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Letters

Comments

To: Rippin JD, Patel A, Belyaev ND, Gill GV, Barnett AH, Bain SC (2003) Nitric oxide synthase gene polymorphisms and diabetic nephropathy. Diabetologia 46:426–428

To the Editor: Endothelial dysfunction has been implicated in the development of diabetic nephropathy, primarily resulting from a decreased availability of vasodilator nitric oxide. Reduced nitric oxide concentrations could be attributed to its impaired synthesis or its increased inactivation in the diabetic state, the latter possibly as a result of quenching by advanced glycation endproducts [1]. Thus, genes involved in nitric oxide metabolism could be involved in conferring genetic susceptibility to this microvascular complication.

Recently, Rippin et al. [2] reported a case-control study that evaluated whether the a-deletion/b-insertion polymorphism located in intron 4 of the endothelial nitric oxide synthase (*eNOS*) gene was associated with diabetic nephropathy. This study was carried out on Caucasian patients with Type 1 diabetes residing in the United Kingdom, and diabetic nephropathy was diagnosed primarily on the basis of persistent proteinuria. No significant association was detected when the genotype distributions of patients (n=464) and control subjects (n=396) were compared. The authors thus suggested that conflicting results of modern genetic studies are related to a variety of issues including limited sample sizes, and an inadequate definition of the phenotype of interest.

In response to this report, we would like to draw attention to our own previous work published over 2 years ago, which had addressed the same hypothesis [3]. A population-based case-control study was also done in which the patients were Type 1 diabetic Caucasians with diabetic nephropathy as evidenced by persistent proteinuria (n=74), or who had developed end-stage renal disease (ESRD, n=78) because of this microvascular complication. The control subjects (n=195) were Type 1 diabetic patients who had remained normoalbuminuric despite having a long duration of diabetes (>15 years). Four polymorphisms were tested including the T-786C in the promoter, G894T (in codon 298), $(CA)_n$ dinucleotide repeat in intron 13, as well as the abovementioned a-deletion/b-insertion.

In comparing control subjects with the proteinuric patients, we note that the latest findings [2] have provided independent replication of our earlier results since we also did not find evidence to support any association between the a-deletion /b-insertion marker and the presence of proteinuria. Indeed, carriage of the a-deletion allele occurred at comparable frequencies among proteinuric patients as well as the control subjects from both studies.

However, the most salient finding from our previous study was the association between the a-deletion allele and the presence of ESRD, which was termed 'advanced diabetic nephropathy' [3]. Notably, carriers of this risk allele were 2.3 times more likely to have ESRD compared to non-carriers (p=0.003). In addition, we managed to confirm this result using the transmission disequilibrium test which is a family-based test for association [4]. In this analysis, the a-deletion was observed to be preferentially transmitted from heterozygous parents to their offspring with ESRD (65% vs 50% expected under the null hypothesis of no association, p=0.03). On the other hand, excess transmission of this risk allele from heterozygous parents to diabetic offspring with proteinuria was not detected (p=NS) [3].

Taken together, our findings which were obtained using two different but complementary approaches, clearly support a hypothesis that the a-deletion allele (or a marker in tight linkage disequilibrium with it) confers a significant risk of ESRD resulting from diabetic nephropathy. This genetic effect seems to manifest itself by influencing the progression from proteinuria to ESRD, rather than the appearance of proteinuria per se. As such, it is disappointing that Rippen et al. have elected not to pursue this important issue in their recent study.

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