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PPARgamma Promoter Polymorphisms and Acute Coronary Syndrome

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ABSTRACT

Background: PPARgamma (PPARg) is a nuclear transcription factor involved in the control of lipid and glucose homeostasis. Two PPARg common polymorphisms, Pro12Ala and 161C>T, have been found to be associated with cardiovascular disease. In this study, in addition to PPARg coding region, we looked for genetic variations in promoters and their association with acute coronary syndrome (ACS).
Methods: We studied 202 Italian patients with ACS, and 295 healthy Italian subjects by dHPLC (denaturing high-performance liquid chromatography), heteroduplex analysis and direct sequencing or RFLP (restriction fragment length polymorphism) analysis for screening mutations.
Results: We identified 7 new and 2 already published polymorphisms in PPARg promoters. The C>T93695 (promoter 4) mutation showed significantly different genotype distribution and allele frequency between controls and ACS patients ($p<0.001$); the T allele conferred a protection against ACS at both univariate (OR: 0.45, 95% CI 0.29–0.69: $p<0.001$) and multivariate analysis adjusted for sex, age and traditional cardiovascular risk factors (OR: 0.44, 95% CI 0.25–0.76: $p<0.005$). Moreover, the 161C>T polymorphism allele frequency ($p=0.03$) and genotype distribution ($p=0.015$) resulted to be different in ACS group if compared to healthy controls.
Conclusions: The protective role of 93695C>T polymorphism in PPARg promoter in ACS suggests that PPARg genetic variants may affect the susceptibility to atherosclerotic diseases.

1. Introduction

Peroxisome proliferator-activated receptor-gamma (PPARg) is a nuclear transcription factor involved in the control of energy, as well as in the lipid and glucose homeostasis [1].

The PPARg gene is located at chromosome 3p25 and produces 4 different PPARg mRNAs by alternative splicing and promoter usage: the presence of 4 different promoters is thought to allow a finer tuning in the control of gene expression. PPARg1, PPARg3, and PPARg4 mRNAs encode the same protein [2–4], whereas PPARg2 mRNA product has an additional 30 amino acids (exon B) at the N terminus [5].

PPARg1 mRNA is rather ubiquitously expressed, PPARg3 mRNA is restricted to adipose tissue, colon epithelium, and macrophages, PPARg2 mRNA expression is confined to adipose tissue, whereas the location of PPARg4 mRNA expression has not yet been investigated [6]. Much evidence exists indicating that PPARg is an important determinant of gene expression in the adipocyte differentiation [7], a key process in the development of obesity, and, in turn, a risk factor for type 2 diabetes. In addition, PPARg may act directly on local vasculature in critical aspects of atherogenesis, e.g. interfering with vascular smooth muscle cells (VSMC) migration and plaque destabilization, reducing the expression of matrix metalloproteinase-9 in macrophages and VSMC, transforming macrophages in foam cells via oxidized-low density lipoproteins (ox-LDL) [8–15].

Two common polymorphisms of PPARg coding region have been widely studied: the Pro12Ala substitution a C>G change in exon B and 161C>T (Hys477Hys) in exon 6. Other authors found the Pro12Ala polymorphism associated with type 2 diabetes [16–18], insulin resistance [19], obesity [20], and cardiovascular diseases [21], while the T allele of the 161C>T polymorphism has been shown to be associated with reduced severity of coronary artery disease (CAD), measured as number of narrowed major coronary arteries [22].

On the other hand, few studies concerning PPARg promoter polymorphisms have been performed: a C>G substitution at position −681 from exon A2 (promoter 3) has been found to be associated with plasma low-density lipoprotein cholesterol concentrations

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[23]. Furthermore, an association between PPARg GTGC haplotype (corresponding to the P3-681C > G, P2-689C > T, Pro12Ala, C > G, and 1431C > T polymorphisms in this order) and the metabolic syndrome in the French population has been reported [24].

However, no definite data regarding the role of genetic variants on the occurrence of coronary atherosclerosis are available. Thus, we designed the present study in order to: (1) look for genetic variations in PPARg promoters between 2 Italian populations, acute coronary syndrome (ACS) patients and clinically healthy subjects; (2) investigate the possible relationship between the Pro12Ala and 161C > T polymorphisms of PPARg and ACS.

2. Materials and methods

2.1. Study population

The study population included 202 consecutive Italian patients (143 males and 59 females) recruited between March and September 2005 with a diagnosis of ACS and underwent primary percutaneous coronary intervention (PCI) at the Catheterization Laboratory of the Heart and Vessels Department of the Azienda Ospedaliero-Universitaria Careggi, Florence, Italy. All patients underwent coronary angiography performed by the Judkins' method and PCI with bare metal or drug-eluting stent implantation for *de novo* stenosis in ≥1 native coronary artery. Coronary vessels with at least 75% stenosis were defined as diseased.

Acute myocardial infarction was diagnosed according to the criteria established by the American College of Cardiology [25]. Unstable angina was defined as a history of new-onset, more frequent, more persistent or rest episode of chest pain, without typical changes of myocardial enzymes. The subjects were classified as having hypertension according to the guidelines of the European Society of Hypertension/European Society of Cardiology or if they reported taking antihypertensive medications, as verified by the physician [26]. Diabetic subjects were defined according to the criteria of the American Diabetes Association or on the basis of self-report data (if confirmed by medication or chart review) [27]. Dyslipidemia was defined according to the Third report of the National Cholesterol Education Program or if they reported taking antidyslipidemic drugs, as verified by the physician [28]. Body mass index was calculated as weight (kg)/height (m^2). A positive family history of cardiovascular diseases was defined as the presence of at least one first-degree relative who had developed a cardiovascular disease before the age of 55 years for men and 65 years for women. All patients signed an informed consent; the study complies with the Declaration of Helsinki and was approved by the local ethic committee. All patients received standard therapy that included aspirin, clopidogrel, low-molecular-weight heparin, intravenous nitrates, statins, β-blockers, angiotensin converting enzyme inhibitors, and so forth when appropriate. The control group consisted of 295 Italian unrelated subjects recruited from a population study conducted in Florence, Italy, between 2002 and 2004 [29]. A detailed interview addressed to personal and familial history was performed in the frame of a physical examination by expert physicians, in order to identify symptom-free subjects and to exclude those who were suspected of having some form of vascular disease.

All subjects signed an informed consent; the study complies with the Declaration of Helsinki and was approved by the local ethic committee.

2.2. Molecular analysis of the PPARg gene

Genomic DNA was isolated from peripheral blood with a QIAmp Blood Kit (QIAGEN, Hilden, Germany). Mutation screening analysis of PPARg genomic fragments was performed by amplification of 8 fragments covering promoter 2 (Pr2), promoter 3 (Pr3), promoter 4 (Pr4), exon B and exon 6 by using primers and temperatures described in Supplementary material. The promoter amplified products were analyzed by electrophoresis on a 2% agarose gel searching for short insertions/deletions. The denaturing high-performance liquid chromatography (dHPLC) analysis was carried out as previously described [30] by using the WAVE DNA Fragment Analysis System (Transgenomic, Cheshire, UK) after fast denaturing and slow re-naturing cycle of the amplified fragments.

Sequencing of the heteroduplexes was performed on an ABI310 sequencer by using the BigDye chemistry (Applied Biosystems, Warrington, Cheshire, UK).

2.3. Genotype typing

Genotyping of the following sequence variation in PPARg gene was carried out by restriction fragment length polymorphism (RFLP). Ten microliters of PCR products were digested at 37 °C for the first 5 fragments and at 65 °C for the sixth fragment for 6 h with 1 unit of restriction enzyme.

The 25819C > G sequence variation in Pr3 was digested with MboII which cuts the wild type allele into two fragments (133 + 195 bp). The 26233T > A sequence variation in Pr3 was digested with Pdm I restriction enzyme which cuts the wild type allele into two fragments (321 + 54 bp). The 93695T > C sequence variation in Pr4 was digested with Sdu I, which cuts the mutated allele into two fragments (240 + 141 bp). The Pro12Ala variation in Exon B was digested with Msp I, which cuts the wild type allele (20 + 217). The 161C > T sequence variation in Exon 6 was digested with Eco72 I, which cuts the wild type allele (105 + 194). The 93673T > C sequence variation in Pr4 was digested with Tai I which cuts the mutated allele into two fragments (215 + 166 bp). Where genotyping analysis by RFLP was not possible, we detected homozygous by dHPLC analysis: the amplicons of each subject (patients and controls) were mixed with the corresponding amplicons of a sequenced wild type, in a 1:1 quantitative ratio before undergoing a second heteroduplex analysis. Heteroduplexes were sequenced.

2.4. Statistical analysis

Statistical analysis was performed by using the Statistical Package of Social Sciences (SPSS, Chicago, IL) software for Windows (Version 11.5). Data are expressed as median and range. The χ^2 test was used to test for proportions and deviations of genotype distribution from Hardy–Weinberg equilibrium, the Fisher's exact test was used in the analyses of genotype frequencies where numbers below five were present. All variations in allele frequencies were obtained by direct count. The nonparametric Mann–Whitney test was used for comparisons between single groups. Kruskal–Wallis test was used to compare the different groups. To assess different models at each locus, genotypes at each position were coded as recessive model (e.g., Pr3 25819 GG vs 25819 CG + CC) and as dominant model (e.g., Pr3 25819 GG + CG vs CC). The relationship between the six polymorphisms and ACS was determined by univariate logistic regression analysis. Variables showing, at univariate analysis, a statistically significant association with ACS were introduced in a multivariate model to evaluate the association with the disease after adjustment for traditional cardiovascular risk factors. Odd ratio (OR) with 95% confidence interval (CI) was determined. $p < 0.05$ was considered to be statistically significant.

3. Results

Demographic characteristics and traditional risk factors for ACS are described in Table 1. As expected, patients showed a

Table 1
Demographic characteristic and traditional risk factors for cardiovascular disease.

Variable	ACS patients (n = 202)	Healthy subjects (n = 295)	p
Age (years)*	66 (25–89)	64 (20–89)	0.4
Males, n (%)	143 (70.8)	205 (69.6)	0.8
Females, n (%)	59 (29.2)	90 (30.4)	0.8
Hypertension, n (%)	120 (59.4)	51 (17.2)	<0.0001
Dyslipidemia, n (%)	96 (47.5)	60 (20.3)	<0.0001
Smoking habit, n (%)	105 (52)	68 (23)	<0.0001
BMI (kg/m^2)*	27 (17–39.6)	26 (15.7–40.9)	0.04
Diabetes, n (%)	54 (26.7)	14 (4.7)	<0.0001
Family history of CAD, n (%)	56 (27.7)	18 (6.1)	<0.0001

* Median (minimum and maximum value).

significantly higher prevalence of traditional cardiovascular risk factors with respect to clinically healthy subjects.

Table 2 shows genotype distributions and allele frequencies for all the identified PPARg mutations in ACS patients and clinically healthy subjects. All the frequency distributions are in Hardy–Weinberg equilibrium.

In promoter 3 of PPARg4 DNA variants were identified, 2 of them are novel; in addition 2 new variants were identified in 5′-UTR (untranslated region) of PPARg2 gene. For these mutations no difference between patients and clinically healthy subjects was found.

In promoter 4 of PPARg, never scanned for variations, 3 variants were found: the 93695C > T mutation showed a significantly different distribution between control subjects and ACS patients (genotype distribution: $p = 0.03$; allele frequency: $p = 0.006$).

With regard to the common polymorphism 161C > T, T allele frequency ($p = 0.03$) and genotype distribution ($p = 0.015$) were statistically different in ACS group with respect to controls. Finally, no different distribution between ACS patients and clinically healthy subjects for Pro12Ala polymorphisms was found.

In order to evaluate if these polymorphisms were significantly associated with ACS, we carried out a logistic regression analysis which showed a significant protection of the rare 93695T allele of the promoter 4 polymorphism (OR: 0.6, 95% CI 0.4–0.9, $p = 0.01$) against ACS. This result was confirmed even when we adjusted the analysis for the traditional cardiovascular risk factors (i.e. gender, age, smoking habit, diabetes, dyslipidemia, family history of ACS) (OR: 0.5, 95% CI 0.3–0.9, $p = 0.02$) (Table 3).

No association for metabolic and lipid profiles according to genotypes of all studied polymorphisms (25819C > G, 26233 T > A, 93673 T > C, 93695 C > T, Pro12Ala C > G, 161C > T) was found (Table 4).

Moreover, we sub-grouped our patient population according to the clinical severity of the disease, i.e. acute myocardial infarction and unstable angina, and we observed a statistically significant difference between the two groups of patients for the two common polymorphisms 161C > T (0.07 vs 0.22, $p = 0.0086$) and Pro12Ala (0.06 vs 0.16, $p = 0.02$), for acute myocardial infarction and unstable angina, respectively, while no differences for the 93695C > T mutation were found.

The two groups of patients did not show significant differences in terms of demographic characteristics and traditional risk factors for ACS, apart from gender (data not shown).

4. Discussion

Several authors extensively investigated PPARg coding region, but few data are available about the regulatory regions, such as the promoters. In the present study we have identified several polymorphisms by carrying out a molecular screening analysis of the PPARg promoters 2, 3 and 4.

Among the variants observed in promoter 4, we identified the 93695C > T substitution; interestingly, this novel polymorphism exhibited a significant difference in genotype distribution and allele frequency between patients and control subjects and showed an independent protective role against ACS in a logistic regression model adjusted for traditional cardiovascular risk factors in the Italian population. Further studies are required to verify the presence and the association of this polymorphism with ACS in other populations.

Sequence analysis of the promoter 4 genomic DNA revealed the existence of putative regulatory elements, some of which (Ap1, RORE, TATA) were studied by Sundvold and Sigbjørn [4]. Interestingly, the 93695C > T polymorphism is located at a binding consensus site for the transcription factor Zeste (searching by Tess-software). Zeste is a DNA binding protein that binds to enhancers and promoters of a number of developmentally important genes [31]. So far no Zeste-like proteins have been found in mammals, thus suggesting that the sequence variation in this binding site may have a role in modulating transcription of PPARg gene.

The fact that metabolic parameters did not differ between the 93695C/T genotypes may suggest that the 93695C > T or the 161C > T DNA variation affect the predisposition to ACS via one of the PPARg actions not involved in lipid and glucose metabolism.

Modulation of PPARg expression in vascular cells (e.g. VSMC, macrophages, endothelial cells) might influence vascular remodelling and inflammatory processes, being critical steps in CAD development. These effects are mediated in part by the regulation of transcription factors AP-1, STAT and NF-kB [8–15]. In particular PPARg activators inhibit proliferation and migration of vascular cells [14] and leukocyte-endothelial cell interaction [13]. Moreover, PPARg inhibits the synthesis of matrix metalloproteinase MMP9 and scavenger receptor A in macrophages [11] while promoting monocyte/macrophage differentiation and gene expression by influencing the uptake of oxidized LDL [8,9].

Further studies on the functional consequences of the 93695C > T polymorphism are required to clarify the role of this variant in ACS.

Regarding promoter 3, Meirhaeghe et al. [23] described the 25819C > G polymorphism in this regulatory region. In a French population, the authors found an allele frequency (0.25) significantly different ($p = 0.03$) from the one detected in the Italian group (Table 2). Moreover, their results showed a correlation between the presence of the G allele with higher total cholesterol, LDL cholesterol and body weight. Our findings did not show a similar trend in the Italian population.

The 26233T > A variant, detected in promoter 3, is already present in an international database (accession number AY157024).

Two new mutations, 65597T > C and 65600G > T, in 5′-UTR of PPARg2 with a very low allele frequency (<1%) have been detected; in particular, the 65597T > C is located at the end of an AP-1 binding site (nt + 1 from the 3′ end of the recognition sequence). The presence of both these mutations only in control subjects will require further investigation on a larger number of subjects to verify if these variants may play a protective role against ACS development.

Several studies about 161C > T polymorphism have been published. A French study [16] reported that in obese subjects 161C > T polymorphism influences circulating leptin levels. Recently, Chao et al. [32] observed an association between the 161TT genotype and premature acute myocardial infarction in Chinese. Therefore, PPARg seems to be involved in the genesis of ACS and our data showed that the T allele is statistically more frequent in ACS patients than in healthy subjects. On the other hand, Wang et al. [22] found a reduced CAD risk in 161CT heterozygous Australian patients, although their findings were based only on a selected high-risk hospital cohort of patients. Moreover, in our CAD patients the prevalence of T allele resulted to be significantly higher in those who were affected by angina with respect to those with myocardial infarction. This result suggests a relationship between the

Table 2
Genotype distribution and allele frequencies of PPARg polymorphisms. (Novel mutations are in grey-shadowed lines.).

			ACS Patients	Control Subjects	
Promoter 3	25819 CC		132 (65.3)	185 (62.7)	
	25819 CG		63 (31.2)	98 (33.2)	
	25819 GG		7 (3.5)	12 (4.1)	0.8*
		25819G	0.19	0.21	0.5§
	25924 CC		197	290	
	25924 CT		5	7	
	25924 TT		-	-	
		25924T	0.012	0.012	
	26233 TT		132 (65.3)	186 (63.1)	
	26233 TA		63 (31.2)	99 (33.6)	
	26233 AA		7 (3.5)	10 (3.4)	0.8*
		26233A	0.19	0.20	0.6§
	26348 AA		202	294	
	26348 AC		-	1	
	26348 CC		-	-	
		26348C	-	0.002	
5'-UTRPPARg 2	65597 TT		202	293	
	65597 TC		-	2	
	65597 CC		-	-	
		65597C	-	0.003	
	65600 GG		202	294	
	65600 GT		-	1	
	65600 TT		-	-	
		65600T	-	0.002	
Promoter 4	93640 TT		200	294	
	93640 TC		2	2	
	93640 CC		-	-	
		93640C	0.005	0.003	
	93673 TT		195 (96.5)	288 (97.3)	
	93673 TC		7 (3.5)	8 (2.7)	
	93673 CC		-	-	0.7*
		93673C	0.017	0.013	0.6§
	93695 CC		161 (79.7)	205 (69.3)	
	93695 CT		37 (18.3)	79 (26.7)	
	93695 TT		4 (2)	12 (4.1)	**0.03***
		93695T	0.11	0.17	**0.006§**
Exon B	P12A CC		169 (83.7)	258 (87.2)	
	P12A CG		30 (14.9)	38 (12.8)	
	P12A GG		3 (1.5)	-	0.085*
		P12A G	0.09	0.06	0.141§
Exon 6	161 CC		161 (79.7)	251 (85.1)	
	161 CT		36 (17.8)	44 (14.9)	
	161 TT		5 (2.5)	-	**0.015***
		161T	0.11	0.07	0.034§

*Genotype distribution and §allele frequency.

Table 3
Multivariate logistic regression.

Multivariate analysis	OR	95% CI	*p*
Gender (males vs females)	0.5	0.3–1.1	0.9
Age	1.01	0.9–1.026	0.2
Hypertension	6.5	3.9–10.7	<0.0001
Diabetes	7.2	3.3–15.6	<0.0001
Dyslipidemia	4.9	2.9–8.2	<0.0001
Family history of CAD	5.9	2.9–12.1	<0.0001
Smoking habit	3.8	2.3–6.3	<0.0001
PPARg Pr4 93695 CT + TT	0.5	0.3–0.9	0.02

Table 4
Lipid and metabolic profiles according to the PPARg polymorphisms genotypes in healthy subjects and patients.

Genotype	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL cholesterol (mg/dL)	Blood glucose (g/L)	BMI (kg/m^2)
25819 CC (n = 318)	199.5 ± 44.2	55.1 ± 13.7	119.9 ± 86.1	112.7 ± 30.9	0.94 ± 0.28	26.9 ± 3.9
25819 CG (n = 161)	191.3 ± 42.2	56.7 ± 13.3	109.2 ± 55.0	110.3 ± 33.4	0.88 ± 0.15	25.7 ± 3.6
25819 GG (n = 19)	191.9 ± 32.5	49.2 ± 11.4	150.3 ± 94.0	112.2 ± 25.0	0.97 ± 0.23	26.5 ± 4.5
p	0.4	0.1	0.1	0.9	0.7	0.3
26233 TT (n = 318)	199.7 ± 44.1	55.1 ± 13.6	119.8 ± 85.9	112.9 ± 31.1	0.94 ± 0.28	26.9 ± 3.9
26233 TA (n = 162)	191.2 ± 42.2	56.8 ± 13.3	109.4 ± 55.0	110.1 ± 33.2	0.88 ± 0.15	25.7 ± 3.6
26233 AA (n = 17)	189.7 ± 32.6	48.1 ± 10.8	153.7 ± 92.6	109.0 ± 24.7	0.98 ± 0.24	26.8 ± 4.5
p	0.2	0.08	0.3	0.07	0.1	0.8
93673 TT (n = 483)	196.7 ± 43.3	55.2 ± 13.4	118.1 ± 78.9	112.5 ± 31.5	0.92 ± 0.24	26.5 ± 3.8
93673 TC (n = 15)	190.6 ± 38.3	58.3 ± 14.6	106.4 ± 24.6	96.4 ± 29.9	1.00 ± 0.18	27.4 ± 4.4
93673 CC (n = 0)	–	–	–	–	–	–
p	0.8	0.5	0.8	0.2	0.2	0.5
93695 CC (n = 366)	195.7 ± 44.7	54.9 ± 13.5	119.2 ± 84.5	111.2 ± 32.0	0.92 ± 0.24	26.6 ± 3.9
93695 CT (n = 116)	198.7 ± 39.3	56.2 ± 13.8	113.3 ± 55.6	114.6 ± 31.2	0.92 ± 0.23	26.2 ± 3.8
93695 TT (n = 16)	200.5 ± 33.4	57.6 ± 10.2	115.8 ± 44.6	113.6 ± 23.4	0.98 ± 0.33	27.4 ± 3.9
p	0.8	0.6	0.9	0.9	0.7	0.7
P12A CC (n = 427)	197.1 ± 43.6	55.4 ± 13.7	117.1 ± 78.9	112.3 ± 31.7	0.93 ± 0.25	26.7 ± 3.9
P12A CG (n = 68)	193.5 ± 41.4	54.7 ± 12.8	122.0 ± 71.8	110.1 ± 31.7	0.89 ± 0.15	25.6 ± 3.4
P12A GG (n = 3)	181.0 ± 21.2	52.0 ± 11.2	73.0 ± 15.2	114.0 ± 34.2	0.88 ± 0.16	31.1 ± 12.0
p	0.2	0.5	0.9	0.9	0.8	0.6
161 CC (n = 412)	197.1 ± 43.9	55.2 ± 13.7	118.4 ± 80.2	112.5 ± 32.3	0.93 ± 0.26	26.6 ± 3.9
161 CT (n = 80)	195.4 ± 40.6	55.7 ± 13.2	113.3 ± 55.5	108.8 ± 28.7	0.87 ± 0.14	26.1 ± 3.7
161 TT (n = 5)	169.7 ± 28.4	55.0 ± 6.8	115.2 ± 71.9	108.5 ± 12.0	0.90 ± 0.16	26.1 ± 3.2
p	0.3	0.9	0.6	0.8	0.8	0.7

Data are expressed as mean ± SEM.

presence of this polymorphism and the severity of cardiovascular disease.

As regards the common polymorphism Pro12Ala, Ridker et al. [21] reported that the presence of Ala allele was associated with a reduced incidence of myocardial infarction in North-American men; in our study no different distribution for Pro12Ala was found between ACS patients and controls for both allele frequency and genotype distribution. These conflicting results might be due to the different clinical severity of pathology; indeed, by dividing our patient population in subgroups according to the clinical severity of disease, the prevalence of Ala allele resulted to be significantly higher in patients affected by angina with respect to those with myocardial infarction. On the other hand, our population comprised a higher number of males in the angina group with respect to the myocardial infarction group. Thus it is likely that the role of these polymorphisms (C > T161 and Pro12Ala) becomes evident in milder pathological conditions where the traditional cardiovascular risk factors are weakly present, or in a specific gender, as otherwise previously reported [21].

On the other hand, the conflicting results might also be related to the different genetic background: the population recruited by Ridker et al. [21] was predominantly made up by white U.S. males (but white may correspond to a pool of subjects with high genetic variability) and a low percentage of non-Caucasian subjects; moreover, in our Italian population there are 25% female subjects.

Recent papers did not find any association between Pro12Ala polymorphism and myocardial infarction [33,34] except for a minor risk of myocardial infarction in subjects affected by diabetes type 2 [35] and a higher risk of myocardial infarction in Chinese Han population [36].

We hypothesized that the Pro12Ala polymorphism *per se* may not be sufficient to significantly affect the risk of ACS. Rather, it is likely to play a role by interacting with additional environmental and supposedly genetic factors, which may be different in different populations.

Wolford et al. [37] reported an association of PPARg variants with the response to PPARg agonist (troglitazone), suggesting a new role for these variants in PPARg ligand therapy which stresses the importance of studies searching for new genetic variants for therapeutic interventions. The identification of mutations acting as modulators of PPARg gene expression may enlighten one aspect of the complex functional role of this gene and suggest new perspectives for the PPARg ligand therapy in cardiovascular diseases. However, the present finding needs to be strengthened by both increasing samples size and functional studies.

5. Conclusions

We have identified a new PPARg polymorphism (93695C > T) localized in a regulatory region and demonstrated the protective role of this polymorphism against ACS.

These results add further weight to the possible role of PPARg on the occurrence of clinical manifestations of atherosclerotic diseases and stimulate the need for studies addressed to investigate the association of this factor with different clinical manifestations of the coronary artery disease as well as with different localizations of the atherothrombotic process.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2008.11.009.

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