Association between 894G>T polymorphism within endothelial nitric oxide synthase (eNOS) gene and coronary artery disease in young adults

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Abstract

Background: Nictric oxide (NO), synthesized by endothelial nitric oxide synthase (eNOS) is important for maintaining of endothelial homeostasis. It mediates vasodilatation and supresses smooth muscle cells proliferation. Defects in the production of NO may influence progression of atherosclerosis and in turn coronary artery disease (CAD). Thus, polymorphisms within *eNOS* (*NOS3*) gene are suggested to be associated with the CAD development. However, data are often contradictory. The aim of the present study was to perform meta-analysis of available data addressing possible association between 894G>T polymorphism (Glu298Asp) within *NOS3* gene and coronary artery disease in younger adults.

Methods: Pubmed, Medline and Google Scholar, were searched using specific keywords. Ten case-control studies with a total number of 1473 patients with angiographically confirmed CAD and 1174 controls were included according to the inclusion/exclusion criteria. Statistical analyses were conducted using MedCalc software. Heterogeneity between the studies was evaluated using the Dersimonian and Laird's Q test. The pooled ORs were estimated with a random or fixed effects models in dependence to heterogeneity.

Results: In case of dominant model analysis (GT+TT vs GG) significant heterogeneity between the analysed studies was observed, thus pooled OR was analysed using random effects model. Carrier-state of 894T allele was found to be

Key words:

coronary artery disease, nitric oxide synthase, eNOS, NOS3, polymorphism, Glu298Asp, premature CAD

related to coronary artery disease (p=0.014, OR=1.421 95%CI 1.07-1.88). In the allelic model (T vs G) random effects model was also used. In this case similar relation with the disease was observed (p=0.011, OR=1.463 95%CI 1.06-1.96).
Conclusions: The carrier state of T allele of 894G>T polymorphism in *NOS3* gene is related to coronary artery disease in younger adults.

Introduction

Coronary artery disease (CAD) is still one of the leading causes of death worldwide despite the fact that in the years 1980–2000 the number of deaths due to CAD decreased in the USA and nearly 50% of this number decreased as a result of the treatment [1].

The progression of atherosclerotic lesions, leading to CAD and myocardial infarction (MI), arises from the interactions between many risk factors, i.e. environmental, biochemical as well as inherited [2-4]. Among genetic risk factors, it has been suggested that almost 60 genetic loci are associated with CAD [5]. The data however indicate that less than 10% of the heritability of CAD can be explained by these factors [6]. The role of genetic risk factors seems to have particularly significant impact on aetiology of CAD in younger patients due to the shorter exposure time to environmental factors. Determining possible interplay of gene-candidate and the disease is also of great importance although it may rise some difficulties in the reliable assessment since the particular polymorphism may increase the risk of CAD in one population while not in another.

Nitric oxide (NO) is a molecule synthesized enzymatically from l-arginine (l-Arg) by three isoforms of NO synthase (NOS), i.e. neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) [7]. One of the NOS inhibitors is asymmetric dimethylarginine (ADMA) which can compete with l-arginine as a substrate. In a situation of elevated level of ADMA the bioavailability of NO decreases. NO synthesized by eNOS is important for maintaining of endothelial homeostasis. It mediates vasodilatation and supresses smooth muscle cells proliferation. In addition, NO exerts an antioxidant effect because it reduces the production of free radicals in the vessels and protects low density lipoprotein (LDL) from the oxidation. Low concentrations of NO promotes among others aggregation and proliferation of thrombocytes or inhibition of apoptosis of myocytes as well as may influence progression of atherosclerosis and in turn CAD. Thus, polymorphisms within eNOS gene are suggested to be associated with the CAD development. The gene encoding eNOS (NOS3) is located on chromosome 7 at q35–q36 and consists of 26 exons [8]. The 894G>T polymorphism in exon 7 is one of the common polymorphisms within *NOS3* gene which results in substitution of glutamate by aspartate at codon 298 (Glu298Asp). However, data regarding the role of *NOS3* 894G>T polymorphism are often contradictory. Some papers demonstrated an association between polymorphic variant of *NOS3* 894G>T polymorphism and CAD [9, 10]. On the other hand, there are also data showing no correlation [11, 12].

In the present study, a meta-analysis of available data addressing possible association between 894G>T polymorphism in *NOS3* gene and coronary artery disease in younger adults was performed.

Materials and Methods

Search strategy

Databases (Pubmed, Medline, Google Scholar) were searched using the following keywords: ("NOS3 polymorphism" or "894G>T polymorphism" or "Glu-298Asp polymorphism") and ("coronary artery disease" or "atherosclerosis" or "myocardial infarction") and ("premature" or "young aults" or "adults") (last search was performed on March 2020). The publication was included in the meta-analysis when the following criteria were met: a) CAD diagnosed in adult patients with angiographic confirmation, b) case-control study c) age of the patients approximately 60 years or younger and d) full-length paper written in

English. In turn, publications were excluded in case of: a) unavailability of genotyping results, b) type of the publication other than case-control study (i.e. conference proceedings, review articles, case reports, meta-analyses or animal studies) c) mean age of CAD patients above 60 years and d) language of the article other than English.

Eventually, 10 case-control studies [10-19] with a total number of 1473 patients with angiographically confirmed CAD and 1174 controls met the inclusion criteria.

Data extraction and methodological quality

From the studies included to the present meta-analysis the following data were extracted: first author's name, year of publication, population, age of cases and control subjects, size of analysed group of patients and controls, and number of particular genotype in the group. The Newcastle-Ottawa scale (NOS) for case-control studies was used to establish methodological quality of the studies included [20, 21]. The scores of this scale ranges from 0 points to 11 points. The study was accepted as a high quality one when the NOS score was 5 or higher. The calculation of Hardy-Weinberg equilibrium (HWE) in control subjects was additionally performed for each study.

Statistical analyses

Statistical analyses were conducted twice using MedCalc software. Heterogeneity between the studies was evaluated using the Dersimonian and Laird's Q test with the assessment of I^2 metric which describes the percentage (from 0% to 100%) of the observed between-study variability resulting from heterogeneity. In case of significant heterogeneity observed between the studies, the pooled OR was estimated with a random effects model, otherwise, a fixed effects model was used. The strength of the correlation between the *NOS3* polymorphism and CAD was assessed with the pooled OR together with the 95% confidence interval (CI) in the dominant (GT+TT vs GG), recessive (TT vs. GG + GT), additive (TT vs. GG) as well as allelic (T vs G) models. The result was considered to be statistically significant when the p value was below 0.05.

Results

Characteristics of the studies included

Characteristics of the studies included to the present meta-analysis along with the NOS quality scores is shown in Table 1. Different racial populations were analysed in all studies included. The youngest patients were recruited by Zigra et al. [16] from Greece population as well as from Egypt population [13]. The largest groups of CAD patients and control subjects were recruited by Salimi et al. [10], Vasilakou et al. [14], and Higorani et al. [17]. In turn, the lowest numbers of patients and control subjects were recruited by Saini et al. [15]. Saini et al. [15] observed also no TT allele in patient group nor in controls. One of the studies [19] demonstrated lack of agreement with HWE in the frequency of genotypes in control group however, according to an assumption of Minelli et al. [22], it was not excluded from the meta-analysis. The correlation between NOS3 polymorphism and CAD was demonstrated in five studies [10, 13, 15, 17, 18] with the values of OR ranging from 1.83 to 17 (Table 2).

Association between *NOS3* polymorphism and CAD

The mutant TT genotype of *NOS3* gene was present in almost 18% of CAD patients studied in the meta-analysis while only in 8% of healthy controls. Also, carriers of T allele (subjects with GT or TT genotypes) were more frequent in cases than in controls (50% vs 41%).

In case of *NOS3* GT+TT vs GG analysis significant heterogeneity between the analysed studies was observed (I^2=64.23%, p=0.003), thus pooled OR was calculated using random effects model. It was observed that carrier-state of 894T allele may be related to coronary artery disease (p=0.014, OR=1.421 95%CI 1.07-1.88) (Fig. 1). In case of recessive model (TT vs. GG + GT) the significant heterogeneity was also demonstrated (I^2=75.49%, p<0.001) and again random effects model was used to calculate the strength of the association. The pooled OR equalled

Table 1.
Characteristics of the studies included to the meta-analysis regarding relation between NOS3 894G>T polymorphism and CAD

Study (year)	PAIENTS WITH CORONARY ARTERY DISEASE						CONTROLS					HWE (for controls) (Σ^2; p)	QUALITY (Newcastle-Ottawa scale)
	Age	Population	N	Genotypes of NOS3 894G>T polymorphism			Age	N	Genotypes of NOS3 894G>T polymorphism				
				GG	GT	TT			GG	GT	TT		
Salimi et al. (2010)	Mean age: 53.32 years	Iran	241	112	103	26	Mean age: 51.8 years	261	160	84	17	1.679; 0.19	7
Mathew et al. (2008)	Mean age: 50.56 years	Tamilian	100	72	26	2	Mean age: 49.33 years	100	79	18	3	2.182; 0.14	6
Abdel-Aziz et al. (2013)	Mean age: 42.4 years	Egipt	116	48	46	22	Mean age: 41.9 years	119	68	39	12	2.974; 0.09	7
Vasilakou et al. (2008)	Below 58 years	Greek	209	109	86	14	Age matched to patients	161	76	74	11	1.555; 0.21	7
Saini et al. (2011)	Mean age: 59.46 years	India	60	45	15	0	Age matched to patients	50	44	6	0	0.204; 0.65	5
Zigra et al. (2013)	Mean age: 32.1 years	Greek	107	50	46	11	Mean age: 31.8 years	103	50	42	11	0.237; 0.63	8
Higorani et al. (1999)	Mean age: 58.9 years	United Kingdom	298	120	71	107	Mean age: 58.1 years	138	66	58	14	0.057; 0.81	6
Joshaghani et al. (2018)	Mean age: 58.0 years	Iran	93	58	34	1	Age matched to patients	93	60	31	2	0.770; 0.38	7
Cam et al. (2005)	Mean age: 48.1 years	Turkey	115	44	37	34	Mean age: 44.6 years	83	57	24	2	0.080; 0.78	6
Kacmaz et al. (2019)	Mean age: 56.7 years	Turkey	134	72	17	45	Mean age: 52.9 years	66	32	10	24	31.646; <0.05	5
		TOTAL	1473	730	481	262	TOTAL	1174	692	386	96		

Table 2.
The results of the correlation between *NOS3 894G>T* polymorphism and CAD obtained by authors of the included studies

Study (year)	Relationship between NOS3 polymorphism and CAD (yes/no)	OR; p
Salimi et al. (2010)	Yes	1.83; 0.001
Mathew et al. (2008)	No	0.66; 1.0
Abdel-Aziz et al. (2013)	Yes	2.6; 0.025
Vasilakou et al. (2008)	No	NA ; 0.669
Saini et al. (2011)	Yes	2.44; <0.05
Zigra et al. (2013)	No*	NA ; >0.05
Higorani et al. (1999)	Yes	4.2; <0.0001
Joshaghani et al. (2018)	No	0.51; 0.650
Cam et al. (2005)	Yes	17.0; 0.0001
Kacmaz et al. (2019)	No	NA ; 0.697

NA – not available; * the authors observed however significant correlations in subgroups' analysis.

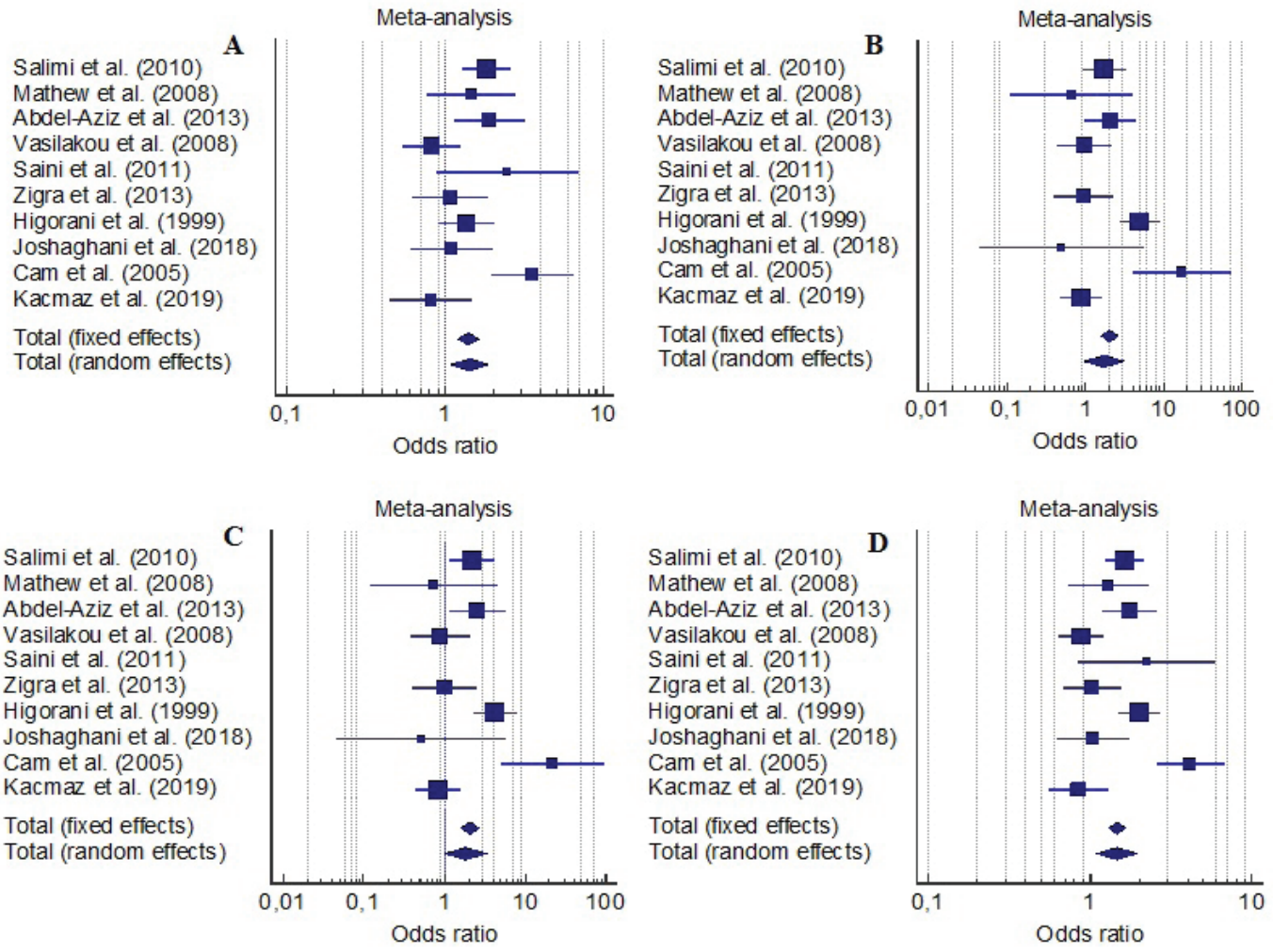

Fig. 1.
Forest plots of association between the *NOS3* 894G>T polymorphism and CAD in younger adults in four statistical models: dominant (A), recessive (B), additive (C) and allelic (D)

1.743 (95% CI 0.95-3.18) however was on bound of significance (p=0.071). Similarly, in the additive model (TT vs GG) the results was on the border of statistical significance (OR=1.835 95% CI 0.99-3.39, p=0.052). In the case of allelic model (T vs G), the T allele was found to be related to CAD in younger adults (p=0.011, OR=1.463 95%CI 1.06-1.96) (Fig. 1).

Sensitivity analysis

Sensitivity analysis, performed by sequential excluding of each study, was used to evaluate the stability of the results. I found no change in the OR value in the case of both dominant and allelic models. Thus, these analyses were stable. However, in the recessive model after omitting the study by Kacmaz et al. [19], a significant association in the prevalence of 894TT genotype of *NOS3* gene between the analysed groups (OR 1.948 95% CI 1.02–3.74, p = 0.045 in the random effects model) was observed. The results of additive model (TT vs. GG) should be treated with caution since some instability appeared during the sensitivity analysis. When omitting studies by Joshaghani et al. [11], Mathew et al. [12], Vasilakou et al. [14], Zigra et al. [16], and Kacmaz et al. [19] positive correlations were observed. Removing all the above mentioned studies from additive analysis allowed to obtain the pooled OR=3.884 (95% CI 1.91-7.88, p<0.001).

Discussion

Previously, the 894G>T polymorphism in *NOS3* gene was suggested to be related to ischemic stroke also in young adults [23, 24]. The meta-analysis by Zhao et al. [25] based on 17 studies confirmed that 894G>T polymorphism may be considered as a risk factor for venous thrombosis, and the polymorphism may increase the risk of developing thrombotic disease in Asia. On the other hand, the *NOS3* polymorphism was not related to gestational diabetes mellitus among pregnant Iranian women [26]. In the study of Campedelli et al. [27] the GG genotype of 894G>T polymorphism in *NOS3* gene showed greater susceptibility to atherosclerosis. However there are also data showing that T allele of the analysed *NOS3* polymorphism may be a protective factor for diabetic foot ulcer in diabetic patients [28].

Although available data show that assessment the role of a single gene-candidate in a development of CAD is insufficient, most case–control studies focus to analyse only one genetic polymorphism in regarding to the disease. To draw meaningful conclusions in the field several polymorphisms with specific gene–gene as well as gene-environment interactions should be studied. This has been confirmed by earlier researches. Data from Poland showed that specific multi-genotype patterns within the genes encoding intercellular adhesion molecule-1 (ICAM1), apolipoprotein E (APOE), peroxisome proliferator-activated receptor alpha (PPARA) and plasminogen activator inhibitor-1 (PAI-1) increased the risk of CAD in patients from Upper Silesia [29]. Other studies indicate that the carrier of four polymorphic gene variants for: *APOE*, butyrylcholinesterase K (*BChE*), receptor activated by γ2 peroxisome proliferators (*PPARγ2*) and *eNOS* significantly increases the risk of premature CAD compared to contribution of a single gene in the development of the disease [30]. In a study by Berdeli et al. [31] synergistic effect of the renin-angiotensin system genes and the *eNOS* gene in the development of premature CAD has also been demonstrated. In turn, Jia et al. [32] found an additive interactions between the mutant genotypes of *eNOS* T-786C polymorphism and the fast form of *ADH2* Arg47His polymorphism in the risk of premature CAD in the Chinese population.

Most often case-control studies regarding the role of genetic risk factor are performed in a small number of patients and controls which may reflect the results (in a positive or negative way). This may result from the fact that in most studies CAD patients are recruited in one medical centre. Thus, meta-analysis enables, as an objective and quantitative statistical method, to pool data from many studies analysing small groups to overcome this problem. It has also a great statistical power.

The present meta-analysis aimed to analyse the role of the 894G>T polymorphism in *NOS3* gene and coronary artery disease in younger adult patients. The results based on a sizeable groups of patients

with coronary artery disease (n=1473) and healthy controls (n=1174) showed higher frequency of *NOS3* TT genotype as well as carrier-state of T allele in cases than in controls. Statistical analyses confirmed that younger adults carrying the 894T allele (subjects with GT and TT genotypes) had over 1.4-fold higher risk of CAD. Similarly, 894T allele itself increases the risk of premature CAD almost 1.5-fold.

In four of the studies included to the meta-analysis no relation between the 894G>T polymorphism in *NOS3* gene and CAD in younger patients was demonstrated [11, 12, 14, 19]. In the study by Zigra et al. [16] no association was found in the case of analysing the whole CAD group. However, the authors demonstrated that prevalence of TT homozygotes were significantly more common only in patients with 'normal' or 'near normal' coronary arteries compared to controls (21%vs.10%, $p<0.001$) [16]. In turn, a strong significant association between *NOS3* 894TT genotype and the disease was found by Cam et al. [18]. The authors obtained results of the multivariate analysis with other confounding variables which indicated that *NOS3* TT genotype was an independent risk factor for premature CAD with OR=15.356. In one study, analysing patients from India, no TT genotypes, both in cases and controls were genotyped [15]. This finding is interesting since it confirms the heterogeneity of the disease and differences in genotypes distribution in various populations. Among studies included, Saini et al. [15] observed also that mean NO level was significantly lower in subject with GT genotype than in those bearing GG genotype. However, the mean NO levels in the CAD patients with GT genotype were lower (7.727 ± 4.452 μmol/L) comparing to the control group (11.833 ± 3.868 μmol/L). According to authors, the T allele of 894G>T polymorphism in *NOS3* gene may be a marker for endothelial dysfunction which is characteristic for CAD.

The mean age of the CAD patients analysed in the present meta-analysis was below 60 years. The study of Jaramillo et al. [33] was excluded from the meta-analysis since it analysed both, patients and controls with wide range of age from 33 years to 74 years. In the literature patients aged 50 years [34] are considered as "young adults". However, data are available which indicates that even patients under the age of 60 can be considered as "young adults" [35]. Thus, due to increasing lifetime, the assumption of Fromm et al. [35] was adopted, and in the current meta-analysis the results obtained from patients below 60 years of age were included.

The present study has some limitations, patients were inhabitants of different sides of the world with possible genetic differences, which may bring plausible selection biases between the studies. In addition, no information about environmental factors in particular patient can be extracted from particular study included. Performing meta-analyses of interactions between particular gene and factors present simultaneously would be more accurate in understanding the role of the polymorphism in the development of the disease. However, very precise data from each included study would be then needed.

In conclusion, the carrier-state of T allele of 894G>T polymorphism within *NOS3* gene is related to coronary artery disease in younger adults.

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