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Review Article

Role of endothelial nitric oxide synthase gene in vascular diseases

M.S. Akhter[a], Arijit Biswas[b] , Renu Saxena[*]

[a] *Department of Hematology, All India Institute of Medical Sciences, New Delhi-110029*
[b]*Institute of Hematology and Transfusion Medicine, Bonn, Germany*

Abstract. The endothelial nitric oxide synthase (eNOS or NOS 3) is expressed in the endothelium where it produces Nitric Oxide (NO) from L-arginine. NO a powerful short lived vascular substance, plays a key role to maintain vascular homeostasis. Association of endothelial nitric oxide synthase gene variants and nitric oxide has been found in many vascular diseases chiefly stroke, coronary artery disease and hypertension. The variants have been associated with low plasma nitric oxide concentrations and reduced vascular reactivity, however difficulties in measuring those phenotypes implicates that their functions remain unclear. While a large no of studies report the association of NOS3 gene with vascular diseases, a few are contradictory to such reports. So a need for large scale genetic association studies using tagging polymorphisms to confirm or refute the role of NOS3 gene in vascular diseases is strongly warranted.

Key words: Vascular diseaser, eadotelial nitric oxide

1. Introduction

Endothelial nitric oxide gene has a pivotal role in the maintenance of vascular homeostasis because of its ability to generate nitric oxide (NO) and this feature of the gene makes it a logical candidate gene for vascular diseases. Nitric oxide (NO) is a short lived vasoactive substance of prime importance constitutively produced from L-arginine by the enzyme nitric oxide synthase (NOS). Three isoforms of NOS have been identified and described as inducible nitric oxide (iNOS), neuronal nitric oxide (nNOS) and endothelial nitric oxide (eNOS) (1). An unstimulated state of the cell may not lead to the expression of the inducible NOS but any abnormal processes in cells, such as infection and inflammation will lead to the induction of iNOS leading to high NO production. The neuronal NOS is expressed in neurons and skeletal muscles where it is responsible for the physiological production of NO in these tissues. The data to support the relevance of nNOS with vascular diseases is relatively scarce (2).The eNOS occurs primarily in the endothelium and at low levels in calmodulin and platelets (3). The eNOS gene was first cloned in 1993 and was localized to chromosome 7q35-36. The eNOS gene comprises of 26 exons that encode a 135- KD protein containing 1,203 amino acids (4). eNOS produces NO which has a cogent vasodialating action and plays a key role in the regulation of blood pressure (5). Besides NO produced by eNOS is known to cause the inhibition of the adhesion of platelets and leukocytes to the endothelium, reduction in the vascular smooth muscle cell migration/proliferation and limiting the oxidation of atherogenic low density lipoproteins (6). An accelerated atherosclerosis in animal model has been attributed to inhibition of eNOS while atherosclerosis in humans was reported with abnormality in the endothelial NO pathway (7, 8).There is evidence suggesting the development of venous diseases with decreased NO production (9). Several features characteristic of small vessel diseases including cerebral hypo perfusion, (10) impaired cerebral auto regulation endothelial damage with breakdown of the blood brain barrier and vessel remodeling (11) are known to occur with a lack of endothelium derived NO.

[*]Correspondence: Dr. Renu Saxena, MD,
Professor & Head Department of Hematology, first floor,
Room No. 151, IRCH Building,
All India Institute of Medical Sciences
AIIMS, New Delhi-110029 India
Tel-0091-11-26494670
E.mail- renusax@hotmail.com

2. Molecular genetics of nitric oxide synthase

Marsden et al. (4) isolated genomic clones encoding human eNOS and determined the structural organization of the gene. The entire gene comprises of 26 exons, spans approximately 21 kb of genomic DNA and encodes an mRNA of 4,052 nucleotides. Characterization of the 5-prime-flanking region indicated that the eNOS gene is TATA-less and exhibits proximal promoter elements consistent with a constitutively expressed gene, namely, SP1 and GATA motifs. Janssens et al. (12) isolated a cDNA encoding a human vascular NOS. The translated human protein was 1,294 amino acids long and shared 52% of its amino acid sequence with brain NOS. They showed that the cDNA encoded a calcium-regulated, constitutively expressed eNOS, capable of producing EDRF in blood vessels. Similarly Marsden et al. (13) cloned and sequenced human eNOS. Their cDNA clones predicted a protein of 1,203 amino acids with about 60% identity with the rat brain NO synthase isoform. NOS have been assigned to 2 classes: a constitutively expressed, calcium-regulated class identified in brain, neutrophils, and endothelial cells, and a calcium-independent class identified in endotoxin- or cytokine-induced macrophages and endothelial cells.

3. SNPs in the eNOS gene

With eNOS gene having 26 exons spanning about more than 21kb of the genome the eNOS gene has been the focus of intensive research so as to see for potentially functional polymorphisms or mutations which may have an influence on its expression or activity. Till date more than 100 polymorphisms have been identified within the gene itself or in its vicinity (NCBI SNP database, http//www.ncbi.nlm.nih.gov/SNP/). In the promoter region more than 15 polymorphisms are known to exist and amongst these, three polymorphism -1468T /A, -922 A /G and -786C/T are found more abundantly in different populations which may have an influence on mRNA transcription and reduce the gene expression. The –786T/C promoter polymorphism is reported to influence transcriptional activity in vitro in a luciferase/reporter assay system and has been associated with coronary arterial spasm in Japanese subjects (14). The –786T/C polymorphism was however neither shown consistently associated with functional measures nor with clinical disease end points. A recent meta-analysis of studies involving 4882 cases and 9366 controls provided marginal evidence of increased risk among CC subjects (odds ratio 1.30, 95% confidence interval (CI) 1.01 to 1.66; p = 0.04) (15). It has been suggested that sequences extending only to -144 bp are essential for promoter activity, although the region between -3500 bp and -1193 bp is found to down-regulate eNOS expression in endothelial cells. Transcriptional factors including Ets-1, Sp1, Sp3, MAZ, and Elf-1 appear to regulate eNOS promoter activity either positively or negatively (16).

Within the gene SNPs in intron 2, IVS2 1 42G > A (G561A); intron 11, IVS11 + 174A > G (A3185G); intron 12, IVS12 + 52G > T 242 (G3411T); intron 18, IVS18 + 27A > C (A27C); intron 22, IVS22 +15A >G (A6007G); and intron 23, IVS23 + 11G >T (G6247T) are known to occur (17). Introns 2 and 8 (32-bp repeats), intron 4 (27-bp repeat), and intron 13, IVS13 + 81(CA) 17- 44 are known to have tandem repeats (4, 18). Some of these variants are common and vary in different ethnic populations (19). No regulational significance has been assigned to these introns : polymorphisms in exons, including 5557G >T (894G/T) in exon 7 (E298D, a Glu to Asp change) and 5172C/T in exon 6.(17,20) A Polymorphism occurring within the coding region of the eNOS gene is known to alter NOS enzymatic activity. The only common polymorphism identified thus far that encodes an amino acid substitution—Glu298Asp (glutamate to aspartate at position 298) is eNOS polymorphisms (894G/T) within exon 7 and is one of the most studied polymorphism occurring in eNOS (21). A debate revolves as to whether this polymorphism is functional or not as two studies have shown that eNOS Asp298 is subjected to selective proteolytic cleavage in endothelial cells and vascular tissues that might account for reduced vascular NO generation (22). While, other studies suggest that this finding might be artefactual (23). A meta-analysis of 6372 cases and 6591 controls identified an odds ratio for ischemic heart disease of 1.33 (95% CI 1.15 to 1.54; p = 0.0001) amongst individuals homozygous for eNOS Asp29819 (24). Considering such inconsistencies, it is required that additional molecular studies are carried out, as are very large scale genetic association studies of endothelial function or clinical outcomes, so as

to exclude the possibility of inconsistent results of small studies .

4. Association between NOS 3 polymorphisms and vascular diseases

4.1. Nitric oxide synthase and stroke

Stroke was observed as a consequence of impaired endothelial dysfunction in studies with stroke-prone SHR rat and was identified as an important predisposing factor leading to stroke (25). Studies with knockout mice deficient in eNOS have revealed that such mice are highly sensitive to focal cerebral ischaemia (26) and have marked vessel wall abnormalities (27). Increased risk of significant coronary artery disease and myocardial infarction in smokers was attributed to a functional variant of nitric oxide synthase (eNOS 4a) in a study by (Wang et al.) (28). Two promoter SNPs -922A/G and -786C/T were found significantly associated with stroke in young black women but not the white populations (E). This association was attributable to an increased prevalence of the -922A allele (OR= 3.0 95%CI = 1.3to 6.8 p=0.005) and the -786C allele (OR= 2.9, 95% CI 1.3 to 6.4, P=0.005) in cases versus controls (29). The intron 4ab insertion/deletion genotype was associated with isolated lacunar infraction. Protective effect of the 4a variant could be mediated through changes in eNOS promoter activity and increased NO levels has been suggested through Haplotype and functional studies (30). Another study by Akar et al., (31**)** of a Turkish population has shown that carriers of the minor 'a' allele of intron 4 VNTR had significantly elevated risk for stroke (a). An over representation of 4c allele of intron 4 VNTR in ischemic stroke patients was suggested in a study involving African American population. In pooled analysis of all patients, intron 4c, but not intron 4a, intron 4b, or 894G/T alleles are associated with stroke (P<0.01). In subgroup analysis by race, the intron 4c allele is most strongly associated with large artery ischemic stroke in African Americans. (P<0.01). (32). The association of SNP in exon 7 with stroke was seen only in the study of Hoffmann et al.,(33), while other studies indicated negative finding in white population. Further more Markus et al., (34) also measured carotid stiffness in stroke patients and found it was unrelated to the exon 7 variant.

Patients with lacunar infarction have generalized endothelial dysfunction was confirmed in a study with diminished Flow-mediated dilatation but normal nitroglycerin response. Intron 4aa genotype of eNOS gene seems to be protective for isolated lacunar infarction and the effect was potentiate by the absence of -786C/T polymorphism in any allele of the promoter region (35).

5. Coronary artery disease (CAD)

Yoon et al (36) reported that plasma NOx in CAD patients with hypertension was increased, however plasma NOx in CAD patients without hypertension did not differ significantly from that of the controls. They also found an association of the G-allele frequency of the polymorphism G10-T in intron 23 in the CAD group than in the controls. A study with smokers by Wang et al., (28) detected an association between homozygosity for the 4a allele in the intron 4 VNTR polymorphism of the eNOS gene and an increased risk of CAD in current and ex- smokers in the Australian population. The 894G/T polymorphism in exon 7 of the NOS 3 gene has been reported to be a strong risk factor for CAD (37) . The risk of developing CAD was 2.5 fold higher for 786C homozygotes with respect to individuals who were homozygous for the 786T allele of -786C/T polymorphism of eNOS gene and this result was conformed by multivariate analysis which demonstrated that this association was independent of other factor possibly related to CAD risk (38). A decreased promoter activity approximately by 50% by the -786C/T polymorphism was reported by Nakayama et al., (14) suggesting that in many carriers of the mutant allele, the L-arginine/NO pathway does not function properly, leading to endothelial dysfunction . A study involving Tamalian Indian CAD patients showed no significant association between the 894G/T polymorphism and CAD in this population group (39) eNOS gene transfection is a valuable approach to augment angiogenic properties of ex vivo expanded endothelial progenitor cells and eNOS-modified endothelial progenitor cells may offer significant advantages than endothelial progenitor cells alone in terms of their clinical use in patients with myocardial ischemia (40).

6. Hypertension

The role of eNOS polymorphisms in relation to hypertension has also been studied extensively since NO controls vascular tone and regulates arterial wall remodeling, and both of these action are relevant to hypertension. Uwabo et al., (41) reported a strong association between the 27-bp repeat polymorphism in intron 4 and essential hypertension among Japanese population. While three (33,42,) studies found that the 894G /T

variant in exon 7 was related to hypertension in both Japanese and white populations, the studies by Benjafield and Morris in an Australian white population (43), and Pulkkinen et al., in a Finnish population (44) reported a negative relationship. Nakayama et al., (45) also reported a significant association between the (CA) repeat polymorphism in intron 13 and hypertension.

7. Conclusion

The candidature of eNOS is strongly recommended as a gene for maintaining the integrity of the arterial wall and the pathological development of atherosclerosis. A number of studies have supported that polymorphisms occurring in the eNOS gene can be predisposing factors for the development of various vascular diseases. Still studies with large populations are required to be performed so as to minimize the errors occurring out of small sample size.

Altered eNOS expressions occurring due to the functional DNA variants in eNOS gene can be modified by the environmental factors, such as cigarette smoking. It is further required that the modifications of expressions of the eNOS gene be studied in relation to other stress developing environmental factors, so as to provide the medical fraternity with interventional strategies.

An exhaustive research into the eNOS gene has to be undertaken in order to provide an insight into the factors both genetic and environmental that are leading to the development of vascular diseases with an aim to develop therapeutic strategies to combat such disorders.

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