Nitric Oxide Regulating Proteins as Biochemical and Genetic Markers of Coronary Artery Disease

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Abstract

Cardiovascular disease (CVD) remains the leading cause of death worldwide. Despite huge efforts and great advances in studying the genetic component of CVD, there is still a great need for exploring the genetic and environmental factors contributing to the development of this disease. Among these factors evolve modulation of nitric oxide (NO) homeostasis and oxidative stress as central players according to recent reports. A wide range of biochemical disturbances, including reduced bioavailability of NO and oxidative stress, has been shown to be associated with endothelial dysfunction (ED). Many studies described the contribution of ED in the predisposition of CVD, particularly coronary artery disease (CAD). Recent evidence indicates that ED may be genetically determined. This chapter points out to the key players that influence vascular NO levels and their role in the protection against and/or predisposition to CAD.

Keywords

Nitric oxide • SNP • Nitric oxide synthase • Coronary artery disease • DDAH • Paraoxonase • NADPH oxidase • Endothelin • Ox-LDL

Abbreviations

ADMA	Asymmetric dimethylarginine
AMI	Acute myocardial infarction
ARE	Arylesterase
CABG	Coronary artery bypass grafting
CVD	Cardiovascular disease
DDAH	Dimethylarginine dimethylaminohydrolase
ED	Endothelial dysfunction
EDN-1	Endothelin-1 gene
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
HDL	High-density lipoprotein
hs-CRP	High-sensitivity C-reactive protein
IHD	Ischemic heart disease
iNOS	Inducible nitric oxide synthase
LDL	Low-density lipoprotein
L-NMMA	Levo-N-monomethyl arginine
MI	Myocardial infarction
NADPH	Hydrogenated nicotinamide adenine dinucleotide phosphate
NHI	National Heart Institute
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
O_2^-	Superoxide radical
$ONOO^-$	Peroxynitrite
Ox-LDL	Oxidized low-density lipoprotein

PCI	Percutaneous coronary interventions
PCR	Polymerase chain reaction
PKC	Protein kinase C
PON	Paraoxonase
RFLP	Restriction fragment length polymorphism
ROSs	Reactive oxygen species
SDMA	Symmetric dimethylarginine
sGC	Soluble guanylate cyclase
SNP	Single nucleotide polymorphism
TG	Triacylglycerols
VSMCs	Vascular smooth muscle cells

Key Facts of Nitric Oxide

- Nitric oxide (NO) is the smallest, lightest molecule – and the first gas – known to act as a biological messenger in mammals.
- NO is identical to EDRF (endothelial-derived relaxing factor), well described in literature before the identification of NO.
- NO participates in the control of vascular tone as an antagonist of the adrenergic regulatory system.
- NO prevents atherosclerosis by vascular smooth muscle relaxation as well as inhibiting platelet aggregation, leukocytes migration and adhesion, and vascular smooth muscle proliferation.
- Intracellular NO production, from L-arginine, is catalyzed by several isoforms of an enzyme termed nitric oxide synthase (NOS, EC1.14.13.39)
- Three major NOS isoforms have been identified in humans: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS).
- The most obvious effector pathway for NO is activation of soluble guanylate cyclase (sGC).
- NO has dual roles in the human body; it has several physiological roles in cardiovascular, respiratory, GIT, and reproductive systems and in immunity, while overproduction of NO is incriminated in the pathogenesis of several conditions such as septic shock, epilepsy, tissue damage, inflammation, and nerve damage left by the stroke.
- NO reacts with reactive oxygen species to form toxic peroxynitrite anions ($ONOO^-$).
- Therapeutic strategies related to NO involve both increasing and decreasing NO.

Definitions

Acute A disease with a rapid onset and/or a short course, mostly presented in severe form. However, not all acute diseases or injuries are severe.

Allele One of a number of alternative forms of the same gene may be reflected in a different phenotype.

Asymmetric dimethylarginine (ADMA) Endogenous physiological inhibitor of nitric oxide synthase.

Cardiovascular disease A class of diseases involving the heart and blood vessels.

Coronary artery bypass grafting (CABG) Also known as heart bypass. It involves "open" heart operation which bypass stenotic arteries by grafting vessels from elsewhere in the body.

Also known as ischaemic heart disease (IHD) and coronary heart disease (CHD). It is a group of disease that includes stable angina, unstable angina, and myocardial infarction.

Genotype Genetic makeup of a cell, an organism, or an individual.

Genotyping Process of assessing the differences in genetic makeup by examining DNA sequence.

Myocardial infarction (MI) Death of heart cells as a result of coronary ischemia

A free radical. It acts in mammals, including human, as an important cellular signaling molecule involved in many physiological and pathological processes.

Nitric oxide synthase (NOS) Enzyme that uses L-arginine and molecular oxygen as substrates to produce NO and the amino acid L-citrulline.

Percutaneous coronary intervention (PCI) Commonly known as coronary angioplasty. It is a nonsurgical procedure used to treat narrowed coronary arteries by inserting a stent or deflated balloon to open the artery.

Polymorphism The occurrence of more than one form in the same population or species.

Single nucleotide polymorphism (SNP) A variation between individuals of a specific population at a single nucleotide in their DNA. By definition, it occurs at above 1 % of the population.

Introduction: Key Players in the Control of Vascular NO Levels

In the blood vessels, NO is produced from the endothelium mainly by the constitutively expressed endothelial nitric oxide synthase (eNOS), which is activated by shear stress of the flowing blood or agonists such as bradykinin and acetylcholine.

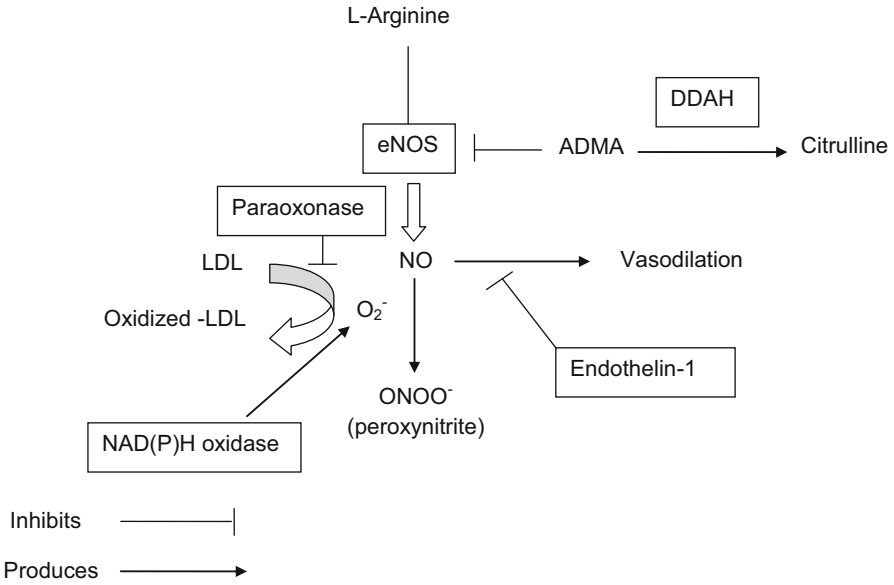

Fig. 1 Enzymes and proteins that control vascular NO levels. This figure comprises several enzymes and proteins that affect NO homeostasis including, asymmetric dimethylarginine (ADMA), oxidized LDL, paraoxonase activity, NAD(P)H oxidase activity, and dimethylarginine dimethylaminohydrolase (DDAH) activity. Dimethylarginine dimethylaminohydrolase (DDAH)

Besides its role as relaxing factor, NO protects blood vessels from thrombosis, by inhibiting platelet aggregation and adhesion. In addition, endothelial NO possesses multiple anti-atherosclerotic properties, which include (I) prevention of leukocyte adhesion to vascular endothelium and leukocyte migration into the vascular wall; (II) decreased endothelial permeability, reduced influx of lipoproteins into the vascular wall, and inhibition of low-density lipoprotein (LDL) oxidation; and (III) inhibition of DNA synthesis, mitogenesis, and proliferation of vascular smooth muscle cells. Reduced bioavailability of eNOS-derived NO or reduction of its biosynthesis markedly contributes to atherogenesis and thereby to MI (Jones and Hingorani 2005). The interrelationship and regulatory mechanisms that control vascular NO levels are quite complex. Several biochemical parameters and enzymes that contribute in these mechanisms are shown in Fig. 1.

In the early 1990s, an endogenous inhibitor to the nitric oxide synthase (NOS) pathway has been identified, namely, asymmetric dimethylarginine (ADMA). Accumulation of ADMA in the plasma of patients in several diseases, including CVD, reduces the release of endothelium-derived NO (Vallance et al. 1992a).

After uptake from the circulation, ADMA is degraded mainly by an intracellular enzyme termed dimethylarginine dimethylaminohydrolase (DDAH). DDAH degrades ADMA to citrulline and dimethylamine. Two isoforms of DDAH have been identified, DDAH-1 and DDAH-2, which regulate to a great extent the level of

ADMA in the blood and tissues. Thus, DDAH, through catabolism of ADMA, regulates the activity of NOS. Consequently, DDAH dysfunction may be a crucial unifying feature of increased cardiovascular risk. Lieper et al. have shown that loss of DDAH-1 activity leads to accumulation of ADMA and reduction in NO signaling (Leiper et al. 2007). This in turn causes vascular pathophysiology, including endothelial dysfunction, increased systemic vascular resistance, and elevated systemic and pulmonary blood pressure.

The serum high-density lipoprotein (HDL) concentration is inversely correlated with risk of MI. The mechanism for this continues to be the subject of considerable debate. High-density lipoproteins are thought to protect LDL from oxidation due to the presence of antioxidant enzymes, among which paraoxonase (PON) [EC.3.1.8.1, aryldiakylphosphatase] seems to be of major importance (Durrington et al. 2001). Paraoxonase represents an endogenous defense mechanism against vascular oxidative stress, thereby contributing to the prevention of atherosclerosis (Horke et al. 2007).

Oxidative stress in the vasculature induced by superoxide anion has been implicated in the pathogenesis of coronary artery disease (CAD). In two studies from our lab, it has been demonstrated in the first evidence that oxidative stress and ADMA are associated with cardiovascular complications in hemodialysis patients (El-Mesallamy et al. 2008), whereas it has been provided in the second evidence that free radicals are implicated in the development of atherosclerosis induced by hypercholesterolemia (Gad et al. 2014).

The sources of superoxide production in the vasculature are diverse and include vascular smooth muscle cells (VSMCs), endothelial cells, and macrophages. Although NADPH oxidase enzyme was originally described in phagocytes, it has recently become evident that the NADH/NADPH oxidase system is an important enzymatic origin of superoxide radical in nonphagocytic cells such as VSMCs and endothelial cells. NADPH oxidase is a major cause of atherosclerosis, and NADPH oxidase inhibitors may reverse atherosclerosis. NADPH oxidase produces reactive oxygen species (ROSs). These ROSs activate an enzyme that makes the macrophages adhere to the artery wall. This process is counterbalanced by NADPH oxidase inhibitors and by antioxidants. It is postulated that atherosclerosis is primarily mediated through the oxidation of LDL (Park et al. 2009).

Vascular tone is regulated by vasodilators and vasoconstrictors. Endothelin-1 (ET-1) is the predominant vasoconstrictor peptide that constricts vascular smooth muscle, whereas NO is the primary vasodilator peptide that relaxes vascular smooth muscle. Kurita et al. inferred in their study the importance of plasma NO/ET-1 ratio as a useful biological marker for predicting CAD (Kurita et al. 2005). High levels of ET-1 impair endothelial NO production via an isoform-specific PKC-mediated inhibition of eNOS expression (Ramzy et al. 2006). Thus, the endothelin system plays a central role in the control of myocardial function and its pathophysiology.

NO Key Players and CAD

Myocardial infarction (MI) is a complex multifactorial and polygenic disorder which is thought to result from an interaction between a person's genetic makeup and various environmental factors. Conventional risk factors for MI include hypertension, diabetes mellitus, and hypercholesterolemia. Although each risk factor is partly under genetic control, a family history of MI is also an independent predictor, suggesting the existence of additional susceptibility genes for this condition.

Furthermore, some patients who have suffered a MI do not have any conventional risk factors, suggesting the contribution of an uncharacterized genetic component. Genetic-linkage studies and candidate-gene analyses have implicated several candidate genes in the predisposition to MI. Among the genetic variants known to increase the risk of MI are those of angiotensin-converting enzyme, platelet aggregation IIIa, coagulation factor VII, and cholesteryl ester transfer protein (Yamada et al. 2002). Few studies were done on enzymes and regulatory proteins that control vascular NO metabolism.

The eNOS/NO Arm

Genetic Variation in the eNOS Gene

To date more than 100 polymorphisms have been identified in, or in the vicinity of, the eNOS gene (NCBI SNP database, http://www.ncbi.nlm.nih.gov/SNP/). Among them, 15 polymorphisms exist in the eNOS promoter that might influence mRNA transcription and reduce gene expression (Jones and Hingorani 2005). However, the Glu298Asp (rs1799983) polymorphism in exon 7 was shown to be the only common variation that leads to amino acid substitution in the mature protein (Hingorani et al. 1999). In this polymorphism the guanine at position 894 is substituted by thymine, leading to a change in the amino acid at position 298 from glutamate to aspartate.

A meta-analysis of the Glu298Asp polymorphism in 19 different population study (9252 subjects) reported that the wild-type GG is the predominant genotype representing 67 %, while the GT and TT genotypes are present in 28 % and 4 % of the subjects, respectively (Zintzaras et al. 2006). In the Egyptians, it was found that the wild-type GG genotype is prevalent in 58.4 % of the healthy controls, while GT and TT are present in 33.7 % and 7.9 %, respectively (Gad et al. 2012). The allele frequencies of the G and T alleles were 75.3 % and 24.7 %, respectively. No significant differences in the eNOS genotype distribution pattern (Mann-Whitney test, $p = 0.12$) or in the allele frequencies (Mann-Whitney test, $p = 0.09$) between female and male subjects were observed. An earlier study conducted using only ten healthy Egyptian subjects showed genotype frequencies of GG (50 %), GT (40 %), and TT (10 %) (Nagib El-Kilany et al. 2004).

Results of the two Egyptian studies are generally comparable to a study on healthy Caucasians ($n = 171$), which showed that GG is the genotype found in

highest frequency (50.3 %), GT frequency was 39.6 %, and TT was 8.2 % (Walch et al. 2008). Analogous genotype distributions were also demonstrated in other studies for populations of European origin: Germany ($n = 190$; GG 50.5 %, GT 40 %, and TT 9.5 %) (Krex et al. 2006), Turkish ($n = 150$; GG 49.3 %, GT 41.3 %, and TT 9.3 %) (Afrasyap and Ozturk 2004), English ($n = 331$; GG 47.8 %, GT 42 %, and TT 10.2 %) (Hingorani et al. 1999), and in the European HapMap-CEU study ($n = 120$; GG 40.0 %, GT 51.7 %, and TT 8.3 %). The allele frequencies in all these studies ranged from 65.8 % to 71.1 % for the G allele and from 29.0 % to 34.2 % for the T allele.

A remarkably different genotype distribution appeared in Asians where the wild-type GG predominates in around 75 % of the population, while the homozygous Asp variant (TT genotype) is nearly absent. Representative examples are studies from Japan ($n = 513$; GG 84.4 %, GT 17.4 %, and TT 0 %) (Kato et al. 1999) and Korea ($n = 411$; GG 97.6 %, GT 19.5 %, and TT 0.9 %) (Moon et al. 2002). A similar pattern was seen in African American ($n = 60$; GG 70.4 %, GT 23.9 %, and TT 5.6 %) (Li et al. 2004).

Controversial results were reported in the literature with regard to the influence of eNOS Glu298Asp polymorphism on the incidence of CAD. While several studies did not provide enough evidence that this polymorphism influences the risk for CAD in Egyptian, Turkish, and British subjects (Jeerooburkhan et al. 2001; Aras et al. 2002; Gad et al. 2012), others observed an association of the Glu298Asp with the risk of MI in British and Japanese subjects (Shimasaki et al. 1998; Hingorani et al. 1999). These findings further support the previously reported role of ethnicity in determining the prevalence of genetic polymorphisms and their subsequent putative impacts in a given population.

Serum NO Levels

Interest in the measurement of serum NO concentration is increasing since it has been reported that NO levels are influenced by several diseases, including diabetes, heart failure, sepsis, and liver cirrhosis; however, little is known about the normal range and the physiological changes of serum NO concentrations in healthy population.

Comparable average serum levels of NO were seen in Egyptians (30.3 μM) (Gad et al. 2012) and Turkish subjects (32.6 μM) (Afrasyap and Ozturk 2004). A large study utilizing 1983 healthy Iranian subjects showed that the mean serum NO was 24.4 μM (Ghasemi et al. 2008). The mean serum NO was 55 μM in Japanese (Higashino et al. 2007) and 53.11 μM in Korean individuals (Moon et al. 2002). In African Americans, the mean serum NO concentration was reported to be 8.8 μM in subjects with dominant eNOS genotype (GG) and 9.9 μM in subjects with recessive eNOS genotypes (GT + TT) (Li et al. 2004). A comparison between the mean serum NO concentrations of healthy Egyptian female versus male subjects revealed a nonsignificant difference (Gad et al. 2012). Also, no statistical significance was detected when comparing serum NO concentrations of the different age groups (<20, 21–30, 31–40, and >40 years old) and comparing the serum NO concentrations among different Glu298Asp genotypes.

A highly significant increase in the serum levels of NO has been observed in the MI patients (Bermudez Pirela et al. 2000; Gad et al. 2012). The reason for this finding may be attributed to the fact that MI results in an increased myocardial inducible nitric oxide synthase (iNOS) expression and NO production and higher nitrotyrosine levels, leading to myocardial dysfunction and increased mortality (Feng et al. 2001). There was no association between eNOS genotypes and the serum levels of NO in the MI patients of Egyptian and South Indian population (Angeline et al. 2010; Gad et al. 2012). Sanchez et al. concluded from their study of 49 Spanish MI patients that neutrophils from patients during MI showed an increased production of NO and a marked expression of the iNOS isoform (Sanchez De Miguel et al. 2002).

The DDAH/ADMA Arm

Genetic Variation in the DDAH-2 Gene

Genes for DDAH-1 and DDAH-2 are located on chromosomes 1p22 and 6p21.3, respectively (Tran et al. 2000). They are differentially regulated through development (Redel et al. 2015). It is apparent from gene-silencing studies in rats that DDAH-1 plays an important role in regulating serum ADMA levels, whereas DDAH-2 appears to control NO-mediated functions of the endothelium. The DDAH-2 isoform predominates in tissues expressing eNOS, such as the endothelium (Jones and Hingorani 2005). Thus, DDAH, through catabolism of ADMA, regulates the activity of NOS. Few studies have focused on the possibility that the DDAH gene polymorphisms may contribute to the inheritable risk for CVD in humans. No one has identified specific differences among ethnic groups.

The discovery of a functional polymorphism within DDAH2 gene that might promote individual differences in the ability to metabolize ADMA in vivo and, in turn, underlies susceptibility to CVD has been previously addressed by Jones et al. (2003). In this study, the researchers identified several DDAH2 gene polymorphisms, two of them are the subjects of a previous study from our lab: SNP1 (−1151 C/A) present in the promoter region upstream of the noncoding exon 1 and SNP2 (−499 C/G) localized within intron 2 of the gene (Gad et al. 2011).

O'Dwyer et al. (2006) observed that carriage of a G allele at position −449 in the promoter region of DDAH2 gene is associated with increased ADMA levels, which suggests that the DDAH2 gene expression with a G allele of this position is lower than that with a C allele. Maas et al. (2009) have indicated that −1151 A/C (SNP1) and −449 G/C (SNP2) polymorphisms in the DDAH2 promoter region are associated with an increased prevalence of hypertension.

In the Egyptian study, evidence has been provided that DDAH2 (−1151 C/A) or (−499 C/G) polymorphisms are associated with increased risk of early MI (Gad et al. 2011). It was also revealed that DDAH2 SNP1 (−1151 A/C) and SNP2 (−449 G/C) are in complete linkage disequilibrium. An interesting finding in this study is the difference in frequency of DDAH2 SNP1/SNP2 polymorphisms for the studied sample of Egyptians from those reported for other populations. This finding

addresses the inquiry about the evolutionary course of this gene polymorphism among Egyptians.

Data that belong to the HapMap project [http://www.hapmap.org/] infer that for DDAH2 SNP1 (−1151 C/A, rs805304), Europeans (Utah residents with northern and western European ancestry) have the lowest CC variant (8.3 %), as compared to CA (46.7 %) and AA (45 %) variants. Asian (Han Chinese in Beijing, P. R. China) had 13.3 % CC, 55.6 % CA, and 31.1 % AA. Sub-Saharan African (Yoruban in Ibadan, Nigeria) had 78 % CC, 18.6 % CA, and 3.4 % AA. The results of the Egyptian study indicated that the genotype distribution of SNP1 control subjects was 28 % CC, 54 % CA, and 18 % AA (Gad et al. 2011), which is somewhat different from Europeans as well as sub-Saharan African figures. It seems from an evolutionary point of view that geographical distribution affects DDAH2 SNP1 genotype pattern: the farther you go from Africa, the lower frequency of CC and higher AA genotype are manifested. Not surprisingly, the same conclusion applies to DDAH2 SNP2 (−449 C/G, rs805305) site that has polymorphisms strongly associated with those of SNP1. Similar data were displayed for SNP1 and SNP2 polymorphisms in the HapMap project.

An interesting part of the aforementioned Egyptian study is the association of DDAH2 polymorphisms with the severity of coronary insufficiency (unpublished data). In this study, CAD patients were subclassified according to severity of coronary insufficiency, as verified by coronary angiography, into (a) patients under conservative medical treatment (Med, $n = 12$), (b) patients directed for percutaneous coronary interventions (PCI, $n = 41$), (c) patients advised to do coronary artery bypass grafting operation (CABG, $n = 36$), and (d) patients suffering from acute myocardial infarction (AMI, $n = 11$).

Results shown in Fig. 2 demonstrate a noticeable increase in AA/GG (SNP1/SNP2) genotype frequencies moving from the least (controls and Med) to the most severe (CABG and AMI) coronary insufficiency. AA/GG frequencies in CABG and AMI were more than twofolds (38.95 % and 36.4 %) higher than the control values (18 %). The same trend was applied to allele distribution (Fig. 3). An increase in A/G (SNP1/SNP2) was observed moving from controls (45 %) to CABG and AMI (58.3 % and 68.2 %).

No significant correlation was perceived between the serum levels of ADMA, SDMA, L-arginine, and hs-CRP and carriage of specific DDAH2 allele or genotype. A trend of higher, but not significant, ADMA, SDMA, creatinine, and hsCRP and lower L-arginine and L-arginine/ADMA was observed in the AA/GG group as compared to the other two genotypes (Gad et al. 2010).

Serum ADMA and SDMA Levels

ADMA is one among three methylarginines physiologically found in all human tissues and biological fluids. The other two are N-monomethylarginine (L-NMMA) and symmetric dimethylarginine (SDMA) (Fig. 4). Methylarginines are generated by the posttranslational methylation of arginine residues in proteins. Following proteolysis, free methylarginines are released into the cytosol where they accumulate before being removed to the plasma and cleared into the urine by the kidney (Tran et al. 2003). A study by Murray-Rust et al. (2001) established that DDAH

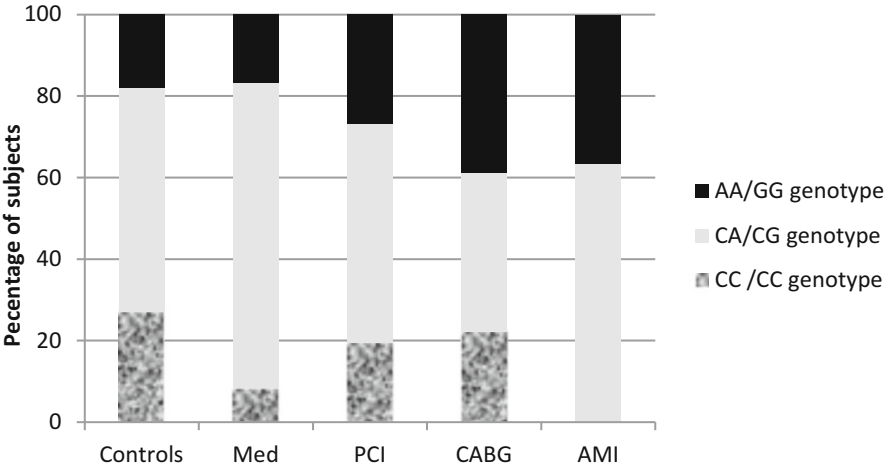

Fig. 2 DDAH2 SNP1/SNP2 genotype distribution in CVD groups as compared to controls. SNP1 = (−1151 C/A, rs805304) and SNP2 (−449 C/G, rs805305). A significant increase in AA/GG genotypes of DDAH2 SNP1/SNP2s, respectively, was observed moving from the controls and med (patients under medication) to the most severe coronary artery bypass grafting (CABG) and acute myocardial infarction (AMI) coronary insufficiency

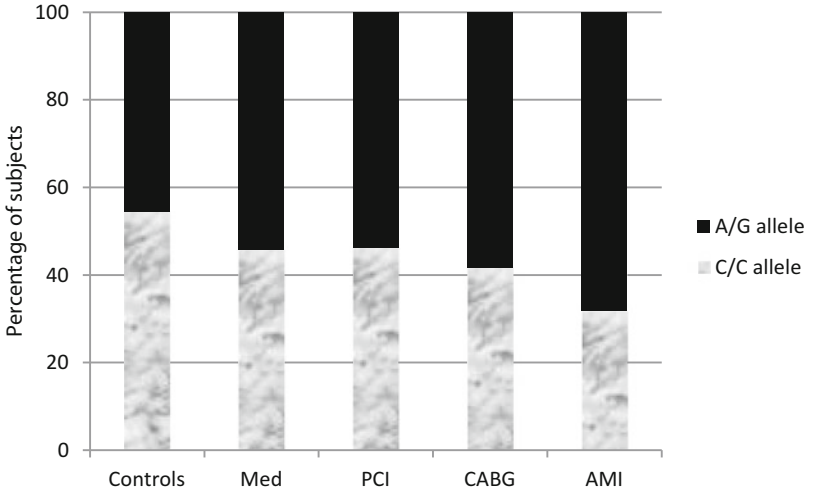

Fig. 3 DDAH2 SNP1/SNP2 allele distribution in CVD groups as compared to controls. SNP1 = (−1151 C/A, rs805304) and SNP2 (−449 C/G, rs805305). A significant increase in A/G alleles of DDAH2 SNP1/SNP2s, respectively, was observed moving from the controls and med (patients under medication) to the most severe coronary artery bypass grafting (CABG) and acute myocardial infarction (AMI) coronary insufficiency

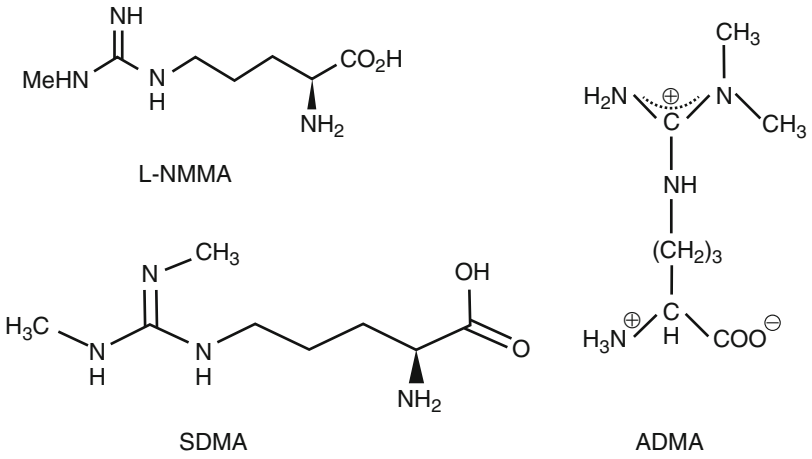

Fig. 4 Methylated arginine identified in eukaryotes. Different forms of methylated arginine physiologically identified in eukaryotes, including the human body. *L-NMMA*, levo-N-monomethylarginine; *ADMA*, asymmetric dimethylarginine; *SDMA*, symmetric dimethylarginine

metabolizes ADMA intracellularly, whereas SDMA is not a substrate for DDAH. Thus, serum ADMA will be dependent primarily on factors that affect DDAH expression and activity, whereas serum SDMA will depend on the rate of renal excretion (Palm et al. 2007). SDMA accumulates to a greater degree (eightfold increase) and more closely parallels creatinine concentration than ADMA. In contrast to ADMA, SDMA does not act as an inhibitor of NO synthase (Vallance et al. 1992b).

Despite the fact that several factors affect the amount of ADMA in tissues, and consequently in the blood, including oxidative stress, hypercholesterolemia, renal function, and DDAH activity, evidence has emerged that ADMA might be a novel cardiovascular risk factor (Boger 2003). However, no significant difference between serum levels of ADMA in controls and CAD patients was observed in the studies of Gad et al. (2010) and Wang et al. (2006) who showed that there was no significant difference in plasma ADMA levels between patients with triple vessel disease and subjects with no detectable coronary disease. Also, levels of SDMA, L-arginine, and L-arginine/ADMA did not differ. In contrast, levels of SDMA in the Egyptian study were surprisingly higher in the CAD patients than controls (Gad et al. 2010).

The ROS/Antioxidants Arm

Genetic Variation in the NADPH Oxidase Gene

All cell types within the heart, including cardiomyocytes, endothelial cells, vascular smooth muscle cells (VSMCs), fibroblasts, and infiltrating inflammatory cells, generate ROS. Potential sources of ROS in these cell types include the mitochondrial electron transport chain, xanthine oxidases, “uncoupled” nitric NOSs, cytochrome

P450, and NADPH oxidase. Among these sources, the NADPH oxidase may be considered unique in that they generate ROS in a highly regulated manner whereas ROSs are generated as by-products of enzymatic activity for all the other sources (Wang et al. 2006).

In the last decade, five NADPH oxidase isoforms each encoded by a separate gene and with distinct tissue distribution have been identified. These isoforms are distinguished by the presence of distinct catalytic subunits, Nox1–Nox5, which mediate the electron transfer process. In addition to the core catalytic Nox subunit, the enzymatic activity of the oxidase depends on additional subunits, which vary according to the isoform. These subunits include gp91phox and p22phox and a cytosolic component composed of subunits p47phox, p40phox, p67phox, and a G protein, Rac (Lassegue and Clempus 2003). In vessels from patients with CAD, expression of Nox2 and Nox4 is enhanced (Guzik et al. 2006). During restenosis of the carotid artery after balloon injury, Nox1, Nox2, and Nox4 are upregulated sequentially at 3, 7–15, and >15 days after injury, respectively (Szocs et al. 2002).

The p22phox subunit is essential to this enzyme's activity, and activation of NADPH through this membrane-bound subunit protein has been shown in vascular cells. Furthermore, many of the stimuli found to activate NADPH oxidase increase expression of the p22phox subunit. The p22phox protein is encoded by the cytochrome b-245, a (CYBA) gene. The CYBA gene is located on the long arm of chromosome 16 (at q24), encodes the alpha subunit of the membrane-bound component, spans 8.5 kilo base (kb), and contains five introns and six exons.

Several CYBA gene variants have been associated with CVD. The C242T (rs4673) CYBA polymorphism has been previously found to influence NADPH oxidase gene expression. This CYBA C242T gene variant is in exon 4 and causes a structural modification in the protein from the histidine-to-tyrosine substitution at residue 72 in a heme-binding site. The resulting structural change in p22phox from this C242T polymorphism has been related to CVD, hypertension, and endothelial function (Feairheller et al. 2009).

Online, Hashad et al. reported an association of a C242T polymorphism of NADPH oxidase p22phox gene with the incidence of AMI (Hashad et al. 2014). The genotype CC in AMI patients was higher by 45 % than controls. This increase was associated with a corresponding rise in ox-LDL (Fig. 5). The study concluded that the wild genotype CC is considered a risk factor of MI and C242T polymorphism of p22phox gene of NADPH oxidase is a novel genetic marker associated with reduced susceptibility to AMI.

Similar results were shown in Asian (Inoue et al. 1998; He et al. 2007) and Finnish populations (Fan et al. 2006). In harmony, Schachinger et al. (2001) observed a significant increase in the flow-dependent dilation in patients bearing the T allele of the C242T polymorphism and an impaired coronary arterial dilator response to nitroglycerin in patients carrying the CC genotype.

Serum Oxidized Low-Density Lipoprotein (ox-LDL) Levels

In 1989, Steinberg et al. (1989) put forward the original oxidative modification hypothesis based on the notion that oxidation represents a biologic modification

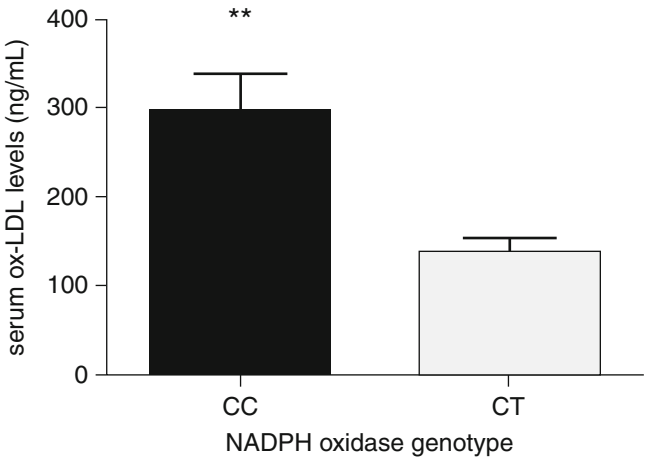

Fig. 5 Correlation between NADPH oxidase genotype distribution and serum levels of ox-LDL in MI patients. TT distribution in MI patients was 0 %. Levels of oxidized LDL were highly elevated in the CC genetic variant of C242T polymorphism of NADPH oxidase p22phox gene in myocardial infarction patients

analogous to chemical modification discovered by Goldstein et al. (1979) that gives rise to foam cells. Since then, numerous studies have supported the ox-LDL hypothesis which says ox-LDL can promote foam cell formation through the so-called "scavenger receptor" pathway. The current oxidative modification or stress hypothesis of atherosclerosis predicts that LDL oxidation is an early, essential event in atherosclerosis that leads to MI and that ox-LDL does contribute to both initiation and progression of atherosclerosis and CAD. Increased levels of ox-LDL have been demonstrated in patients with AMI and unstable angina (Ehara et al. 2001).

Experimental studies have identified several mechanisms through which ox-LDL may contribute to the development of atherosclerosis. Oxidized LDL may cause intimal inflammation by activating expression of adhesion molecules on endothelial cells, stimulating leukocyte chemotaxis, and by inducing release of growth factors from macrophages. A substantial body of evidence suggests that most, if not all, of the atherogenic effects of ox-LDL are derived from the oxidized lipid components. The "active" lipids include both esterified and unesterified peroxidized lipids, lysophosphatidylcholine, cholesterol oxidation products, aldehydes derived from breakdown of both esterified and unesterified oxidized fatty acids, and perhaps proteolipids that may have peroxidized lipids bound to fragmented apoB-10 (Young and Parthasarathy 1994).

Many studies coincide with the above conclusion (Holvoet et al. 1998, 2001; Hashad et al. 2014; Ehara et al. 2001; Fredrikson et al. 2003) suggesting that ox-LDL plays an important role in the progression of CAD and AMI. The question arises regarding what causes high levels of ox-LDL in AMI patients. Are systemic changes involved that alter the lipid profile or is it the atherosclerotic process itself that could be held responsible? At this stage, it is fair to state that this remains speculative.

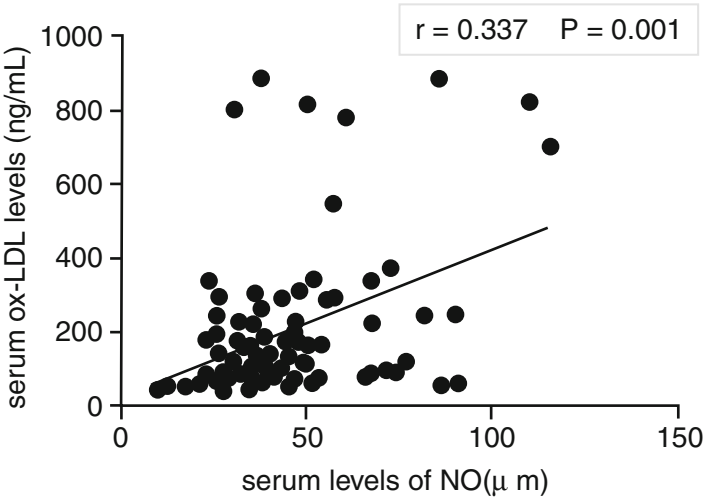

Fig. 6 Correlation between serum NO levels and serum ox-LDL levels in AMI patients. Levels of ox-LDL in the AMI patients are well correlated with serum levels of NO

Previous in vitro studies have documented that macrophages and lymphocytes are capable of oxidizing LDL (Ehara et al. 2001).

It was found in Hashad et al. study (Hashad et al. 2014) that high levels of ox-LDL in the AMI patients are well correlated with serum levels of NO (Fig. 6). NO reacts with O_2^- anion to form ONOO−, a potent oxidant (Beckman and Koppenol 1996). Therefore, NO plays a prooxidant role when present simultaneously with O_2^- anion, which is implicated in the mechanisms of LDL oxidation. Endothelial cells, smooth muscle cells, and macrophages generate O_2^- anion, and thereby ONOO− or other reactive nitrogen intermediates could be formed in the artery wall and lead in part to cell-mediated LDL oxidation (Yoshida and Kisugi 2010).

Genetic Variation in the PON-1 Gene

The serum HDL concentration is inversely correlated with risk of AMI (Durrington et al. 2001). HDL is thought to protect LDL from oxidation due to the presence of antioxidant enzymes, among which paraoxonase (PON) seems to be of major importance (Horke et al. 2007). Paraoxonase represents an endogenous defense mechanism against vascular oxidative stress, thereby contributing to the prevention of atherosclerosis.

The PON gene family in mammals includes at least three members: PON1, PON2, and PON3 (Gupta et al. 2009). The three PON genes share about 65 % similarity at the amino acid level and are located adjacent to each other on chromosome 7 (7q21.3) in humans. Both PON2 and PON3 possess antioxidant properties and lactonase activity, but unlike PON1, they lack the paraoxon or phenyl acetate-hydrolysing activity. PON1 is synthesized in the liver and is closely associated with

Table 1 The genotype distributions and allele frequencies of PON1 Q192R in AMI and control groups. A significant difference was observed in both PON1 genotype distribution patterns ($p = 0.0001$) and the allele frequencies ($p = 0.0002$) between the AMI patients and the controls. The number of subjects is shown in brackets. Patients were randomly employed for the study from the intensive care unit of the National Heart Institute, Imbaba, Egypt. Patients were included if they had a diagnosis of an acute single- or multivessel CAD verified by clinical presentation, ECG changes, and/or cardiac marker elevation. The AMI patients (age range 35 and 55 years) were comprised of 32 females and 52 males. Controls were age and sex matched

	QQ (%)	QR (%)	RR (%)	*p* value
AMI patients ($n = 84$)	34 (40.5 %)	40 (47.6 %)	10 (11.9 %)	0.0001
Control subjects ($n = 100$)	71 (71.0 %)	21 (21.0 %)	8 (8.0 %)	
	Q (%)		**R** (%)	
AMI patients ($n = 84$)	108 (64.3 %)		60 (35.7 %)	0.0002
Control subjects ($n = 100$)	163 (81.5 %)		37 (18.5 %)	

HDL. This most likely explains its ability to metabolize lipid peroxides and to protect against their accumulation on LDL (Durrington et al. 2001).

Several polymorphisms have been reported in the PON1 structure, including Q192 R polymorphism. In this polymorphism the adenine at position 575 is substituted by guanine, leading to a change in the amino acid at position 192 from glutamine to arginine. Numerous studies have been conducted to assess the effect of the PON1Q/R192 polymorphism on susceptibility to CAD. While some studies reported people with the PON1 192 R alloenzyme are more prone to CAD than are those with the Q alloenzyme, others reported no association between PON1Q/R192 polymorphism and CAD (Ombres et al. 1998).

PON1 has esterase and more specifically paraoxonase activity. It was postulated that a single serum enzyme, with both paraoxonase and arylesterase activity, exists in two different isozymic forms with qualitatively different properties and that paraoxon is a "discriminating" substrate (having a polymorphic distribution of activity) and phenylacetate is a "nondiscriminating" substrate for the two isozymes The average activities of serum of individuals of a specific PON1 (Q192) genotype showed higher arylesterase and lower paraoxonase activity than the PON1 (R192) genotype.

The results displayed in Table 1 (unpublished data) showed that the genotype distribution of PON1 gene was significantly different between AMI patients and controls. The corresponding allele frequencies were also significantly different. The odds ratio between QQ genotype and QR+RR genotypes was 3.231 ($p < 0.001$), while the odd ratio between the Q allele and the R allele was 2.256 ($p = 0.001$). The genotypes QR and RR showed higher risk of AMI compared to the homozygous QQ (odds ratio = 3.231, $p < 0.001$). Average PON/ARE ratio showed a significant difference between different genotypes in both AMI patients (QQ 0.91 ± 0.11, QR 1.09 ± 0.11, and RR 2.65 ± 0.4) ($p = 0.0002$) and controls (QQ 0.68 ± 0.1, QR 1.07 ± 0.11, and RR 4.89 ± 2.84) ($p < 0.0001$).

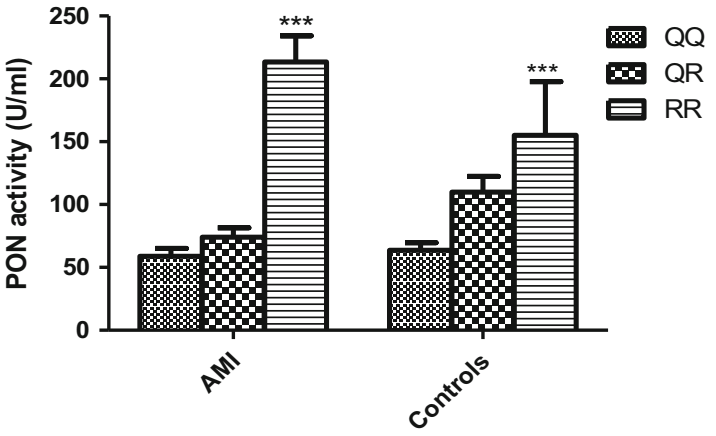

Fig. 7 PON activities among different genotypes in MI patients and controls. Results are expressed as mean ± SEM. ***: Significant difference among various PON1 genotypes at $p < 0.001$. Serum PON activities are significantly different among various PON genotypes in both AMI patients ($p = 0.0009$) and control subjects ($p < 0.0001$) where PON activity is highest in RR genotype then QR genotype and lowest in QQ genotype. Description of subjects is shown in Table 1 legend

Serum PON Activity

PON1 polymorphisms are important in determining the capacity of HDL to protect LDL against oxidative modification in vitro, which may explain the relationship between the PON1 alleles and CAD in case-control studies (Mackness et al. 2001). However, it was suggested that the PON1 phenotype (enzyme activity) is a better predictor of vascular disease than PON1 genotype (Jarvik et al. 2000).

The results displayed in Fig. 7 showed a significant difference in the PON activities among the different genotypes in both AMI patients and control subjects. Similarly, PON/ARE ratios showed a significant difference between different genotypes in both AMI and control subjects. Meanwhile, no significant difference was observed in the ARE activities.

Serum hs-CRP Levels

Inflammation has been proposed to contribute to different stages in the pathogenesis of CHD, including the lifelong process of atherogenesis; the acute atherothrombotic event, which causes ischemic necrosis in AMI; and the myocardial damage following ischemia. Accumulation, aggregation, and oxidative modification of LDL are believed to play an important role in the activation of this inflammation (Entman et al. 1991; Lowe and Pepys 2006).

CRP is the most extensively studied systemic marker of inflammation. CRP is a non-glycosylated circulating plasma protein, which together with the distinct but closely related protein, serum amyloid P component, comprise the pentraxin family of proteins (Pepys and Baltz 1983). CRP is an acute phase reactant that responds as a

sensitive, though nonspecific, marker of systemic inflammation. The pentameric globular protein is synthesized by the liver in response to stimuli from circulating inflammatory cytokines. CRP has traditionally been used as a systemic marker of infection and tissue injury. An expanding body of research now indicates that CRP likely plays a direct, active inflammatory role in blood vessels, leading to the development of atherosclerosis (Szmitko et al. 2003).

Despite many claims and assertions in the literature, neither the normal functions of human CRP nor its possible role in disease is known. This is because neither deficiency or even structural polymorphism of human CRP has yet been reported nor is any drug or other therapeutic maneuvering yet available which specifically inhibits or depletes human CRP in vivo. Any function proposed for human CRP must be consistent with the remarkable speed and dynamic range of its plasma concentration, which can rise by over 1000-fold in 24–48 h after a strong acute stimulus, such as sepsis or AMI, and can fall with a half time of about 24 h when the stimulus is removed. These dramatic changes are not associated with any local or systemic vascular or inflammatory effects in patients other than those related to the pathology or treatment, which respectively triggered or alleviated the acute phase response.

In patients with established coronary disease, CRP has been shown to predict adverse clinical events. In addition, prospective studies have consistently shown that CRP is a strong predictor of future coronary events in apparently healthy men and women. The relative risk associated with CRP is independent of other CVS risk factors.

Gad et al. reported a specific elevation pattern of CRP that copes with the severity of CAD (Gad et al. 2010). In the same study, the authors noticed the explicit difference in the levels of biomarkers between chronic and AMI CAD patients. Acute patients showed higher serum levels of ADMA, SDMA, and hsCRP and lower serum levels of L-arginine and L-arginine/ADMA ratio. The positive association of SDMA with ADMA in AMI was previously noticed by Korandji et al. (2007), who addressed the suggestion that SDMA could be a good risk indicator for CAD in AMI patients.

The Counter Effect (Vasoconstriction) Arm

Genetic Variation in the Endothelin-1 Gene

Following the discovery of endothelium-derived relaxing factor (EDRF) by Furchgott in 1980 (Furchgott and Vanhoutte 1989), Hickey et al. (1985) published a report that described an endothelium-derived contractile factor. Later, in 1988, this factor was successfully purified, identified as a novel peptide, and named endothelin (ET) (Yanagisawa et al. 1988).

ET-1, which was initially isolated and identified from conditioned medium of cultured porcine endothelial cells, is a potent vasoconstrictive peptide comprising 21 amino acid residues. This peptide has a molecular weight of 2492, free amino and carboxyl termini, and two intramolecular disulfide bonds. ET-1 is present in many mammalian species, including humans. Two additional human endothelin

isopeptides, endothelin-2 and endothelin-3, encoded by separate genes were also detected (Inoue et al. 1989).

As ET-1 plasma concentration is very low; it is not a circulating hormone, but it may be a paracrine/autocrine mediator. ET-1 is released from vascular endothelium and acts on the underlying smooth muscles to increase peripheral vascular resistance. In isolated cardiac muscle, ET-1 induces contraction and exerts a potent positive inotropic action. ET-1 is also reported to induce positive chronotropic action via ETB receptors and negative chronotropic action via ETA receptors. It also controls vascular tone and contraction of myocytes (Miyauchi and Masaki 1999).

However, the precise pathophysiologic effects of ET-1 in AMI patients remain uncertain. In 1994, Omland et al. (1994) reported that the plasma ET-1 level is a prognostic indicator of 1-year mortality after AMI. Animal model also demonstrated that ET-1 may contribute to microvasculature dysfunction due to its potent vasoconstrictive property, thus having adverse effects to AMI by restricting myocardial blood flow following reperfusion (Kelly et al. 1996).

An SNP in exon 5 of endothelin gene (EDN-1) and a G-to-T transversion that causes the Lys-to-Asn substitution at codon 198 have been reported (Tiret et al. 1999). Several association studies tried to explore the relationship between EDN lys198Asn (K198N) polymorphism and cardiovascular diseases, some studies reported an association between this polymorphism and the incidence of hypertension and coronary artery diseases in hypertensives (Tiret et al. 1999; Popov et al. 2008), but others reported lack of association between endothelin gene variants and cardiovascular diseases (Palacin et al. 2009).

Serum Endothelin-1 Levels

Elevated levels of serum ET-1 in MI patients were previously reported (Stewart et al. 1991; Miyauchi and Masaki 1999). ET-1 is increased in accordance with cardiac and pulmonary circulatory distress in AMI patients, which may further aggravate circulatory dysfunction. Stewart et al. demonstrated the early elevation of ET-1 levels after the onset of MI even before the elevation of creatine kinase (Stewart et al. 1991). Several studies showed that the ET-1 level is an important prognostic marker following MI as high ET-1 levels were associated with a higher mortality rate (Omland et al. 1994; Yip et al. 2005). Plasma NO/ET-1 ratio also proved to be efficient for prediction of CAD (Kurita et al. 2005) as the cardiovascular functions are regulated by the balance between the vasodilator NO and the vasoconstrictor ET-1. High ET-1 levels impair endothelial NO production via an isoform-specific PKC-mediated inhibition of eNOS expression (Ramzy et al. 2006).

The origin of the elevated plasma ET-1 post-AMI remains unclear. There is a reason to believe that at least part of this increase originates from the heart, as, in vitro, the ischemic heart releases ET-1 (Brunner et al. 1992), and in the rat occlusion–reperfusion model, plasma ET-1 increases after 50 min of coronary occlusion (Watanabe et al. 1991). ET-1 can be secreted from the atherosclerotic plaque as atherosclerosis precedes AMI. ET-1 is released from activated macrophages and smooth muscle cells, which are abundant in atherosclerotic arteries (Zeiher et al. 1995).

Finally, here are some flashing conclusions of the above reports:

1. *The association of eNOS Glu298Asp polymorphism with CVD is variable among different populations*.
2. *No enough evidence for the correlation between the genotypes of eNOS Glu298Asp and mean serum NO levels*.
3. *Mean serum NO concentrations are different among different populations*.
4. *No age- or sex-related differences in mean serum NO concentrations were observed in healthy subjects*.
5. *A allele/AA genotype for DDAH2 SNP1* (*−1151 C/A*, *rs805304*) *and G allele/GG genotype for SNP2* (*−449 C/G*, *rs805305*) *are associated with early incidence of CAD in Egyptian patients*.
6. *DDAH2 SNP1 and SNP2 are in complete linkage disequilibrium. Association between C/C and A/G alleles for SNP1/SNP2 and CC/CC*, *CA/CG*, *and AA/GG for the genotypes was evident*.
7. *Frequency of SNP1 A allele/AA genotype and SNP2 G allele/GG genotype is directly proportional with the severity of coronary insufficiency.*
8. *No direct association between DDAH2 genotype and serum levels of ADMA*, *SDMA*, *L-arginine*, *and hs-CRP.*
9. *There is a tendency that serum levels of ADMA*, *SDMA*, *L-arginine*, *and hsCRP are correlated with the severity and incidence of CAD.*
10. *C242T polymorphism of the p22 phox gene of NAD(P)H oxidase may reduce susceptibility to MI and that T allele exerts protective effect from CAD*.
11. *Patients having MI had elevated mean serum levels of NO and ox-LDL suggesting the role of inflammation and oxidative stress*, *respectively*, *in the incidence of MI*.
12. *There is a significant correlation between serum levels of NO and ox-LDL in the MI patients*.
13. *Carrying PON1 192R allele represents an independent risk factor for early onset AMI. The PON1 R192 isoform is associated with a higher PON/ARE ratio*.
14. *The PON1 Q192 polymorphism appears to modify the PON-1 enzyme activity.*
15. *Serum hsCRP levels correlate with the severity and incidence of CAD*.
16. *Acute MI patients showed higher serum levels of ADMA*, *SDMA*, *and hsCRP and lower serum levels of L-arginine and L-arginine/ADMA ratio than chronic MI patients*.
17. *There is no enough evidence to prove that EDN Lys198Asn polymorphism is a risk factor for early onset AMI*.
18. *Serum endothelin concentration is higher in MI patients than control subjects*.

Potential Applications to Prognosis, Other Diseases, or Conditions

Until the beginning of the 1980s, nitric oxide (NO) has been just a toxic molecule of a lengthy list of environmental pollutants such as cigarette smoke and smog. In fact, NO had a very bad reputation of being destroyer of ozone, suspected carcinogen, and precursor of acid rain.

However, over the last three decades, the picture has been totally changed. Diverse lines of evidence have converged to show that this sometime poison is a fundamental player in the everyday business of the human body. NO activity was probed in the brain, arteries, immune system, liver, pancreas, uterus, peripheral nerves, lungs, and almost every system in the human body. It exhibits diverse vital roles in the human body. It is now clearly recognized that NO participated in the control of vascular tone as an antagonist of the adrenergic regulatory system. It inhibits aggregation of platelets and their adhesion on a vascular wall. NO causes smooth muscle relaxation not only at the vascular wall but also on the gastrointestinal tract wall. NO functions both in the central and peripheral nervous systems. In brain, it acts as a neurotransmitter and may be a long-sought mystery molecule that aids in learning and remembering. In males, it is the messenger that translates sexual excitement into an erect penis. NO regulates the activity of respiratory system organs and also of the digestive tract and genitourinary systems via efferent nerves. It also influences the functioning of secretory tissues and cells and play roles in vision, feeding behavior, and olfaction. In addition, NO is produced in large quantities during host defense and immunologic reactions.

On the other hand, overproduction of NO is incriminated in the pathogenesis of several conditions such as septic shock, epilepsy, tissue damage, inflammation, and nerve damage left by the stroke. This "split" personality for NO imparted great excitement for more research in this area.

Research on NO has already had a measurable impact on the practice of medicine and drug design, and the impact will increase after understanding all the mechanisms related to NOS activity. Therapeutic strategies related to NO involve both increasing and decreasing NO production. Reduced generation of NO has been implicated in a number of clinical conditions. In these, or even in some situations in which NO production is unimpaired, it may be desirable to mimic or enhance the physiological generation of NO. This may be achieved in several ways, including direct administration of NO, the use of compounds that will donate NO, stimulating receptors linked to the L-arginine–NO pathway, augmenting the action of endogenous NO, or providing additional substrate for its synthesis. On the other hand, inhibition of the synthesis of NO may be desirable in situations in which there is overproduction of NO, as a result of overactivity of any of the NOS isoforms. The analogs of L-arginine are the most commonly used inhibitors of NOS for determining the involvement of NO in a physiological or pathophysiological process. By virtue of their structure similarity to L-arginine, they bind at the substrate-binding site of NOS.

Hundreds of NOS inhibitors are now under development and consideration for their therapeutic potential, especially for inhibition of iNOS that is thought to contribute to the pathophysiology of a number of human diseases, such as arthritis, asthma, inflammatory bowel disease, glaucoma, and psoriasis. However, the world is waiting for a breakthrough in the area of selective NOS inhibition. NOS inhibitors known to date do not possess the desired pharmacological selectivity, pharmacokinetic, and pharmacodynamic profiles to render them therapeutic agents. Clearly, these selective agents will be needed not only as potential therapeutics but also as probes to allow new directions to emerge from the NO research field. Therapeutic

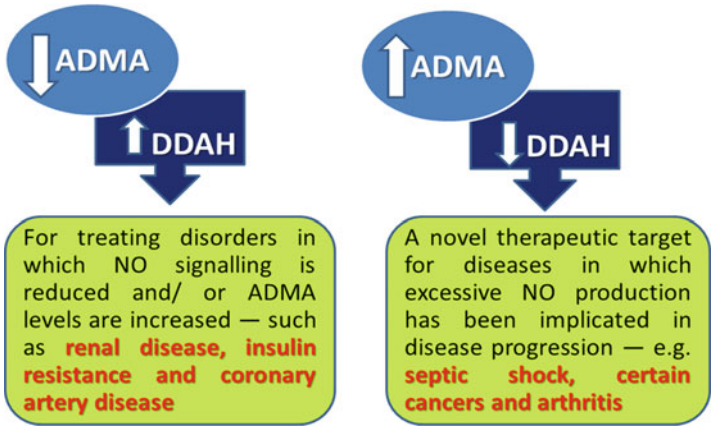

Fig. 8 Therapeutic strategies related to DDAH/ADMA modulation. Asymmetric dimethylarginine (ADMA) is a physiological inhibitor to all nitric oxide synthase isoforms and is degraded by dimethylarginine dimethylaminohydrolase (DDAH). Thus, therapeutic research is going in two directions either by increasing NO as a therapy, through reducing ADMA levels, or by decreasing NO in other conditions by decreasing ADMA levels

strategies related to DDAH/ADMA modulation are also under extensive research (Fig. 8).

Worth mentioning, new directions in this area include trials for gene therapy related to NO generation or inhibition, which have been initiated since two decades and still underway. An example of these trials was done by Kibbe and Tzeng for examining the role of iNOS gene transfer to the vasculature in preventing the development of vascular injury response (Kibbe and Tzeng 2000). Moreover, the importance of NO and ox-LDL is extended from just being targets for prevention, diagnosis, and therapy of CVD to also being candidate biomarkers in evaluating the human biological age (Gradinaru et al. 2015). Association studies of polymorphisms in genes coding for enzymes and proteins metabolizing NO with variable diseases, other than the CVS, are also extensively running (Li et al. 2015; Martinez-Barquero et al. 2015; Polat et al. 2015).

At last, there are still numerous unanswered questions and areas of less understanding that need more research in the next decade, such as:

1. What is the interrelationship between molecular oxygen, NO, and superoxide in normal homeostatic states and in disease?
2. What role does iNOS play in numerous disease pathologies?
3. What is the physiological role of ADMA?
4. What are the reactive nitrogen species directly produced from L-arginine by NOS (NO, nitroxyl, peroxynitrite, or all three) and the stoichiometry of the reaction?
5. What is the significance and the basis of the subcellular localization of the NOSs?

6. What is the molecular and structural basis of the high isoform selectivity of some NOS inhibitors?
7. Will selective iNOS, nNOS, or iNOS + nNOS inhibitors prove to be of value in the treatment of human diseases, and if so, which diseases and what side effects might result?
8. What other physiological and pathological roles of NO in the human body that have not been discovered yet?
9. What are the factors that regulate the metabolism of arginine, the precursor of NOS, and its distribution to different pathways?
10. Which biochemical and genetic biomarkers will be of value in the assessment of cardiovascular health and which of them can be used as early markers of CVD?

Summary Points

- In the blood vessels, nitric oxide (NO) is produced from the endothelium mainly by the constitutively expressed endothelial nitric oxide synthase (eNOS).
- Endothelial NO possesses multiple anti-atherosclerotic properties.
- A range of biochemical disturbances, including reduced availability of NO and oxidative stress, has been shown to be associated with endothelial dysfunction.
- There are several key players that influence vascular NO levels and their role in the protection and/or predisposition to cardiovascular disease (CVD), including the cellular activities of eNOS, dimethylarginine dimethylaminohydrolase (DDAH), paraoxonase, and NAD(P)H oxidase as well as the production levels of asymmetric dimethylarginine (ADMA), oxidized low-density lipoprotein (ox-LDL), C-reactive protein (CRP), and endothelin.
- Alleles or genotypes associated with early incidence of coronary artery disease (CAD) included A allele/AA genotype for DDAH2 SNP1 (−1151 C/A, rs805304), G allele/GG genotype for SNP2 (−449 C/G, rs805305), and carrying PON1 192R allele, whereas C242T polymorphism of the p22 phox gene of NAD(P)H oxidase confers protection from CAD. In contrast, there is no enough evidence to support the association of eNOS Glu298Asp and EDN Lys198Asn polymorphisms on CAD incidence.
- There is a tendency that serum ADMA, symmetric dimethylarginine (SDMA), L-arginine, and hsCRP levels correlate with the severity and incidence of CAD.
- Serum ox-LDL, hs-CRP, NO, and endothelin levels are elevated in CAD patients.

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