Lack of association of *NAMPT* rs9770242 and rs59744560 polymorphisms with disease susceptibility and cardiovascular risk in patients with rheumatoid arthritis

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Abstract

Objective

Visfatin is an adipokine encoded by the NAMPT (PBEF1) gene. In this study we assessed the potential association of two NAMPT gene polymorphisms with disease susceptibility and cardiovascular (CV) risk in patients with rheumatoid arthritis (RA).

Methods

A total of 1,395 patients fulfilling the 1987 ACR classification criteria for RA and 1,230 matched controls, were genotyped for the NAMPT rs9770242 and rs59744560 gene polymorphisms, located within the proximal promoter, using predesigned TaqMan single nucleotide polymorphism genotyping assay. Also, HLA-DRB1 genotyping was performed using molecular based methods. In a second step, 1,196 patients in whom full information was available were assessed to determine the influence of NAMPT rs9770242 and rs59744560 polymorphisms in the development of CV events. Also, the potential influence of these polymorphisms in the development of subclinical atherosclerosis was assessed in a subgroup of patients with no history of CV events by brachial artery reactivity to determine flow-mediated endothelium-dependent and endothelium-independent vasodilatation (n=125) and by B-mode ultrasonography to determine the carotid artery intima-media thickness (n=105).

Results

No statistically significant differences in the allele or genotype frequencies for the NAMPT gene polymorphisms between RA patients and controls were found. A modest non significant lower frequency of the minor allele G of rs9770242 polymorphism was observed among patients with CV disease (20.62%) compared to those without CV disease (22.83%) (p=0.39). Also, a slight nonsignificant lower frequency of the minor allele T of rs59744560 polymorphism in patients with CV events (9.81%) compared with those RA patients who did not experience CV disease (13.07%) (p=0.11) was observed. Likewise, no significant association between the NAMPT polymorphisms with surrogate markers of subclinical atherosclerosis was found in patients with RA.

Conclusion

NAMPT rs9770242 and rs59744560 polymorphisms are not markers of disease susceptibility and CV disease in RA.

Key words

rheumatoid arthritis, atherosclerosis, cardiovascular disease, genetics, NAMPT rs9770242 (-1001T>G) and rs59744560 (-948G>T).

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease associated with accelerated atherosclerosis and increased incidence of cardiovascular (CV) events (1, 2). Besides classic CV risk factors (3, 4) and chronic systemic inflammation (5, 6), recent studies have emphasised the potential influence of genetic factors in the development of endothelial dysfunction (7-9), and CV events in RA (5, 9-11).

Visfatin, also known as pre-B cell colony-enhancing factor (PBEF) (12) or nicotinamide phosphoribosyl transferase (NAMPT), (13) is an adipokine produced by visceral adipose tissue and also by other cells such as macrophages and neutrophils. It is induced by inflammation and immune activation (14) and has immunomodulatory properties including enhancement of B cell differentiation, induction of cytokines and matrix metalloproteinases (15), and inhibition of neutrophil apoptosis (16). Visfatin synthesis is regulated by several factors, including glucocorticoids, TNF-α, IL-6 and growth hormone. Visfatin has been shown to induce chemotaxis and the production of IL-1β, TNF-α, IL-6 and costimulatory molecules by $CD14^+$ monocytes, and to increase alloproliferative responses in lymphocytes, effects which are mediated intracellularly by p38 and MEK1 (17). This adipokine promotes vascular smooth muscle cell maturation being associated with a potential role in vascular dysfunction and inflammation associated with some metabolic disorders (18). It has pro-inflammatory and atherogenic effects and it has been associated with insulin resistance (19, 20). Visfatin is upregulated in models of acute lung injury and sepsis (21).

Expression of visfatin is increased in rheumatoid synovial fibroblasts, particularly at sites of invasion of cartilage (16). Visfatin concentrations are elevated in patients with RA (22) and are associated with increased radiographic joint damage (23). This 52-kilodalton adipokine is encoded by the *NAMPT (PBEF1)* gene. There are number of polymorphic markers in the *NAMPT* gene. Some polymorphisms in this gene have been reported to be associated with levels of expression of the protein. One of these SNPs, the *NAMPT* rs9770242 (-1001T>G) is physically close (53 base pairs) to and in moderate linkage disequilibrium with the rs59744560 (-948G>T) polymorphism (r^2=0.47), raising the possibility that these two SNPs are markers of the same functional polymorphism(s) placed in regulatory elements in the 5´-flanking region and influencing visfatin expression (24). Bailey *et al*. disclosed a significant association between 2 SNPs that are in perfect linkage disequilibrium (rs9770242 and rs1319501) and fasting insulin levels (25). On the other hand, Johansson *et al*. observed that obese individuals carrying the T allele of *NAMPT* rs59744560 (-948G>T) polymorphism had significantly higher levels of HDL-cholesterol than those GG homozygous (26). Interestingly, patients with RA often have a dyslipemic pattern, a metabolic syndrome and HDL-cholesterol levels are generally decreased, in particular in those with severe and active disease.

Taking into account all these evidences, the purpose of the present study was to determine if *NAMPT* rs9770242 (-1001T>G) and rs59744560 (-948G>T) polymorphisms might be associated with susceptibility to RA. In a second step we assessed the potential implication of the *NAMPT* rs9770242 and rs59744560 polymorphisms in the development of CV disease in patients with RA.

Material and methods

Patients and controls

Between March 1996 and September 2008, 1,395 consecutive patients that fulfilled the 1987 American College of Rheumatology classification criteria for RA (27) were recruited from the Rheumatology Outpatient Clinics of Hospital Xeral-Calde (Lugo), Hospital Clínico San Carlos (Madrid), Hospital Universitario La Paz (Madrid) and Hospital Universitario La Princesa (Madrid), Spain. Patients and 1,210 bone marrow and blood donors from National Repository DNA Bank, matched by age, sex and ethnicity, from the corresponding regions, were assessed for differences in the *NAMPT* rs9770242 (-1001T>G)

and rs59744560 (-948G>T) gene polymorphisms. A DNA sample (see below) was extracted from these patients at the time of recruitment.

Study Protocol

Between December 2009 and January 2010 patient's clinical records for whom information was available, were examined until patient's death, loss of follow-up or December 1st, 2009. Clinical definitions for CV events and classic CV risk factors were established as previously described (5, 28). In this regard, smoking habit was considered to be present in those patients who smoked at the time of disease diagnosis, during the follow-up or who had smoked within the 10 years before the onset of RA symptoms or the disease diagnosis (5). A CV event was considered to be present if the patient had ischaemic heart disease, heart failure, cerebrovascular accident or peripheral arteriopathy.

The definition of ischaemic heart disease (IHD) included acute coronary syndromes with or without persistent ST-segment elevation and chronic coronary heart disease. IHD was diagnosed if any of the following criteria were satisfied: a recorded diagnosis of ischaemic cardiopathy, on account of some acute coronary syndrome (acute myocardial infarction or unstable angina), the presence of pathological Q waves in the electrocardiogram, and coronary images showing >50% stenosis of at least one coronary vessel (5). Data regarding the clinical presentation of heart failure were also collected using the Framingham criteria (28). A patient was considered to have a cerebrovascular accident when he/she had a stroke and/or transient ischaemic attacks (TIAs). Strokes were classified according to their clinical features and they were confirmed by computed tomography and/or magnetic resonance imaging. TIAs were diagnosed if the symptoms were self-limited in less than 24 hours, without residual neurological damage (5). Peripheral arterial disease was considered to be present if it was confirmed by Doppler and arteriography (29).

To determine the potential association between the *NAMPT* rs9770242 and rs59744560 polymorphisms and the presence of subclinical atherosclerosis, between March 2007 and September 2009 a random subgroup of patients from the Lugo cohort with no previous history of CV events was selected. Presence of endothelial dysfunction was assessed by a brachial artery reactivity study in 125 patients. Flow-mediated endothelium-dependent vasodilatation-FMD (post-ischaemia) and endothelium-independent-NTG (post-nitroglycerin) vasodilatation were measured by brachial ultrasonography as previously reported (30, 31). A value of FMD less than 7% was considered pathologic, indicating the presence of endothelial dysfunction (31). Intra-observer variability for FMD and NTG was 1.3% and 1.9%, respectively, based on the repeat of the brachial ultrasonography in 32 healthy controls. Assessment of endothelial function of patients undergoing anti-TNF therapy was performed 24–48 hours before drug administration. Also, carotid ultrasonography studies were performed in 105 patients to determine the carotid artery intima-media thickness (IMT). It was assessed in the right common carotid artery as previously reported (32, 33). Based on a second carotid ultrasonography performed to 20 RA patients and 20 healthy controls within a week after the first assessment the correlation coefficient for carotid IMT was 0.98.

The subjects' written consent was obtained according to the declaration of Helsinki, and the design of the work was approved by the Ethics Committee of Galicia (Spain). The Ethics Committees of the Hospital Clinico San Carlos (Madrid), Hospital La Paz (Madrid) and Hospital de la Princesa (Madrid) also approved the study.

Genotyping

– NAMPT genotyping

DNA was obtained from peripheral blood, using standard methods. Subjects were genotyped to determine *NAMPT* rs9770242 (-1001T>G) and rs59744560 (-948G>T) polymorphisms status, both located within the promoter region (25), using TaqMan Assays-on-Demand and TaqMan Genotyping Master Mix, and analysed using the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

– Shared epitope determination

Several *HLA-DRB1* alleles (*HLA-DRB1**0401, *0404, *0405, *0408, *0101, *0102, *1001, *1402) are associated with susceptibility to RA. These alleles encode a conserved amino acid sequence (QKRAA, QRRAA, or RRRAA), called the shared epitope, at position 70–74 in the third hypervariable region of the HLA-DRβ1 molecule (34). *HLA-DRB1*-shared epitope alleles are also implicated in the severity of the disease (35). *HLA-DRB1* typing was carried out using a reverse dot-blot kit with sequence-specific oligonucleotide (SSO) probes (Dynal RELITM SSO HLA-DRB1 typing kit; Dynal Biotech, Bromborough, UK).

Statistical analysis

All genotype data were checked for deviation from Hardy-Weinberg equilibrium (HWE) using http://ihg.gsf.de/cgi-bin/hw/hwa1.pl. Neither controls nor RA patients with CV disease or without CV disease had a deviation from HWE for the *NAMPT* polymorphisms.

Comparison of proportions was carried out using χ^2 test or Fisher test, when required. Strength of associations between CV events and genotypes or alleles of *NAMPT* polymorphisms were estimated using odds ratios (OR) and 95% confidence intervals (CI), via multiple logistic regression; estimates were further adjusted by gender, age at RA diagnosis, time of follow-up, presence or absence of the rheumatoid shared epitope, and classic CV risk factors (hypertension, diabetes mellitus, dyslipidemia, obesity and smoking habit) as potential confounders.

The association between genotypes of the *NAMPT* polymorphisms and surrogate markers of subclinical atherosclerosis: carotid IMT, FMD%-endothelium dependent vasodilatation or NTG%-endothelium independent vasodilatation were tested using unpaired *t*-test, to compare between 2 groups, and one-way analysis of variance (ANOVA) to

compare among more than two groups. Moreover, we also tested association between these parameters and alleles using analysis of covariance (ANCOVA) adjusting for gender, age and duration of the disease at the time of the ultrasonographic study, and presence or absence of shared epitope and traditional CV risk factors. Statistical significance was defined as $p<0.05$. Calculations were performed with STATA 10 (STATA Corporation, College Station, Texas, USA).

Estimation of the power of the study was performed using the CaTS-Power Calculator (Ann Arbor, Michigan, USA) (36) that allowed detecting small effects like OR 1.25 in our cohort for both polymorphisms assuming the estimated prevalence of RA in the general population of Spain (37).

Table I. Genotype and allele frequencies of *NAMPT* rs9770242 (-1001T>G) and rs59744560 (-948G>T) polymorphisms in healthy controls and patients with rheumatoid arthritis (RA).

NAMPT rs9770242		RA Patients		Controls				
		n	%	n	%	*p*-value	OR	(IC 95%)
Genotypes	TT	829	60.91	717	60.05	0.66	1.04	0.88-1.22
	TG	466	34.24	402	33.67	0.76	1.03	0.87-1.21
	GG	66	4.85	75	6.28	0.11	0.76	0.54-1.07
Alleles	T	2,124	78.03	1,836	76.88			
	G	598	21.97	552	23.12	0.33	0.94	0.82-1.07
NAMPT rs59744560		RA Patients		Controls				
		n	%	n	%	*p*-value	OR	(IC 95%)
Genotypes	GG	1,051	77.51	934	77.19	0.85	1.02	0.85-1.23
	GT	279	20.58	254	20.99	0.80	0.97	0.81-1.18
	TT	26	1.92	22	1.82	0.85	1.05	0.59-1.85
Alleles	G	2,381	87.79	2,122	87.69			
	T	331	12.21	298	12.31	0.91	0.99	0.84-1.17

Results

Allele and genotype frequencies of the NAMPT rs9770242 (-1001T>G) and rs59744560 (-948G>T) polymorphisms in RA patients and controls

We had enough statistical power to detect small effects like OR 1.25 in our cohort for both polymorphisms: assuming prevalence of RA in Spanish population 0.005, and the minor allele frequency obtained in our study we reached a power of 95% and 82% for rs9770242 and rs59744560. Genotype frequencies of the *NAMPT* rs9770242 and rs59744560 variants studied were in Hardy-Weinberg equilibrium in patients and controls.

No statistically significant differences in the genotype or allele frequency of the *NAMPT* rs9770242 (-1001T>G) gene polymorphism between RA patients and controls were seen. It was also the case when allele of genotype frequencies of the rs59744560 (-948G>T) polymorphism in patients were compared with those observed in the controls (Table I). Also, no significant differences in the age at the onset of the disease, rheumatoid factor, anticyclic citrullinated peptide antibodies, shared epitope, and age at the time of disease diagnosis were observed according to the different *NAMPT* rs9770242 and rs59744560 genotypes in this series of RA patients (data not shown).

We estimated the linkage disequilibrium (LD) between both variants and their allelic combinations (r^2=0.40) using the UNPHASED software (38).

NAMPT rs9770242 and rs59744560 polymorphisms and CV events in patients with RA

Information on the main demographic characteristics, CV risk factors and CV events of patients in whom clinical information was available at the time of the study is shown in Table II. One hundred and sixty-one (18%) of these 1,196 patients with RA experienced clinically evident CV events.

Table III shows the genotype frequencies of the *NAMPT* rs9770242 and rs59744560 gene polymorphisms in this series of RA patients stratified according to the presence or absence of CV events. We found a lower frequency of the minor allele G of rs9770242 polymorphism among patients with CV disease (20.62%) compared to those without CV disease (22.83%) but the difference was not statistically significant (p=0.39). We also observed a lower frequency of the minor allele T of rs59744560 in patients with CV events (9.81%) compared with those RA patients who did not experience CV disease (13.07%) but the difference did not reach statistical significance (p=0.11). In a further step, we specifically assessed the influence of these polymorphisms in the occurrence of cardiac ischaemic events or cerebrovascular accidents. Again, no significant associations for rs9770242 were found in both the unadjusted (p=0.78, p=0.28; respectively) or in the adjusted (p=0.61, p=0.22; respectively) analyses. Likewise, no association was found for rs59744560 and the risk cardiac ischaemic events or cerebrovascular accidents (unadjusted p=0.16, p=0.89; adjusted p=0.13, p=0.67; respectively).

Moreover, a logistic regression model to determine the presence of CV disease in patients with RA according to *NAMPT* rs9770242 or rs59744560 allele distribution did not disclose statistically significant differences (rs9770242 p=0.39, adjusted p=0.59; rs59744560 p=0.13, adjusted p=0.42). Analyses were adjusted for gender, age at RA diagnosis, follow-up time and presence or absence of shared epitope, hypertension, diabetes mellitus, dyslipidemia, obesity and smoking habit as confounder factors. In a further step, to assess the independency of both polymorphisms in their association with clinically evident CV disease, we performed a conditional analysis. However, no significant association was observed (data not shown). Likewise, no epistatic interactions of

Table II. Demographic characteristics of the patients with rheumatoid arthritis included in the study for whom clinical information was available at the time of study.

Variables	n. (%)	With CV events (%)	Without CV events (%)	*p*-value
Females	885 (74%)	(58%)	(77%)	<0.01
Age of patients at the time of disease diagnosis, years, median (IQR)	56 (45-65)	62 (55-69)	53 (43-63)	<0.01
Time follow-up, years, median (IQR)	12.7 (6.5-17.7)	14 (7.5-19)	12 (6-17)	0.08
Anti-CCP positive (n=870)	529 (61%)			
Rheumatoid Factor positive (n=1,104)	822 (74%)			
Shared epitope, presence (n=922)	582 (63%)			
Cardiovascular events	161 (18%)			
Ischaemic heart disease	92 (12%)			
Cerebrovascular accidents	42 (5%)			
Heart failure	37 (5%)			
Peripheral arteriopathy	18 (2%)			
Hypertension (n=928)	397 (43%)	(68%)	(36%)	<0.01
Diabetes mellitus (n=902)	129 (14%)	(26%)	(11%)	<0.01
Dyslipidemia (n=1,004)	466 (46%)	(59%)	(42%)	<0.01
Obesity (n=795)	98 (12%)	(9%)	(12%)	0.28
Smoking habit (n=947)	219 (23%)	(24%)	(22%)	0.70

Values are expressed as n (%), except where indicated otherwise.
IQR: Interquartile Range. Anti-CCP: anti-cyclic citrullinated peptide antibodies.

Table III. Differences in the genotype and allele frequencies of *NAMPT* rs9770242 and rs59744560 polymorphisms between rheumatoid arthritis patients with cardiovascular (CV) events or without CV events.

NAMPT	With CV events	Without CV events	*p*-value	OR [95% CI]
rs9770242				
TT	98 (61.25)	436 (60.14)		1
TG	58 (36.25)	247 (34.07)	0.81	1.04 (0.72-1.52)
GG	4 (2.50)	42 (5.79)	0.10	0.42 (0.11-1.21)
T	254 (79.38)	1119 (77.17)		1
G	66 (20.63)	331 (22.83)	0.39	0.88 (0.65-1.19)
rs59744560				
GG	129 (81.65)	557 (76.62)		1
GT	27 (17.09)	150 (20.63)	0.27	0.78 (0.48-1.25)
TT	2 (1.27)	20 (2.75)	0.25	0.43 (0.05-1.82)
G	285 (90.19)	1264 (86.93)		1
T	31 (9.81)	190 (13.07)	0.11	0.72 (0.47-1.10)

CV: Cardiovascular; OR [95% CI]: Odds Ratio with 95% Confidence Interval.

the *NAMPT* polymorphisms with *HLA-DRB1*-shared epitope we observed (data not shown).

NAMPT rs9770242 and rs59744560 gene polymorphisms and subclinical atherosclerosis

We analysed potential differences in the carotid IMT in 105 patients without history of CV events stratified according to the genotype and allele distribution. However, as shown in Tables IV.A and IV.C, no significant differences were observed for this parameter in our series.

Endothelial function was studied in 125 RA patients with no history of CV events at the time of the brachial ultrasonographic assessment. The mean value of FMD% in this series of RA patients was lower than 7%. This result confirmed the previously reported presence of endothelial dysfunction in long-standing RA patients from Northwest Spain (7, 31).

With respect to the *NAMPT* rs9770242 and rs59744560 gene polymorphisms, RA patients carrying *NAMPT* rs9770242 TT had lower values of FMD% (5.17±4.48) than those carrying the rs9770242 TG (FMD%: 6.87±5.84) or the rs9770242 GG (FMD%: 5.41±4.15) genotype but the difference was not statistically significant (p=0.20). Likewise, RA patients carrying the *NAMPT* rs59744560 T allele had a more severe endothelial dysfunction (5.63±4.80%) than those carrying the *NAMPT* rs59744560 G allele (6.91±6.08%) but this did not reach statistical significance (Tables IV.B and IV.C).

No statistically significant association between genotypes or alleles of the *NAMPT* polymorphisms and NTG%-endothelium independent vasodilatation was found (data not shown).

Discussion

Visfatin plays a role in the modulation of inflammation and immune responses (13, 21). This protein is expressed predominantly in macrophages from human visceral fat (39). It is also expressed in other tissues, where it has been linked to a variety of functions such as inflammatory response and inhibition of apoptosis (16). Concentrations of visfatin were higher in patients with systemic lupus erythematosus than in controls (40), although they were not associated with coronary atherosclerosis in these patients. Increased plasma visfatin concentrations were also observed in subjects with RA (22). However, we recently reported that in RA patients with severe disease undergoing treatment with TNF-α-infliximab there was no correlation between circulating visfatin levels and clinical and laboratory parameters of disease activity, adiposity or metabolic syndrome. In addition, serum visfatin concentrations did not show significant changes upon anti-TNF-α-infliximab administration (41).

Increased visfatin levels were described to be associated with coronary artery disease (42) and increased expression of visfatin was found in macrophages of human unstable carotid and coronary atherosclerosis (43). Also, visfatin was reported to be an independent risk factor for augmented carotid IMT (44). However, our results show that *NAMPT* rs9770242 (-1001T>G) and rs59744560 (-948G>T) polymorphisms do no seem to be associated with the susceptibility to RA and the development of CV disease in these patients. In this regard, although this gene has not been well-covered in RA, none of the GWAS performed to date in RA have

Table IV. A. Comparison of carotid artery intima-media thickness (IMT) according to *NAMPT* rs9770242 and rs59744560 polymorphisms. **B**. Comparison of brachial flow-mediated endothelium dependent (post-ischaemia) vasodilatation (FMD) according to *NAMPT* rs9770242 and rs59744560 polymorphisms. **C**. Comparison of carotid artery IMT and FMD vasodilatation, according to *NAMPT* rs9770242 and rs59744560 alleles in an adjusted ANCOVA model.

A

NAMPT rs 9770242	IMT mm, mean (SD)	*p*-value	*NAMPT* rs59744560	IMT mm, mean (SD)	*p*-value
TT (n=60)	0.72 (0.19)		GG (n=77)	0.73 (0.19)	
TG (n=39)	0.75 (0.15)		GT (n=26)	0.74 (0.16)	
GG (n=6)	0.78 (0.19)		TT (n=2)	0.77 (0.14)	
Model		0.54			0.95
T (n=159)	0.73 (0.18)		G (n=180)	0.74 (0.18)	
G (n=51)	0.76 (0.16)	0.27	T (n=30)	0.75 (0.16)	0.78

B

NAMPT rs 9770242	FMD%, mean (SD)	*p*-value	*NAMPT* rs59744560	FMD%, mean (SD)	*p*-value
TT (n=69)	5.17 (4.48)		GG (n=87)	5.24 (4.34)	
TG (n=48)	6.87 (5.84)		GT (n=34)	7.58 (6.48)	
GG (n=8)	5.41 (4.15)		TT (n=4)	4.10 (2.87)	
Model		0.20	Model		0.06
T (n=186)	5.61 (4.90)		G (n=208)	5.63 (4.80)	
G (n=64)	6.50 (5.45)	0.22	T (n=42)	6.91 (6.08)	0.13

C

		IMT	FMD
NAMPT rs9770242,	p G *vs*. T*	0.90	0.18
NAMPT rs59744560,	p T *vs*. G*	0.92	0.19
NAMPT rs9770242,	p G *vs*. T**	0.75	0.64
NAMPT rs59744560,	p T *vs*. G**	0.63	0.52

*Analyses adjusted for gender, age at brachial ultrasonography performance, follow-up time and presence or absence of shared epitope, hypertension, diabetes, dyslipidemia, obesity and smoking habit. **Conditional analysis of the *NAMPT* rs9770242 and rs59744560 polymorphisms.

disclosed any association with the region 7q22.3, *NAMPT (PBEF1)* gene or with any of the polymorphisms tested in our study.

We previously observed that interactions between *NOS* gene polymorphisms and *HLA-DRB1* alleles confer an increased risk of developing CV events in patients with RA (45). Moreover, we disclosed an association of the *TNFA* rs1800629 gene polymorphism with predisposition to CV complications in patients with RA. This predisposition was restricted to individuals carrying the rheumatoid shared epitope (46). However, we could not find interaction between *NAMPT* polymorphisms and *HLA-DRB1* alleles.

In keeping with the lack of association of *NAMPT* polymorphisms with CV disease in RA, we did not observe association with other gene variants located outside the MHC region (*PTPN22*, *STAT4* and *TRAF1/C5*) that have also been associated with increased susceptibility to RA (47, 48). It was also the case for some gene polymorphisms associated with inflammation such as *MIF -173* (49) or *VEGFA* polymorphisms (50) or with other adipokine polymorphisms such as *ADIPOQ* rs266729 and rs1501299, *LEP* rs2167270 (19G>A) and *RETN* gene rs1862513 (51-53) and the endogenous ligand for the growth hormone secretagogue receptor *GHSR* rs509035, rs512692 and rs2922126 gene variants (54).

In conclusion, our results show that *NAMPT* rs9770242 (-1001T>G) and rs59744560 (-948G>T) polymorphisms do no seem to be associated with the susceptibility to RA and the development of CV disease in patients with RA.

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