary outpatient retina clinic in Israel. All provided informed consent to participate in the study. Excluded were patients with other diseases of the retina (diabetic retinopathy, angioid streaks, high myopia, central serous chorioretinopathy, or retinal dystrophies) or with media opacities that prevented precise examination of the retina.

All patients underwent a complete clinical eye examination, funduscopy, and optical coherence tomography (OCT). All eyes with AMD were treated with intravitreal injections of bevacizumab; ranibizumab was added after bevacizumab if the event of no response to three injections of bevacizumab. One eye per patient was analyzed for the study. If both eyes were treated for AMD, the eye with complete documentation and vision of 0.1–1.4 logMar was selected. Response to treatment was defined as a statistically significant reduction in by OCT or an improvement or no change in best corrected visual acuity (BCVA) at 3 months after onset of treatment and at the end of the follow-up period.

Genotyping

Blood samples were collected from all patients. Genomic DNA was extracted from peripheral blood leukocytes using the iPrep™ Purification Instrument (Life Technologies, Invitrogen, Grand Island, NY) and iPrep™ PureLink® gDNA Blood Kit (Invitrogen), according to the manufacturer's instructions.

Genotyping was performed by allele-specific polymerase chain reaction using reagents and conditions from SNP genotyping assays (Taqman; Applied Biosystems, Courtaboeuf, France). The representative genotypes were ascertained by direct DNA sequencing for quality-control purposes. Primers and probes relevant for SNP genotyping assays (rs699947, rs2010963, rs3025039, rs1136287, rs9913583, rs800292, rs11200638, and rs10490924) were designed by Rhenium Ltd., Applied Biosystems (Israel). Real-time PCR amplification was performed with the TaqMan Genotyping MasterMix (Applied Biosystems). The primers for the genotyping probes are presented in Table 1.

Statistical analysis

One-way analysis of variance, χ^2 test, Fisher exact test, and unpaired t-test were used to analyze the data. Statistical significance was defined as $P < 0.05$.

Results

The study group consisted of 54 male and 46 female patients aged 60–91 years (mean ± SD, 77.6 ± 7.5). Fifty-one right eyes and 49 left eyes were evaluated. The number of intravitreal injections per eye ranged from 3 to 60 (mean 21.6). Forty-five eyes received 1.25 mg bevacizumab alone and 55 received combined 1.25 mg bevacizumab, followed by 0.5 ranibizumab [Table 2]. The duration of follow-up was 3–123 months (mean 40.43 ± 24.7). Vision improved in 56 patients. central retinal thickness (CRT) improved in 61 patients. Mean (±SD) BCVA (Logmar) was 0.686 (± 0.4658) at entrance to the study and 0.7738 (± 0.69303) at the end of the study. Corresponding values of CRT were 378.49 (± 132.806) and 314.91 (± 122.491). There was no significant difference in patient age, duration of follow-up, or mean number of injections between responders and non-responders. As expected, there were more non-responders in the combined-therapy subgroup. There was no association of age at diagnosis with

Table 1: Primers for genotyping probes for 8 SNPs

	dbSNP	Context sequence [VIC/FAM]
VEGFA	rs699947	GCCAGCTGTAGGCCAGACCCTGGCA[A/C]GATCTGGGTGGATAATCAGACTGAC
VEGFA	rs2010963	CGCGCGGGCGTGCGAGCAGCGAAAG[C/G]GACAGGGGCAAAGTGAGTGACCTGC
VEGFA	rs3025039	GCATTCCCGGGCGGGTGACCCAGCA[C/T]GGTCCCTCTTGGAATTGGATTCGCC
PEDF (SERPINF1)	rs1136287	GACCTGTACCGGGTGCGATCCAGCA[C/T]GAGCCCCACGACCAACGTGCTCCTG
PEDF (SERPINF1)	rs9913583	CTGGATTAGAAGGCAGCAAAAAAAG[A/C]TCTGTGCTGGCTGGAGCCCCCTCAG
CFH	rs800292	CCCTGGATATAGATCTCTTGGAAAT[A/G]TAATAATGGTATGCAGGAAGGGAGA
HTRA1	rs11200638	AGCTCCGCGGACGCTGCCTTCGTCC[A/G]GCCGCAGAGGCCCCGCGGTCAGGGT
ARMS2	rs10490924	TTTATCACACTCCATGATCCCAGCT[G/T]CTAAAATCCACACTGAGCTCTGCTT

SNPs: Single-nucleotide polymorphisms, VEGFA: Vascular endothelial growth factor A, PEDF: Pigment epithelium derived factor, CFH: Complement factor, HTRA1: High temperature requirement A-1 genes, ARMS2: Age-related maculopathy susceptibility 2

Table 2: Genotype distribution of 8 SNPs in 100 patients treated for AMD

Gene	Risk allele	Genotype	Frequency	Percent
VEGFA SNP rs699947*	C	AA	18	18.4
		AC	50	51
		CC	30	30.6
VEGFA SNP rs2010963*	G	CC	22	22.4
		CG	35	35.7
		GG	41	41.8
VEGFA SNP rs3025039	T	CC	74	75.5
		CT	23	23.5
		TT	1	1
PEDF (SERPINF1) SNP rs1136287	T	CC	14	14.3
		CT	40	40.8
		TT	44	44.9
PEDF (SERPIN1) SNP rs9913583	C	AA	0	0
		AC	6	6.1
		CC	92	93.9
CFH SNP rs800292	G	AA	0	0
		AG	16	16.3
		GG	82	83.7
HTRA1 SNP rs11200638	G	AA	20	21.3
		AG	41	43.6
		GG	33	35.1
ARMS2 SNP rs10490924	T	GG	35	35.7
		GT	42	42.9
		TT	21	21.4

*These SNPs were statistically significantly associated with outcome.
SNPs: Single-nucleotide polymorphisms, VEGFA: Vascular endothelial growth factor A, PEDF: Pigment epithelium derived factor, CFH: Complement factor, HTRA1: High temperature requirement A-1 genes, ARMS2: Age-related maculopathy susceptibility 2

response to treatment by either drug protocol (bevacizumab alone or with ranibizumab) or outcome measure (BCVA and CRT).

The genotype distribution for the 8 SNPs studied is shown in Table 2. Each SNP was retrieved for 94–98 patients; there were no cases of AA homozygosity for CFH rs800292 or PEDF (SERPINF) rs9913583. The association of the genotypes with changes in BCVA is shown in Table 3. A correlation with change in BCVA was found for two of the eight SNPs investigated: VEGFA rs699947 and VEGFA rs2010963, which achieved trend level at 3 months and statistical significance at study end ($P < 0.05$). Specifically, the AA genotype of VEGFA rs699947 was more frequently represented in responders at both 3 months ($\chi^2 = 5.935$, $p = 0.051$, trend level) and at the end of the study ($\chi^2 = 11.738$, $p = 0.03$). For VEGFA rs2010913, CG (heterozygous) genotype was more frequently represented in nonresponders at 3 months ($\chi^2 = 6.961$, $p = 0.031$), and the GG (homozygous mutant) genotype was more frequently represented in responders at the end of the study ($\chi^2 = 6.225$, $p = 0.044$).

None of the tested SNPs was significantly associated with changes in CRT [Table 4]. However, treatment led to a more prominent reduction in CRT in patients homozygous for the non-risk allele of VEGFA rs2010963 than in patients heterozygous for the non-risk allele or homozygous for the risk allele CRT thinning was also greater in patients homozygous for the risk allele of VEGFA rs699947 than in the other genotype subgroups. Like for BCVA, SNP associations for CRT were more significant at the end of the study than at 3 months. For all SNPs examined, the changes in CRT were less significant than the changes in BCVA. There was no association of age with SNP variability.

Discussion

All SNPs evaluated in the present study were reported in the literature to be significant predictors of treatment response or risk patients with AMD.[12,17-19] In the present study, two of them were found to be relevant to predicting outcome, VEGFA rs2010963 and VEGFA rs699947, at the end of follow-up in Israeli patients treated with an anti-VEGF agent. Response to treatment was measured by changes in BCVA and CRT. The correlation with BCVA reached trend level at 3 months and statistical significance at the end of the study. The correlation

Table 3: Association of SNPs with change in BCVA at study time points

SNP	3 months after treatment		End of follow-up	
	χ^2	P	χ^2	P
VEGFA				
SNP rs699947	5.935	0.051	11.738	0.003*
SNP rs2010963	6.961	0.031*	6.225	0.044*
SNP rs3025039	0.921	0.631	4.304	0.116
SERPINF1				
SNP rs1136287	4.695	0.096	1.099	0.577
SNP rs9913583	1.438	0.230	1.159	0.282
CFH				
SNP rs 800292	0.67	0.770	0.897	0.344
HTRA1				
SNP rs 11200638	1.146	0.564	1.424	0.491
ARMS2				
SNP rs 10490924	1.621	0.445	1.641	0.440

Table 4: Association of SNPs with change in CRT at study time points

SNP	3 months after treatment		End of follow-up	
	χ^2	P	χ^2	P
VEGFA				
SNP rs699947	4.849	0.089	3.756	0.153
SNP rs2010963	2.520	0.284	5.304	0.071
SNP rs3025039	0.036	0.849	0.001	0.977
SERPINF1				
SNP rs1136287	3.929	0.140	0.090	0.956
SNP rs9913583	1.711	0.191	1.171	0.679
CFH				
SNP rs 800292	0.713	0.398	0.195	0.659
HTRA1				
SNP rs 11200638	2.977	0.226	1.095	0.578
ARMS2				
SNP rs 10490924	2.558	0.278	0.708	0.702

with CRT was weaker: not significant at 3 months and trend level at the end of the study. The responder and non-responder groups were similar for age, duration of follow-up, and number of injections.

In an earlier study of AMD, Nakata *et al.* (2011) found that the VEGFA gene polymorphism rs699947 was associated with response to intravitreal bevacizumab and triple therapy (photodynamic therapy with intravitreal triamcinolone acetonide and intravitreal bevacizumab). Imai *et al.*,[18] in a study of SNPs of VEGF and other genes, reported a correlation of response to treatment (improved vision) with VEGFA rs699947 at 1 month and with PEDF rs1136287 at 3 months, with no correlation for rs2010963. In the present study, both VEGFA rs699947 and VEGFA rs2010963 were associated with a trend-level improvement in BCVA at 3 months; the correlation reached statistical significance by the end of follow-up. We did not check results at 1 month for comparison with the findings of Imai *et al.* (2010) although at 3 months, there was no correlation of PEDF rs1136287 with changes in vision.

Boltz *et al.*,[12] in a study from Austria, genotyped 7 VEGFA polymorphisms (rs2010963 and rs699947, as well as rs1413711, rs3025039, rs833061, rs3024997, and rs1005230) in 141 patients (185 eyes) with AMD. On univariate analysis, rs2010963 was correlated with treatment response but only for G/G homozygotes, who had significantly better BCVA by the end of treatment than C/G heterozygotes. Accordingly, in the present study, vision improved in 69% of G/G homozygotes compared to only 45.5% of C/C homozygotes and 42.9% of C/G heterozygotes. The rs3024997 polymorphism was associated with significantly lower BCVA at treatment end than the other 5 SNPs.[12]

Considering that we noted a significant improvement only at the end of follow-up, the difference in findings between the study of Boltz *et al.*[12] and the present study (for rs699947 and rs3025039) might be attributable to the shorter treatment period in the earlier work (1–15 bevacizumab injections administered over 42–1182 days) than in ours (mean 26 injections administered over 90–3690 days). Boltz *et al.*[12] also analyzed both eyes per patient whereas we included only one. Furthermore, on multivariate analysis, including time, baseline BCVA, and number of reinjections, none of the 7 SNPs showed a significant correlation with outcome.

It is also noteworthy that the correlation of polymorphism rs3024497 with treatment outcome in the study of Boltz *et al.*[12] was not supported by a recent meta-analysis[20] based on a search of PubMed, Embase, Wanfang (Chinese), VIP (Chinese), and the Chinese National Knowledge Infrastructure database up to October 2011.

Two studies suggested a possible association also of the other VEGFA polymorphisms (rs94380, rs1413711, and rs833061) with treatment response,[20,21] but the findings were not supported by others.[22] Therefore, these SNPs were not included in the present study.

None of the patients in our study had the AA genotype for CFH rs800292, a common allele in exudative AMD in the Japanese population (9.3%)[23] or for PEDF (SERPINF1) rs9913583, a common allele (11.7%) in the Korean population.[24] We were unable to find information on the frequency of PEDF rs9913583 AA in the general population. By contrast to the present study and others,[18,25] studies from China reported a significant association of rs800292 with response to treatment.[19,26] Analysis of data for 144 patients from 13 centers yielded a change in BCVA of 4.4 letters in those with the CC genotype, 8.7 letters in those with the CT genotype, and 15.5 letters in those with the CT or TT genotype ($p = 0.009$).[26]

The same multicenter studies[19,26] also found that in patients with the ARMS2 rs10490924 polymorphism, mean BCVA changed by 3.6 letters in carriers of the TT genotype, 12.1 letters in carriers of the TG genotype, and 9.6 letters in carriers of the GG genotype (p = 0.001). The TT genotype was significantly associated with greater BCVA improvement[26] whereas the GG (homozygote risk) genotype was significantly associated with poorer BCVA outcome.[19] A similar association with poorer BCVA was found for the risk allele of HTRA1 rs11200638 (AA) in a study of 224 patients in Australia.[25] Both these studies, however, are in disagreement with ours in which neither genotype was significantly correlated with either improvement or deterioration in vision. However, most of the patients in the Australian study[25] received only ranibizumab whereas all of ours started with bevacizumab, and ranibizumab was added only when there was no response to bevacizumab after 3 injections (approximately 3 months). Furthermore, in our study, the decision to treat was followed by three injections, one per month, and not a single injection as in the Australian study.[25-28]

Our findings are in line with the recent CATT study[23] of 834 patients of multiethnic origin from 43 clinical center in the United States which found no association of ARMS2 rs10490924 or HTRA1 rs11200638 with outcome of anti-VEGF treatment in neovascular AMD.

ARMS2 and HTRA1 were considered good candidate genes for predicting the response to treatment in AMD as both lie in the susceptibility locus identified on chromosome 10q26 and their products are expressed in the retina. Furthermore, ARMS2 A69S has been found to increase the risk of oxidative damage and aging in photoreceptor cells, and HTRA1 rs11200638 lies in the promotor region and was suspected to increase expression levels of the gene, thereby possible altering the integrity of Bruch's membrane and inducing CNV development. However, the precise mechanisms by which these genetic variants affect AMD are still not fully understood.[23]

Study limitations

The CRT values in the present study were derived by OCT. However, due to the long follow-up, different instruments (Cirrus, Heidelberg) were used in different patients and sometimes in the same patients. However, the results for CRT did not achieve statistical significance. Furthermore, some patients (n = 15) had undergone photodynamic therapy before enrollment, but since the study focused on the genetic response, we did not exclude them. We limited our analysis to one eye per patient because the genetic effect is expected to be the same bilaterally, and we measured the change in vision, not the worst or best visual acuity. Nevertheless, overall, the patients had uniform characteristics in terms of inclusion criteria and vision. Finally, the Israeli population includes individuals of many ethnic origins. However, the SNP frequencies were similar to those in the database of the world European population according to the genome 1000 project.

Conclusion

The role of polymorphisms in the visual outcome of patients with exudative AMD is supported by the present study which found 2 VEGFA SNPs to be independent predictors of response to anti-VEGF treatment in the Israeli population. The association between AMD genetics and response to treatment illustrates how pharmacogenetic factors may help determine treatment modality and dosing. This could ultimately provide basic data for personalized medicine in AMD, with genetic variants serving as biomarkers of outcome.

Acknowledgments

Authors' Contributions

G.T. Conducted, analyzed and wrote the MS.
S.W. Conducted and analyzed the results.
R.A.S. Designed, revised the MS.
N.G.C. Designed, conducted, analyzed and wrote the MS.
All authors approved the final article.

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