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# DDAH gene and cardiovascular risk

Veli-Pekka Valkonen<sup>a</sup>, Tomi-Pekka Tuomainen<sup>a</sup> and Reijo Laaksonen<sup>b</sup>

**Abstract:** The crucial role of nitric oxide (NO) for normal endothelial function is well known. In many conditions associated with increased risk of cardiovascular diseases such as hypercholesterolemia, hypertension, abdominal obesity, diabetes and smoking, NO biosynthesis is dysregulated, leading to endothelial dysfunction. The growing evidence from animal and human studies indicates that endogenous inhibitors of endothelial NO synthase such as asymmetric dimethylarginine (ADMA) and *N*<sup>G</sup>-monomethyl-L-arginine (L-NMMA) are associated with the endothelial dysfunction and potentially regulate NO synthase. The major route of elimination of ADMA is metabolism by the enzymes dimethylarginine dimethylaminohydrolase-1 and -2 (DDAH). In our recent study 16 men with either low or high plasma ADMA concentrations were screened to identify DDAH polymorphisms that could potentially be associated with increased susceptibility to cardiovascular diseases. In that study a novel functional mutation of DDAH-1 was identified; the mutation carriers had a significantly elevated risk for cardiovascular disease and a tendency to develop hypertension. These results confirmed the clinical role of DDAH enzymes in ADMA metabolism. Furthermore, it is possible that more common variants of DDAH genes contribute more widely to increased cardiovascular risk.

**Key words:** ADMA; DDAH; cardiovascular disease; gene mutation

## Introduction

The development of atherosclerosis is associated with endothelial dysfunction, which is linked closely to various other pathological conditions that predispose to atherosclerosis, including hypercholesterolemia,<sup>1,2</sup> hypertension,<sup>3</sup> types 1 and 2 diabetes,<sup>4,5</sup> hyperhomocyst(e)inemia,<sup>6</sup> chronic renal failure,<sup>7</sup> obesity (abdominal),<sup>5</sup> infection,<sup>8</sup> aging<sup>9</sup> and smoking.<sup>10</sup> A major cause of the endothelial dysfunction is decreased bioavailability of nitric oxide (NO), a potent biological vasodilator synthesized in vascular endothelium from L-arginine by the action of endothelial NO synthase (eNOS). Endothelium-derived NO maintains vascular/endothelial functions in various ways. NO is a critical mediator in vasodilation and antithrombotic processes, as well as in growth inhibition and inflammation.

Asymmetric dimethylarginine (ADMA) is one of three known endogenously produced circulating methylarginines (i.e. ADMA, *N*<sup>G</sup>-monomethyl-L-argi-

nine (L-NMMA) and symmetrically methylated *N*<sup>G</sup>, *N*'<sup>G</sup>-dimethyl-L-arginine). ADMA is formed by the action of protein arginine methyltransferases that methylate arginine residues in proteins and after which free ADMA is released.<sup>11,12</sup> ADMA and L-NMMA can competitively inhibit NO elaboration by displacing L-arginine from NO synthase (NOS).<sup>13,14</sup> The amount of methylarginines is related to overall metabolic activity and the protein turnover rate of cells. Although methylarginines are excreted partly by the kidneys,<sup>13</sup> the major route of elimination of ADMA in humans is metabolism by the dimethylarginine dimethylaminohydrolase (DDAH) enzymes.<sup>15–17</sup> Inhibition of DDAH leads to the accumulation of ADMA<sup>16,18,19</sup> and consequently to inhibition of NO-mediated endothelium-dependent relaxation of blood vessels.

## ADMA and cardiovascular diseases

Several studies have shown that elevated ADMA levels are present in patients with different types of coronary heart disease. The potential role of ADMA in angina pectoris has been evaluated by Piatti and co-workers, who reported ADMA levels to be higher in patients with cardiac syndrome X (angina pectoris with normal coronary arteriograms) than in controls.<sup>20</sup> According to Japanese data in patients with vasospastic angina there is an increased ADMA concentration

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that relates to a decrease in NO concentrations in the coronary circulation.<sup>21</sup> Together with other evidence<sup>22,23</sup> showing the endothelial dysfunction to be one essential phenomenon in the development of clinically significant coronary artery disease, it seems likely that ADMA could be a potent marker for cardiovascular risk.

In the results of a few prospective studies there has been a clear indication that a raised ADMA level is related to increased cardiovascular events in patients with end-stage renal disease<sup>24</sup> and in previously healthy middle-aged men.<sup>25</sup> According to preliminary results from the CARDIAC (Coronary Artery Risk Determination investigating the influence of ADMA Concentration) study, patients with coronary heart disease ( $n = 816$ ) had a higher median ADMA plasma concentration than age and sex matched controls (median 0.91 vs. 0.70  $\mu\text{mol/l}$ ;  $p < 0.0001$ ).<sup>26</sup> It is interesting that, in a prospective Chinese study, a high plasma ADMA level independently predicted subsequent cardiovascular adverse events (cardiovascular death, myocardial infarction, and repeated revascularization of a target vessel).<sup>27</sup>

### DDAH and cardiovascular diseases

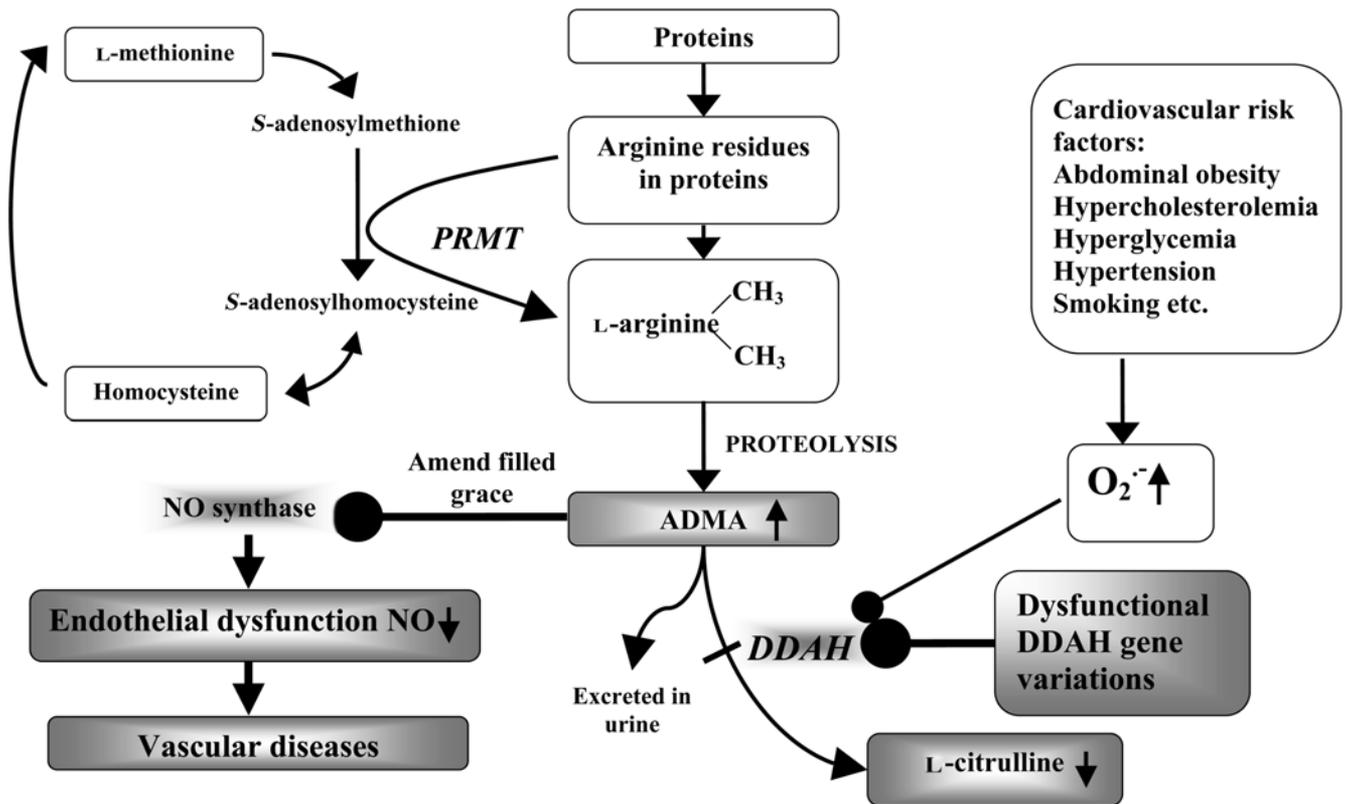
There are only few published findings concerning variations in human DDAH. However, polymorphisms in other genes potentially related to risk factors for endothelial dysfunction and cardiovascular events have been studied. Reduced NO synthesis has been implicated in the development of atherosclerosis.<sup>28</sup> For example, there are some functionally important variants of the NOS3<sup>29,30</sup> that could affect individual vulnerability to atherosclerosis by changing the amount of NO generated by the endothelium.<sup>31–34</sup> In a British population study, the relationship between G3T polymorphism in exon 7 of the NOS3 gene variant and atherosclerotic coronary artery disease was investigated using two independent case-controlled studies.<sup>34</sup> The authors reported that homozygosity for a common NOS3 polymorphism (894 G3T) that encodes a Glu298Asp amino acid substitution in eNOS is a risk factor for angiographic coronary artery disease and recent myocardial infarction. In another British study, this NOS3 gene variant did not influence significantly the level of plasma NO end products (nitrate+nitrite) or the risk of ischaemic heart disease in middle-aged British men.<sup>35</sup>

There have been some animal and human studies in which a critical role for DDAH activity in regulating NO synthesis *in vivo* was demonstrated. In one mouse model, overexpression of DDAH-1 did increase NOS activity *in vitro* and *in vivo*.<sup>36</sup> In the same study, the human DDAH-1 transgenic animals exhibited reduced systolic blood pressure, systemic vascular resistance and cardiac stroke volume. Further evidence that the

metabolism of endogenous ADMA and DDAH plays an important role in the regulation of NOS activity was shown in a human study. Vallance and his group found a functional insertion/deletion polymorphism variant in the DDAH-2 enzyme among unrelated individuals.<sup>37</sup> A variant in the core promoter region was associated with increased basal DDAH-2 expression. The prevalence of the variant was about 1% in the population studied. These studies show that there could be functional variants in DDAH genes and that, when other population samples are studied, it is likely that new functional polymorphisms in genes coding DDAH enzymes will be found, and that these variations in expression or activity of the DDAH enzymes may affect the risk of vascular events (Figure 1). This hypothesis was tested in our recent population study in 1609 middle-aged Finnish men, who were participants in the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD), which is an ongoing population-based study designed to investigate risk factors for cardiovascular diseases, type 2 diabetes and related outcomes in men from eastern Finland.<sup>23</sup> In this study we identified several variants of DDAH. One variant was found in the DDAH-2 and six variants were observed in the DDAH-1 gene. The identified gene variants were, however, very rare in our study population. We were able to confirm statistical analyses for one DDAH-1 variant. This mutation was found in 13 men. No homozygous mutation carriers were identified. In a logistic regression model adjusting for age, serum triglyceride, hypertension and smoking status, the occurrence of coronary heart disease was over 50-fold for mutation carriers versus noncarriers. In a risk factor (age, smoking status and body mass index) adjusted model, the mutation carriers had almost a five-fold prevalence of hypertension. Haplotype analysis gave similar results to the above mentioned single mutation analysis. All 13 DDAH mutation carriers were used as probands for a family study of 55 relatives (spouses and siblings). About 50% of the relatives of the probands were carriers of this mutation. The prevalence of hypertension was almost six-fold higher among the DDAH mutation carriers compared with the noncarriers. There were no significant differences between other cardiovascular diseases in the family study. Plasma ADMA levels were higher in the DDAH-1 mutation carriers compared with the noncarrier relatives.

### Conclusions

There are probably several functional variations in genes coding DDAH enzymes in different populations. Some of them could confer protection against the harmful effects of elevated ADMA and others impair enzyme function causing accumulation of ADMA in cytosol and/or blood. We have recently



**Figure 1** Possible pathways by which variants of the DDAH gene could affect ADMA degradation, increasing susceptibility to cardiovascular diseases.

found a rare variation in the DDAH-1 gene, which is associated with elevated plasma concentrations of ADMA in heterozygous mutation carriers. There was also an increased prevalence of CHD and a tendency to hypertension among individuals with this DDAH-1 mutation. These observations highlight the importance of ADMA as a possible risk factor and emphasize the essential role of DDAH in regulating ADMA levels.

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