A FUNCTIONAL POLYMORPHIC VARIANT OF DDAH2 IN A BASAL PROMOTER ELEMENT

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Synthesis of the vasodilator nitric oxide (NO) can be inhibited by the endogenously produced methylarginines monomethyl L-arginine (L-NMMA) and asymmetric dimethylarginine (ADMA). Cellular L-NMMA and ADMA levels are determined in part by the activity of dimethylarginine dimethylaminohydrolase (DDAH) enzymes DDAH1 and DDAH2.

We analysed the DDAH2 promoter to identify mechanisms of transcriptional regulation. A luciferase promoter/reporter assay was used to measure DDAH2 promoter activity in ECV 304 cells. 5' deletions of the promoter and other constructs were made by PCR. Constructs were cotransfected with a β-galactosidase expression vector and luciferase activity was normalised to β-galactosidase activity. We also screened the DDAH2 promoter for common genetic polymorphisms by single-strand conformational polymorphism analysis. Two polymorphisms were identified and investigated using promoter/reporter constructs carrying the different alleles for each variant in primary human umbilical vein endothelial cells (HUVECs).

5' deletions of the promoter identified a 270-bp region that was required for basal transcription. The activity of this isolated region was 2.24±0.25-fold greater (n=16, $P<0.01$) than that of the full promoter construct. Site-specific deletions of Sp1 and IRF-1 binding sites in this basal region reduced transcriptional activity by 64.2±7.7% and 74.9±2.2% (n=16, $P<0.01$), respectively, suggesting important functional roles for these binding sites. In ECV 304 cells and in primary HUVECs, a 6/7G insertion/deletion polymorphism at –871 (relative to translation start site) was functional. The 7G variant gave 1.54±0.16-fold higher activity (n=12, $P<0.05$) than the 6G variant.

These studies have therefore identified a region within the DDAH2 promoter that is required for basal promoter activity and specific binding sites within this region that are likely to drive this activity. Furthermore, a single nucleotide polymorphism within this region is capable of enhancing promoter activity. Since DDAH expression may be modified by drug treatments, these data provide a basis for further genotype-phenotype studies in conditions or drug treatments in which altered ADMA levels have been reported.