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Commentary

Endothelial nitric oxide synthase (eNOS) variants in cardiovascular disease: pharmacogenomic implications

The DNA-based information provided by the Human Genome Project, International HapMap Project and the Human Variome project gave an impetus to understanding the nature and consequences of human genetic variations, specifically in conferring susceptibility to common multifactorial diseases[1-3]. Approximately 20 million sequence variants have been identified by extensive genotyping of common variations among ethnically diverse human populations. The most common human sequence variations are differences in individual base pairs, termed single nucleotide polymorphisms (SNPs). Researchers have discovered many associations between genetic variants in the relevant candidate genes and clinical outcome in diseases such as diabetes, hypertension and cancer[4,5]. Genome-wide association studies by the Wellcome Trust Case Control Consortium (WTCCC) highlighted the association of SNP markers on chromosome 9p21.3 with coronary artery disease[6]. This was the most significant association seen across the genome for this disease and has been independently replicated in different populations. A number of genetic association studies examined SNPs as these are considered as the primary genetic determinants of the inter-individual variability in susceptibility to disease, response to treatment (pharmacogenetics) and clinical outcomes[7-9].

Human cardiovascular disease (CVD) comprises a group of complex diseases which claim over ten million lives annually world wide[10,11]. CVD includes multifactorial heterogenous conditions such as hypertension, coronary heart disease, stroke, and congestive heart failure. Marked variation in CVD pattern and susceptibility is observed in different populations which can be attributed to genetic and environmental factors. Ethnic differences in responses to cardiovascular drugs such as β-blockers and angiotensin converting enzyme (ACE) inhibitors have been recognized for several decades, and in some cases ethnicity is used in drug therapy decision-making. Studies have reported differential effects of commonly used cardiovascular medications, such as lipid-lowering agents and antihypertensives based on individual variation. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement is well documented across a variety of ethnic populations. Genetic variation can influence both pharmacokinetic and pharmacodynamic mechanisms underlying variation in drug response. Using a candidate gene approach researchers have identified genetic polymorphisms that influence the pharmacodynamic determinants of antihypertensive response. Polymorphisms that have been shown to influence the BP response to diuretics have been identified in candidate genes encoding alpha-adducin (ADD1), subunits of G-proteins (GNB3 and GNAS1), endothelial nitric oxide synthase (eNOS), and ACE[12-14].

Vascular endothelial dysfunction is a common characteristic of various cardiovascular diseases. The maintenance of regular vascular tone substantially depends on the bioavailability of endothelium-derived nitric oxide (NO) synthesized by eNOS. The essential role of NO, as the elusive endothelium-derived relaxing factor (EDRF), was the topic of research that won the 1998 Nobel Prize in Physiology or Medicine[15-17]. The *eNOS* gene, as a candidate gene in the investigations on hypertension genetics, has attracted the attention of several researchers because of the established role of NO in vascular homeostasis. The *eNOS* variants located in the 7q35-q36 region have been investigated for their association with CVD, particularly hypertension[18,19]. Considering the functional roles, relatively high

occurrence of minor alleles in different ethnic groups and clinical relevance, three variants, *viz.*, (*i*) G894T substitution in exon 7 resulting in a Glu to Asp substitution at codon 298 (rs1799983), (*ii*) an insertion-deletion in intron 4 (4a/b) consisting of two alleles (the a*-deletion which has four tandem 27-bp repeats and the b*-insertion having five repeats), and (*iii*) a T786C substitution in the promoter region (rs2070744), have been extensively studied[20-22]. Individual SNPs often cause only a modest change in the resulting gene expression or function. It is, therefore, the concurrent presence of a number of SNPs or haplotypes within a defined region of the chromosome that determines susceptibility to disease development and progression, particularly in case of polygenic diseases[23].

In the current issue, Shankarishan *et al*[24] analysed for the first time the prevalence of eNOS exon 7 Glu298Asp polymorphism in tea garden community of North Eastern India, who are a high risk group for CVD. This study also included indigenous Assamese population and found no significant difference between the distribution patterns of *eNOS* exon *7* Glu298Asp variants between the communities. They have rightly mentioned that for developing public health policies and programmes it is necessary to know the prevalence and distribution of the candidate genes in the population, as well as trends in different population groups. They have also observed that the *eNOS* exon *7* homozygous GG wild genotype (75.8%) was predominant in the study population followed by heterozygous GT genotype (21.5%) and homozygous TT genotype (2.7%). The frequency distribution of the homozygous GG, heterozygous GT and homozygous mutant TT genotypes were comparable to that of the north Indian and south Indian population. However, the prevalence of GG genotype was found to be low in the study population when compared with other Asian populations. The prevalence of GT genotype was highest among the Italian population (51.8%) and the lowest among the Chinese population (8.3%) and the 'T' allele frequency was found to be relatively higher among the Caucasians and lower among Asian populations[24]. Although this polymorphism is associated with several diseases, indicating its impact on eNOS function and subsequent NO production, its functional significance remains to be better understood. As glutamate and aspartate are conservative replacements, this polymorphism is thought to be a marker for a functional locus elsewhere in the gene. Moreover, there is no consensus on impact of this polymorphism on the enzyme function. Some studies have showed that the Asp variant is more susceptible to inactivation by proteolytic cleavage, possibly due to a tighter turn of the alpha helix, suggesting that homozygosity for this variant may result in impaired catalytic function of eNOS[25]. Conversely, some studies obtained no evidence for altered enzyme function associated with this polymorphism[26]. Therefore, the haplotype analysis considering other polymorphisms within the gene will give better understanding on the implication of this gene as a marker for cardiovascular diseases[27].

Polymorphisms in the endothelial nitric oxide synthase gene have been associated inconsistently with cardiovascular diseases. Varying distribution of *eNOS* variants among ethnic groups may explain inter-ethnic differences in nitric oxide mediated vasodilation and response to drugs[28]. Different population studies showed association of *eNOS* polymorphisms with variations in NO formation and response to drugs[29,30]. Cardiovascular drugs including statins increase eNOS expression and upregulate NO formation and this effect may be responsible for protective, pleiotropic effects produced by statins[31]. With respect to hypertension, studies have reported interactions between diuretics and polymorphisms in *eNOS* gene. Particularly, the Glu298Asp polymorphism made a statistically significant contribution to predicting blood pressure response to diuretics[32,33]. Further progress in this line of research may lead to development of cardiovascular drugs that provide individual based treatment for each patient and the disease mechanism that applies to that patient rather than on the basis of symptoms or in a population based manner.

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