Associations between Genetic Polymorphisms of Insulin-like Growth Factor Axis Genes and Risk for Age-Related Macular Degeneration

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Purpose. To investigate whether insulin-like growth factor (IGF) axis genes, together with a novel dietary risk factor, the dietary glycemic index (dGI), and body mass index (BMI) affect the risk for age-related macular degeneration (AMD).

Methods. This case–control study involved 962 subjects originally recruited through the Age-Related Eye Disease Study (AREDS) Genetic Repository. After those with missing covariates or invalid calorie intake (n = 23), diabetes (n = 59), and non-Caucasian race (n = 16) were excluded, 864 participants were used, including 209 AREDS category 1 participants (control group), 354 category 2 or 3 participants (drusen group), and 301 category 4 participants (advanced AMD group). A total of 25 single-nucleotide polymorphisms (SNPs) selected from *IGF-1* (n = 9), *IGF-2* (n = 1), IGF binding protein 1 (*IGFBP1*; n = 3), *IGFBP3* (n = 3), acid-labile subunit of IGFBP (*IGFALS*; n = 2), IGF1 receptor (*IGF1R*; n = 4), and *IGF2R* (n = 3) were genotyped. SNP-AMD associations were measured with genotype, allele χ^2 tests and Armitage's trend test. Odds ratios (OR), 95% confidence intervals (CIs), and SNP-exposure interactions were evaluated by multivariate logistic regression.

Results. One SNP (rs2872060) in *IGF1R* revealed a significant association with advanced AMD (P-allele = 0.0009, P-trend = 0.0008; the significance level was set at 0.05/25 = 0.002 for multiple comparisons). The risk allele (G) in the heterozygous and homozygous states (OR, 1.67 and 2.93; 95% CI, 1.03–2.71 and 1.60–5.36, respectively) suggests susceptibility and an additive effect on AMD risk. Further stratification analysis remained significant for both neovascularization (OR, 1.49 and 2.61; 95% CI, 0.90–2.48 and 1.39–4.90, respectively) and geographic atrophy (OR, 2.57 and 4.52; 95% CI, 0.99–6.71 and 1.49–13.74, respectively). The G allele interaction analysis with BMI was significant for neovascularization (P = 0.042) but not for geographic atrophy (P = 0.47). No significant interaction was found with dGI.

Conclusions. These data suggest a role of *IGF1R* on the risk for advanced AMD in this group of subjects. (*Invest Ophthalmol Vis Sci.* 2011;52:9099–9107) DOI:10.1167/iovs.11-7782

Age-related macular degeneration (AMD) is the major cause of irreversible vision loss in the Western world,[1] affecting approximately 15% of the elderly. At present, there is no widely practicable treatment for AMD. It is believed that AMD is a multifactorial disease, and the risk of AMD is determined by multiple genetic and environmental (including nutritional) factors.[2,3] In recent studies, we observed a link between glycemic index (GI) and increased risk for AMD in two American cohorts: the Nutrition and Vision Project (NVP) substudy of the Nurses' Health Study (NHS) and the Age-Related Eye Diseases Study (AREDS).[4–6] Both studies indicate that consuming diets that cause higher blood glucose loads (i.e., diets with higher glycemic index [GI]) is associated with higher risk for AMD in otherwise healthy, nondiabetic individuals. The findings were also replicated in the Blue Mountain Eye Study (BMES) cohort, Australia.[7]

The GI is a physiological measure of the "glycemic quality" of carbohydrate-containing foods.[8] Intake of high-GI foods results in rapid elevation of postprandial blood glucose levels relative to low-GI foods. The clinical and public health implication of GI is that it can help people to choose foods. The dietary glycemic index (dGI) for each subject was calculated as Σ ($GI_i \times W_i$)/W: the weighted average of the GI values for each food item i (GI_i) with the amount of carbohydrate consumed from each food item i as the weight W_i/W.[9] Thus, dGI can be thought of as a description of a dietary pattern that describes the glycemic quality of a diet. Our recent evaluations indicated that the dGI is a better correlate of AMD than is total carbohydrate intake (quantity).[4–6] Our analyses also found that the association with dGI is stronger in individuals with bilateral

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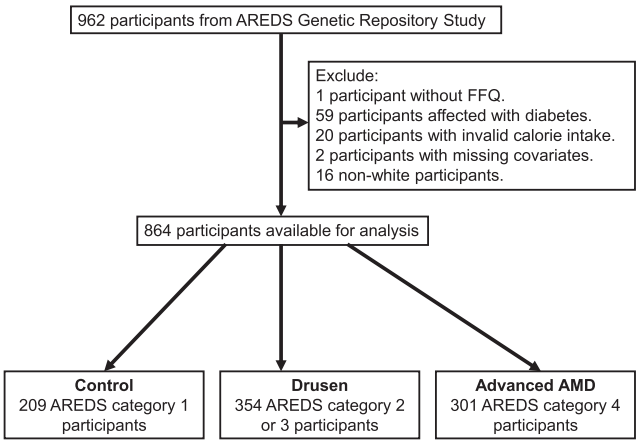

FIGURE 1. Exclusion criteria and eligible participants from the AREDS Genetic Repository Study.

AMD progression (more susceptible to AMD progression).[6] Since bilateral AMD progression suggests a stronger genetic influence, we hypothesized that genetic variants play a epistatic or modifier role in the novel association between dGI and AMD risk.[6] Specifically, we speculate that gene–carbohydrate interactions are etiologic factors for AMD.[3] In this study, we investigated roles for insulin-like growth factor (IGF)–related (often referred to as the IGF axis) genes because carbohydrate nutrition has been shown to affect postprandial circulating levels of IGFs and their responses[10–12] and several mechanistic studies have related these genes to choroidal neovascularization, a late-stage manifestation of AMD.[13–15] To test our hypothesis, we used a candidate gene approach in a association study, to evaluate whether genetic polymorphisms in specific IGF-axis genes alter the risk of AMD.

MATERIAL AND METHODS

Study Subjects

The subjects were participants from the AREDS Genetic Repository Study (n = 962; age range, 55–80 years; median, 69 years; 56% female) who had reliable dietary data and genomic DNA samples (n = 864; Fig. 1). To avoid potential bias from population stratification, we excluded nonwhite participants in our main analyses. After excluding those without dietary information, missing covariates or invalid calorie intake (n = 23), diabetes (n = 59), and non-Caucasian race (n = 16), the following remained in the sample: 209 AREDS category 1 participants (control group), 354 category 2 or 3 participants (drusen group), and 301 category 4 participants (advanced AMD group). The protocol complied with the Declaration of Helsinki.

Case and Control Definitions

The baseline AMD category was assessed according to AREDS AMD grading procedures.[16,17] Persons in category 1 were free of AMD and had a total drusen area of less than five small drusen (<63 μm in diameter) and visual acuity (VA) of 20/32 or better in both eyes. Category 2 participants had mild age-related macular lesions (multiple small drusen, nonextensive (<20) intermediate drusen (63–124 μm in diameter), pigment abnormalities, or a combination thereof) in the most advanced eye and VA of 20/32 or better in both eyes. Category 3 required the absence of advanced AMD in both eyes and at least 1 eye with VA of 20/32 or better with at least one large druse (≥125 μm in diameter) and extensive (as measured by drusen area) intermediate drusen, or geographic atrophy that did not involve the center of the macula, or a combination thereof. In category 3a, both eyes met these criteria, whereas in category 3b, one eye either had reduced VA not resulting from AMD or a disqualifying ocular condition. Category 4 participants had VA of 20/32 or better and no advanced AMD (geographic atrophy involving the center of the macula or features of choroidal neovascularization) in the study eye, and the fellow eye had either lesions of advanced AMD (category 4a) or VA less than 20/32 and AMD abnormalities sufficient to explain reduced VA (category 4b), as determined by examination of photographs at the reading center. Persons aged 55 to 59 years were eligible for the study only if they met the criteria for categories 3 or 4.

Single-Nucleotide Polymorphism (SNP) Identifying and Genotyping

Genotyping data were derived from genomic DNA for the SNPs of interest. In addition to functional or previously studied polymorphisms,[18–24] tagging SNPs were selected for each gene that included coverage of the promoter and 3′ flanking regions (Table 1). Tagging SNPs were identified from build 36 of the European-derived (CEU) HapMap database using a minor allele frequency of ≥20% and an $r^2 \geq 0.80$. A total of 25 SNPs selected from *IGF-1* (n = 9), *IGF-2* (n = 1), IGF binding protein 1 (*IGFBP1*; n = 3), *IGFBP3* (n = 3), acid-labile subunit of *IGFBP* (*IGFALS*; n = 2), IGF-1 receptor (*IGF1R*; n = 4), and *IGF2R* (n = 3) were genotyped. Because of the large number of tagging SNPs necessary to evaluate *IGF1R* and *IGF2R*, only the polymorphisms previously studied were genotyped for these two genes.[23,24] The National Center for Biotechnology Information (Bethesda, MD; PubMed web site (http://www.ncbi.nlm.nih.gov/pubmed) was used to search for candidate SNPs with keywords: "igf1r AND polymorphism" and "igf2r AND polymorphism." SNPs with significant associations with diseases were preferred. Four SNPs were selected for *IGF1R* and three SNPs were selected for *IGF2R*. The search was performed at the end of 2007, when the study was initiated.

All SNPs were genotyped by 5′ exonuclease allele discrimination (*Taq*man, on a 7900HT with SDS 2.0 software; Applied Biosystems, Foster City, CA). Positive controls were used to assure plate-to-plate consistency of genotypes, and all genotype calls were conducted using the double-masked genotype assignments and discrepancies addressed using raw data.

Defining Potential Covariates

Data on possible risk factors for AMD were obtained from a baseline general physical and ophthalmic examination, a detailed questionnaire

TABLE 1. The 25 SNPs Selected from 7 IGF Axis Genes

Gene	Functional or Significant SNPs	Tagging SNPs
IGF-1	rs2162679, rs7965399	rs6214, rs5742678, rs7956547, rs12821878, rs5742632, rs7136446, rs9989002
IGF-2	Not done	rs3213221
IGFBP1	rs1995051, rs3793344	rs4619
IBGBP3	Not done	rs2453839, rs2471551, rs3110697
IGFALS	rs3751893	rs17559
IGF1R	rs1319868, rs1567811, rs8041224, rs2872060	Not done
IGF2R	rs1805075, rs8191754, rs629849	Not done

on basic characteristics and demographic data, and a validated food-frequency questionnaire (FFQ).[25]

The 90-item modified block FFQ collected information about usual dietary intakes over the previous year and classified them into nine possible response categories, ranging from never or less than once per month to two or more times per day. The daily total nutrient intake of an individual was calculated by summing the product of the frequency, serving size, and carbohydrate content per serving from individual food items derived from the nutrition database of the Nutrition Coordinating Center at the University of Minnesota.

The GI values for foods in the FFQ were either derived from published values with white bread used as the reference food, or imputed from GI values of comparable foods.[26] Indigestible fiber content was subtracted from the carbohydrate content. The dGI was adjusted for total energy intake by using the residuals method.[27]

In addition, the following were considered as potential covariates in our analyses: age, sex, education level (college graduate, and high school or less), body mass index (BMI, computed from weight [in kilograms] divided by height [in meters squared]), smoking status (ever or never), sunlight exposure (hours per day),[28] and hypertension history.

Statistical Analysis

We tested the hypothesis that genetic polymorphisms of IGF-axis genes and their interactions with dGI and BMI affect the risk for AMD in nondiabetic individuals from the AREDS. For each genotyped SNP (Table 1), we first examined whether the genotype frequencies were in Hardy-Weinberg equilibrium (*P*-HWE[b], exact *P* value for HWE) and evaluated the relationships with AMD status using a genotype case–control test (*P*-genotype),[29] allele case–control test (*P*-allele),[30] and Armitage's trend test (*P*-trend)[31] (calculations by SAS/Genetics; SAS, Cary NC). The trend test and allele case–control test are most useful when there is an additive allele effect on the disease susceptibility. When HWE holds in the combined sample of cases and controls, these statistics are approximately equal and have an asymptotic χ_1^2 distribution. However, if the assumption of HWE in the combined sample is violated, then the variance for the allele case–control statistic is incorrect; only the trend test remains valid under this violation. If dominance effects of alleles were also suspected to contribute to disease susceptibility, the genotype case–control test was used. The standard 2×3 contingency table analysis was used to form the χ_2^2 statistic for the genotype case–control test, which tests for both additive and dominance (nonadditive) allelic effects.

For those with significant associations, we further calculated the multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) estimated from logistic models. Age, sex, education level, BMI, smoking status, sunlight exposure, hypertension history, and dGI were included as covariates in multivariate models. The genotype with the lowest OR served as the referent group. Allele-specific ORs and 95% CIs were calculated in a similar way. The interactions between markers and dGI and BMI were evaluated by incorporating an interaction term in the model.[32,33] We estimated ORs and CIs by logistic regression analysis (PROC LOGISTIC and PROC CASECONTROL; SAS).

Population Stratification

Population stratification occurs when there are allele frequency differences between cases and controls due to systematic ancestry differences that can cause spurious associations. To assess whether population stratification confounds the associations between the 25 SNPs and advanced AMD, we used a method based on principal component analysis (PCA).[34] The PCA was performed using the data for the 25 SNPs from advanced AMD cases and controls in our sample ($n = 485$, all white; see Study Subjects section).

Results

Compared with the control group (Table 2), cases in the drusen group were significantly older, less educated, more likely to be a female, and more likely to be smokers. In addition to those characteristics, cases in the advanced AMD group, had

Table 2. Baseline Characteristics by AREDS AMD Categories*

	Control ($n = 209$)	Drusen ($n = 354$)	Advanced AMD ($n = 301$)
Age, y, mean ± SE	66.3 ± 0.29	68.6 ± 0.27 $P < 0.0001$†	69.4 ± 0.30 $P < 0.0001$†
Education			
Some high school or less	47 (22.49)	120 (33.90)	143 (47.51)
Some college or higher	162 (77.51)	234 (66.10) P = 0.004‡	158 (52.49) $P < 0.0001$‡
Female	104 (49.76)	213 (60.17) $P = 0.02$‡	178 (59.14) $P = 0.04$‡
Smoking status			
Never	104 (49.76)	146 (41.24)	119 (39.53)
Current or ever	105 (50.24)	208 (58.76) $P = 0.05$‡	182 (60.47) $P = 0.02$‡
Hypertension history	69 (33.01)	128 (36.16) $P = 0.45$‡	113 (37.54) $P = 0.29$‡
Body mass index, kg/m^2, mean ± SE	27.1 ± 0.25	27.4 ± 0.31 $P = 0.42$†	28.3 ± 0.30 $P = 0.01$†
Sunlight exposure, h/d, mean ± SE	1.0 ± 0.07	1.2 ± 0.06 $P = 0.13$†	1.1 ± 0.07 $P = 0.62$†
Dietary glycemic index, mean ± SE	77.9 ± 0.35	77.8 ± 0.25 $P = 0.85$†	79.0 ± 0.27 $P = 0.01$†

Data are *n* (%) unless otherwise indicated.

* Controls, AREDS category 1; Drusen cases, AREDS category 2 and 3; advanced AMD cases: AREDS category 4.

† *P* values are for the distributional differences compared with control groups and were derived by *t*-tests.

‡ *P* values are for the distributional differences compared with control groups and were derived by χ^2-tests.

TABLE 3. Association of SNP-rs2872060 in *IGF1R* with Risk for Advanced AMD and Drusen*

	Controls	Cases	*P*-HWE	*P*-Genotype	*P*-Allele	*P*-Trend
Drusen	201	345	0.73	0.12	0.047	0.045
Advanced AMD	201	284	0.72	0.004	0.0009*	0.0008*

"Determined" rate = 96.1%; *P*-HWE = 0.73.
* Statistically significant, with α set at 0.05/25 = 0.002.

higher BMI and dGI. These two factors were further evaluated in the interaction analysis.

In our scanning of 25 SNPs one SNP (rs2872060) in *IGF1R* revealed a significant association with advanced AMD (*P*-allele = 0.0009, *P*-trend = 0.0008; α-level was set at 0.05/25 = 0.002 for multiple comparisons; Table 3). This result suggests that SNP rs2872060 may have an additive allele effect on the risk for advanced AMD. The other 24 SNPs (Table 1) showed no significant associations with either drusen or advanced AMD.

Further logistic analysis estimates that the risk G allele is associated with a 1.5-fold increased risk for advanced AMD whereas GG genotype confers threefold increased risk (OR, 1.67 and 2.93; 95% CI, 1.03–2.71 and 1.60–5.36, respectively; *P*-trend = 0.0005; Table 4). Further stratification analysis remained significant for both neovascularization (OR, 1.49 and 2.61; 95% CI, 0.90–2.48 and 1.39–4.90, respectively; *P*-trend = 0.003) and geographic atrophy (OR, 2.57 and 4.52; 95% CI, 0.99–6.71 and 1.49–13.74, respectively; *P*-trend = 0.007; Table 5).

The G allele interaction analysis with BMI was significant for neovascularization (*P* = 0.049; Fig. 2) but not for geographic atrophy (*P* = 0.47). The OR per BMI unit (kilogram/meters squared) was higher in the subjects with the G allele than in the subjects with the T allele and in overall subjects as shown in Figure 2. To evaluate whether the interaction between G allele and BMI for neovascularization was modified by sex or smoking, we further stratified the analysis by sex and smoking. None of the *P* values was significant, probably because of the small sample size in each stratum. However, it appears that the interaction between the G allele and BMI was more dominant in the men (*P* = 0.07) than in the women (*P* = 0.24). The significance of the interaction did not differ between smokers (*P* = 0.10) and nonsmokers (*P* = 0.10).

No significant interaction was found between the risk G allele and dGI.

In the analysis of population stratification, we plotted the first two principal components. The scatterplot shows no obvious cluster in our study sample, indicating no significant confounding effect from population stratification on our results.

TABLE 4. Logistic Analysis of Association between SNP-rs2872060 in *IGF1R* and Advanced AMD

	Controls	Cases	OR	95% CI	*P*
Allele					
T	65	62	1	—	—
G	136	222	1.97	1.24–3.12	0.004
Genotype					
TT	65	62	1	—	—
GT	103	144	1.67	1.03–2.71	0.038
GG	33	78	2.93	1.60–5.36	0.0005
P-trend–logistic			0.0005		

DISCUSSION

In this study on nondiabetic individuals from the AREDS, the SNP rs2872060 in *IGF1R* was significantly associated with the risk for advanced AMD and the association remained significant after stratification by the two types of the disease: neovascularization and geographic atrophy. The risk allele (G) showed an additive effect and a significant interaction with BMI on the risk for neovascularization, but not for geographic atrophy.

Genetic variants in IGF axis genes and differential expression of such genes have been related to diabetes or its complications,[35,36] cardiovascular diseases,[36] cancers,[21,37–40] open angle glaucoma,[41] and Alzheimer's disease,[42,43] all of which share risk factors with AMD. To our knowledge, there has been no publication relating genetic polymorphisms of IGF axis genes to the risk for AMD. Because we had found that higher dGI is associated with increased risk for AMD,[4–6,44–46] we hypothesized that the genetic polymorphisms of the IGF axis affects the risk for AMD and that this association may be modified by dGI. In this study, except for rs2872060 in *IGF1R*, we did not find any association between the 25 SNPs and early (drusen) or late AMD risk. We also did not find that the status of dGI modifies the association between rs2872060 and risk for advanced AMD. However, these results do not exclude the possibility that other genetic variants in the IGF axis may be responsible for the hypothesized associations.

The IGF axis is an important regulator of metabolic function and cellular development and growth that cells in almost every bodily organ use to communicate with the physiologic environment.[10] This complex system consists of two cell-surface receptors (IGF1R and IGF2R), two growth factor ligands (IGF-1 and IGF-2), a family of six high-affinity IGF binding proteins (IGFBP 1–6), and associated IGFBP degrading enzymes, referred to collectively as IGFBP proteases.[47,48]

IGFs and IGFRs share high amino acid sequence homologies with insulin and insulin receptor (IR), respectively.[49] Like

TABLE 5. Logistic Analysis of Associations between SNP-rs2872060 in *IGF1R* and Two Types of Advanced AMD

Allele	Controls	Cases	OR	95% CI	*P*
Neovascular AMD					
T	65	55	1	—	—
G	136	182	1.76	1.09–2.86	0.02
Genotype					
TT	65	55	1	—	—
GT	103	119	1.49	0.90–2.48	0.12
GG	33	63	2.61	1.39–4.90	0.003
P-trend–logistic			0.003		
Geographic AMD					
T	65	7	1	—	—
G	136	40	2.97	1.17–7.52	0.02
Genotype					
TT	65	7	1	—	—
GT	103	25	2.57	0.99–6.71	0.05
GG	33	15	4.52	1.49–13.74	0.008
P-trend–logistic			0.007		

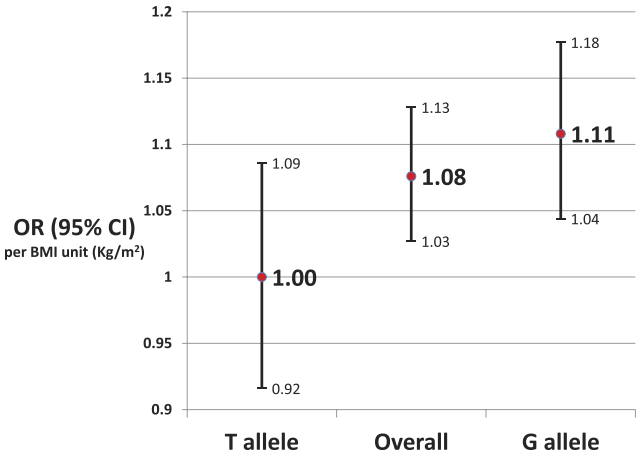

FIGURE 2. BMI-associated risk of neovascular AMD is highest in persons with the G allele in the rs2872060 polymorphism of *IGF1R* (*P* interaction = 0.042).

insulin, the binding of IGF-1 to its receptors results in the activation of the intrinsic tyrosine kinase, and postreceptor phosphorylation of members of the insulin receptor substrate (IRS) family.[50] The IR preferentially phosphorylates IRS-1, whereas IGF1R preferentially phosphorylates IRS-2.[51] This factor may partly differentiate their activities: IGF-1 is a more potent mitogen with stronger antiapoptotic activity than insulin and plays a major role in regulating cell replication, differentiation, and survival,[52] whereas insulin has stronger metabolic activity than does IGF-1.[53] For example, ligand activation of IGF1R by IGF-1 was shown to result in activation of intracellular signaling pathways including Ras/mitogen-activated protein kinase (MAPK; a synonym for extracellular-signal-regulated kinase [ERK]) and phosphatidylinostiol-3 kinase (PI3K)/AKT, indicating an IGF-1-mediated cell proliferation (1 and 2 in Fig. 3),[54,55] such as angiogenesis in diabetic retinopathy (DR) and AMD.[56,57] Through different signal transduction mediators, ligand activation of IGF1R by IGF-1 may also lead to the ubiquitin-mediated degradation of IGF1R (3 in Fig. 3).[58]

Unlike insulin, which is mainly present in free form, nearly 99% of IGF-1 in the circulation is bound to one of the six IGFBPs (4 in Fig. 3).[59] By binding IGF-1, IGFBPs can prevent the proteolysis and extend the half-life of IGF-1, while also reducing its bioavailability. Thus, IGFBP proteases may regulate the IGF-1 half-life and play a critical role in modulating IGF availability at the cellular level. IGFBPs also have biological activity independent of IGF-1.[60] For example, IGFBP3 can directly bind to the ribonucleic acid polymerase II binding subunit 3, suggesting a possible role of IGFBP3 in directly regulating gene transcription and the cell cycle.[61] However, the effects of IGFBP3 on the cell cycle are largely opposite those of IGF-1, since IGFBP3 is proapoptotic and antiproliferative.[62]

Although total circulating IGF-1 and IGFBP3 levels appear to show little or no intraindividual variation,[63] there is extensive interindividual variation in levels of total IGF-1 and the IGFBPs.[64] "Free" IGF-1 levels (about approximately 1% of IGF-1 in circulation) are also highly variable between individuals.[65] Genetic factors may, at least partially, explain these interindividual variations. It is reasonable to assume that these interindividual differences could play a role in disease risk. Like some other hormones (e.g., estradiol and thyroid hormone), free IGF-1 has been proposed to be the main bioactive component of IGF-1 in circulation.[66] However, unlike total IGF-1, free IGF-1 levels may vary significantly in the postprandial state, largely due to the regulation of IGFBP1 by insulin.[67,68] Heritability studies have shown that genetic and environmental factors contribute equally to the variation in circulating IGF-1 levels.[69–72]

Nutrition is an important modifiable determinant of circulating IGF-1 levels.[10,11] In a metabolic study, it was suggested that a high-GI diet is implicated in the risk of age-related diseases through modulating the IGF axis.[12] The data

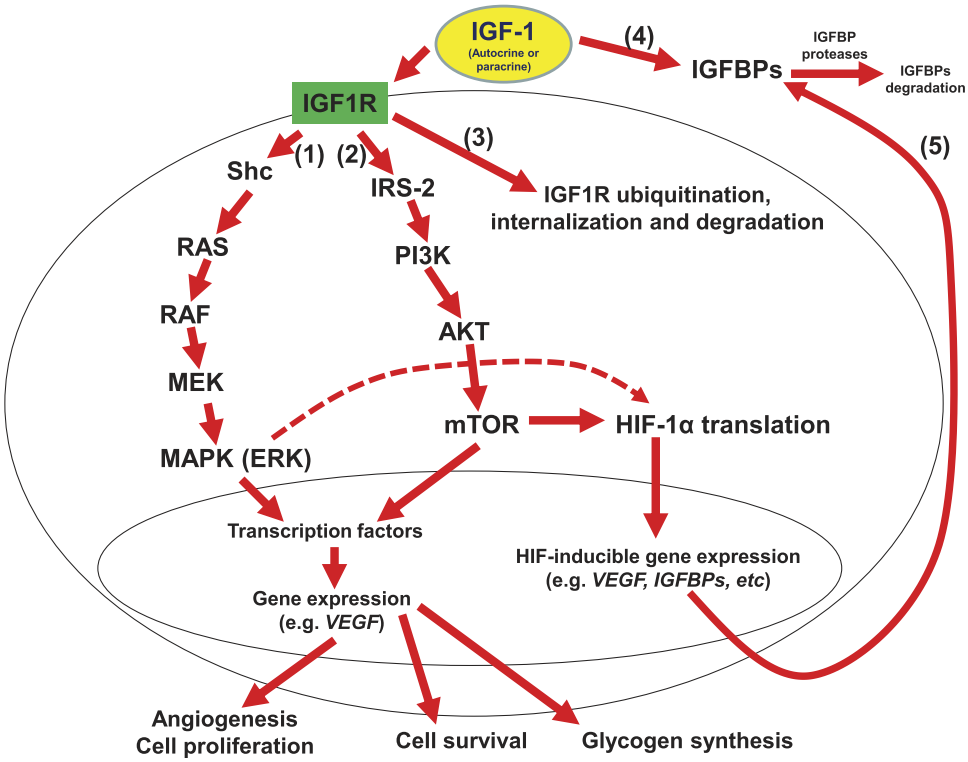

FIGURE 3. Signal transduction pathways demonstrate the different molecular consequences of ligand activation of IGF1R by IGF-1, including the activation of MAPK and PI3K/AKT cascades, which, in turn, induces expression of genes, such as *VEGF*, and ubiquitin-mediated IGF1R degradation. The activation of HIF-1 by mTOR in the PI3K/AKT cascade may result in the expression of IGFBPs and offer a feedback control on the IGF-1 activity. By binding IGF-1, IGFBPs can prevent proteolysis and extend the half-life of IGF-1, while reducing its bioavailability. Therefore, IGFBP proteases can regulate IGF half-life and play a critical role in modulating IGF availability at the cellular level.

implied that high-GI diets regulate IGF-1 levels through inhibiting IGFBP-3 but not directly through stimulating IGF-1 itself.

We recently proposed a novel hyperglycemic pathway: the hypoxia-inducible factor (HIF) pathway. In this model, in addition to hypoxia, hyperglycemia (e.g., the high postprandial hyperglycemia induced by high-GI diets) may also affect risk for DR, AMD, and other diseases through the activation of HIF-inducible genes, such as *VEGF* and VEGF receptor.[46] Studies have also shown that IGF-1 can lead to HIF-1 activation and *VEGF* expression by a mechanism different from hypoxia.[73–75] Interestingly, several IGF axis genes which are associated with the regulation of IGF-1, such as *IGFBP1*, *2*, and *3*, are also among the HIF-inducible genes.[76] This appears to be part of the feedback control of HIF on the IGF axis (5 in Fig. 3).

Several lines of evidence from basic research suggest mechanistic explanations of the association between IGF axis genes and AMD risk, especially for neovascular AMD. IGF-1 protein and IGF1R appear to be colocalized in the retina. This suggests an autocrine function of IGF-1 in the normal human retina and may point toward a role for the IGF axis in the pathogenesis of neovascular AMD.[15] It has also been shown that the IGF axis can contribute to angiogenesis directly by increasing proliferation of retinal endothelial cells,[77,78] or indirectly through inducing vascular endothelial growth factor (*VEGF*) gene expression of cultured RPE cells.[79] Furthermore, inhibitors of IGF1R, such as somatostatin and picropodophyllin,[14,80] reduce IGF-1-dependent VEGF expression in human RPE cells and in vivo mouse model, respectively. VEGF is a potent inducer of postnatal neovascularization and angiogenesis and anti-VEGF (e.g., such as Lucentis and Avastin; Genentech, South San Francisco, CA; and Macugen, Pfizer, New York, NY) has been used in clinics to treat exudative AMD.[81,82]

Inflammation is associated with AMD. The IGF axis may also affect AMD risk through modulating inflammatory responses. Data suggest that intraocular IGF-I, but not systemic IGF-I, is sufficient to trigger processes leading to blood–retinal barrier breakdown and increased retinal vascular permeability.[83–85] SNPs in *IGF1R* have also been implicated in differential immune response, such as lymphocyte recruitment and proliferation, resulting in difference in disease risk. For example, the TT genotype of rs2872060 was found to be associated with increased risk for bacteremia in sickle cell disease.[23]

Since SNP rs2872060 is located in the intron region of *IGF1R*, it is unlikely that this SNP directly affects the activity or stability of IGF1R protein. Instead, this SNP may affect the efficiency of transcription or RNA splicing for *IGF1R* and hence influence the risk for AMD.

Some epidemiologic studies have related higher BMI to increased risk for cancers, and it has been suggested that obesity-related tumorigenesis may be modulated by IGF1R.[86] In Japanese breast cancer patients, it has been suggested that the molecular consequence of the increased BMI include increased expression of *IGF1R*, which, through modulation of the cell cycle and apoptosis, results in development and progression of postmenopausal breast cancer.[87] In a case–control study of esophageal cancer, it was concluded that the common *IGF1R* gene polymorphism G1013A modulates the risk of obesity for esophageal cancer by influencing gene transcription or mRNA stability.[86,88] Although BMI has long been recognized as a risk factor for AMD and,[89] as discussed previously, the IGF axis has been implicated in the pathogenesis of AMD, our finding of an interaction between BMI and genetic polymorphism of *IGF1R* needs further confirmation and a mechanistic link underlying the interrelationship between obesity, the IGF axis, and AMD pathogenesis warrants further study.

There are several limitations in this study. First, to reduce cost, we used a candidate gene approach and subjectively selected 25 SNPs from 7 IGF axis genes for study. The coverage and information of our SNP list highly depended on the data from the HapMap project. Furthermore, other types of genetic polymorphisms, such as copy number variants,[90] may be responsible for the underlying association with AMD risk. Therefore, false-negative findings are possible. On the other hand, because of the small sample size for geographic atrophy (Table 5), false-positive findings are also possible. In addition, we used Bonferroni's correction for our hypothesis testing on the 25 SNPs. Furthermore, multiple tests were performed for each SNP. This may also increase the likelihood of false-positive findings. Although rs2872060 is implicated in AMD risk, no study has related it to major systemic diseases or mortality. However, polymorphisms in IGF axis genes are related to risk for some diseases and aging, and this may create bias. If the relationship with the other diseases is stronger and subjects with these diseases had excluded from this study, it may have create false associations with AMD or attenuated existing ones. This survival bias could be assessed by comparing disease risk or survival time between different IGF axis gene genotypes. Because the primary interest of the AREDS is eye diseases and data regarding mortality are not available, it is difficult to assess the potential survival bias. Furthermore, it was found that survival bias results in no more than a 20% effect size erosion in cohorts with a mean age of <75 years,[91] similar to our study sample. Finally, we included only white subjects in this study of the association between the 25 SNPs and advanced AMD. While previous genome-wide association studies (GWAS) in subsets of white participants from the AREDS showed inconsistencies in population stratification,[92,93] we did not find obvious population stratification in our study sample. Unlike in GWAS, which must consider the confounding effects of population stratification on the associations between diseases and SNPs from across the whole genome, in this study of the 25 candidate SNPs we are not interested in the overall population stratification. Instead, we are interested in systematic ancestry differences in allele frequencies in the 25 SNPs between cases and controls. Although PCA can be applied to assess population stratification in GWAS and candidate gene association studies as well, its power depends on both the number of SNPS and the sample size.[94] Based on the data from our advanced AMD case and controls ($n = 485$), we may have had inadequate power for detecting the underlying population stratification.

In summary, the findings in this study support an association between the IGF axis and risk for AMD in the AREDS cohort. More extensive screens of IGF axis genes, preferably in other cohorts, are needed to confirm the association. Elucidating the biochemical mechanisms by which the IGF axis affect AMD risk is also warranted. These insights will be helpful in designing intervention strategies and furthering our understanding of the underlying pathogenesis and would inform us about the designs of new therapeutics for AMD.

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