Angiotensin-converting enzyme gene variants interact with the renin–angiotensin system pathway to confer risk and protection against type 2 diabetic retinopathy

The angiotensin-converting enzyme (ACE) gene has been found to be associated with pathogenesis and progression of diabetic retinopathy (DR). A recent meta-analysis comprising 2,224 Chinese patients showed moderate evidence of a relationship between the ACE insertion/deletion (I/D) polymorphism and proliferative diabetic retinopathy (PDR)\(^1\); however, the role of other renin–angiotensin system (RAS) polymorphisms and their possible interactions with ACE I/D genotypes are less clear.

Table 1 | Genotype frequencies of the renin–angiotensin system variants in type 2 diabetes patients (diabetes mellitus vs non-proliferative diabetic retinopathy vs proliferative diabetic retinopathy)

| Polymorphisms | Cohort 1 | Cohort 2 |
|---------------|----------|----------|
|               | DM (213) | NPDR (205) | PDR (92) | OR (95% CI) | *P*-value |
|               | DM (391) | NPDR (377) | PDR (168) | OR (95% CI) | *P*-value |
| AGT: rs699 (M235T) | MM = 85 (0.40) | MM = 109 (0.51) | MM = 88 (0.43) | 1.87 (1.18-2.96) | 0.002 |
|               | MT = 105 (0.49) | MT = 105 (0.51) | MT = 94 (0.46) | 0.002 |
|               | TT = 23 (0.11) | TT = 23 (0.11) | TT = 18 (0.20) | 0.002 |
| ACE: rs13447447 (I/D) | MM = 164 (0.42) | MM = 164 (0.42) | MM = 155 (0.41) | 2.68 (1.45-4.93) | 0.002 |
|               | MT = 188 (0.48) | MT = 188 (0.48) | MT = 181 (0.48) | 0.002 |
|               | TT = 39 (0.10) | TT = 39 (0.10) | TT = 41 (0.11) | 0.002 |
| AGTR1: rs5186 (A1166C) | MM = 25 (0.27) | MM = 25 (0.25) | MM = 23 (0.25) | 3.75 (1.98-7.08) | <0.0001 |
|               | MT = 49 (0.53) | MT = 49 (0.53) | MT = 49 (0.53) | 3.75 |
|               | TT = 18 (0.11) | TT = 18 (0.11) | TT = 18 (0.11) | 3.75 |
|               | AA = 122 (0.57) | AA = 109 (0.51) | AA = 116 (0.57) | 3.11 (1.75-5.53) | 0.0001 |
|               | AC = 72 (0.34) | AC = 72 (0.34) | AC = 72 (0.34) | 0.0001 |
|               | CC = 19 (0.09) | CC = 19 (0.09) | CC = 19 (0.09) | 0.0001 |

Multivariate logistic regression was used to compute the odds ratio for developing diabetic retinopathy by adjusting for potential confounders, which include age, sex, glycated hemoglobin, duration of diabetes, smoking, systolic blood pressure and triglyceride levels, angiotensin converting enzyme inhibitor/angiotensin II type 1 receptor blocker, serum creatinine and estimated glomerular filtration rate. Genomic DNA was isolated from peripheral blood using the proteinase K chloroform-phenol method. Genotyping was carried out using allele-specific oligonucleotide polymerase chain reaction and polymerase chain reaction restriction fragment length polymorphism assays. The study was approved by the Postgraduate Institute of Medical Education and Research, Chandigarh ethics committee and written consent was obtained from participating subjects. Cohort 1 and 2 were two independently ascertained cohorts of North Indian origin, which cannot be merged. ACE, angiotensin-converting enzyme; AGT, angiotensigen; AGTR1, angiotensin II receptor type 1; DM, diabetes mellitus; NPDR, non-proliferative diabetic retinopathy; OR, odds ratio; PDR, proliferative diabetic retinopathy.

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clearly defined. Thus, we investigated the association and interaction between RAS gene polymorphisms and the development and progression of DR in 1,446 north Indian type 2 diabetic patients.

DR patients were divided into two groups, non-proliferative diabetic retinopathy (NPDR) and PDR. DR was diagnosed by dilating the pupils with mydriatics and then carefully examining the retina. Retinal photography or fluorescein angiography tests were also carried out.

A total of 1,720 type 2 diabetic patients were screened for the presence of DR, of which, 1,446 patients were finally included for the present study. Patients receiving antihypertensive drug treatment, with any retinopathy other than DR, cataracts and cataract surgery, and age-related macular degeneration were excluded from the study. All the participants in both the cohorts were age (≥35 years), sex and ethnicity matched.

Significant deviation from the Hardy-Weinberg equilibrium of genotype distribution in the present population in ACE I/D and angiotensinogen (AGT) variant rs5050 might be a result of the moderate population size or the rare allele, which can cause a random change in allele frequencies. Furthermore, we excluded the possibility of a typing error (logarithmic odds ratio score >0). The present sample size had the power of 90% at a small effect size (0.1) and alpha level (0.05). In both the cohorts, we observed a consistently higher prevalence and increased risk of PDR in patients with the DD genotype of ACE I/D, TT genotype of AGTrs699 and the CG genotype of angiotensin II receptor type 1 A1166C (Table 1).

The biological mechanism through which the ACE I/D polymorphism might be related to an increased risk of PDR is unclear. Serum ACE concentrations are significantly higher in those carrying the DD genotype. Also, the final component of RAS, which is converted by ACE, has a vasoconstrictive effect, promotes the accumulation of extracellular matrix and induces plasminogen activator inhibitor-1 production in endothelial cells. Furthermore, DR is associated with impaired balance of retinal RAS. Increased expression of ACE/AGT overcomes this imbalance and confers protection against DR.

The present results suggest RAS polymorphism as a significant risk factor for DR in Asian Indians.

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