Multiple VEGF-A Variants Linked to Severe DR in Patients With Type 1 Diabetes

Addressing the mechanisms by which VEGF-A variants exert effects on diabetic retinopathy should be a priority for further research.

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As described in detail in numerous previous publications, The Diabetes Control and Complications Trial (DCCT)[1] established the salutary effects of intensive insulin treatment on the development and progression of diabetic retinopathy (DR), neuropathy, and nephropathy in patients with type 1 diabetes. The DCCT cohort continues to be followed in the Epidemiology of Diabetes Interventions and Complications (EDIC), a long-term observational study.[2]

We sought to determine if any common variants in the gene for vascular endothelial growth factor (VEGF-A) are associated with long-term renal and retinal complications in diabetes.[3]

A total of 1,369 white patients with type 1 diabetes from the DCCT/EDIC Study had an average of 17 retinal photographs and 10 renal measures over 15 years.[4] Among the patients in the DCCT/EDIC, our group studied 18 single nucleotide polymorphisms (SNP) in VEGF-A that represent all common sets of genetic variations across the gene and tested them for association with time to development of severe DR, DR progression of three or more steps, clinically significant macular edema, persistent microalbuminuria, and severe nephropathy.

GLOBAL MULTI-SNP TEST

We found in a global multi-SNP test, there was a highly significant association of VEGF-A SNPs with severe retinopathy (P=6.8 X 10^{-5}). The other four outcomes we found to be nonsignificant. In survival analyses controlling for covariate risk factors, eight SNPs showed significant association with severe retinopathy (P<.05). The most significant single SNP was rs3025021 (hazard ratio, 1.43, 95% confidence interval [1.16-1.76], P=8.6 X 10^{-4}). Significant excess transmission of the T allele of rs3025021 to probands with severe DR was also observed in family-based analysis (P=.013), making it unlikely that the result in the probands was due to confounding by population stratification.

Backward selection of 15 markers, and allowing for pairwise interactions, selected six SNPs that are independently significant in the model (P<.05), suggesting that multiple variants in VEGF-A are associated with severe DR. These data demonstrate the genetic involvement of VEGF-A in predisposition to the development of severe retinopathy type 1 diabetes.

BACKGROUND

DR affects most patients who have diabetes >15 years. The risk factors for DR are well known, and they include poor glycemic control (as measured by A1C), longer diabetes duration, earlier age at diagnosis, higher waist-to-hip ratio, fasting triglyceride, body mass index, blood pressure, and serum/plasma fibrinogen. DR's essential features include capillary microaneurysms and increased vascular permeability leading to macular edema, vascular occlusion and ischemia, neovascularization, and contraction of fibrovascular proliferation in the vitreous.

VEGF-A is a mitogen that targets endothelial cells and has effects that include mediating increased vascular permeability and inducing angiogenesis, cell growth, migration, and inhibition of apoptosis, all of which have been well described

Retinal cells that produce VEGF-A include pigment epithelial cells, capillary pericytes, endothelial cells, Müller

cells, ganglion cells, and glial cells. Hypoxia increases VEGF-A expression in retinal cells by three- to 30-fold. VEGF-A is significantly upregulated in diabetic retinopathy, particularly in retinal pigmented epithelial cells, glial cells, and vitreal fibroblasts. Higher plasma and vitreous VEGF-A levels can be found in patients with proliferative DR when compared with nondiabetic control individuals or with individuals with nonocular diseases.

GENETIC CROSS-SECTIONAL STUDIES

Several cross-sectional studies have investigated the genetic involvement of VEGF-A in DR. Three studies tested the SNP rs833061 (also known as the promoter SNP −460) for association with DR in patients with type 2 diabetes. One observed an association with the C allele and the C/C genotype, another found association with the C/T genotype, whereas the third study found no association.

Two studies investigated the association of an 18-bp deletion also in the promoter with diabetic retinopathy in type 1 and type 2 diabetes but produced conflicting results. All of these studies used a case-control design with various phenotype definitions and investigated only markers in the promoter region in relatively small sample sizes of Japanese type 2 diabetic patients or whites with a mix of type 1 and type 2 diabetes. Any available covariates were measured at a single time point when the phenotype was also determined.

IMPLICATIONS

We examined the association between VEGF-A SNPs and time-to-event for three retinal and two renal outcomes in the DCCT/EDIC cohort. Our approach, capturing associations due to the most common variations in VEGF-A and taking into account factors known to be associated with the development of complications, particularly long-term measures of glycemic control, is more comprehensive than previous studies of DR that examined a single or few variations in only the promoter region of VEGF-A.

Our use of tagSNPs, SNPs which act as proxies for a number of other highly correlated SNPs, facilitates detection of association between polymorphic variation in and around the gene with relative efficiency in genotyping. This strategy has been made possible by the data generated by the International Haplotype Map (HapMap) project.[5] We note the importance of including known risk factors in analyses aiming to identify gene effects. Single SNP analyses identified eight SNPs that were significantly associated with severe retinopathy in the multivariate analyses ($P<.05$), while only three of these were significant in the univariate analysis, underscoring the importance of inclusion of measured covariates.

In conclusion, our study extends previous reports in several respects. The retinal phenotypes in the DCCT/EDIC Study have been measured 17 times over 15 years, leading to a more precise measure of when the complication arises. A strength of prospective studies such as DCCT/EDIC is the reliability of outcome and covariates measured repeatedly over an extended period of time. In contrast to case-control studies, this allows inclusion of important covariates measured before development of the outcome. All known risk factors for retinopathy were measured and included in the multivariate time-to-event analysis.

As expected, longitudinal measures of A1C over the period at risk had a strong relationship with the development of severe DR. This is in contrast to cross-sectional studies that typically only have a single measure at the time of observation. Moreover, unlike previous studies that focused only on variants in the promoter region, we investigated SNPs that cover the entire gene. Under the assumption of prior evidence for association with VEGF-A, the current study provides strong evidence that more than one SNP in VEGF-A is independently associated with the risk of developing severe DR in patients with type 1 diabetes. This suggests that allelic heterogeneity is operating through one or more pathological mechanisms or that an as yet unidentified variant is underlying the observed association.

The generalizability of these findings must be interpreted with some caution, however, given the extensive inclusion and exclusion criteria of the original DCCT Study, specifically the exclusion of severe DR at DCCT baseline. These findings add to the growing literature concerning the important role that VEGF-A plays in this diabetes complication. Addressing the mechanisms by which VEGF-A variants exert effects on DR should be a priority for further research. ■

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