Cardiovascular Events Are Not Associated with *MTHFR* Polymorphisms, But Are Associated with Methotrexate Use and Traditional Risk Factors in US Veterans with Rheumatoid Arthritis

Lisa A. Davis, Grant W. Cannon, Lauren F. Pointer, Leah M. Haverhals, Roger K. Wolff, Ted R. Mikuls, Andreas M. Reimold, Gail S. Kerr, J. Steuart Richards, Dannette S. Johnson, Robert Valuck, Allan Prochazka, and Liron Caplan

ABSTRACT. ***Objective.*** C677T and A1298C polymorphisms in the enzyme methylenetetrahydrofolate reductase (*MTHFR*) have been associated with increased cardiovascular (CV) events in non-rheumatoid arthritis (RA) populations. We investigated potential associations of *MTHFR* polymorphisms and use of methotrexate (MTX) with time-to-CV event in data from the Veterans Affairs Rheumatoid Arthritis (VARA) registry.

Methods. VARA participants were genotyped for *MTHFR* polymorphisms. Variables included demographic information, baseline comorbidities, RA duration, autoantibody status, and disease activity. Patients' comorbidities and outcome variables were defined using International Classification of Diseases-9 and Current Procedural Terminology codes. The combined CV event outcome included myocardial infarction (MI), percutaneous coronary intervention, coronary artery bypass graft surgery, and stroke. Cox proportional hazards regression was used to model the time-to-CV event.

Results. Data were available for 1047 subjects. Post-enrollment CV events occurred in 97 patients (9.26%). Although there was a trend toward reduced risk of CV events, *MTHFR* polymorphisms were not significantly associated with time-to-CV event. Time-to-CV event was associated with prior stroke (HR 2.01, 95% CI 1.03–3.90), prior MI (HR 1.70, 95% CI 1.06–2.71), hyperlipidemia (HR 1.57, 95% CI 1.01–2.43), and increased modified Charlson-Deyo index (HR 1.23, 95% CI 1.13–1.34). MTX use (HR 0.66, 95% CI 0.44–0.99) and increasing education (HR 0.87, 95% CI 0.80–0.95) were associated with a lower risk for CV events.

Conclusion. Although *MTHFR* polymorphisms were previously associated with CV events in non-RA populations, we found only a trend toward decreased association with CV events in RA. Traditional risk factors conferred substantial CV risk, while MTX use and increasing years of education were protective. (J Rheumatol First Release April 1 2013; doi:10.3899/jrheum.121012)

Key Indexing Terms:
RHEUMATOID ARTHRITIS METHOTREXATE SINGLE-NUCLEOTIDE POLYMORPHISM METHYLENETETRAHYDROFOLATE REDUCTASE CARDIOVASCULAR DISEASES

From the Department of Rheumatology, University of Colorado School of Medicine, Denver, in conjunction with the Department of Veterans Affairs, Denver, Colorado, USA.

The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs.

Supported by Veterans Affairs Health Services Research & Development (HSR&D) grant SHP 08-172 and the Division of Rheumatology, University of Colorado Denver. Dr. Davis is supported by NIH/NIAMS T32 AR007534-24, National Institutes of Health Loan Repayment Award, the American College of Rheumatology Research and Education Fund Rheumatology Scientist Development Award, and the Colorado Clinical and Translational Science Institute. Dr. Caplan is supported by VA HSR&D Career Development Award CDA 07-221.

L.A. Davis, MD, Denver Veterans Affairs Medical Center (VAMC) and University of Colorado School of Medicine; G.W. Cannon, MD, George E. Wahlen VAMC and University of Utah, Salt Lake City, Utah; L.F. Pointer, MS; L.M. Haverhals, MA, Denver VAMC and University of Colorado School of Medicine; R.K. Wolff, PhD, George E. Wahlen VAMC and University of Utah, Salt Lake City, Utah; T.R. Mikuls, MD, MSPH, Omaha VAMC and Nebraska Arthritis Outcomes Research Center, University of Nebraska Medical Center, Omaha, Nebraska; A.M. Reimold, MD, Dallas VAMC and Texas Southwestern University Medical Center, Dallas, Texas; G.S. Kerr, MD; J.S. Richards, MD, Washington, DC VAMC and Georgetown University, Washington, DC; D.S. Johnson, DO, Jackson VAMC and University of Mississippi, Jackson, Mississippi; R. Valuck, PhD, University of Colorado Skaggs School of Pharmacy, Denver, Colorado; A. Prochazka, MD, MSc; L. Caplan, MD, PhD, Denver VAMC and University of Colorado School of Medicine.

Address correspondence to Dr. L. Davis, University of Colorado School of Medicine, Barbara Davis Center, 1775 Aurora Ct Mail Stop B-115, Denver, CO 80045-2536, USA. E-mail: lisa.davis@ucdenver.edu

Accepted for publication January 25, 2013.

In rheumatoid arthritis (RA), cardiovascular (CV) disease represents the most important cause of mortality[1]. Further, the rate of CV disease in RA exceeds that of the general population[2]. Traditional risk factors such as age, sex, smoking, hypertension, and diabetes have not fully explained this increased risk of CV disease, leading investigators to search for novel biologic or genetic explanations[3]. Some of these theories include an increased genetic susceptibility inherent in RA, enhanced systemic inflammation, or a modification of traditional CV risk factors by RA.

Methotrexate (MTX) is the "cornerstone" drug in treatment of RA[4]. MTX affects the folic acid pathway, and has been associated with hyperhomocysteinemia[5]. Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that also participates in the folic acid pathway[6] and catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Two *MTHFR* gene polymorphisms, C677T and A1298C [single-nucleotide polymorphism (SNP) identification numbers rs1801133 and rs1801131, respectively], result in an enzyme with reduced activity[6]. These *MTHFR* polymorphisms have been linked to the occurrence of MTX adverse drug reactions in a number of reports[7,8,9,10].

MTHFR polymorphisms have also been implicated in the occurrence of CV disease in the general population. Specifically, patients with the *MTHFR* C677T polymorphism may have an increased risk of CV disease[11], premature myocardial infarction (MI) in the setting of angiographically normal coronary arteries[12], and ischemic stroke[11,13]. Further, individuals who are homozygous for *MTHFR* C677T polymorphism demonstrate increased levels of homocysteine[6], and elevated concentrations of this amino acid may impart additional risk for CV and cerebrovascular disease[11].

Recognizing the biologic plausibility of these associations, reports linking *MTHFR* polymorphisms with CV risk have been inconsistent in their findings. Of 2 metaanalyses examining *MTHFR* C677T in non-RA populations, one supported an increased risk of CV disease[14], while the other did not[15]. In RA — a condition known to accelerate CV disease — a single modest-size case-control study (n = 612 RA cases) investigating *MTHFR* polymorphisms and CV disease supported an association of *MTHFR* A1298C with increased CV risk[16]. However, that study did not control for important covariates such as MTX use.

Because CV disease is the most common cause of morbidity and mortality in patients with RA, *MTHFR* polymorphisms may carry an increased risk of CV events, and MTX may further complicate the effects of the *MTHFR* polymorphisms, we hypothesized that RA patients with *MTHFR* C677T and A1298C polymorphisms would be at greater risk for CV events (decreased time-to-CV event) compared to RA patients without these polymorphisms, after controlling for important risk factors.

MATERIALS AND METHODS

Study design. We evaluated a prospective observational registry supplementing with data recorded in patients' electronic medical records.

Setting. This substudy of the Veterans Affairs Rheumatoid Arthritis (VARA) registry included data from 6 US Department of Veterans Affairs medical centers: Denver, CO; Omaha, NE; Salt Lake City, UT; Washington, DC; Dallas, TX; and Jackson, MS. VARA is a prospective longitudinal clinical registry and biologic repository. Details of the registry are described elsewhere[17,18].

Participants. Inclusion criteria consisted of patient participation in the VARA registry, patients meeting the 1987 American College of Rheumatology criteria for RA[19], and the availability of genotyping data for *MTHFR* C677T and/or A1298C polymorphisms (n = 1047).

Each participating VARA site obtained Institutional Review Board approval and all subjects underwent an informed consent procedure. The Colorado Multiple Institutional Review Board granted approval for this VARA substudy, as did the VARA Scientific Ethics Advisory Committee.

Genotyping. DNA samples were derived from whole blood for all study subjects collected at enrollment into VARA. Patients were genotyped for the *MTHFR* C677T (rs1801133) and A1298C (rs1801131) polymorphisms. For amplification of A1298C, a TaqMan assay was performed on a GeneAmp 9700 machine (Applied Biosystems) with endpoint analysis on a Prism 7900HT Sequence Detection System (Applied Biosystems). For C677T, the genotype was generated by a BeadExpress platform (Illumina). These 2 markers were part of a larger set of 113 markers run on these 2 platforms. Ninety-one samples (8.7%) were run as duplicates to assess genotype quality. Call rates among duplicate samples were 99.98% concordant. In addition, 4 markers were run on both platforms, yielding an inter-platform concordance of 99.95%.

Administrative and clinical data. All administrative data were derived from the Veterans Affairs inpatient and outpatient patient treatment files. Patient characteristics and RA severity measures were derived from the VARA clinical database.

Predictor variables. We assembled covariates from 3 domains: patient characteristics, RA severity/disease activity measures, and genetic characteristics, as follows.

1. Patient and exposure characteristics included age; sex; race (white vs non-white); education level (years); tobacco use (never, former, current) upon enrollment; MTX exposure at time of enrollment (yes/no); glucocorticoid ever-user (yes/no) during the time of enrollment; body mass index (BMI) upon enrollment (kg/m^2); a prevalent diagnosis of alcohol abuse, diabetes mellitus, hypertension, or hyperlipidemia [based on *International Classification of Diseases*, 9th Revision, Clinical Modification (ICD-9-CM) in any coding position in inpatient and outpatient records] in the 7 years prior to enrollment in VARA (Appendix 1); urban vs rural residence (defined using the patient's zip code and metropolitan statistical area); and modified Charlson-Deyo comorbidity index (excluding diabetes mellitus and CV codes, which were modeled separately)[20]. We included covariates to indicate a personal history of CV-related conditions/events in the 7 years prior to registry enrollment. These conditions/events consisted of MI, heart failure (HF), peripheral vascular disease (PVD), percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG), or stroke. We defined these baseline comorbidities using ICD-9-CM codes and Current Procedural Terminology (CPT) codes in any position in the inpatient and outpatient VA patient treatment files occurring in the 7 years prior to enrollment in the VARA registry. The coding for each condition/event was based on previously validated algorithms, when available[21,22], and if unavailable, algorithms appearing in peer-reviewed publications[23,24,25,26]. ICD-9-CM and CPT codes used to define the baseline comorbidities are shown in Appendix 1.

2. RA severity/disease activity characteristics consisted of RA duration prior to enrollment (years); radiological changes present in hand, wrist, or foot radiographs (ever); mean 28-joint 4-variable Disease Activity Score (DAS-28) using erythrocyte sedimentation rate (ESR) since the time of

enrollment[27]; rheumatoid factor (RF) positivity (ever); anticitrullinated protein antibody (ACPA) positivity (ever); C-reactive protein (CRP) level at enrollment; and presence of rheumatoid nodules (ever). For this VARA substudy, antibody results were based upon the clinical laboratory results reported at each study site for participating subjects.

3. Genetic characteristics were evaluated by a genotypic analysis with variables for *MTHFR* 677 TT and 677 CT and *MTHFR* 1298 CC and 1298 AC. Additionally, we used HLA-DRB-1 shared-epitope (SE) status as a control variable[28], and performed DRB-1 genotyping as described[29].

Outcome variable. We defined time-to-CV event as the number of days from enrollment into VARA until the first occurrence of any one of the following conditions/events (both fatal and nonfatal): MI, stroke, PCI, or CABG. These events were defined by ICD-9-CM or CPT coding in the primary coding position of either inpatient or outpatient treatment files for MI (ICD-9-CM 410.x) and stroke (ICD-9-CM 433.11, 434.91, 435.x, 438.x), or in any coding position for PCI (ICD-9-procedure: 36.06, 36.07, 0.66; CPT: 92973, 92980, 92995) or CABG (ICD-9-procedure: 36.1x)[30]. If no event was identified, subjects were censored 1 day after their last inpatient or outpatient observation.

Statistical methods. For missing variables, education (163 subjects) and BMI (103 subjects), we used the SAS Multiple Imputation procedure to impute missing values based on race, sex, and age. Twenty imputation datasets were created and the average value for each subject was used. Patient characteristics for subjects sustaining a CV event and those without a CV event were compared using the Student t test and chi-square test where appropriate. Data were tested such that they met the assumptions of the Cox proportional hazards model by graphing the hazards. Cox proportional hazards regression was used to model time-to-CV event, with the time beginning with enrollment in VARA. We first identified significant predictor variables using backward stepwise selection and an alpha level of 0.2. All predictors identified in the initial model were included in the final backward stepwise selection using a 2-tailed alpha level of 0.05. As the variables of interest, A1298C and C677T polymorphisms were forced into the final model. We also tested for interaction terms between *MTHFR* polymorphisms and MTX use.

Secondary analyses. Two secondary analyses were performed. The first secondary analysis used Cox proportional hazards regression to predict time-to-CV event, but this analysis examined only those participants without a prior CV event (MI, stroke, CABG, PCI). Because of lower numbers of observations, the *MTHFR* polymorphisms were analyzed in a dominant model (0 or ≥ 1 copy of the minor allele) for this particular analysis. The second analysis used Cox proportional hazards regression to identify significant predictors of time-to-event all-cause mortality (as specific causes of death were not known). Both models were performed using the methods described above. All analyses were performed using SAS version 9.2 or Stata version 11.

RESULTS

Descriptive data and univariate analysis. Patient characteristics for those included in the analysis are summarized in Table 1. Data were available for 1047 patients covering a mean observation period of 1550 days, or an average of 4.25 years, with a total of 3743 person-years of followup. From this cohort, 97 patients (9.26%) sustained CV events post-enrollment, producing a crude event rate of 2.59 events/100 person-years (95% CI 2.11–3.15). Of the 1047 patients observed, 252 (24.07%) died during the followup period. The cause of death was not known (i.e., all-cause mortality). However, of these 252 patients, 24 (2.29%) died within 30 days of the CV event.

The preponderance of the cohort was male, white, former or current smokers, having a diagnosis of hypertension and hyperlipidemia, with well-established RA. Most were seropositive for RF (89.1%) or ACPA (81.4%). Compared to those without a CV event occurring during observation, the CV event group included a higher percentage of males, lower education status, higher rates of hypertension and hyperlipidemia diagnoses, higher modified Charlson-Deyo index, and higher rate of prior CV events (combined, MI, HF, PVD, and stroke). The CV event group also contributed a longer period of observation (1738 vs 1530 days; $p < 0.0001$) and demonstrated a higher rate of CC or CT polymorphisms in *MTHFR* C677T (homozygous major allele and heterozygous allele, respectively).

The homozygous minor allele genotypes, *MTHFR* 1298 CC and 677 TT, were present in 10.8% and 10.1% of the cohort, respectively (Table 1). While the minor allele frequencies did not differ between the CV event and no-CV event groups for *MTHFR* A1298C (rs1801131), there was a statistically significant difference between the 2 groups for *MTHFR* C677T, with a lower frequency of the homozygous minor (TT) genotype in those experiencing a CV event compared to those in the no-CV event group ($p = 0.023$). The cohort, stratified by ethnicity[31], were in Hardy-Weinberg equilibrium for both *MTHFR* A1298C (white: Pearson chi-square = 3.70, $p = 0.054$; African American: Pearson chi-square = 0.053, $p = 0.818$) and *MTHFR* C677T (white: Pearson chi-square = 0.107, $p = 0.744$; African American: Pearson chi-square = 0.329, $p = 0.566$).

Multivariate analysis. Results of the multivariate Cox proportional hazards regression are shown in Table 2. The *MTHFR* A1298C and C677T variables were not significant in the genetic domain model, but were forced into the final model. In the final model, although *MTHFR* 677 minor allele homozygotes trended toward significance (HR 0.24, 95% CI 0.06–1.02, $p = 0.053$), the variable did not achieve significance of $p < 0.05$. The other *MTHFR* polymorphisms were nonsignificant. Interaction terms between the *MTHFR* polymorphisms and MTX were also not statistically significant (data not shown). Prior stroke (HR 2.01, 95% CI 1.03–3.90), prior MI (HR 1.70, 95% CI 1.06–2.71), hyperlipidemia (HR 1.57, 95% CI 1.01–2.43), and increasing modified Charlson-Deyo index (HR 1.23, 95% CI 1.13–1.34) were associated with a higher hazard ratio of a CV event. MTX use (HR 0.66, 95% CI 0.44–1.00) and higher education status (HR 0.87, 95% CI 0.80–0.95) were associated with a lower hazard ratio for CV events.

There was no significant difference between the survival curves of the major homozygous forms (*MTHFR* 1298 AA and 677 CC) in comparison with *MTHFR* 1298 CC and 677 TT minor homozygotes.

Secondary analyses. In secondary analyses, we first excluded those who had a history of CV events and performed backward stepwise analysis, as described above.

Table 1. Patient characteristics.

Characteristic	All, n = 1047	CV Event, n = 97	No CV Event, n = 950	p
Age, yrs, mean (range)	63.7 (21–91)	64.6 (48–88)	63.6 (21–91)	0.325
Male, % (n)	90.9 (952)	96.9 (94)	90.3 (858)	0.031
White, % (n)	78.3 (819)	81.44 (79)	78.0 (740)	0.430
Education, yrs (SD)	12.7 (2.4)	11.9 (2.1)	12.8 (2.5)	0.001
Body mass index, kg/m^2* (SD)	28.3 (5.8)	27.9 (6.2)	28.3 (5.7)	0.551
Alcohol abuse, % (n)	8.69 (91)	10.31 (10)	8.53 (81)	0.553
Hypertension, % (n)	71.35 (747)	82.5 (80)	70.21 (667)	0.011
Hyperlipidemia, % (n)	52.1 (545)	63.9 (62)	50.8 (483)	0.014
Diabetes, % (n)	25.2 (264)	30.9 (30)	24.6 (234)	0.174
Urban, % (n)	71.4 (748)	77.3 (75)	70.8 (673)	0.179
Modified Charlson-Deyo index (SD)	1.7 (2.0)	2.7 (2.5)	1.6 (1.9)	< 0.0001
Prior cardiovascular event, % (n)				
MI	15.7 (164)	35.1 (34)	13.7 (130)	< 0.001
HF	13.3 (139)	20.6 (20)	12.5 (119)	0.025
PVD	14.7 (154)	25.77 (25)	13.6 (129)	0.001
PCI	2.6 (27)	5.2 (5)	2.3 (22)	0.096
CABG	1.0 (10)	2.0 (2)	0.84 (8)	0.235
Stroke	4.58 (48)	11.34 (11)	3.89 (37)	0.003
RA duration, yrs (SD)	14.5 (12.2)	15.1 (12)	14.4 (12.2)	0.614
Radiological changes, % (n)	71.2 (730)	71.3 (67)	71.2 (663)	0.990
Mean DAS28 (SD)	3.7 (1.18)	3.9 (1.2)	3.7 (1.2)	0.225
Rheumatoid factor-positive, % (n)	89.1 (930)	94.9 (92)	88.5 (838)	0.056
ACPA-positive, % (n)	81.4 (745)	84.7 (72)	81.1 (673)	0.414
Mean CRP, mg/dl (SD)	13.1 (20)	14.9 (20)	12.9 (20)	0.354
Rheumatoid nodules, % (n)	59.1 (574)	62.0 (57)	58.8 (517)	0.560
Observation time (days), mean (SD)	1549.6 (483.1)	1738.3 (443.0)	1530.3 (483.1)	< 0.0001
Smoking status, % (n)				
Never	20.3 (212)	15.5 (15)	20.8 (197)	0.290
Former	52.4 (548)	51.55 (50)	52.5 (498)	
Current	27.3 (286)	33.0 (32)	26.8 (254)	
Methotrexate use, % (n)	51.2 (536)	49.5 (48)	51.4 (488)	0.724
Glucocorticoid ever-user, % (n)	63.9 (669)	69.1 (67)	63.4 (602)	0.265
MTHFR A1298C† (rs1801131), % (n)				
AA	50.1 (523)	43.1 (47)	50.2 (476)	0.689
AC	39.1 (409)	38.1 (37)	39.24 (372)	
CC	10.8 (113)	13.4 (13)	10.6 (100)	
MTHFR C667T† (rs1801133), % (n)				
CC	50.1 (524)	54.6 (53)	49.7 (471)	0.023
CT	39.8 (416)	43.3 (42)	39.5 (374)	
TT	10.1 (105)	2.1 (2)	10.86 (103)	
HLA-DRB-1 SE*, % (n)				
2 copies	21.0 (218)	18.75 (18)	21.2 (200)	0.724
1 copy	51.0 (530)	50.0 (48)	51.1 (482)	
0 copies	28.1 (292)	31.25 (30)	27.8 (262)	

† Polymorphisms are listed in order of major allele homozygous, heterozygous, minor allele homozygous. * Missing data for HLA-DRB-1 SE = 26.4% (n = 276). MI: myocardial infarction; CV: cardiovascular; DAS28: 4-variable Disease Activity Score using 28-joint count and erythrocyte sedimentation rate; ACPA: anticitrullinated protein antibody; MTHFR: methylenetetrahydrofolate reductase; DRB-1: HLA subtype; HF: heart failure; PVD: peripheral vascular disease; PCI: percutaneous coronary intervention; CABG: coronary artery bypass graft; RA: rheumatoid arthritis; CRP: C-reactive protein.

Data were available for 844 patients with a total of 3029 person-years. From this cohort, 55 patients (6.52%) sustained CV events post-enrollment, producing a crude event rate of 1.82 events/100 person-years (95% CI 1.39–2.36). In the multivariate Cox analysis (Table 3), increasing modified Charlson-Deyo index (HR 1.26, 95% CI 1.12–1.42, p < 0.0001) and ACPA positivity (HR 4.59, 95% CI 1.10–19.08, p = 0.036) were associated with a higher hazard ratio of a CV event, while increasing years of education (HR 0.84, 95% CI 0.76–0.94, p = 0.003) and prevalent diagnosis of diabetes (HR 0.43, 95% CI 0.19–0.96, p = 0.039) were associated with a lower hazard

Table 2. Predictors of time to cardiovascular event, using multivariate Cox regression.

Variable	HR (95% CI)	p
MTHFR A1298C* (rs1801131)		
AA	ref	
AC	0.77 (0.49, 1.21)	0.262
CC	0.67 (0.33, 1.34)	0.259
MTHFR C677T* (rs1801133)		
CC	ref	
CT	1.16 (0.74, 1.80)	0.523
TT	0.24 (0.06, 1.02)	0.053
Methotrexate use	0.66 (0.44, 1.00)	0.048
Modified Charlson-Deyo index	1.23 (1.13, 1.34)	< 0.0001
Prior myocardial infarction	1.70 (1.06, 2.71)	0.027
Hyperlipidemia	1.57 (1.01, 2.43)	0.046
Prior stroke	2.01 (1.03, 3.90)	0.040
Education	0.87 (0.80, 0.95)	0.003
Smoking status		
Never	ref	
Former	0.92 (0.51, 1.69)	0.796
Current	1.29 (0.68, 2.47)	0.437

* Polymorphisms are listed in order of major allele homozygous, heterozygous, minor allele homozygous. MTHFR: methylenetetrahydrofolate reductase; ref: referent.

Table 3. Predictors of time to CV event, excluding those with prior CV events, using multivariate Cox regression.

Variable	HR (95% CI)	p
MTHFR A1298C; ≥ 1 copy minor allele	1.28 (0.71, 2.32)	0.414
MTHFR C677T; ≥ 1 copy minor allele	1.07 (0.59, 1.93)	0.832
Education	0.84 (0.76, 0.94)	0.003
Diabetes	0.43 (0.19, 0.96)	0.039
Modified Charlson-Deyo index	1.26 (1.12, 1.42)	< 0.0001
ACPA	4.59 (1.10, 19.08)	0.036

MTHFR: methylenetetrahydrofolate reductase; ACPA: anticitrullinated protein antibody; CV: cardiovascular.

ratio for CV events. *MTHFR* 1298 and 677 minor alleles were not significant in this model (HR 1.28, 95% CI 0.71–2.32, p = 0.414; HR 1.07, 95% CI 0.59–1.93, p = 0.832, respectively).

In a second secondary analysis, we examined predictors of time-to-all-cause mortality. Data were available for 1047 patients, with a total of 3800 person-years. From this cohort, 252 (24.07%) patients died during the followup period, producing a crude rate of 6.63 deaths/100 person-years (95% CI 5.86–7.50). In the multivariate Cox analysis (Table 4), variables associated with increased hazard ratio of death were increasing age (HR 1.08, 95% CI 1.06–1.09, p < 0.0001), increasing modified Charlson-Deyo index (HR 1.13, 95% CI 1.07–1.20, p < 0.0001), increasing DAS-28 (HR 1.38, 95% CI 1.23–1.55, p < 0.0001), increasing CRP (HR 1.01, 95% CI 1.00–1.01, p = 0.011), current smoking (HR 1.93, 95% CI 1.26–2.96, p = 0.003), and prevalent

Table 4. Predictors of time to all-cause mortality, using multivariate Cox regression.

Variable	HR (95% CI)	p
MTHFR A1298C* (rs1801131)		
AA	ref	
AC	0.98 (0.74, 1.32)	0.916
CC	0.92 (0.58, 1.45)	0.717
MTHFR C677T* (rs1801133)		
CC	ref	
CT	0.85 (0.64, 1.14)	0.280
TT	0.87 (0.52, 1.44)	0.578
Age	1.08 (1.06, 1.09)	< 0.0001
Methotrexate use	0.75 (0.58, 0.97)	0.027
Body mass index	0.96 (0.93, 0.98)	0.002
Modified Charlson-Deyo index	1.13 (1.07, 1.20)	< 0.0001
Heart failure	1.52 (1.13, 2.06)	0.006
DAS28	1.38 (1.23, 1.55)	< 0.0001
CRP	1.01 (1.00, 1.01)	0.011
Smoking status		
Never	ref	
Former	1.35 (0.94, 1.93)	0.100
Current	1.93 (1.26, 2.96)	0.003

* Polymorphisms are listed in order of major allele homozygous, heterozygous, minor allele homozygous. MTHFR: methylenetetrahydrofolate reductase; ref: referent; DAS28: 4-variable Disease Activity Score using 28-joint count and erythrocyte sedimentation rate; CRP: C-reactive protein.

diagnosis of heart failure (HR 1.52, 95% CI 1.13–2.06, p = 0.006). MTX use (HR 0.75, 95% CI 0.58–0.97, p = 0.027) and increasing BMI (HR 0.96, 95% CI 0.93–0.98, p = 0.002) were associated with decreased hazard ratio of all-cause mortality.

DISCUSSION

In our study, we used a prospective registry of US veterans with RA to study potential association between *MTHFR* polymorphisms and time-to-CV event. After adjusting for multiple patient demographic and RA severity measures, we found that traditional risk factors for CV disease, including hyperlipidemia, prior MI, and prior stroke, were associated with an increased hazard ratio for time-to-combined CV event (MI, stroke, PCI, or CABG), while MTX use was associated with a lower risk for time-to-CV event. We did not find a statistically significant association of the *MTHFR* polymorphisms A1298C or C677T with time-to-CV events, but rather a trend toward the minor alleles being protective. In 2 secondary analyses, the first excluding those with prior CV events and the second examining all-cause mortality, we again did not find association of the *MTHFR* polymorphisms with the outcomes of interest (time-to-CV event and time-to-all-cause mortality).

CV disease is of high concern to patients and rheumatologists because it results in greater mortality among patients with RA than any other cause of death[1]. Several studies have examined the associations between CV risk factors, markers

of RA severity, and atherosclerosis. However, most of these studies have not used CV events as an outcome, but rather have used proxies such as carotid intimal thickness and carotid artery plaque[3,32]. In this study, we chose to use time-to-CV event as the outcome variable, as we believe that this represents a more clinically durable endpoint.

MTHFR polymorphisms are an example of a potentially additive nontraditional CV risk in patients with RA. In the general population, *MTHFR* C677T has been shown to increase homocysteine[6] and has been associated with increased risks of CV disease[11], cerebrovascular disease[11], premature MI[12], and ischemic stroke[11,13]. It has been theorized that these multiple CV events may result from increased circulating homocysteine levels[12]. MTX, which interacts with the folic acid pathway, has been shown to improve CV outcomes in patients with RA[33,34]; however, the interaction between *MTHFR* polymorphisms, RA, and MTX use has not previously been delineated.

We did not find convincing support for our hypothesis — that *MTHFR* homozygous polymorphisms are associated with time-to-CV event — nor did we find a decreased time-to-CV event in patients with the *MTHFR* polymorphisms who were taking MTX. In a posthoc power analysis based on a Cox regression model, a sample of 1047 observations achieved 62% power at an alpha level of 0.05 to detect a hazard ratio of 1.5. Under similar assumptions, 1047 observations achieved 98% power to detect a hazard ratio of 2.0. Thus, this study was well powered to detect moderate-size effects. As our study did not detect an association between *MTHFR* status and CV events, if there were a true effect, it would likely be quite modest.

To date, only 1 publication has focused specifically on *MTHFR* polymorphisms in RA patients and CV events[16]. In that prospective observational study, there was an association between CV events and endothelial dysfunction related to the *MTHFR* A1298C genotype, but not with C677T. The study cohort differed from our population. The mean age was younger (mean age 53 yrs), fewer patients with RA were followed (n = 612), and the period of followup (14 yrs) was longer. In addition, because the cohort was from Spain, the genetic background of the participants may be different from our US population. Although our 2 studies have arrived at divergent conclusions, with certain considerations, our findings may be similar. The association between the *MTHFR* A1298C minor allele and CV disease may be associated with early-onset disease, which was not observed in our older cohort. An additional potential difference is that of folic acid supplementation. While we could not accurately track folic acid supplementation in the VA system (as it is typically given over the counter), US cereal products are widely supplemented with folic acid, while European cereal products are not fortified[35]. This background folic acid supplementation could potentially blunt the possible effects of the *MTHFR* polymorphisms. The differences in our studies also could be explained by methodological differences, CV outcome variable, sample sizes, or variance in the underlying populations. Replication of these studies in other populations may resolve these questions.

Not surprisingly, CV events were associated with previous MI, prior stroke, hyperlipidemia, and increasing modified Charlson-Deyo comorbidity indices. Previous MI or stroke and hyperlipidemia are traditional CV risk factors[3] and are consistent with findings in the general population. These associations are consistent with the existing literature and lend face validity to our findings. As the Charlson-Deyo comorbidity index is a measure of multiple comorbidities, it is unsurprising that an increasing measure of comorbidities would be associated with increasing risk of CV disease. The decreased risk of CV events associated with MTX use might reflect the antiinflammatory effects of MTX, as documented[33,34]. These reports suggest that MTX not only reduces the inflammation in RA, but improves associated diseases, such as atherosclerosis.

The decreased risk of CV events associated with increasing years of education may be explained in one of many ways. One possibility is that as education status increases, patients become more "health literate" and are thus more likely to adhere to medication regimens, diets, and exercise as prescribed by their doctors[36]. Alternatively, education status may be a proxy marker for income status. In a recent report by Maynard, *et al*[37], low socioeconomic status was associated with increased CV risk factors and related outcomes in patients with systemic lupus erythematosus. While this may not apply to our inflammatory disease of interest, RA, it is a point to be considered and further investigated.

Of interest, we did not find an association of CV events with measures of disease activity. This is similar to other studies[38,39] that found no association of measures of disease activity with carotid intimal thickness, and found no association between disease activity and CV events in a case-control study, respectively.

In our secondary analyses, we first examined time-to-CV event in those without prior CV events. The findings in this secondary analysis were similar to the primary analysis in that increasing modified Charlson-Deyo index was associated with an increased hazard ratio and increasing education was associated with a decreased hazard ratio of CV event. After excluding those with prior CV events, those with positive ACPA had an increased risk of a CV event, which is similar to findings by Hjeltnes, *et al*[40], who associated ACPA with impaired endothelial function. Interestingly, prevalent diagnosis of diabetes was associated with a decreased hazard ratio of CV event. This may be due to preferential removal of "high-risk" (i.e., poorly controlled) diabetic patients from the cohort through removal of those with prior CV events, leaving only those

patients with well-controlled disease, who are appropriately managed for CV risk factors.

In the second secondary analysis, we examined time to all-cause mortality. As expected, increasing age, increasing modified Charlson-Deyo index, prevalent diagnosis of heart failure, and current smoking were associated with increased hazard ratio of all-cause mortality. Elevated CRP has been associated with increased risk of all-cause mortality in men with cancer[41] and with early mortality in hospitalized nursing home residents[42], and thus is not unexpected. Improvement in DAS-28 has previously been associated with reduction in risk of CV event[43], and it may be presumed that the reverse may be true, similar to what we found in our study. MTX use was associated with decreased mortality, similar to the findings of Ajeganova, *et al* in an early disease cohort[43]. Of note, increasing BMI was associated with decreased mortality. This may be because our outcome was all-cause mortality, which presumably would include cachexia-associated diseases such as cancers, etc.

Our study has several potential limitations. Because our cohort consisted primarily of elderly men with longstanding RA, it is possible that *MTHFR*-associated CV events preferentially occurred early in life[12], thus eliminating from our study those who died from these events (survival bias). However, this population with high risk for CV events represents an excellent cohort in which to study these genetic factors if they are not related to early death. Another potential limitation is that we cannot currently comment on the amount of time required for MTX to affect the CV system. It is possible that there is an interaction between *MTHFR* polymorphisms and MTX after a certain cumulative dose or after a certain period of taking the medication. Additionally, as our study was primarily in a male cohort, there is the potential for a female-*MTHFR* interaction that we could not identify with our population. While *MTHFR* polymorphisms have been associated with such disparate outcomes as recurrent fetal loss[44] and breast cancer[45] in women, the studies that do exist that associated *MTHFR* polymorphisms with increased risk for CV disease in women[11] were focused on women, and did not compare female to male populations. In a study that contrasts the polymorphisms in male and female populations[46], the investigators, to the contrary, found an association between *MTHFR* C677T polymorphisms and carotid artery compliance in men, but not women. Thus, this would potentially argue that men's CV system may be more influenced by *MTHFR* polymorphisms than women's, and our population was actually a well-selected group to study for these effects. Another potential limitation is that patients may have received care outside the VA system. Patients who may not have been identified in our database are those who received care outside the VA system and died from a CV event. To contain costs, VA policy dictates patients hospitalized at non-VA facilities be transferred to VA facilities. We attempted to control for this with a variable that categorized patients as urban versus rural, under the assumption that the likelihood of being hospitalized at a VA facility may vary according to whether patients reside in rural or urban areas[47]. A further potential limitation was our inability to track certain medications such as over-the-counter preparations (folic acid and nonsteroidal antiinflammatory drugs; data not collected in the VA system) and certain prescription medications such as tumor necrosis factor inhibitors (data are collected in a different pharmacy system). While it is possible that these medications had a significant effect on the cardiovascular system and potentially interact with the *MTHFR* polymorphisms, they are not the primary emphasis of this report.

Our study took place within a comprehensive healthcare system, the Veterans Health Affairs, using a prospective registry, thereby increasing the likelihood that the relevant clinical data were captured during the period of observation. Additionally, the sample size for our study was the largest to date to evaluate CV events and *MTHFR* polymorphisms in patients with RA[16]. Finally, the outcome we examined represents a more robust clinical outcome (CV events) compared with other studies, which used proxies for CV disease.

We were able to confirm the association of CV disease with multiple established traditional CV risk factors; however, we found no evidence that *MTHFR* status influences CV events in patients with RA in a time-to-event analysis.

REFERENCES

1. Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Williams CA, et al. The mortality of rheumatoid arthritis. Arthritis Rheum 1994;37:481-94.
2. Watson DJ, Rhodes T, Guess HA. All-cause mortality and vascular events among patients with rheumatoid arthritis, osteoarthritis, or no arthritis in the UK General Practice Research Database. J Rheumatol 2003;30:1196-202.
3. del Rincon ID, Williams K, Stern MP, Freeman GL, Escalante A. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. Arthritis Rheum 2001;44:2737-45.
4. Gaujoux-Viala C, Smolen JS, Landewe R, Dougados M, Kvien TK, Mola EM, et al. Current evidence for the management of rheumatoid arthritis with synthetic disease-modifying antirheumatic drugs: A systematic literature review informing the EULAR recommendations for the management of rheumatoid arthritis. Ann Rheum Dis 2010;69:1004-9.
5. Kremer JM. Toward a better understanding of methotrexate. Arthritis Rheum 2004;50:1370-82.
6. Hider SL, Bruce IN, Thomson W. The pharmacogenetics of methotrexate. Rheumatology 2007;46:1520-4.
7. Ranganathan P, Culverhouse R, Marsh S, Mody A, Scott-Horton TJ, Brasington R, et al. Methotrexate (MTX) pathway gene polymorphisms and their effects on MTX toxicity in Caucasian and African American patients with rheumatoid arthritis. J Rheumatol 2008;35:572-9.

Appendix 1. ICD-9-CM, ICD-9 procedure, and CPT codes used to define baseline comorbidities and cardiovascular (CV) events.

Operational Term	Source	ICD-9-CM/ ICD-9-procedure/ CPT codes Prevalent Disease	ICD-9/CPT codes CV event
Alcohol abuse	Quan[22]	265.2 291.1 291.2 291.3 291.5 291.8x 291.9 303.0 303.9 305.0 357.5 425.5 535.3 571.0 571.1 571.2 571.3 980.x v11.3	
Diabetes	Birman-Deych[21]	250. x	
Hypertension	Birman-Deych[21]	401.x 402.x 403.x 404.x 405.x 437.2	
Myocardial infarction	Birman-Deych[21]	410.x 411.x 412.x 413.x 414.x 429.2 v45.81	410.x
Heart Failure	Birman-Deych[21]	398.91 402.01 402.11 402.91 404.01 404.03 404.11 404.13 404.91 404.93 428.x	
PVD	Nowygrod[25]/ Egorova[23]	250.7 440.2 440.3 440.31 440.32 440.9 442.3 443.9 444.0 444.22 445.02 447.1 707.1 785.4 996.74 38.08 38.18 38.38 38.48 38.88 39.29 39.50 39.90 84.114 84.13 84.14 84.15 84.16 84.17	
PCI	Modification of Nallamothu[24] (CPT codes)	36.01 36.02 36.05 36.06 36.07 36.09 0.66* 99.10* 92973 92980 92995	36.06 36.07 0.66 92973 92980 92995
CABG	Nowygrod[25]/ Egorova[23]	36.1x	36.1x
Stroke	Birman-Deych[21]	433.01 433.11 433.21 433.31 433.81 433.91 434.01 434.11 434.91 435.x 436.x 437.1x 437.9x 438.x	433.11 434.91 435.x 438.x
Hyperlipidemia	Szeto[26]	272.0 272.1 272.2 272.3 272.4	

*Modification of original coding. ICD-9-CM: *International Classification of Diseases*, 9th Revision, Clinical Modification; ICD-9 procedure: *International Classification of Diseases*, 9th Revision, procedure; CPT: current procedural terminology; PVD: peripheral vascular disease; PCI: percutaneous coronary intervention; CABG: coronary artery bypass graft.

8. Taniguchi A, Urano W, Tanaka E, Furihata S, Kamitsuji S, Inoue E, et al. Validation of the associations between single nucleotide polymorphisms or haplotypes and responses to disease-modifying antirheumatic drugs in patients with rheumatoid arthritis: A proposal for prospective pharmacogenomic study in clinical practice. Pharmacogenet Genomics 2007;17:383-90.
9. Urano W, Taniguchi A, Yamanaka H, Tanaka E, Nakajima H, Matsuda Y, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene were associated with both the efficacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analyses. Pharmacogenetics 2002;12:183-90.
10. van Ede AE, Laan RF, Blom HJ, Huizinga TW, Haagsma CJ, Giesendorf BA, et al. The C677T mutation in the methylenetetrahydrofolate reductase gene: A genetic risk factor for methotrexate-related elevation of liver enzymes in rheumatoid arthritis patients. Arthritis Rheum 2001;44:2525-30.
11. Fan AZ, Yesupriya A, Chang MH, House M, Fang J, Ned R, et al. Gene polymorphisms in association with emerging cardiovascular risk markers in adult women. BMC Med Genet 2010;11:6.
12. Rallidis LS, Gialeraki A, Komporozos C, Vavoulis P, Pavlakis G, Travlou A, et al. Role of methylenetetrahydrofolate reductase 677C->T polymorphism in the development of premature myocardial infarction. Atherosclerosis 2008;200:115-20.
13. Al Allawi NA, Avo AS, Jubrael JM. Methylenetetrahydrofolate reductase C677T polymorphism in Iraqi patients with ischemic stroke. Neurol India 2009;57:631-5.
14. Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG. MTHFR 677C—>T polymorphism and risk of coronary heart disease: A meta-analysis. JAMA 2002;288:2023-31.
15. Lewis SJ, Ebrahim S, Davey SG. Meta-analysis of MTHFR 677C->T polymorphism and coronary heart disease: Does totality of evidence support causal role for homocysteine and preventive potential of folate? BMJ 2005;331:1053.
16. Palomino-Morales R, Gonzalez-Juanatey C, Vazquez-Rodriguez TR, Rodriguez L, Miranda-Filloy JA, Fernandez-Gutierrez B, et al. A1298C polymorphism in the MTHFR gene predisposes to cardiovascular risk in rheumatoid arthritis. Arthritis Res Ther 2010;12:R71.
17. Curtis JR, Jain A, Askling J, Bridges SL Jr, Carmona L, Dixon W, et al. A comparison of patient characteristics and outcomes in selected European and U.S. rheumatoid arthritis registries. Semin Arthritis Rheum 2010;40:2-14.
18. Mikuls TR, Kazi S, Cipher D, Hooker R, Kerr GS, Richards JS, et

al. The association of race and ethnicity with disease expression in male US veterans with rheumatoid arthritis. J Rheumatol 2007;34:1480-4.
19. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
20. Deyo RA, Cherkin DC, Ciol MA. Adapting a clinical comorbidity index for use with ICD-9-CM administrative databases. J Clin Epidemiol 1992;45:613-9.
21. Birman-Deych E, Waterman AD, Yan Y, Nilasena DS, Radford MJ, Gage BF. Accuracy of ICD-9-CM codes for identifying cardiovascular and stroke risk factors. Med Care 2005;43:480-5.
22. Quan H, Sundararajan V, Halfon P, Fong A, Burnand B, Luthi JC, et al. Coding algorithms for defining comorbidities in ICD-9-CM and ICD-10 administrative data. Med Care 2005;43:1130-9.
23. Egorova N, Vouyouka AG, Quin J, Guillerme S, Moskowitz A, Marin M, et al. Analysis of gender-related differences in lower extremity peripheral arterial disease. J Vasc Surg 2010;51:372-8.
24. Nallamothu BK, Lu X, Vaughan-Sarrazin MS, Cram P. Coronary revascularization at specialty cardiac hospitals and peer general hospitals in black Medicare beneficiaries. Circ Cardiovasc Qual Outcomes 2008;1:116-22.
25. Nowygrod R, Egorova N, Greco G, Anderson P, Gelijns A, Moskowitz A, et al. Trends, complications, and mortality in peripheral vascular surgery. J Vasc Surg 2006;43:205-16.
26. Szeto HC, Coleman RK, Gholami P, Hoffman BB, Goldstein MK. Accuracy of computerized outpatient diagnoses in a Veterans Affairs general medicine clinic. Am J Manag Care 2002;8:37-43.
27. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44-8.
28. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30:1205-13.
29. Mikuls TR, Gould KA, Bynote KK, Yu F, Levan TD, Thiele GM, et al. Anti-citrullinated protein antibody (ACPA) in rheumatoid arthritis: Influence of an interaction between HLA-DRB1 shared epitope and a deletion polymorphism in glutathione S-transferase in a cross-sectional study. Arthritis Res Ther 2010;12:R213.
30. Davis LA, Cannon GH, Reimold A, Kerr G, Mikuls T, Caplan L. Validation of diagnostic and procedural codes for identification of incident cardiovascular disease in subjects with rheumatoid arthritis [abstract]. Arthritis Rheum 2011;63 Suppl: S48.
31. Chang MH, Lindegren ML, Butler MA, Chanock SJ, Dowling NF, Gallagher M, et al. Prevalence in the United States of selected candidate gene variants: Third National Health and Nutrition Examination Survey, 1991-1994. Am J Epidemiol 2009;169:54-66.
32. Dessein PH, Norton GR, Woodiwiss AJ, Joffe BI, Wolfe F. Influence of nonclassical cardiovascular risk factors on the accuracy of predicting subclinical atherosclerosis in rheumatoid arthritis. J Rheumatol 2007;34:943-51.
33. Choi HK, Hernan MA, Seeger JD, Robins JM, Wolfe F. Methotrexate and mortality in patients with rheumatoid arthritis: A prospective study. Lancet 2002;359:1173-7.
34. Westlake SL, Colebatch AN, Baird J, Kiely P, Quinn M, Choy E, et al. The effect of methotrexate on cardiovascular disease in patients with rheumatoid arthritis: A systematic literature review. Rheumatology 2010;49:295-307.
35. 50th Annual Meeting of the Society for Research into Hydrocephalus and Spina Bifida, Cambridge, UK, 30 August–2 September 2006 [abstracts]. Cerebrospinal Fluid Res 2006;3 Suppl 1:S1-S62.
36. Pincus T, Keysor J, Sokka T, Krishnan E, Callahan LF. Patient questionnaires and formal education level as prospective predictors of mortality over 10 years in 97% of 1416 patients with rheumatoid arthritis from 15 United States private practices. J Rheumatol 2004;31:229-34.
37. Maynard JW, Fang H, Petri M. Low socioeconomic status is associated with cardiovascular risk factors and outcomes in systemic lupus erythematosus. J Rheumatol 2012;39:777-83.
38. Radovits BJ, Popa-Diaconu DA, Popa C, Eijsbouts A, Laan RF, van Riel PL, et al. Disease activity as a risk factor for myocardial infarction in rheumatoid arthritis. Ann Rheum Dis 2009;68:1271-6.
39. Ristic GG, Lepic T, Glisic B, Stanisavljevic D, Vojvodic D, Petronijevic M, et al. Rheumatoid arthritis is an independent risk factor for increased carotid intima-media thickness: Impact of anti-inflammatory treatment. Rheumatology 2010;49:1076-81.
40. Hjeltnes G, Hollan I, Forre O, Wiik A, Mikkelsen K, Agewall S. Anti-CCP and RF IgM: Predictors of impaired endothelial function in rheumatoid arthritis patients. Scand J Rheumatol 2011;40:422-7.
41. Ko YJ, Kwon YM, Kim KH, Choi HC, Chun SH, Yoon HJ, et al. High-sensitivity C-reactive protein levels and cancer mortality. Cancer Epidemiol Biomarkers Prev 2012;21:2076-86.
42. Alrawi YA, Parker RA, Harvey RC, Sultanzadeh SJ, Patel J, Mallinson R, et al. Predictors of early mortality among hospitalized nursing home residents. QJM 2013;106:51-7.
43. Ajeganova S, de Faire U, Jogestrand T, Frostegard J, Hafstrom I. Carotid atherosclerosis, disease measures, oxidized low-density lipoproteins, and atheroprotective natural antibodies for cardiovascular disease in early rheumatoid arthritis — An inception cohort study. J Rheumatol 2012;39:1146-54.
44. Canda MT, Demir N, Sezer O. Impact of Factor V Leiden, prothrombin and methylenetetrahydrofolate reductase gene mutations on infant birth weight in women with recurrent fetal loss and women with successful pregnancies. Clin Exp Obstet Gynecol 2012;39:359-61.
45. Papandreou CN, Doxani C, Zdoukopoulos N, Vlachostergios PJ, Hatzidaki E, Bakalos G, et al. Evidence of association between methylenetetrahydrofolate reductase gene and susceptibility to breast cancer: A candidate-gene association study in a South-eastern European population. DNA Cell Biol 2012;31:193-8.
46. Collings A, Raitakari OT, Juonala M, Rontu R, Kahonen M, Hutri-Kahonen N, et al. Associations of methylenetetrahydrofolate reductase C677T polymorphism with markers of subclinical atherosclerosis: The Cardiovascular Risk in Young Finns Study. Scand J Clin Lab Invest 2008;68:22-30.
47. Landrum MB, Guadagnoli E, Zummo R, Chin D, McNeil BJ. Care following acute myocardial infarction in the Veterans Administration Medical Centers: A comparison with Medicare. Health Serv Res 2004;39(6 Pt 1):1773-92.