Impact of Ethnicity and MTHFR Genotype on Age at Onset of Coronary Artery Disease in Women in Israel

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Abstract

Background: The C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene is associated with early onset of coronary artery disease in some populations with certain ethnic backgrounds. However, data on its effect on CAD development in women are limited and conflicting.

Objectives: To investigate the effects of the MTHFR C677T mutation and ethnicity on the development and age at onset of CAD in women in Israel.

Methods: The sample included 135 Jewish women with well-documented CAD (62 Ashkenazi, 44 Oriental and 29 of other origins) in whom CAD symptoms first developed at age ≤ 65 years. DNA samples from 235 women served as the control.

Results: CAD symptoms developed later in Ashkenazi than in Oriental women or women of other origins (51.0 ± 7.0 years vs. 48.3 ± 7.5 and 46.3 ± 7.7 years, respectively, $P = 0.024$). Among Ashkenazi women, the T/T genotype was less common in patients in whom CAD symptoms appeared after age 50 (6.4%) than in patients with earlier CAD symptoms (25.8%, $P = 0.037$) and Ashkenazi control subjects (23.3%, $P = 0.045$). Among women from other origins, these differences were not significant. On logistic regression analysis, the T/T genotype was associated with a nearly fourfold increase in the risk of early onset (age < 45 years) of CAD (odds ratio 3.87, 95% confidence interval 1.12–13.45, adjusted for risk factors and origin) and a trend towards an influence of ethnicity ($P = 0.08$). Compared to Ashkenazi women, the risk of early development of CAD associated with the T/T genotype among Oriental ones was 0.46 (95%CI 0.189–1.114) and in women of other origins, 5.84 (95%CI 1.76–19.34). Each additional risk factor increased the risk of earlier onset of CAD by 42% (OR 1.42, 95%CI 1.06–1.89).

Conclusions: The age at onset of CAD in Israeli women is influenced by the MTHFR genotype, ethnic origin and coronary risk factors.

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An elevated fasting plasma homocysteine level is associated with increased risk of coronary artery disease [1-3] and with mortality in patients with ischemic heart disease [4]. Fasting plasma homocysteine level is regulated by methylenetetrahydrofolate reductase (MTHFR), a key enzyme in homocysteine remethylation. Homozyosity for the C677T mutation in the MTHFR gene, found in 5–15% of western populations, results in decreased activity of this enzyme [5,6] and is associated with hyperhomocysteinemia [6], particularly in subjects with low plasma levels of folate [7], a co-factor in homocysteine remethylation.

Studies of some populations with certain ethnic backgrounds – from Ireland [8], The Netherlands [9,10], Japan [11,12] and Israel [13] – found an association between the C677T mutation and both with CAD [8-12], and with early onset of CAD [13]. However, the data on its effect on CAD development in women are limited and conflicting. In investigations of the risk of myocardial infarction in young women, one study from The Netherlands [14] found a higher risk in T/T homozygotes with low plasma folate levels, whereas another study from North America did not [15].

In an earlier study from Israel, which included a predominantly male group of patients, we found an association between the T/T genotype and early onset of CAD [13]. However, the role played by this genotype in Israeli women has not been examined. The purpose of the present study was to examine the possible effects of the T/T genotype on age at onset of CAD among Jewish women of different ethnic backgrounds in Israel.

Patients and Methods

The study sample included 135 consecutive Israeli Jewish women with well-documented CAD attending our coronary clinic in whom disease symptoms first developed at age ≤ 65 years. Symptoms were defined as typical anginal pain or a symptomatic acute myocardial infarction. The diagnosis of CAD was based on the coronary angiographic finding of > 50% stenosis in at least one epicardial coronary artery or a well-documented history of myocardial infarction diagnosed by clinical, electrocardiographic and biochemical criteria. All the patients were interviewed and their hospital charts were reviewed for data on age at onset of CAD symptoms, ethnic origin, smoking habits, body weight, premature CAD in first-degree relatives, hypertension (blood pressure > 140/90 mmHg or receiving antihypertensive treatment), diabetes mellitus, dyslipidemia (low density lipoprotein-cholesterol > 130 mg/dl or high density lipoprotein-cholesterol < 40 mg/dl or triglycerides > 300 mg/dl, or on lipid-lowering treatment), serum creatinine, and use of medications including vitamins. Those excluded were patients with renal failure, hypothyroidism, monogenic familial hypercholesterolemia, patients taking folic acid, vitamin B6 or B12, and patients who failed to give informed

CAD = coronary artery disease
CI = confidence interval
OR = odds ratio

consent. All participants underwent coronary angiography for reasons unrelated to this study.

Because of the possible effect of genetic background on the association between the C677T mutation and CAD, and the marked ethnic differences in the frequency of this mutation in Israel, we also divided the patients and controls according to ethnic origin. The patients' ethnic origin was defined according to their parents' ethnic origin, as follows: Ashkenazi Jews are Jews originating from France and Germany who immigrated to Israel from the United States, Western Europe, and Eastern Europe including the former USSR. Oriental Jews are those originating from Spain, Portugal and the Near East who immigrated to Israel from Near and Far East countries including Turkey, Iraq, Iran and Yemen. The origin of women whose parents' origin was unknown or did not match any of these groups and women whose maternal and paternal parents were of different ethnic groups was defined as "other." All the data on ethnic origin were obtained by interview.

For control purposes, DNA samples were collected from 235 random Jewish women with well-defined ethnic origin (women whose two parents were of the same ethnic origin) who underwent prenatal screening at the genetic clinic in our center. All were younger than 45 years and had no history of CAD.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in approval by the Human Research Committee of the Rabin Medical Center, and informed consent was obtained from each participant.

Genetic analysis

DNA was isolated from peripheral leukocytes with a DNA Isolation Kit for Mammalian Blood (Boehringer Mannheim, Germany). Screening for the 677C →T substitution was performed by polymerase chain reaction of genomic DNA, followed by Hinf I digestion and agarose gel electrophoresis as described by Frosst et al. [5].

Statistical analysis

Comparison between means was performed with the ANOVA test and comparison between frequencies with the Pearson chi-square test. The effect of the interaction of biological parameters according to age at onset of CAD (early vs. late) and MTHFR genotype (T/T vs. non-T/T) was assessed by two-way ANOVA. Logistic regression analysis was used to calculate the odds ratio and associated 95% confidence intervals for the risk associated with the T/T genotype, ethnic origin and number of risk factors. A P value < 0.05 was considered significant.

Results

The patients' characteristics are listed in Table 1. The age at onset of CAD was well defined in all the patients. CAD developed significantly later in Ashkenazi patients than in Oriental patients or patients of other origins. The Ashkenazi patients also had dyslipidemia and hypertension more often and a greater total number of risk factors per patient.

The genotypes conformed with the Hardy-Weinberg equilibrium. Figure 1 shows the frequency of the T/T genotype by ethnic origin, and age at onset of CAD symptoms. Within the Ashkenazi group, the prevalence of the T/T genotype was highest in the younger patients and decreased with increasing age at onset. A similar trend was noted for patients of Oriental origin and for those of other origins, although the frequency of the T/T genotype peaked later in the Oriental group than in the other two ethnic groups.

Table 1. Patient characteristics

	Ashkenazi (n=62)	Oriental (n=44)	Other (n=29)	*P*
Age at CAD onset (yrs)	51.0 (7.0)	48.3 (7.5)	46.3 (7.7)	0.024
T/T	16%	11%	14%	0.78
Cigarette smoker	50%	53%	75%	0.07
Diabetes	34%	37%	28%	0.75
Dyslipidemia	70%	53%	48%	0.099
Hypertension	64%	30%	32%	0.001
Family history	66%	65%	40%	0.19
Obesity	52%	23%	41%	0.02
NRF (n)	3.4 (1.3)	2.7 (1.6)	2.7 (1.6)	0.045

Data on age and number of risk factors are presented as mean (SD). All other data are percentages.
NRF = number of risk factors per patient

Table 2. Distribution of the T/T genotype among patients and control subjects

	Patients							
	Age ≤ 50 years		Age > 50 years			Control		
	n	T/T (%)	n	T/T (%)	*P**	n	T/T (%)	*P***
Ashkenazi	31	8 (25.8)	31	3 (6.4)	0.037	77	18 (23.3)	0.045
Oriental	27	3 (11.1)	17	2 (11.8)	NS	158	10 (6.3)	NS
Other	22	4 (18.2)	7	0 (0)	NS	–	–	–

* ≤ 50 years vs. > 50 years, ** >50 years vs. control

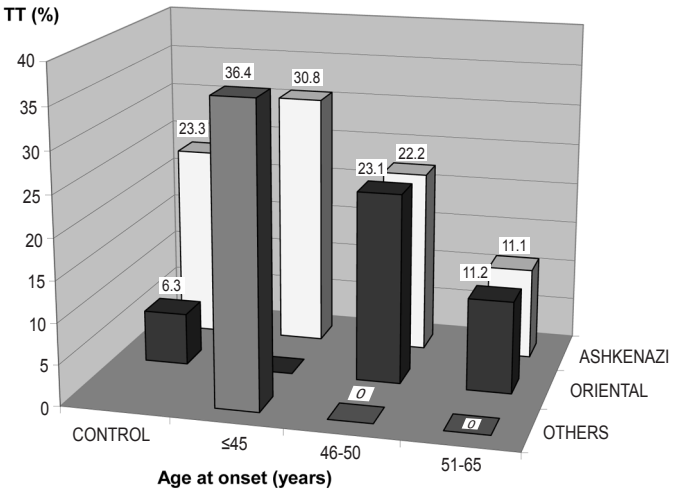

Figure 1. Distribution of the T/T genotype among the patients according to ethnic origin and age at onset of CAD

Table 2 presents the frequency of the T/T genotype in the control group according to ethnic origin, and in the patients according to ethnic origin and age at onset of CAD. The frequency of the T/T genotype was markedly higher in the control subjects who were of Ashkenazi origin than in the control subjects of Oriental origin ($P < 0.001$). The frequency of the T/T genotype was significantly lower in Ashkenazi patients in whom CAD symptoms appeared at age > 50 years compared to Ashkenazi patients who experienced CAD symptoms earlier, and compared to Ashkenazi control subjects. A similar trend was observed in the other ethnic groups as well but the differences did not reach statistical significance.

Logistic regression analysis of a model that included the MTHFR genotype (T/T vs. non-T/T), ethnic origin and the number of risk factors per patient showed that after adjustment for ethnic origin and coronary risk factors the T/T genotype was associated with a nearly fourfold increase in the risk of early onset of CAD (age < 45 years) in women (OR 3.87, 95%CI 1.12–13.45) and a trend towards an influence of the ethnic origin ($P = 0.08$). Compared to Ashkenazi women, the risk of early development of CAD associated with the T/T genotype in Oriental women was 0.46 (95%CI 0.189–1.114) and in women of other origins, 5.84 (95%CI 1.76–19.34). Each additional risk factor increased the risk of earlier onset of CAD by 42% (OR 1.42, 95%CI 1.06–1.89).

Discussion

The results of this study show, for the first time, an association between the MTHFR genotype and age at onset of CAD in women, and that in Israeli Jewish women the age at onset of CAD is influenced also by ethnic origin. Together with our previous findings, these results show that in Israel, homozygosity for the C677T mutation in the MTHFR gene is associated with earlier onset of CAD in both women and men.

The frequency of the TT genotype markedly increased with decreasing age in all three ethnic groups [Figure 1]. Interestingly, the frequency of the TT genotype peaked later in Oriental women than in the other ethnic groups. However, it then declined with increasing age, as in the other two groups. This relatively late peak is probably explained by the age of the youngest Oriental cases and, as our results show, by the effect of concomitant coronary risk factors and the ethnic background itself. The age at which certain concomitant acquired risk factors developed could also have played a role in this context. The older age of the Ashkenazi group is probably explained by the same factors. The number of risk factors was higher in the Ashkenazi group, owing mainly to the higher prevalence of hypertension and obesity and a trend towards higher prevalence of dyslipidemia, all of which may reflect genetic predisposition as well as specific nutritional habits. In addition, it has been suggested that the T/T genotype may predispose to obesity [16] and hypertension [17]. Although these associations have not been confirmed in women, it is possible that the high frequency of the T/T genotype in Ashkenazi patients contributed to the higher frequency of obesity and hypertension in these patients. However, the results of regression analysis clearly showed, despite the relatively small number of cases, a significant association between the T/T genotype and early CAD that characterized the entire study population and was independent of ethnic background and the prevalence of coronary risk factors. The finding that the frequency of the T/T genotype increased with decreasing age in three different ethnic groups [Figure 1] further supports the conclusion that this genotype is associated with early onset of CAD in Jewish women, and reduces the possibility of an effect of chance.

Our results may appear to conflict with those of Schwartz et al. [15], who found no association between the MTHFR genotype and risk of myocardial infarction in young North American women. However, it is noteworthy that none of the studies that examined the relationship between the MTHFR genotype and risk of CAD in a North American population has yielded a positive result, in contrast to the positive findings in some other populations, including our findings in Israeli men [8-13]. Thus our results, together with previous findings, suggest that the effect of the MTHFR genotype on CAD development is similar in men and women depending on their ethnic background.

We found marked ethnic differences in frequency of the T/T genotype in our control subjects, with a particularly high frequency in the Ashkenazi control group. These findings are highly consistent with the data reported previously from Israel [18-21]. Of note, previous reports from Israel also showed a high consistency in the frequency of the T/T genotype in different age groups within each ethnic group [18-21], indicating that the frequency of the T/T genotype in healthy subjects in this population does not vary with age.

Recently, an association between the C677T mutation, endothelial cell dysfunction and cardiac syndrome X has been reported in women from Israel [22]. This finding shows that in women the T/T genotype is associated with symptoms of myocardial ischemia also in the absence of coronary atherothrombotic lesions. However, because evidence for significant coronary lesions was found in all our patients on coronary angiography and they underwent the procedure immediately or shortly after the onset of symptoms, it is unlikely that symptomatic endothelial cell dysfunction could have played an important role in determining the time of onset of symptoms.

Examination of the effects of the mutation on CAD in women was important because of the reported gender differences in the effect of the mutation on plasma homocysteine. Russo and colleagues [23] examined the relationship among MTHFR genotype, age, gender and fasting plasma homocysteine in participants of the Framingham Offspring study, and found lower geometric mean plasma homocysteine and higher folate levels in women, and higher plasma homocysteine levels in subjects > 55 years old and in T/T participants than in participants with C/C or C/T genotypes. However, the association between genotype and plasma homocysteine was confined to men < 55 years old with low plasma folate. Importantly, they found no gender difference in the frequency of the T allele or the T/T genotype. The similarity in the frequency of the T/T genotype in our control group, which included only women, and other control groups from Israel, which included mixed or male populations, agrees

with this finding. The lower homocysteine levels found in their women underscores the importance of examining the effect of the C677T mutation on CAD in women. However, because in their and other reports of North American Caucasian populations, the T/T genotype was found to have only a mild effect on plasma homocysteine, and no effect on CAD, in contrast to the marked effect of this mutation on plasma homocysteine [22] and on CAD onset [13] reported for Israeli CAD patients, and absence of age effect on plasma homocysteine in Israeli control subjects [24], their findings on the relationship between the MTHFR genotype and plasma homocysteine do not necessarily contradict our findings.

All the DNA samples used for control in the present work were from unselected women at childbearing age. Because the exact age at the time of genotyping was not recorded, we could not match our patients and control subjects for age. Nevertheless, in view of the well-documented consistency in Israel in the frequency of the T/T genotype within each ethnic group by age [18-21], it is unlikely that matching for age in the present study would alter the results.

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