Polymorphism of genes related to cardiovascular disease in patients with rheumatoid arthritis

L. Ärlestig, S. Wållberg Jonsson, B. Stegmayr, S. Rantapää-Dahlqvist

Department of Public Health and Clinical Medicine/Rheumatology and Medicine, University Hospital, Umeå, Sweden.

Abstract

Objective

To analyze candidate genes, related to cardiovascular disease (CVD) in general, and potentially involved in the inflammatory process, in RA patients from northern Sweden.

Methods

Four hundred and sixty-seven individuals (345 females; 122 males) with RA (ACR criteria), having a mean age of 61.8 ± 13.0 years and mean disease duration of 16.2 ± 12.1 years, were consecutively recruited and followed-up for 3 years. The prevalence of CVD, [(ischemic heart disease (IHD), deep vein thromboses/pulmonary embolism (DVT/PE) and/or stroke/TIA] and hypertension was registered. Candidate genes encoding for β-fibrinogen (G-455A), Factor XIIIA (Val34Leu), plasminogen activator inhibitor type-1 (PAI-1 4G/5G), and tumor necrosis factor receptor (TNFR)II (M196R) were analysed. Controls (n = 672) were randomly selected according to age and gender from the Medical Biobank of Northern Sweden. Polymorphisms were genotyped using a TaqMan 9700HT and the 5'nuclease allelic discrimination assay.

Results

The genotypes, carriers and alleles did not differ in distribution between patients and controls. Carriage of the TNFRII R variant was more frequent among patients with hypertension (p = 0.018). The genotype distribution of PAI-1 in patients with IHD differed significantly (p = 0.002) because carriage of 4G was more frequent (p = 0.024). Combined carriage of TNFRII 196R variant and β-fibrinogen-455A was a stronger predictor for hypertension than each genotype separately. The distribution of FXIIIA genotypes deviated significantly in RA patients with DVT/PE (p = 0.028) with an increased frequency of the Leu34 variant.

Conclusion

The unusual alleles of TNFRII, PAI-1 and FXIIIA were associated with CVD in RA patients. The combination of several of the rare types further increased the predictive values for CVD.

Key words

Rheumatoid arthritis, β-fibrinogen (G-455A), factor XIIIA G163T(Val34Leu), tumor necrosis factor receptor (TNFR)II T587G (M196R).

Lisbeth Ärlestig, BSc;
Solveig Wållberg Jonsson, MD, PhD;
Birgitta Stegmayr, PhD;
Solbritt Rantapää-Dahlqvist, MD, PhD.

This work was supported by grants from Swedish Research Council (K2003-74XD-14705-01), King Gustaf V's 80-Year Fund, the Swedish Heart-Lung Foundation, and the Medical Faculty of Umeå University, Umeå, Sweden. The Northern Sweden MONICA project and the funds supporting it.

Please address correspondence to:
Solbritt Rantapää-Dahlqvist, Department of Rheumatology, University Hospital, SE-901 85 Umeå, Sweden.
E-mail: Solbritt.Rantapaa.Dahlqvist@medicin.umu.se

Received on February 22, 2007; accepted in revised form on June 7, 2007.

Competing interests: none declared.

Introduction

Increased mortality due to cardiovascular disease (CVD) in patients with rheumatoid arthritis (RA) has been reported in several studies (1-3). Furthermore epidemiological (4) and physiological (5) studies show morbidity due to CVD to be more prevalent in patients with RA compared with control population. The etiopathogenic mechanisms leading to this increased morbidity are not well defined. We have identified inflammatory activity in RA to be of importance for the development of CVD and that hypofibrinolysis, such as increased PAI-1 activity and decreased tissue plasminogen capacity, predict future development of a cardiovascular event in patients with RA (6). A relationship between inflammation and endothelial dysfunction is strongly suggested (7). Accelerated atherosclerotic progression, as assessed by ultrasound measured intima media thickness, and aortic cusp sclerosis, in patients with RA has also been described (5). We have found significantly higher lipid levels in RA patients with atherosclerotic plaques compared with patients without plaque, despite relatively low cholesterol levels in RA patients with active disease (8, 9). Hypertension has in some reports been identified as one of the traditional risk factors of significant predictive value for CVD in RA (10).

A large number of candidate genes, each interacting with environmental influences, that create the phenotype for CVD have been analysed in the general population (11). In this study on patients with RA, we chose to analyse candidate genes encoding factors that could be involved in an inflammatory disease as well as in cardiovascular disease including thromboembolic disease *e.g.* gene products involved in inflammation (tumor necrosis factor receptor (TNFRII)), coagulation and/or thrombotic disease, (fibrinogen, Factor XIIIA, plasminogen activator inhibitor type-1 (PAI-1)). TNFRII gene polymorphism has been suggested to be involved in insulin resistance and coronary artery disease (12, 13) as well as in predisposing RA (14, 15). We have found in a previous study an association between TNFRII polymorphism and hypertension in patients with RA (16). Variants of the genes of β-fibrinogen (-455G/A), Factor XIIIA (Val34Leu) and PAI-1(4G/5G), which we selected to analyse, have been associated with progression of coronary artery disease in the general population (17-20).

Materials and methods

Study groups

A cohort of 467 patients (345 females and 122 males) with adult rheumatoid arthritis (RA) (21), from northern Sweden and attending the clinic at the Department of Rheumatology were recruited into the study. The first 150 patients were recruited consecutively during a 3 months period and the subsequent 317 patients during a second period of 5 months. A thorough survey of all patient records from disease onset was performed retrospectively according to a structured registration form and the patients were followed prospectively for 3 years. The mean age (± SD) at disease onset was 45.8 ± 14.8 years and the mean disease duration 16.2 ± 12.1 years, with no differences between the sexes. Of the patients 55% were treated with oral corticosteroids (for ≥ 1 year) (prednisolone ≤ 7.5 mg) and 90% were or had been treated with disease-modifying anti-rheumatic drugs (DMARDs) including sulphasalazine, antimalarials, intramuscular or oral gold, d-penicillamine, methotrexate, cyclosporine, azathioprine, chlorambucil and cyclophosphamide (≥ 6 months with each drug); the mean number of DMARDs used for more than 6 months was 2.4 ± 1.7. Total accumulated disease activ-ity analysed from retrospective data by calculation of number of arthritis, erythrocyte sedimentation rate and the physicians global assessment was performed as described by Baecklund (22). The number of patients who had ever smoked was 50% (227/467) being 45% of the female patients and 64% among the males.

The first ever cardiovascular event (CVE) after onset of RA, whether fatal or nonfatal, was registered retrospectively and prospectively according to the following definitions: myocardial infarction (MI) was diagnosed when changes on electrocardiograms, according to the Minnesota code (23), assessed by a clinical physiologist or cardiologist

together with the typical enzymatic pattern and/or verified on echocardiographic examination; ischaemic heart disease (IHD) included in addition to MI, severe verified coronary insufficiency, coronary artery bypass graft surgery and percutaneous coronary intervention; stroke was registered when intra cerebral haemorrhage or infarction had been diagnosed following computerized tomography or magnetic resonance imaging, or when a typical clinical picture with neurological deficits had persisted for more than 24h; transient ischaemic attack (TIA) was registered in cases where focal neurological deficit of presumed ischaemic origin had persisted for less than 24 h; deep vein thrombosis/pulmonary embolism (DVT/PE) was recorded when diagnosis had been verified objectively (*e.g.*, phlebography, sonography, scintigraphy, and/or arteriography) or when clinical signs combined with pulmonary radiography, electrocardiography, and laboratory changes resulted in full time treatment with warfarin. Information on fatal CVE was obtained from autopsy reports. In 2 cases the information on the death certificate, together with medical record information, was relied upon. Hypertension was defined as treatment for hypertension.

As controls, we used a population-based group of 672 individuals who were participating in the Northern Sweden WHO MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) study. They were randomly selected from the population register of the county of Västerbotten. All of the controls had the same ethnic and geographical background (based on the place of birth) as the patients and were selected on the basis of age and gender. The number of controls receiving treatment for hypertension was 150 (22.3%). Of the controls 44% (300/672) had ever smoked; 42% among females and 49% of the males. There were no data available regarding CVD in the control group.This study was approved by the Research Ethics Committee of the University Hospital of Umeå.

DNA extraction and assays

Genomic DNA was extracted from either EDTA preserved blood or from buffy-coats using standard methods. Primers and probes for the polymorphisms described earlier in TNFRII(M196R) (24), β-fibrinogen-455G/A (25), Factor XIIIA Val34Leu(26) and PAI-1 (4G/5G) (27) were designed using Assays-By-Design (Applied Biosystems, Foster City, CA, USA). The polymerase chain reactions (PCR) were carried out according to Applied Biosystems instructions, and detection of the different genotypes using an ABI PRISM 7900HT sequence detector system (Applied Biosystems). Data were processed using SDS 2.1 software (Applied Biosystems).

Statistics

Differences in continuous data between two groups were analyzed using an independent *t*-test. The chi-square test was used for testing categorical data between groups. Logistic regression analyses were used to estimate the odds ratio (OR) for predicting variables for the dependent variable. All *p*-values are two-sided, and *p*-values ≤ 0.05 were considered statistically significant. All calculations were performed using the SPSS for Windows (version 11.5; Chicago, IL, USA). The adjustments in the multiple logistic analyses were chosen based on knowledge from previous reports. With the current sample size of 672 controls and 467 patients, this study had an ability to detect a statistically significant difference in the proportion of carriers of the R allele of 35% (RA-patients) *vs*. 43.6% (controls) with at least 80% power. The power calculation was based on a significance level of 5% and a two-sided test using normal approximation. The selection of genes for analyses in this study is based on preformed hypothesis. However, to compensate for multiple testing a corrected *p*-value (pc) is also given.

Results

The distributions of the genes of PAI-1 4G/5G, FXIIIA Val34Leu, β-fibrinogen -455G/A, and TNF RII M196R (see Table I) in patients and controls were in Hardy-Weinberg equilibrium.

Table I. Genotype and allele frequencies of tumor necrosis factor receptor II (TNFRII M196R), β-fibrinogen -455G/A, plasminogen activator inhibitor type-1 (PAI-1) and Factor XIIIA (Val34Leu) polymorphisms in patients with RA and controls. (Presented as n and %).

Gene	Genotype/allele	RA patients (n = 467)	Controls (n = 672)
TNFRII	MM	278 (59.5)	377 (56.4)†
M196R	MR	159 (34.0)	247 (36.9)†
	RR	30 (6.4)	45 (6.7)†
	M allele	0.7655	0.7481
	R allele	0.2345	0.2519
β-fibrinogen	GG	292 (62.5)	438 (65.5)†
-455	GA	154 (33.0)	206 (30.8)†
	AA	21 (4.5)	25 (3.7)†
	A allele	0.2099	0.19
	G allele	0.79	0.809
PAI-1	5G5G	100 (21.4)	114 (17.0)
	5G4G	227 (48.6)	339 (50.7)
	4G4G	140 (30.0)	219 (32.7)
	5G	0.457	0.422
	4G	0.543	0.578
Factor	ValVal34	272 (58.2)	nt
XIIIA	ValLeu34	166 (35.5)	nt
	LeuLeu34	29 (6.2)	nt
	Leu allele	0.24	nt
	Val allele	0.76	nt

†number of controls analysed: 669, nt: not tested.

There were no significant differences in the distributions between patients and controls except for significant deviation in the distribution of TNFRII in males (data not shown). Carriage of the R variant was significantly more frequent among male patients compared with female patients (χ^2 = 7.17, p = 0.007, pc = 0.042) and compared with male control subjects (χ^2 = 9.11, p = 0.003, pc = 0.018).

The prevalence of CVE and hypertension in the patients with RA is presented in Table II. TNFRII (M196R) differed significantly between RA patients with hypertension compared with those without (χ^2 = 7.41, 2df, p = 0.025, pc = 0.150) due to an excess of heterozygotes. Carriage of the R type was significantly increased in the RA patients with hypertension compared with those without (χ^2 = 5.61, p = 0.018, pc = 0.108). When stratified for sex the difference was only significant for female patients (χ^2 = 4.57, p = 0.029, pc = 0.174). There was no association between TNFRII polymorphism and hypertension in the controls.

There were no significant relationships between the genotypes, alleles or carriage of β-fibrinogen -455 and CVE or hypertension in RA patients. However, when carriage of TNFRII R variant and β-fibrinogen -455 A variant were combined, carriage of both these rare types was a stronger predictor for hypertension than carriage of R variant alone. In multiple regression analysis including age and adjusted for sex, disease duration, smoking, accumulated disease activity score, and treatment with methotrexate, antimalarials or corticosteroids, the OR was slightly higher for the combination (Table III).

In RA patients with IHD the genotypes of PAI-1 deviated significantly from Hardy-Weinberg equilibrium (χ^2= 10.31, p < 0.01). The distribution of the genotypes differed also significantly in patients with IHD from those without (χ^2 = 12.27, 2df, p = 0.002, pc = 0.012). This was mainly due to a significant increase of heterozygotes among the patients with IHD (Table IV). Carriage of 4G predicted IHD (Table V). The OR for carriage of 4G was of the same magnitude, in a multiple logistic regression analysis including sex, hypertension and age with the adjustments of smoking habit, accumulated disease activity score and treatment with methotrexate, antimalarials or corticosteroids (Table V).

In the patients with DVT and/or PE (n = 13) the distributions of FXIIIA genotypes deviated compared with those without (χ^2 = 7.12, 2df, p = 0.028, pc = 0.168) with an increase of the Leu34 variant (OR = 4.855, 95%CI 1.318-17.882). Carriage of Val34 was significantly less frequent in DVT/PE suggesting a protective effect (OR = 0.206, 95%CI 0.056-0.763). The distribution of TNFRII also deviated significantly with increased OR for carriage of the less frequent R allele, (OR = 3.432, 95%CI 1.041-11.311). Carriage of both of these unusual alleles predicted DVT and/or PE with an even higher OR (OR = 6.588, 95%CI 2.149-20.202). In a multiple logistic regression analysis

Table II. Prevalence of cardiovascular disease in 467 patients with RA.

	All patients (n = 467) n (%)	Women (n = 345) n (%)	Men (n = 122) n (%)
Treatment for hypertension	145 (31.0)‡	105 (30.4)	40 (32.8)
Ischaemic heart disease[1]	59 (12.7)	32 (9.3)	27 (22.2)*
Stroke/TIA	40 (8.6)	26 (7.6)	14 (11.5)
DVT/ PE[2]	14 (3.0)	13 (3.8)	1 (0.8)

[1]Ischaemic heart disease was defined as myocardial infarction, severe verified coronary insufficiency, coronary angioplasty and coronary artery bypass graft surgery.
[2]DVT: deep vein thrombosis; PE: pulmonary embolism.
‡ p < 0.001 number of treated patients compared with controls (22.3%).
* p < 0.05 males compared with females.

Table III. Results of simple and multiple logistic regression analyses with hypertension as dependent variable in patients with RA. The adjustments in the mutiple regression analysis were sex, smoking, accumulated disease activity score, methotrexate, antimalarial and/or corticosteroid treatment.

Variable(s)	OR	95% CI
Simple:		
TNFRII, carrier R	1.614	1.084-2.401
F-455, carrier A	1.398	0.936-2.089
TNFRII, carrier R & F-455 carrier A	2.456	1.463-4.121
Multiple:		
TNFRII, carrier R & F-455 carrier A	2.673	1.551-4.607
Age (year)	1.040	1.021-1.059

OR: Odds ratio, 95%CI = 95% confidence intervals.
F-455: β-fibrinogen-455.

Table IV. Genotype and carriage distribution of PAI-1 4G5G in patients with RA with and without ischemic heart disease (IHD).

	RA patients With IHD (n = 59) n (%)	RA patients without IHD (n = 407) n (%)	OR	95%CI
Genotype				
4G4G	12 (20.3)	128 (31.4)	0.557	0.285-1.085
4G5G	41 (69.5)	185 (45.5)	2.773	1.519-4.919
5G5G	6 (10.2)	94 (23.1)	0.377	0.157-0.904
Carriage				
4G	53 (89.8)	313 (76.9)	2.653	1.106-6.365
5G	47 (79.7)	279 (68.6)	1.797	0.922-3.503

OR: Odds ratio; 95%CI: 95% confidence intervals.

Table V. Results of simple and multiple logistic regression analyses with ischemic heart disease (IHD) as dependent variable in patients with RA. The adjustments in the mutiple regression analysis were sex, smoking, accumulated disease activity score, methotrexate, antimalarials and/or corticosteroid treatment.

Variable(s)	OR	95%CI
Simple:		
PAI-1, carrier 4G	2.653	1.106-6.365
Multiple:		
Sex (M/F)	2.913	1.590-5.335
PAI-1, carrier 4G	2.619	1.057-6.489
Hypertension	1.875	1.024-3.432
Age (years)	1.065	1.033-1.097

OR: Odds ratio, 95%CI = 95% confidence intervals.

only treatment with corticosteroids had higher predictive value, than carriage of a combination of the unusual variants (OR = 7.163, 95%CI 2.038-25.17 and OR = 9.203, 95%CI 1.724-49.129, respectively). The adjustments were age, sex, smoking habits, accumulated disease activity score and treatment with methotrexate, or antimalarials.

There were no differences concerning FXIIIA genotypes, alleles or carriage of any type associated with stroke and/or TIA or IHD. However, when stratified for sex, carriage of the ValVal34 genotype in females (n = 26) predicted stroke/TIA (OR = 2.724, 95%CI1.065-6.964). In a multiple logistic regression FXIIIA genotype ValVal 34 had the highest OR (OR = 3.454, 95%CI 1.147-10.402), followed by hypertension (OR = 3.151, 95%CI 1.199-8.282) and age (OR = 1.046, 95%CI 1.013-1.080) with adjustments for smoking habits, accumulated disease activity score, and treatment of methotrexate, antimalarials and/or corticosteroids for stroke.

Discussion

In this study we have shown an impact of polymorphisms in certain genes for CVD in patients with RA. Association between RA and CVD has been evaluated in a number of other studies (28, 29). However, the genes selected for this study were chosen on the basis of their involvement in inflammation, coagulation and/or thrombotic disease, which, considering our previous studies, could be of interest in RA patients (6, 16). Carriage of PAI-1 4G type predicted IHD in both simple and multiple logistic regression analyses. The OR was almost identical in both analyses suggesting that the relationship was independent of variables such as sex, age, hypertension, smoking, disease activity or treatment. Our results are consistent with those published for population based cohort studies on coronary disease. In a recent meta-analysis the per allele relative risk of the 4G variant for MI and coronary stenosis combined was 1.06 (95%CI 1.02-1.10) (20). However, when confined to studies with at least 500 cases the combined relative risk yielded 1.01 (95%CI 0.95-1.07) suggesting publication bias of smaller studies (20). The PAI-1 4G deletion allele is associated with increased levels of circulating PAI-1 (17), which we found previously predicted future CVD in RA patients (6). Whether there is a direct relationship between PAI-1 gene polymorphisms and myocardial infarction or CVD or the relationship is a result of confounder effects is not settled (30, 31).

Findings of a relationship between fibrinogen, and factor XIII in clot formation in CVD or other thrombotic disorders has focused attention on these two proteins (18). The suggested protective role of the Leu 34 allele for MI was lost in the presence of insulin resistance (18) or by smoking (32). There are also studies reporting no association between Leu34 and risk of MI (18). Protective associations, similar to that described for MI have been reported for venous thrombotic disorders, as have studies demonstrating no association (18). A higher prevalence was first shown of the Leu allele in subjects with primary intracranial haemorrhage (33), however, contradictory findings were subsequently reported (18). Our findings favour the interpretations that carriage of Leu allele is associated with DVT and/or PE or that homozygocity of Val34 protects from DVT and/or PE but predisposes for stroke. Our results, albeit seemingly contradictory, may be explained in line with previous findings. We did not find any relationship with MI or IHD, and the number of cases was too low to allow stratification for smoking habits.

The results of our present study, confirming our previous findings, enabled us to substantiate those, indicating an association between TNFRII codon 196 and RA patients with hypertension (16). Associations with the M196R polymorphism of the TNFRII gene and RA patients with a positive family history of RA have been reported by some investigators (14, 15) whilst others have disputed this finding (34). No significant differences were reported in allele or genotype frequencies between individuals with normal coronary arteries or healthy controls and patients with coronary artery disease (35).

Both linkage and association have been shown between micro satellite markers at the TNFRSF1B (TNFRII gene locus) and essential hypertension (36) or coronary artery disease, respectively (12). Another TNFRSF1B marker was associated with obesity, leptin and insulin resistance in type 2 diabetes (13). In this study we found that an association with TNFRII gene polymorphism is confined to the RA patients but not to the controls in line with the results for controls with coronary artery disease (35). Its predictability for hypertension was strengthened by combination with the β-fibrinogen (-455) rare types. It was almost unaffected by sex, smoking and disease related factors favouring the interpretation of a more etiologic relationship for hypertension in RA patients. The β-fibrinogen -455 polymorphism was not alone related to hypertension or CVD in the RA patients. Our results relating the PAI-1 4G type to IHD were also in line with those reported for general populations and the relationship was unaffected by confounding factors *e.g.*, sex, age or hypertension or by disease related factors.

The number of RA patients and controls included in this study was relatively high but after stratification for CVE, IHD, DVT/PE or stroke the number decreased and this could diminish the possibility of detecting differences. However, despite that, we were able to detect significant differences. An advantage of our study is that both patients and the population-based controls are drawn

from the same homogenous population of northern Sweden (37). Unfortunately, the corresponding follow-up co-morbidity data for the controls is unavailable.

In conclusion, the aetiology and comorbidity factors for cardiovascular disease in RA patients are complex. Our results, although the genes analysed in this study do not have a major contribution for CVD in RA, point to direct effects of certain candidate genes some identified in cohorts from the general population and separate from confounding factors and disease related factors.

Acknowledgements

We thank Marie Eriksson, BSc, for helpful discussion regarding statistical analyses.

References

1. WOLFE F, MITCHELL DM, SIBLEY JT *et al*.: The mortality of rheumatoid arthritis. *Arthritis Rheum* 1994; 37: 481-94.
2. MYLLYKANGAS-LUOSUJÄRVI R, AHO K, KAUTIAINEN H, ISOMÄKI H: Shortening of life span and causes of excess mortality in a popultion-based series of subjects with rheumatoid arthritis. *Clin Exp Rheumatol* 1995; 13: 149-53.
3. WÅLLBERG-JONSSON S, ÖHMAN M-L, RANTAPÄÄ-DAHLQVIST S: Cardiovascular morbidity and mortality in patients with seropositive rheumatoid arthritis in Northern Sweden. *J Rheumatol* 1997; 24: 445-51.
4. DEL RINCÓN I, WILLIAMS K, STERN MP, FREEMAN GL, ESCALANTE A: High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional risk factors *Arthritis Rheum* 2001; 44: 2737-45.
5. WÅLLBERG JONSSON S, LUNDSTRÖM E, BACKMAN C, KARP K, RANTAPÄÄ DAHLQVIST S: Increased prevalence of atherosclerosis in patients with medium-term rheumatoid arthritis. *J Rheumatol* 2001; 28: 2597-602.
6. WÅLLBERG JONSSON S, CEDERFELDT M, RANTAPÄÄ DAHLQVIST S: Hemostatic factors and cardiovascular disease in active rheumatoid arthritis-an eight year follow-up study. *J Rheumatol* 2000; 27: 71-5.
7. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, MARTIN J: Inflammation and endothelial dysfunction in rheumatoid arthritis. *Clin Exp Rheumatol* 2006; 24: 115-7.
8. RANTAPÄÄ-DAHLQVIST S, WÅLLBERG JONSSON S, DAHLÉN G: Lipoprotein(a), lipids and lipoproteins in patients with rheumatoid arthritis. *Ann Rheum Dis* 1991; 50: 36-8.
9. PARK YB, LEE SK, LEE WK *et al*.: Lipid profiles in untreated patients with rheumatoid arthritis. *J Rheumatol* 1999; 26: 1701-4.
10. WÅLLBERG JONSSON S, JOHANSSON H, ÖHMAN M-L, RANTAPÄÄ DAHLQVIST S: Extent of inflammation predicts cardiovascular disease and overall mortality in seropositive rheumatoid arthritis. *J Rheumatol* 1999; 26: 2562-71.
11. GRANT PJ: The gentics of atherothrombotic disorders: a clinician's view. Review article. *J Thromb Haemost* 2003; 1: 1381-90.
12. BENJAFIELD AV, WANG XL, MORRIS BJ: Tumor necrosis factor receptor 2 gene (TNFRSF1B) in genetic basis of coronary artery disease. *J Mol Med* 2001; 79: 109-15.
13. FERNANDEZ-REAL JM, VENDRELL J, RICART W *et al*.: Polymorphism of the tumor necrosis factor-alpha receptor 2 gene is associated with obesity, leptin levels, and insulin resistance in young subjects and diet-treated type 2 diabetic patients. *Diabetes Care* 2000 ; 23: 831-7.
14. BAYLEY JP, BAKKER AM, KAIJZEL EL, HUIZINGA TW, VERWEIJ CL: Association of polymorphisms of the tumour necrosis factor receptors I and II and rheumatoid arthritis. *Rheumatology* (Oxford) 2003; 42: 969-71.
15. BARTON A, JOHN S, OLLIER WE, SILMAN A, WORTHINGTON J: Association between rheumatoid arthritis and polymorphism of tumor necrosis factor receptor II, but not tumor necrosis factor receptor I, in Caucasians. *Arthritis Rheum* 2001; 44: 61-5.
16. DAHLQVIST SR, ÄRLESTIG L, SIKSTROM C, LINGHULT S: Tumor necrosis factor receptor type II (exon 6) and interleukin-6 (-174) gene polymorphisms are not associated with family history but tumor necrosis factor receptor type II is associated with hypertension in patients with rheumatoid arthritis from northern Sweden. *Arthritis Rheum* 2002; 46: 3096-8.
17. ERIKSSON P, KALLIN B, VAN'T HOOFT FM, BAVENHOLM P, HAMSTEN A: Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. *Proc Natl Acad Sci USA* 1995; 92: 1851-5.
18. ARIENS RA, LAI TS, WEISEL JW, GREENBERG CS, GRANT PJ: Role of factor XIII in fibrin clot formation and effects of genetic polymorphisms. *Blood* 2002; 100: 743-54 (Review).
19. SCOTT EM, ARIENS RA, GRANT PJ: Genetic and environmental determinants of fibrin structure and function: relevance to clinical disease. *Arterioscler Thromb Vasc Biol* 2004; 24: 1558-66.
20. YE Z, LIU EH, HIGGINS JP *et al*.: Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66,155 cases and 91,307 controls. *Lancet* 2006; 367: 651-8.
21. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
22. BAECKLUND E: *Associations between rheumatoid arthritis and malignant lymphomas. Uppsala University*. Medical dissertation. ISBN 91-554-6335-5 Uppsala 2005.
23. PRINEAS RJ, CROW RJ, BLACKBURN H: *The Minnesota code manual of electrocardiographic findings: standards and procedures for measurement and classification*. Boston (Mass.): John Wright & Son Ltd, 1982.
24. AL-ANSARI AS, OLLIER WE, VILLARREAL J, ORDI J, TEH LS, HAJEER AH: Tumor necrosis factor receptor II (TNFRII) exon 6 polymorphism in systemic lupus erythematosus. *Tissue Antigens* 2000; 55: 97-9.
25. HUMPHRIES SE, YE S, TALMUD P, BARA L, WILHELMSEN L, TIRET L: European Atherosclerosis Research Study: genotype at the fibrinogen locus (G-455-A beta-gene) is associated with differences in plasma fibrinogen levels in young men and women from different regions in Europe. Evidence for gender-genotype-environment interaction. *Arterioscler Thromb Vasc Biol* 1995; 15: 96-104.
26. KANGSADALAMPAI S, BOARD PG: The Val34Leu polymorphism in the A subunit of coagulation factor XIII contributes to the large normal range in activity and demonstrates that the activation peptide plays a role in catalytic activity. *Blood* 1998; 92: 2766-70.
27. DAWSON SJ, WIMAN B, HAMSTEN A, GREEN F, HUMPHRIES S, HENNEY AM: Promoter of the Plasminogen Activator Inhibitor- 1 (PAI-1) Gene Respond Differently to Interleukin-1 in HepG2 Cells. *J Biol Chem* 1993; 268: 10739-45.
28. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, LOPEZ-DIAZ MJ *et al*.: HLA-DRB1 and persistent chronic inflammation contribute to cardiovascular events and cardiovascular mortality in patients with rheumatoid arthritis. *Arthritis Rheum* 2007; 57: 125-32.
29. SWANBERG M, LIDMAN O, PADYUKOV L *et al*.: MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction. *Nat Genet* 2005; 37: 486-94. Epub 2005 Apr 10.
30. DING J, NICKLAS BJ, FALLIN MD *et al*.: Plasminogen activator inhibitor type 1 gene polymorphisms and haplotypes are associated with plasma plasminogen activator inhibitor type 1 levels but not with myocardial infarction or stroke. *Am Heart J* 2006; 152: 1109-15.
31. BONCORAGLIO GB, BODINI A, BRAMBILLA C, CARRIERO MR, CIUSANI E, PARATI EA: An effect of the PAI-1 4G/5G polymorphism on cholesterol levels may explain conflicting associations with myocardial infarction and stroke. *Cerebrovasc Dis* 2006; 22: 191-5. Epub 2006 May 30.
32. FRANCO RF, PAZIN-FILHO A, TAVELLA MH, SIMOES MV, MARIN-NETO JA, ZAGO MA: Factor XIII val34leu and the risk of myocardial infarction. *Haematologica* 2000; 85: 67-71.
33. CATTO AJ, KOHLER HP, BANNAN S, STICKLAND M, CARTER A, GRANT PJ: Factor XIII Val 34 Leu: a novel association with primary intracerebral hemorrhage. *Stroke* 1998; 29: 813-6.
34. DIEUDE P, PETIT E, CAILLEAU-MOINDRAULT S *et al*.: European Consortium on Rheumatoid Arthritis Families. Association between tumor necrosis factor receptor II and familial, but not sporadic, rheumatoid arthritis: evidence for genetic heterogeneity. *Arthritis Rheum* 2002; 46: 2039-44.
35. ALLEN RA, LEE EM, ROBERTS DH, PARK BK, PIRMOHAMED M: Polymorphisms in the TNF-alpha and TNF-receptor genes in patients with coronary artery disease. *Eur J Clin Invest* 2001; 31: 843-51.
36. GLENN CL, WANG WY, BENJAFIELD AV, MORRIS BJ: Linkage and association of tumor necrosis factor receptor 2 locus with hypertension, hypercholesterolemia and plasma shed receptor. *Hum Mol Genet* 2000; 12: 1943-9.
37. EINARSDOTTIR E: *Mapping genetic diseases in northern Sweden, Umeå University Medical Dissertations new series nr. 946*. ISBN 91-7305-836-X Umeå, Sweden, 2005.