Association of Thrombophilic Gene Variant with Smoking as Risk Factors for Early Onset of Acute Coronary Syndrome

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Abstract: *Background*: Coronary heart disease was commonly associated to specific risk factors as smoking, diabetes, dyslipidemia, and hypertension since Framingham study confirmed their role. However, a lot of reports in the Literature are focused to find further risk factor for coronary heart disease in particular for subjects with early onset of disease.

Aim: The aim of the study was to look for an association between inherited thrombophilia and early onset of acute coronary syndrome.

Patients and Methods: We selected 25 patients with acute coronary syndrome with early onset (i.e. 50 yy) and tested them for several thrombophilic gene variants. Other common risk factors for coronary heart disease were also considered in selected patients. Blood samples were taken from antecubital vein of selected patients and in order to extract DNA for gene analysis. Evaluated thrombophilic gene variants were the following: factor V Leiden gene variant, prothrombin A20210G gene variant, MTHFR C677T gene variant, ACE I\D gene variant, APO-B Arg-3500-Trp and Arg-3500-Gln mutations and APO-E Arg-112-Cys and Arg-158-Cys mutations.

Results: Smoking was the only common risk factor for ACS significantly increased in the group with ACS compared to controls. No significant differences were found concerning diabetes, dyslipidemia, and hypertension in both groups. Homozygosis for MTHFR C677T and ACE del\del gene variant were found to be more frequent in the group of subjects with ACS compared to control group.

Discussion: Early onset of ACS seems to be associated to the presence of combined risk factors. In this panel, we may include acquired common risk factors for ACS in particular smoking and such gene variant associated to thrombotic disorders as MTHFR C677T gene variant and ACE del\del genotype. Further studies are needed to confirm this association.

Keywords: Thrombophilia, acute coronary syndrome, MTHFR gene variant, ACE gene polymorphism.

BACKGROUND

Coronary heart disease (CHD) may be present from a clinical point of view as acute or chronic illness. Moreover, acute CHD is divided into three different clinical presentations named acute coronary syndromes (ACS): unstable angina (UA), non-ST elevated myocardial infarction (NSTEMI), and ST elevated myocardial infarction (STEMI) [1, 2].

ACS are the most common cause of mortality in Western Countries and their incidence is really high after 50 years [3]. However, in the last years, an increased number of ACS has been observed before 50 years and are commonly summarised as juvenile ACS [3, 4]. The increase of juvenile ACS may be related to the improvement of diagnostic tools, to an increased surveillance of population and to a better knowledge of risk factors. Common risk factors for CHD, in fact, are well known since the Framingham Study was performed and are smoking, hypertension, dyslipidemia, diabetes, obesity, age and family history of CHD [5], Furthermore, in the last few years, further risk factors for CHD and ACS have been investigated in particular for patients that develop ACS without presence of common risk factors for CHD. A clear relationship between patients with family history of CHD and thrombophilia is still a matter of discussion [4].

Inherited thrombophilia with a trend towards hypercoagulable state is a well known risk factor for vascular disease, in particular, venous thromboembolism, also with early onset [6]. Inherited thrombophilia, in fact, is identified as one of the major risk factors for venous thromboembolism in any clinical presentations (e.g. superficial vein thrombosis, deep vein thrombosis, pulmonary embolism) and recurrent pregnancy loss without further causes of abortion [7], while its role in pathophysiology of arterial thrombosis, as ACS, is still under evaluation. Several studies have been performed on this topic but results are controversial also because a

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1876-5068/09

different inclusion and exclusion criteria of selected patients [8-16].

The aim of this study is to screen inherited thrombophilia through analysis of six gene variants in people with ACS before and after 50 years in order to identify possible markers predictor of ACS.

PATIENTS AND METHODS

Patients

We selected 25 consecutive patients (17 males and 8 females) were selected who were affected by acute coronary syndrome (ACS) with early onset (i.e. < 50 yy) accordingly with the international guidelines of the American Heart Association [1-2].

10 patients showed ACS as ST-elevated myocardial infarction (STEMI), while 11 patients showed ACS as non-elevated ST myocardial infarction and 4 as unstable angina.

As control group, subjects have been selected from the general population of Southern Italy. We excluded from the control group subjects with previous myocardial infarction and\or angina pectoris and women with recurrent pregnancy loss has been excluded because of the strong association of recurrent pregnancy loss and inherited thrombophilia.

Other common risk factors for ACS as smoking, diabetes, hypertension and dyslipidemia were also recorded.

As control group, 25 subjects (16 males and 9 females) were selected without personal and familial history of CHD.

Methods

Blood samples from antecubital vein were taken from each subject selected for the study and collected in EDTA tubes. 7 ml of blood were taken in order to screen gene variants with the trend toward thrombophilia.

Each DNA sample has been amplified by a simple multiple PCR and analysed by reverse dot-blot (Nuclear Medicine, Milan, Italy) for the following gene variants: factor V Leiden, prothrombin A20210G, methylenetetrahydrofolate reductase C677T gene variant, Angiotensin converting enzyme (ACE) insertion\deletion gene variant, apoliprotein B Arg-3500-Trp and Arg-3500-Gln mutations.

Statistical Analysis

Statistical analysis has been performed with chi-square test; moreover for expected variables for which attended value was < 5, Fisher exact test was performed. Results were considered to be significant if p values were < 0.05.

RESULTS

Analysis of Common Risk Factors for ACS

Common risk factors for ACS other than smoking were comparable in both groups and are summarised in Table **1**.

In particular, no difference was found for hypertension (40% of subjects with ACS *vs* 36% of control subjects, p: 0.87, ns), diabetes (12% of subjects with ACS *vs* 12% of control subjects, p: 1, ns), and dyslipidemia (24% of subjects with ACS *vs* 16% of control subjects, p: 1, ns), on the other hand, a significant difference was found for smoking (56% of subjects with ACS *vs* 20% of control subjects, p: 0.22, s).

Table 1. Risk Factors for SCA in Patients with SCA and Control Group

	Patients with SCA (25)	Control Group (25)	*p*
Hypertension	10/25 (40%)	9/25 (36%)	0.87
Diabetes	3/25 (12%)	3/25 (12%)	1
Dyslipidemia	6/25 (24%)	4/25 (16%)	1
Smoking	14/25 (56%)	5/25 (20%)	0.22

Analysis of Gene Variant associated to Thrombotic Disorders

Gene variant associated to thrombotic disorders were summarised in Table **2**, both for patients with ACS and control group.

Table 2. Gene Variants Associated to Thrombotic Disorders in Patients with ACS and in Control Group

	Patients with SCA (25)	Control Group (25)	*p*
MTHFR C677T heterozygosis	17/25 (68%)	14/25 (56%)	0.67, ns
MTHFR C677T homozygosis	8/25 (24%)	0/25 (0%)	0.03, s
PTHRA20210G heterozygosis	1/25 (4%)	0/25 (0%)	1, ns
FVL heterozygosis	0/25 0%	1/25 (4%)	1, ns
ACE del\del homozygosis	10/25 (40%)	3/25 (12%)	0.03, s

Heterozygosis for MTHFR C677T gene variant was present in 68% (17/25) of patients with ACS compared to 56% (14/25) of control subjects (p: 0.67, ns). Homozygosis for MTHFR C677T gene variant was present in 24% (8/25) of patients with ACS compared to none of the control group (p: 0.03, s).

Heterozygosis for prothrombin A20210G gene variant was present in 4% (1/25) of patients with ACS compared to none of the control subjects (p: 1, ns). Homozygosis for prothrombin A20210G was not present in patients with ACS nor in control subjects.

Heterozygosis for FVL gene variant was absent in patients with ACS compared to one subject of control group (p: 1, ns). Homozygosis for FVL was not present in patients with ACS nor in the control subjects.

Homozygosis for insertion/deletion polymorphism of ACE was present in 40% (10/25) of patients with ACS compared to 12% (3/25) of control subjects (p: 0.03, s).

Mutations Arg-3500-Trp and Arg-3500-Gln of apolipoprotein B were absent both in group of patients with ACS and in control group (p: 1, ns) (data not shown in Table **2**).

DISCUSSION

The association between inherited thrombophilia and venous thromboembolism is well known, but the association between inherited thrombophilia and atherosclerosis is still a matter of discussion. In the last years, several studies have

been performed and showed controversial results. In particular, less evidences of this association seems to be present for the association between inherited thrombophilia and acute coronary syndrome with early onset.

Lot of reports available in the Literature, in fact, failed because the different inclusion and exclusion criteria as for statistical power for not a simple evaluation of further common risk factors for atherothrombosis.

The results underlined that patients with early onset of ACS first of all frequently show common risk factors for atherothrombosis, in particular, smoking also raised statistical significance if patients were compared with early onset of ACS *vs* control group (Table **1**).

Moreover, a strong association between the associations of early onset of ACS was not found and compared to control group for the presence of such thrombophilic gene variant as factor V Leiden, A20210G prothrombin and heterozygosis for C677T MTHFR.

On the other hand, a strong relationship was found for the association of inherited thrombophilia and early onset of ACS for homozygosis for C677T gene variant of MTHFR and del\del gene variant of ACE. These data also raised statistical significance if compared with control group. Furthermore, these data may confirm previous data available in the Literature although in most previous reports an association with early onset was controversial.

In conclusion, early onset of ACS seems to be associated to the presence of combined risk factors. A model of risk may be suggested similar to that for venous thromboembolism, in which the presence of acquired risk factors, as smoking, may trigger a genetic predisposition for thrombotic disorders also including ACS. In this panel, such gene variant may be included associated to thrombotic disorders as MTHFR C677T gene variant and ACE del\del genotype. Further studies are needed to confirm this association in larger population of patients with early onset of ACS.

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Received: February 9, 2009 Revised: February 15, 2009 Accepted: February 28, 2009