



## Association of G894T eNOS, 4G/5G PAI and T1131C APOA5 polymorphisms with susceptibility to myocardial infarction in Morocco



Hind Hassani Idrissi<sup>a,\*</sup>, Wiam Hmimech<sup>a,1</sup>, Brehima Diakite<sup>a</sup>, Farah Korchi<sup>b</sup>, Dalila Baghdadi<sup>b</sup>, Rachida Habbal<sup>b</sup>, Sellama Nadifi<sup>a</sup>

<sup>a</sup> Laboratory of Genetics and Molecular Pathology, Medical School, University Hassan II, Casablanca, Morocco

<sup>b</sup> Department of Cardiology, University Hospital Center Ibn Rochd, Casablanca, Morocco

### ARTICLE INFO

#### Article history:

Received 30 December 2015

Revised 30 January 2016

Accepted 23 March 2016

Available online 3 April 2016

#### Keywords:

Myocardial infarction

Susceptibility

G894T eNOS

4G/5G PAI

T1131C APOA5

Moroccan population

### ABSTRACT

**Background:** Myocardial infarction (MI) is a common multifactorial disease. Numerous studies have found that genetic plays an essential role in MI occurrence. The main objective of our case–control study is to explore the association of G894T eNOS (rs1799983), 4G/5G PAI (rs1799889) and T1131C APOA5 (rs662799) polymorphisms with MI susceptibility in the Moroccan population.

**Methods and results:** 118 MI patients were recruited vs 184 healthy controls. DNA samples were genotyped by PCR-RFLP method using MboI, BslI and MseI restriction enzymes respectively for the G894T eNOS, 4G/5G PAI and T1131C APOA5 polymorphisms. Our results show that the G894T eNOS was significantly associated with increased risk of MI under the three genetic transmission models (dominant: OR = 1.64, 95% CI = 1.05–2.58, P = 0.003; recessive: OR = 2.15, 95% CI = 0.74–6.16, P = 0.03; additive: OR = 1.54, 95% CI = 1.06–2.23, P = 0.001). The T1131C APOA5 polymorphism was associated to MI risk in recessive and additive models (OR = 1.53, 95% CI = 0.72–3.2, P = 0.04 and OR = 1.78, 95% CI = 1.26–2.51, P = 0.03 respectively). For the 4G/5G PAI variant, even the cases and controls groups were not in Hardy–Weinberg Equilibrium (HWE), the dominant and additive models show a statistically significant association with MI risk (OR = 7.96, 95%CI = 3.83–16.36, P = 0.01 and OR = 1.96, 95% CI = 1.4–2.72, P = 0.03 respectively).

**Conclusion:** Our results suggest that G894T eNOS and T1131C APOA5 polymorphisms may be considered as genetic markers of MI among the Moroccan population. Further studies including larger sample sizes and exploring more genetic associations are needed to confirm our results and to better understand the susceptibility to MI.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### 1. Background

Heart Diseases, including myocardial infarction (MI), are one of the leading causes of death in the world. They are responsible for approximately 30% of all cases of death around the world (Gaziano, 2008). According to data, around 10% of all patients with (MI) are under 45 years of age (Doughty et al., 2002).

Almost 90% of (MI) cases are the result of an occlusive thrombosis in a coronary artery, occurring after the rupture of an atherosclerosis plaque (Sakowicz et al., 2010b). Consequently, a cascade of events follows: it begins with platelet aggregation, formation of thrombin and fibrin, and at last formation of the thrombus. Myocardial cells irrigated by

the occluded artery will at a first time try to survive with a minimum of energy before dying, due to lack of oxygen (Perlemuter et al., 2005).

In addition to known risk factors for (MI), such as arterial hypertension, smoking, hypercholesterolemia, obesity, diabetes, sedentary, and alcohol consumption, the genetic contribution to heart diseases has been proved (Sakowicz et al., 2010b). As being polygenic, these disorders involve the contribution of more than one gene; they are genes involved in the inflammatory (endothelial Nitric Oxide Synthase, eNOS), coagulation (plasminogen activating inhibitor, PAI) and lipid (APO lipoprotein A5, APOA5) pathways. Each one may act alone or in synergy with others. This makes the understanding of the genetic factors underlying these complex disorders as a very important challenge (Butt et al., 2003).

#### 1.1. eNOS gene

Nitric Oxide (NO) is a molecule synthesized by endothelial cells; it contributes to vascular homeostasis and the regulation of several essential cardiovascular functions (Förstermann and Sessa, 2012). NO is

\* Corresponding author at: 19 Street Tarik Ibnou Ziad, Casablanca BP 9154, Morocco.

E-mail addresses: [hassani-idrissi-hind@hotmail.fr](mailto:hassani-idrissi-hind@hotmail.fr) (H. Hassani Idrissi), [hmimech@hotmail.com](mailto:hmimech@hotmail.com) (W. Hmimech), [br.diakite@yahoo.fr](mailto:br.diakite@yahoo.fr) (B. Diakite), [habbalrachida@hotmail.com](mailto:habbalrachida@hotmail.com) (R. Habbal), [nadifisel@yahoo.fr](mailto:nadifisel@yahoo.fr) (S. Nadifi).

<sup>1</sup> Hind Hassani Idrissi and Wiam Hmimech contributed equally to this work.

involved in vasodilatation process, regulation of blood pressure, and has numerous other vaso-protective and anti-atherosclerotic properties. Its production is controlled by endothelial Nitric Oxide Synthase (eNOS) gene; from L-arginine oxidation in vascular endothelium cells (Williams, 2003–2004). The gene encoding eNOS is mapped on chromosome 7q35–36, and contains 26 exons and 25 introns (Colomba et al., 2008). Several polymorphisms of eNOS gene have been reported. G894T variant, located in exon 7, is the most clinically relevant polymorphism in eNOS gene that has been described, and seems to be associated with reduced NO production, and development of coronary artery diseases (CAD), including (MI) (Dias et al., 2011).

### 1.2. PAI gene

The risk of thrombotic events can also be affected by variations in Plasminogen Activator Inhibitor type 1 activity (PAI-1) (Kholer and Grant, 2000). This molecule belongs to the serpin family inhibitors and is one of the key inhibitors of plasmin generation in the plasma and tissues (Alhenc-Gelas and Aiach, 2001). It acts by inhibiting the activity of the fibrinolytic system, via inhibition of tPA (tissue plasminogen activator) and uPA (urokinase plasminogen activator). Localized on the chromosome 7, PAI-1 gene covers 16 Kb of longer and contains 9 exons. It encodes for a protein of 50 KDa, composed by 379 amino acids (Alhenc-Gelas and Aiach, 2001). A single insertion/deletion of a “G” at position –675 in the promoter region of the gene gives rise to 4G and 5G alleles, which differ by their regulation of PAI-1 activity (Nordt et al., 2001; Isordia-Salas et al., 2009). Numerous studies have found an association between SNPs in PAI-1 gene and the development of many diseases (HTA, stroke...); concerning (MI), results remain contradictory (Ding et al., 2006; Collet et al., 2003; Ye et al., 1995; Iacoviello et al., 1998; Nilsson et al., 2008).

### 1.3. APOA5 gene

High plasma level of TG is also one of the important risk factors of cardiovascular diseases. According to data, about 50% of the final levels are genetically determined (Forrester, 2001). The influence of numerous gene polymorphisms on plasma TG levels has been intensively analyzed in last 15 years (Hubáček et al., 2009). The most promising results were connected with variants of the Apo-lipoprotein gene cluster APOA1/C3/A5. APOA5 was the last member to be discovered (Pennacchio et al., 2001).

The Apo-lipoprotein A5 (APOA5) human gene is located on chromosome 11 (11q23), and consists of 4 exons. The protein encoded by this gene is composed by 369 amino acid (Pennacchio et al., 2001). Many studies have demonstrated that APOA5 enhances the activity of the lipoprotein lipase (LPL), which leads to diminution of TG levels in VLDL particles (Fruchart-najib et al., 2004; Schaap et al., 2004). Numerous variants of APOA5 gene have been detected (Pennacchio et al., 2001). Two mutations of APOA5 gene, resulting in premature stop codons (Gln 145 and Gln 139), were found to have an effect on plasma TG levels, development of extreme hypertriglyceridemia, and development of myocardial infarction (Hubáček et al., 2009).

The main objective of our study is to investigate the potential association of +894G/T, –675 4G/5G and T1131C polymorphisms of the eNOS, PAI and APOA5 genes successively, with (MI) susceptibility, and to explore their frequencies, among Moroccan population.

## 2. Materials and methods

### 2.1. Study population

Blood samples were collected from 118 unrelated MI patients. The control group consisted of 184 unrelated and apparently healthy subjects showing no symptoms of coronary artery diseases. Clinical data concerning risk factors and biological parameters were collected for

each patient in our study, and an informed consent was developed for both patients and controls.

### 2.2. DNA extraction

Venous blood from all participants in this study was collected in tubes containing 3.2% sodium citrate. Samples were stored at –20 °C until extraction of DNA. Genomic DNA was extracted from blood leukocytes using the standard method of salting out (Miller et al., 1988).

### 2.3. Genotype determination

We used PCR-RFLP to genotype samples for all +894G/T eNOS, –675 4G/5G PAI-1 and T1131C APOA5 polymorphisms, as previously described by (Hingorani et al., 1999), (Brown et al., 2001) and (Talmud et al., 2002). Genotyping of each variant was performed by amplification from 50 to 100 ng of genomic DNA, followed by digestion using MboI restriction enzyme for +894G/T eNOS, BslI for –675 4G/5G PAI-1 and MseI for T1131C APOA5. For the +894G/T eNOS polymorphism, digestion gave rise to three profiles: wild GG homozygous (one fragment of 206 bp), GT heterozygote (three fragments of 206, 119 and 87 bp), and mutated TT homozygous (two fragments of 119 and 87 bp). For the –675 4G/5G PAI-1 polymorphism, profiles were: 4G/4G (one fragment of 98 bp), 4G/5G (three fragments of 98, 77 and 23 bp), and 5G/5G (two fragments of 77 and 23 bp). For the T1131C APOA5, profiles were: TT wild type (267 bp, 109 bp, 22 bp), TC (289 bp, 267 bp, 109 bp, 22 bp) and CC (289 bp, 109 bp). The digested products were separated on 3% agarose gel electrophoresis stained with Ethidium Bromide (BET), and visualized with UV rays.

### 2.4. Statistical analysis

Statistical analysis was performed using SPSS 21.0 software. Chi square test ( $\chi^2$ ) was used to determine statistical significance of association/non-association between genotypes and classical risk factors. Hardy–Weinberg Equilibrium test (HWE) was performed in both cases and controls groups for the 3 polymorphisms analyzed. Odd ratio (OR) was calculated to estimate the association between genotypes and MI risk, using a Confidence Interval (CI) of 95%. Significance was approved at P-value less than 0.05.

## 3. Results

The distributions of G894T eNOS and T-1131C APOA5 variants were in accordance with Hardy–Weinberg equilibrium (HWE) among cases and controls. For the 4G/5G PAI-1 polymorphism, distribution was not in (HWE) in both cases and control groups (Table 1). The average age of patients and controls was  $56.27 \pm 2$  and  $54.15 \pm 2$  respectively. Older age patients (age > 50 years) were more frequent in our study sample compared to young subjects (age ≤ 50 years), with a predominance of males (73 male vs 45 female).

Risk factors analysis shows a positive correlation of G894T eNOS with sex and smoking. The T-1131C APOA5 variant was correlated

**Table 1**

HWE equilibrium for the G894T eNOS, 4G/5G PAI and T1131C APOA5 distributions in cases and controls.

Genotypes	EHW cases		EHW controls	
	X <sup>2</sup> square	P-value (P > 0.05)	X <sup>2</sup> square	P-value (P > 0.05)
G894T eNOS	0.32	0.85*	0.63	0.73*
4G/5G PAI-1	7.03	0.003	44.8	<0.05
T-1131C APOA5	4.73	0.09*	1.07	0.59*

\* Significant.

**Table 2**  
Distribution of G894T eNOS, 4G/5G PAI-1 and T-1131C APOA5 polymorphisms according to risk factors among MI patients.

Risk Factors	Patients N (%)	GG (%)	eNOS GT (%)	TT (%)	P-value	4G/4G (%)	PAI 4G/5G (%)	5G/5G (%)	P-value	TT (%)	APOA5 TC (%)	CC (%)	P-value
Age	118				0,07				0,8				0,38
≤50	34	12 (35,3)	18 (53)	4 (11,7)		13 (38,2)	18 (53)	3 (8,8)		7 (20,6)	23 (67,64)	4 (11,76)	
>50	84	48 (57)	32 (38)	4 (5)		28 (33,3)	50 (59,5)	6 (7,2)		27 (32,14)	46 (54,76)	11 (13,1)	
Sex	118				0,01				0,1				0,44
Male	73	32 (44)	38 (52)	3 (4)		22 (30,1)	43 (58,9)	8 (11)		22 (30,13)	44 (60,27)	7 (9,6)	
Female	45	28 (62,2)	12 (26,7)	5 (11,1)		19 (42,2)	25 (55,6)	1 (2,2)		12 (26,7)	25 (55,6)	8 (17,7)	
HTA	118				0,26				0,09				0008
presence	62	33 (55)	27 (54)	2 (25)		25 (61)	35 (51,5)	2 (22,2)		18 (52,9)	31 (44,9)	13 (86,7)	
absence	56	27 (45)	23 (46)	6 (75)		16 (39)	33 (48,5)	7 (77,8)		16 (47,1)	38 (55,1)	2 (13,3)	
Diabetes	118				0,84				0,3				0,79
presence	54	27 (45)	24 (48)	3 (37,5)		20 (48,8)	32 (47,1)	2 (22,2)		17 (50)	31 (44,9)	6 (40)	
absence	64	33 (55)	26 (52)	5 (62,5)		21 (51,2)	36 (52,9)	7 (77,8)		17 (50)	38 (55,1)	9 (60)	
Smoking	118				0,04				0,39				0,76
presence	49	19 (31,7)	29 (58)	1 (12,5)		14 (34,1)	30 (44,1)	5 (55,6)		15 (44,1)	29 (42)	5 (33,3)	
absence	69	41 (68,3)	21 (42)	7 (87,5)		27 (65,9)	38 (55,9)	4 (44,4)		19 (55,9)	40 (58)	10 (66,7)	
Obesity	118				0,66				0,7				0,6
presence	22	13 (21,7)	8 (16)	1 (15,5)		9 (22)	12 (17,6)	1 (11,1)		7 (20,6)	11 (15,9)	4 (26,7)	
absence	96	47 (78,3)	42 (84)	7 (87,5)		32 (78)	56 (82,4)	8 (88,9)		27 (79,4)	58 (84,1)	11 (73,3)	
Dyslipidemia	118				0,37				0,34				0,88
presence	34	16 (26,7)	17 (34)	1 (12,5)		11 (26,8)	22 (32,4)	1 (11,1)		9 (26,5)	20 (29)	5 (33,3)	
absence	84	44 (73,3)	33 (66)	7 (87,5)		30 (73,2)	46 (67,6)	8 (88,9)		25 (73,5)	49 (71)	10 (66,7)	

with HTA and obesity; 4G/5G PAI-1 was not correlated with any one of the risk factors analyzed (Table 2).

Table 3 reports the genotypic and allelic distribution of G894T eNOS, 4G/5G PAI-1 and T-1131C APOA5 polymorphisms, and their transmission models among cases and controls. For the G894T eNOS variant, genotypic frequencies were 50.85% GG, 62.37% GT and 6.78% TT, and 63.04% GG, 33.69% GT and 6.27% TT among cases and controls respectively. They were 7.64% 5G/5G, 57.62% 4G/5G and 34.74% 4G/4G, and 39.68% 5G/5G, 26.63% 4G/5G and 33.69% 4G/4G among cases and

controls respectively for the 4G/5G PAI-1 variant; and 28.81% TT, 58.47% TC and 12.72% CC, and 51.08% TT, 40.21% TC and 8.71% CC among cases and controls respectively for the T-1131C APOA5 polymorphism. Allele frequencies of G894T eNOS variant were 72.03% G versus 27.97% T, and 79.89% G versus 20.11% T in cases and controls groups respectively. They were 36.45% 5G versus 63.55% 4G, and 52.98% 5G versus 47.02% 4G among cases and controls respectively for the 4G/5G PAI-1 variant, and 58.05% T versus 41.95% C, and 71.19% T versus 28.81% C among cases and controls respectively for the T-1131C APOA5 polymorphism.

For G894T eNOS variant, a significant increase of risk of MI was observed in both GT (OR = 1.55, 95% CI = 0.97–2.48, P = 0.02) and TT (OR = 2.57, 95% CI = 0.87–7.52, P = 0.01) genotypes. There was a positive correlation of the 3 models with MI risk (dominant model: OR = 1.64, CI = 1.05–2.58, P = 0.003; recessive model: OR = 2.15, CI = 0.74–6.16, P = 0.03; additive model: OR = 1.54, CI = 1.06–2.23, P = 0.001) (Table 3).

There was a significant correlation between 4G/4G and 4G/5G genotypes and MI risk (OR = 5.36, 95% CI = 3.8–7.2, P < 0.001; OR = 11.2, 95% CI = 8.3–15.08, P < 0.001 respectively). The dominant and additive models were significantly correlated to MI risk (OR = 7.96, 95% CI = 3.83–16.36, P = 0.01; OR = 1.96, 95% CI = 1.4–2.72, P = 0.01 respectively); yet the recessive model was not (OR = 1.04, 95% CI = 0.65–1.66, P = 0.8) (Table 3).

For the T-1131C APOA5 variant, only the mutated genotype CC was correlated to MI risk (OR = 2.6, 95% CI = 1.18–5.66, P = 0.03 versus OR = 2.57, 95% CI = 1.56–4.17, P = 0.3 for the TC genotype). The recessive and additive models show a significant increase of MI risk (OR = 1.53, 95% CI = 0.72–3.2, P = 0.04; OR = 1.78, 95% CI = 1.26–2.51, P = 0.03 respectively); the association was not significant in the dominant model (OR = 2.58, 95% CI = 1.6–4.09, P = 0.07) (Table 3).

#### 4. Discussion

Myocardial infarction (MI) is a complex pathology, determined by numerous factors, including genetic. Several genes were found to be in association with MI susceptibility, they are genes involved in regulation of the vascular system, (eNOS gene), lipid-related pathways (APOA5 gene), fibrinolytic system (PAI gene), DNA repair pathways, renin-angiotensin aldosterone system... (Zhang et al., 2012; Song et al., 2013; Gong et al., 2012; Forstermann and Munzel, 2006; Franco et al., 2007). Our study is the first to explore the association of G894T eNOS, 4G/5G

**Table 3**  
G894T eNOS, 4G/5G PAI-1 and T-1131C APOA5 polymorphisms frequencies and genetic models among cases and controls.

Genotypes/alleles		Cases N = 118 (%)	Controls N = 184 (%)	OR (95% CI)	P-value
eNOS	GG	60 (50,85)	116 (63,04)	1	
	GT	50 (42,37)	62 (33,69)	1,55 [0,97; 2,48]	0,02*
	TT	8 (6,78)	6 (3,27)	2,57 [0,87; 7,52]	0,01*
	GG + GT <sup>a</sup>	110 (93,22)	178 (96,73)	1	
	TT	8 (6,78)	6 (3,27)	2,15 [0,74; 6,16]	0,03*
	GG <sup>b</sup>	60 (50,85)	116 (63,04)	1	
	GT + TT	58 (49,15)	68 (36,96)	1,64 [1,05; 2,58]	0,003*
	G <sup>c</sup>	170 (72,03)	294 (79,89)	1	
	T	66 (27,97)	74 (20,11)	1,54 [1,06; 2,23]	0,001*
	PAI	5G/5G	9 (7,64)	73 (39,68)	1
4G/5G		68 (57,62)	49 (26,63)	11,2 [8,3–15,08]	<0,001*
4G/4G		41 (34,74)	62 (33,69)	5,36 [3,8–7,2]	<0,001*
4G/5G + 5G/5G <sup>a</sup>		77 (65,26)	122 (66,31)	1	
4G/4G		41 (34,74)	62 (33,69)	1,04 [0,65–1,66]	0,8
5G/5G <sup>b</sup>		9 (7,64)	73 (39,68)	1	
4G/4G + 4G/5G		109 (92,37)	111 (60,32)	7,96 [3,83–16,36]	0,01*
5G <sup>c</sup>		86 (36,45)	195 (52,98)	1	
4G		150 (63,55)	173 (47,02)	1,96 [1,4–2,72]	0,03*
APOA5		TT	34 (28,81)	94 (51,08)	1
	TC	69 (58,47)	74 (40,21)	2,57 [1,56; 4,17]	0,3
	CC	15 (12,72)	16 (8,71)	2,60 [1,18; 5,66]	0,03*
	TT + TC <sup>a</sup>	103 (87,28)	168 (91,29)	1	
	CC	15 (12,72)	16 (8,71)	1,53 [0,72; 3,2]	0,04*
	TT <sup>b</sup>	34 (28,81)	94 (51,08)	1	
	TC + CC	84 (71,18)	90 (48,91)	2,58 [1,6; 4,09]	0,07
	T <sup>c</sup>	137 (58,05)	262 (71,19)	1	
	C	99 (41,95)	106 (28,81)	1,78 [1,26; 2,51]	0,03*

OR: Odds Ratio; CI: Confidence Interval; P value: \*: significant; N: Number; %: percentage; a: Recessive model; b: Dominant model; c: Additive model.

PAI and T1131C APOA5 polymorphisms with myocardial infarction (MI) among a sample of Moroccan patients.

In our study sample, older aged patients ( $\geq 50$  years) were more frequent than subjects  $< 50$  year of age, and most of them were male. This was in agreement with Doughty et al.'s (2002) study, reporting that about 90% of MI patients were  $> 45$  years of age.

#### 4.1. eNOS

Regarding its critical role in vascular system and blood flow regulation, NO—the main product of endothelial NOS enzyme—has been implicated in the occurrence of numerous pathologies, such as ischemic stroke (Diakite et al., 2014), HTA (Tang et al., 2008), renal disease (Aldámiz-Echevarría and Andrade, 2012), and cardiovascular diseases (Charalambos et al., 2006). According to the literature, studies exploring the association of G894T eNOS polymorphism with MI predisposition still remain inconclusive and results are contradictory (Luo et al., 2014).

Concerning the association with risk factors, G894T eNOS polymorphism was significantly associated with sex and smoking (Table 2). There was also a significant association of G894T eNOS genotypes and genetic models with MI risk (Table 3). A meta-analysis performed by Luo et al. (2014), involving 21,068 MI patients from 34 studies, also reported that there were significant association of eNOS G894T genotypes and the transmission models with MI risk: OR = 1.41 for TT genotype, OR = 1.12 for GT genotype, OR = 1.35 for the recessive model, and OR = 1.18 for the dominant model. In another study involving a sample of 323 CAD Tunisian patients, Ben Ali et al. (2015) reported that G894T eNOS polymorphism was associated with CAD under the dominant and additive models, but not the recessive one (additive OR: 2.81; 95% CI [2.05–3.85];  $P < 0.001$ , dominant OR: 2.84; 95% CI [2.09–3.86];  $P < 0.001$ , and recessive  $P = 0.09$ ).

Several studies reported that carriers of the 894TT eNOS genotype have decrease of blood pressure comparing to those carrying the other genotypes. NO production-known for its role in regulation of blood pressure variability- is decreased and endothelial dysfunction occurs more often, leading to myocardial infarction development (Zhang et al., 2012; Song et al., 2013; Gong et al., 2012; Forstermann and Munzel, 2006; Franco et al., 2007; Diakite et al., 2014; Tang et al., 2008; Aldámiz-Echevarría and Andrade, 2012; Charalambos et al., 2006; Luo et al., 2014; Ben Ali et al., 2015; Casas et al., 2004; Veldman et al., 2002; Rankinen et al., 2000).

Results relating eNOS G894T polymorphism to MI risk still remain conflicting and inconclusive. Many of them have found that G894T eNOS is associated to MI occurrence (Zhang et al., 2012; Szabo, 2013; Antoniadis et al., 2005; Hingorani et al., 1999). In their meta-analysis, Luo et al., (2014) reported that subgroup analysis stratified according to ethnicity suggested that eNOS G894T variant was significantly correlated to MI susceptibility in the Asian subgroup with  $P < 0.05$  comparing to non-Asian subgroup ( $P > 0.05$ ). These findings suggest that ethnicity may explain some of the conflicting results related to the association between MI risk and G894T eNOS polymorphism, in addition to many other factors, such as differences of studies sample sizes; differences in selection of patients and controls.

#### 4.2. PAI

PAI-1 belongs to the serine proteinase inhibitor family, and is known for its role of regulating the endogenous fibrinolysis activity (Vaughan, 2005). Impaired fibrinolytic function, as being affected by increased plasma concentrations of PAI-1, is a marker of first and recurrent myocardial infarction, especially among young patients ( $< 50$  years of age) (Hamsten et al., 1985, 1987).

The most studied polymorphism in PAI-1 gene is the insertion-deletion 4G/5G. Subjects homozygous for the 4G allele have PAI-1 plasma levels about 25% higher than those carrying double copy of the 5G allele (Eriksson et al., 1995). Available data investigating the potential

association between 4G/5G PAI-1 polymorphism and myocardial infarction are inconclusive and provide divergent results (Li, 2012).

Our results show that 4G/5G PAI-1 polymorphism was not associated with any one of the risk factors analyzed (Table 2). We found that 4G allele was significantly associated with MI risk ( $P = 0.03$ ), this risk became elevated among subjects carrying double copy of the 4G allele (5.36 fold higher) (Table 3). Genetic dominant (4G/5G + 4G/4G vs 5G/5G) and additive (4G vs 5G) models were also significantly correlated to MI risk (OR = 7.96, 95% CI = 3.83–16.36,  $P = 0.01$  and OR = 1.96, 95% CI = 1.4–2.72,  $P = 0.01$  respectively), but not the recessive model (OR = 1.04, 95% CI = 0.65–1.66,  $P = 0.8$ ). In his meta-analysis, Li (2012) reported similar findings concerning the association of the 4G allele with MI risk among a Chinese Han population; he reported that subjects carrying the 4G allele of the PAI-1 4G/5G gene in this population might be predisposed to develop coronary artery disease. Actually, the PAI-1 4G allele shows higher transcriptional activity than the 5G allele; this may explain why subjects homozygous for the 4G allele have elevated plasma PAI-1 concentrations and are more predisposed to thrombotic events such as myocardial infarction (Dawson et al., 1991).

In a stratified analysis by ethnicity, Zhang et al. (2014) noted in his meta-analysis that Asians and Caucasians subjects carrying the 4G allele had higher risk of cardiovascular diseases, when Festa et al. (2003) reported that the PAI 4G/5G genotype explained 0.63% of the variability of circulating PAI-1 levels in non-Hispanic whites, 0.99% in Hispanics, and 2.37% in blacks, and that there interaction analyses did not show any statistical differences in the relation between PAI-1 levels and the 4G/5G genotype by ethnicity.

Our study sample was not in HWG equilibrium for the distribution of 4G/5G PAI polymorphism among cases and controls ( $P = 0.003$  and  $P < 0.05$  respectively). For that reason, we cannot say that the distribution in our sample study describes the real one of this variant among Moroccan MI patients. Thus, we cannot conclude about its association with MI risk among Moroccan MI patients.

#### 4.3. APOA5

APOA5 is a good candidate for the investigation of Gene-cardiovascular diseases (CVD) risk association, as it enhances Lipoprotein Lipase (LPL) activity and activates TG hydrolysis in the blood (Aberle et al., 2005; Merkel and Heeren, 2005). Increased plasma levels of triglycerides (TG) have always been considered as an important risk factor for (CVD) (Lim et al., 2013; Forrester, 2001; Hokanson and Austin, 1996). Literature report that about 50% of their final levels are genetically determined (Hubáček et al., 2009). Numerous studies have reported a significant association between the T1131C APOA5 variant and cardiovascular diseases development, especially myocardial infarction (MI) (Hubáček et al., 2003, 2009; Hubacek et al., 2004; Szalai et al., 2004; Zhou et al., 2013; Ouatou et al., 2014). However, this association was not observed in many other studies (Prochaska et al., 2010; Martinelli et al., 2007; Lee et al., 2004).

In our study, we found that T1131C APOA5 variant was associated with HTA and obesity. There was a significant association between the 1131C allele and MI risk ( $P = 0.03$ ); among subjects carrying double copy of this allele, MI risk increases (2.6 fold higher) (Table 3). These findings were in agreement with what Jing Xia et al. have found among a subgroup of Asian patients, but not among the European subgroup (Xia et al., 2015).

The distribution of genetic models shows an increase of MI risk under the recessive and additive models (Table 3), but not the dominant one. In 2013, Lin et al. (2013) performed a meta-analysis in which they combined the geographic distribution of APOA5 T1131C variant with different studies results, trying thereby to map a potential association of this polymorphism with CVD risk. Results of this study show that this variant was associated with CVD under the dominant, recessive and allelic models in both East Asia ( $P < 10^{-5}$ ) and India ( $P = 0.001$ ), but not in Europe and Brazil ( $P = 0.17$  and 0.613 respectively). In

other Asian ethnic populations such as Korean and Chinese, an elevated prevalence of 1131C APOA5 allele was found (Ratio of the 1131C allele in cases/controls = 1.14 and 1.23 respectively), in contrast to Caucasians (Ratio of the 1131C allele in cases/controls = 1). There is a high prevalence of insulin resistance, CAD, pancreatitis... in these regions where a strong association exists (Chandak et al., 2006), this may explain the heterogeneity of association found across these different geographic regions. All these findings support the idea of relating the inheritance of APOA5 T1131C variant to triglyceride increased levels and cardiovascular diseases risk, especially myocardial infarction (Smith and Ebrahim, 2003).

The divergence of results between studies investigating the association of G894T eNOS, 4G/5G PAI and T1131C APOA5 polymorphisms with MI risk can be explained by numerous factors such as studies sample sizes, differences of allele frequencies distribution among the studied populations, which may be as well affected by cultural reasons, eating habits... Also the ethnicity may explain a part of inconsistency between results found in literature (Lin et al., 2013). All these reasons and others may give rise to genetically differentiated populations, and automatically divergent results.

## 5. Conclusion

Our study is the first to evaluate the association of G894T eNOS, 4G/5G PAI and T1131C APOA5 polymorphisms with myocardial infarction risk among a sample of Moroccan patients. We found that eNOS G894T and APOA5 T1131C were significantly correlated to MI increased risk among Moroccan population. The PAI 4G/5G distribution was not in HWG equilibrium in cases and controls groups, so we cannot decide about its implication in MI susceptibility among MI Moroccan patients. Further studies including larger sample sizes and exploring interactions between these polymorphisms or between the genetic factor and biochemical parameters can provide useful information to better understand the susceptibility to MI and improve the biomedical context.

## Abbreviations

MI	myocardial infarction
eNOS	endothelial nitric oxide synthase
PAI	plasminogen activator inhibitor
APOA5	Apo-lipoprotein A5
CI	confidence interval
OR	odd ratio
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism

## Competing interest

The author(s) declare that they have no financial or non-financial competing interests.

## Authors' contributions

HHI and WH carried out the molecular genetic study and performed the statistical analysis and interpretation of results. BD was involved in the practical part of the study; he also provided substantive intellectual contribution to the study. FK and DB were involved in collection of data and blood samples from the Department of Cardiology, University Hospital Center Ibn Rochd, Casablanca, Morocco. RH and SN are the directors of the study; they critically revised the manuscript, provided important intellectual contribution and gave final approval of the version to be published. All authors read and approved the final version of the manuscript.

## References

- Aberle, J., Evans, D., Beil, F.U., Seedorf, U., 2005. A polymorphism in the apolipoprotein A5 gene is associated with weight loss after short-term diet. *Clin. Genet.* 68, 152–154.
- Aldámiz-Echevarría, Luis, Andrade, Fernando, 2012. Asymmetric Dimethylarginine-Endothelial Dysfunction and Renal Disease.
- Alhenc-Gelas, M., Aiach, M., 2001. Mutations et Polymorphismes génétiques associés Aux Thromboses (EMC – Hématologie 2001; 13-022-B-60, 11-001-G-20, 13 pp.).
- Antoniades, C., Tousoulis, D., Vasiliadou, C., Pitsavos, C., Chrysochoou, C., et al., 2005. Genetic polymorphism on endothelial nitric oxide synthase affects endothelial activation and inflammatory response during the acute phase of myocardial infarction. *J. Am. Coll. Cardiol.* 46, 1101–1109.
- Ben Ali, M., Messaoudi, S., Ezzine, H., Mahjoub, T., 2015. Contribution of eNOS Variants to the Genetic Susceptibility of Coronary Artery Disease in a Tunisian Population.
- Brown, N.J., Murphey, L.J., Srikuma, N., Koschachuhanan, N., Williams, G.H., Vaughan, D.E., 2001. Interactive effect of PAI-1 4G/5G genotype and salt intake on PAI-1 antigen. *Arterioscler. Thromb. Vasc. Biol.* 21, 1071–1077.
- Butt, C., Zheng, H., Randell, E., Robb, D., Parfrey, P., Xie, Y.G., 2003. Combined carrier status of prothrombin 20210A and factor XIII-A Leu34 alleles as a strong risk factor for myocardial infarction: evidence of a gene-gene interaction. *Blood* 101, 3037–3041.
- Casas, J.P., Bautista, L.E., Humphries, S.E., Hingorani, A., 2004. Endothelial nitric oxide synthase genotype and ischemic heart disease. Meta-analysis of 26 studies involving 23028 subjects. *Circulation* 109, 1359–1365.
- Chandak, G.R., Ward, K.J., Yajnik, C.S., Pandit, A.N., Bavdekar, A., Joglekar, C.V., et al., 2006. Triglyceride associated polymorphisms of the apoA5 gene have very different allele frequencies in Pune, India compared to Europeans. *BMC Med. Genet.* 7, 76.
- Charalambos, A., Dimitris, T., Carmen, V., Christos, P., Marina, T., Costas, T., Kyriakoula, M., Christodoulos, S., 2006. Genetic polymorphisms G894T on the eNOS gene is associated with endothelial function and vWF levels in premature myocardial infarction survivors. *Int. J. Cardiol.* 107, 95–100.
- Collet, J.P., Montalescot, G., Vicaut, E., Ankri, A., Walylo, F., Lesty, C., et al., 2003. Acute release of plasminogen activator inhibitor-1 in ST-segment elevation myocardial infarction predicts mortality. *Circulation* 108, 391–394.
- Colomba, D., Duro, G., Corrao, S., Argano, C., Di Chiara, T., Nuzzo, D., Pizzo, F., Parrinello, G., Scaglione, R., Licata, G., 2008. Endothelial nitric oxide synthase gene polymorphisms and cardiovascular damage in hypertensive subjects: an Italian case-control study. *Immun. Ageing* 5, 4.
- Dawson, S., Hamsten, A., Wiman, B., Henney, A., Humphries, S., 1991. Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. *Arterioscler. Thromb.* 11, 183–190.
- Diakite, Brehima, Hamzi, Khalil, Slassi, Ilham, EL Yahyaoui, Mohammed, EL Alaoui, Moulay M.F., Habbal, Rachida, Sellama, Nadiï, GMRAVC, 2014. G894T Endothelial Nitric Oxide Synthase Polymorphism and Ischemic Stroke in Morocco.
- Dias, R.G., Gowdak, M.M., Pereira, A.C., 2011. Genetics and cardiovascular system: influence of human genetic variants on vascular function. *Genes Nutr.* 6, 55–62.
- Ding, J., Nicklas, B.J., Fallin, M.D., de Rekeneire, N., Kritchevsky, S.B., Pahor, M., et al., 2006. Plasminogen activator inhibitor type 1 gene polymorphisms and haplotypes are associated with plasma plasminogen activator inhibitor type 1 levels but not with myocardial infarction or stroke. *Am. Heart J.* 152, 1109–1115.
- Doughty, M., Mehta, R., Bruckman, D., Das, S., Karavite, D., Tsai, T., Eagle, K., 2002. Acute myocardial infarction in the young: the University of Michigan experience. *Am. Heart J.* 143, 56–62.
- Eriksson, P., Kallin, B., van'tHooft, F.M., Båvenholm, P., Hamsten, A., 1995. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. *Proc. Natl. Acad. Sci. U. S. A.* 92, 1851–1855.
- Festa, Andreas, D'Agostino Jr., Ralph, Rich, Steven S., Jenny, Nancy S., Tracy, Russell P., Haffner, Steven M., 2003. Promoter (4G/5G) Plasminogen Activator Inhibitor-1 Genotype and Plasminogen Activator Inhibitor-1 Levels in Blacks, Hispanics, and Non-Hispanic Whites: the Insulin Resistance Atherosclerosis Study.
- Forrester, J.S., 2001. Triglycerides: risk factor or fellow traveler? *Curr. Opin. Cardiol.* 16, 261–264.
- Forstermann, U., Munzel, T., 2006. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* 113, 1708–1714.
- Förstermann, Ulrich, Sessa, William C., 2012. Nitric oxide synthases: regulation and function. *Eur. Heart J.* 33 (7), 829–837 (Apr).
- Franco, E., Palumbo, L., Crobu, F., Anselmino, M., Frea, S., et al., 2007. Reninangiotensin-aldosterone system polymorphisms: a role or a hole in occurrence and long-term prognosis of acute myocardial infarction at young age. *BMC Med. Genet.* 8, 27.
- Fruchart-najib, J., Bauge, E., Niculescu, L.S., Pham, T., Thomas, B., Rommens, C., 2004. Mechanism of triglyceride lowering in mice expressing human apolipoprotein A5. *Biochem. Biophys. Res. Commun.* 319, 397–404.
- Gaziano, J.M., 2008. Global burden of cardiovascular disease. Braunwald's Heart Disease: a Textbook of Cardiovascular Medicine, eighth ed. Elsevier Saunders, Philadelphia.
- Gong, L.L., Peng, J.H., Han, F.F., Zhu, J., Fang, L.H., et al., 2012. Association of tissue plasminogen activator and plasminogen activator inhibitor polymorphism with myocardial infarction: a meta-analysis. *Thromb. Res.* 130, e43–e51.
- Hamsten, A., Wiman, B., de Faire, U., Blombäck, M., 1985. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. *N. Engl. J. Med.* 313, 1557–1563.
- Hamsten, A., de Faire, U., Waldius, G., Dahlen, G., Szamosi, A., Landou, C., Blombäck, M., Wiman, B., 1987. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet* 2, 3–9.
- Hingorani, A.D., Liang, C.F., Fatibene, J., Lyon, A., Monteith, S., Parsons, A., et al., 1999. A common variant of the endothelial nitric oxide synthase (Glu298→Asp) is

- major risk factor for coronary artery disease in the UK. *Circulation* 100, 1515–1520.
- Hokanson, J.E., Austin, M.A., 1996. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J. Cardiovasc. Risk* 3, 213–219.
- Hubáček, J.A., Škodová, Z., Adámková, V., Vrablík, M., Hořínek, A., Lánská, V., Češka, R., Poledne, R., 2003. APOAV polymorphisms (T-1131/C and Ser19/Trp) influence plasma triglyceride levels and risk of myocardial infarction. *Exp. Clin. Cardiol.* 8, 151–154.
- Hubáček, J.A., Škodová, Z., Adámková, V., Lánská, V., Poledne, R., 2004. The influence of APOAV polymorphisms (T-1131 > C and S19 > W) on plasma triglyceride levels and risk of myocardial infarction. *Clin. Genet.* 65, 126–130.
- Hubáček, J.A., Adámková, V., Vrablík, M., Kadlecová, M., Zicha, J., Kuneš, J., Pit'ha, J., Suchánek, P., Poledne, R., 2009. Apolipoprotein A5 in Health and Disease.
- Iacoviello, L., Burzotta, F., Di Castelnuovo, A., Zito, F., Marchioli, R., Donati, M.B., 1998. The 4G/5G polymorphism of PAI-1 promoter gene and the risk of myocardial infarction: a meta-analysis. *Thromb. Haemost.* 80, 1029–1030.
- Isordia-Salas, Irma, Leanos-Miranda, Alfredo, Sainz, Irma M., Reyes-Maldonado, Elba, Borrayo-Sanchez, Gabriela, 2009. Association of the Plasminogen Activator Inhibitor-1 Gene 4G/5G Polymorphism with ST Elevation Acute Myocardial Infarction in Young Patients.
- Luo, Jian-Quan, Wen, Jia-Gen, Zhou, Hong-Hao, Chen\*, Xiao-Ping, Zhang\*, Wei, 2014. Endothelial Nitric Oxide Synthase Gene G894T Polymorphism and Myocardial Infarction: a Meta-Analysis of 34 Studies Involving 21068 Subjects.
- Kholler, H.P., Grant, P.J., 2000. Plasminogen-activator inhibitor type 1 and coronary artery disease. *N. Engl. J. Med.* 342, 1792–1801.
- Lee, K.W., Ayyobi, A.F., Frohlich, J.J., Hill, J.S., 2004. APOA5 gene polymorphism modulates levels of triglyceride, HDL cholesterol and FERHDL but is not a risk factor for coronary artery disease. *Atherosclerosis* 176, 165–172.
- Li, Yan-yan, 2012. Plasminogen Activator Inhibitor-1 4G/5G Gene Polymorphism and Coronary Artery Disease in the Chinese Han Population: a Meta-Analysis.
- Lim, S., Park, Y.M., Sakuma, I., Koh, K.K., 2013. How to control residual cardiovascular risk despite statin treatment: focusing on HDL-cholesterol. *Int. J. Cardiol.* 166, 8–14.
- Lin, Hua, Li, Lin, Zhou, Ping, Yang, Zheng, 2013. Combining Geographic Region with Metaanalysis to Map the Potential Association between Three Genetic Polymorphisms and Coronary Artery Disease.
- Martinelli, N., Trabetti, E., Bassi, A., Girelli, D., Friso, S., Pizzolo, F., Sandri, M., Malerba, G., Pignatti, P.F., Corrocher, R., Olivieri, O., 2007. The -1131T > C and S19W APOA5 gene polymorphisms are associated with high levels of triglycerides and apolipoprotein C-III, but not with coronary artery disease: an angiographic study. *Atherosclerosis* 191, 409–417.
- Merkel, M., Heeren, J., 2005. Give me A5 for lipoprotein hydrolysis! *J. Clin. Invest.* 115, 2694–2696.
- Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16, 1215.
- Nilsson, J.B., Boman, K., Jansson, J.H., Nilsson, T., Nilsson, U., 2008. The influence of acute-phase levels of haemostatic factors on reperfusion and mortality in patients with acute myocardial infarction treated with streptokinase. *J. Thromb. Thrombolysis* 26, 188–195.
- Nordt, T.K., Lohrmann, J., Bode, C., 2001. Regulation of PAI-1 expression by genetic polymorphism. Impact on atherogenesis. *Thromb. Res.* 103 (Suppl. 1), S1–S5.
- Ouatou, S., Ajjemami, M., Charoute, H., Sefri, H., Ghalim, N., Rhaissi, H., Benrahma, H., Barakat, A., Rouba, H., 2014. Association of APOA5 rs662799 and rs3135506 polymorphisms with arterial hypertension in Moroccan patients. *Lipids Health Dis.* 13, 60.
- Pennacchio, L.A., Olivier, M., Hubáček, J.A., Cohen, J.C., Cox, D.R., Fruchart, J.C., Krauss, R.M., 2001. RUBIN EM: an apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science* 294, 169–173.
- Perlemuter, Katy, Montalescot, Gilles, Bassand, Jean Pierre, 2005. *Infarctus Du Myocarde*.
- Prochaska, C.L., Picheth, G., Angheben-Oliveira, M.I., Costantini, C.O., de Souza, E.M., Pedrosa, F.O., Scartezini, M., 2010. The polymorphisms -1131T > C and the S19W of the APOA5 gene are not associated with coronary artery disease in a Brazilian population. *Clin. Chem. Lab. Med.* 48, 419–422.
- Rankinen, T., Rice, T., Perusse, L., Chagnon, Y.C., Gagnon, J., et al., 2000. NOS3 Glu298Asp genotype and blood pressure response to endurance training: the HERITAGE family study. *Hypertension* 36, 885–889.
- Sakowicz, A., Fendler, W., Lelonek, M., Pietrucha, T., 2010b. Genetic variability and the risk of myocardial infarction in Poles under 45 years of age. *Arch. Med. Sci.* 6, 160–167.
- Schaap, F.G., Rensen, P.C., Voshol, P.J., Vriens, C., Van der Vliet, H.N., Chamuleau, R.A., Havekes, L.M., Groen, A.K., Van dijk, K.W., 2004. ApoAV reduces plasma triglycerides by inhibiting very low density lipoprotein triglyceride (VLDL-TG) production and stimulating lipoprotein lipase-mediated VLDL-TG hydrolysis. *J. Biol. Chem.* 279, 27941–27947.
- Smith, G.D., Ibrahim, S., 2003. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int. J. Epidemiol.* 32, 1–22.
- Song, C., Pedersen, N.L., Reynolds, C.A., Sabater-Lleal, M., Kanoni, S., et al., 2013. Genetic variants from lipid-related pathways and risk for incident myocardial infarction. *PLoS One* 8, e60454.
- Szabo, Gabor Viktor, 2013. The Role and Importance of Gene Polymorphisms in the Development of Atherosclerosis.
- Szalai, C., Keszei, M., Doba, J., Proházska, Z., Kozma, G.T., Császár, A., Balogh, S., Almássy, Z., Fust, G., Czinner, A., 2004. Polymorphism in the promoter region of the apolipoprotein A5 gene is associated with an increased susceptibility for coronary artery disease. *Atherosclerosis* 173, 109–114.
- Talmud, P.J., Hawe, E., Martin, S., Olivier, M., Miller, G.J., Rubin, E.M., et al., 2002. Relative contribution of variation within the APOC3/A4/A5 gene cluster in determining plasma triglycerides. *Hum. Mol. Genet.* 11, 3039–3046.
- Tang, W., Yang, Y., Wang, B., Xiao, C., 2008. Association between a G894T polymorphism of eNOS gene and essential hypertension in Hani and Yi minority groups of China. *Arch. Med. Res.* 39, 222–225.
- Vaughan, D.E., 2005. PAI-1 and atherothrombosis. *J. Thromb. Haemost.* 3, 1879–1883.
- Veldman, B.A., Spiering, W., Doevendans, P.A., Vervoort, G., Kroon, A.A., et al., 2002. The Glu298Asp polymorphism of the NOS 3 gene as a determinant of the baseline production of nitric oxide. *J. Hypertens.* 20, 2023–2027.
- Williams, S., 2003–2004. The Association of the Glu298Asp Polymorphism of Endothelial Nitric Oxide Synthase (eNOS) with Outcome after Subarachnoid Hemorrhage. *Doris Duke Medical Students J Vol.* 3, pp. 52–56.
- Xia, Jing, Cai, Weiping, Peng, Caosheng, 2015. Association of APOA5 T1131C Polymorphism and Risk of Coronary Artery Disease.
- Ye, S., Green, F.R., Scarabin, P.Y., Nicaud, V., Bara, L., Dawson, S.J., Humphries, S.E., Evans, A., Luc, G., Cambou, J.P., Arveiler, D., Henney, A.M., Cambien, F., 1995. The 4G/5G genetic polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene is associated with differences in plasma PAI-1 activity but not with risk of myocardial infarction in the ECTIM study. *Thromb. Haemost.* 74, 837–841.
- Zhang, K., Bai, P., Shi, S., Zhou, B., Wang, Y., et al., 2012. The G894T polymorphism on endothelial nitric oxide synthase gene is associated with increased coronary heart disease among Asia population: evidence from a meta-analysis. *Thromb. Res.* 130, 192–197.
- Zhang, Hui Feng, Dong, Pingshuan, Yang, Xuming, Liu, Zhenghao, 2014. Plasminogen Activator Inhibitor-1 4G/5G Polymorphism is Associated with Coronary Artery Disease Risk: a Meta-Analysis.
- Zhou, Jianqing, Xu, Limin, Huang, Rong Stephanie, Huang, Yi, Le, Yanping, Jiang, Danjie, Yang, Xi, Xu, Weifeng, Huang, Xiaoyan, Dong, Changzheng, Ye, Meng, Lian, Jiangfang, Duan, Shiwei, 2013. Apolipoprotein A5 Gene Variants and the Risk of Coronary Heart Disease: a Case-Control Study and Meta-Analysis.