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Autoimmunity and atherosclerosis: functional polymorphism of PTPN22 is associated with phenotypes related to the risk of atherosclerosis. The Cardiovascular Risk in Young Finns Study

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Summary

There is a growing body of evidence attesting the significance of inflammation in the pathogenesis of atherosclerosis. Protein tyrosine phosphate PTPN22 C/T single nucleotide polymorphism (SNP) at +1858 has been identified recently as a susceptibility factor for various inflammatory autoimmune diseases. We hypothesized that data on the genetic polymorphism of the PTPN22 enzyme associated with an increased risk of autoimmunity could also provide insight into the possible role of autoimmunity in the pathogenesis of atherosclerosis. Therefore we analysed the PTPN22 + 1858 C/T polymorphism in a population of young Finnish adults ($n = 2268$) for whom data on carotid artery intima-media thickness (IMT), a presymptomatic predictor of atherosclerosis, and risk factors for atherosclerosis were available. In males carriage of the T allele of PTPN22 + 1858 was associated significantly with IMT in univariate and multivariate analyses, while in females it was associated with several risk factors for atherosclerosis (BMI, waist circumference, waist-to-hip ratio, serum concentrations of C-reactive protein and triglycerides) but not with IMT. Our results indicate that the genetic polymorphism of PTPN22 + 1858 known to predispose to autoimmunity also enhances the development of atherosclerosis and thereby links the genetics of autoimmunity and atherosclerosis.

Keywords: atherosclerosis, autoimmunity, gene, inflammation, polymorphism, PTPN22

Introduction

Several recent studies have identified the protein tyrosine phosphate PTPN22 C/T single nucleotide polymorphism (SNP) at +1858, leading to the R620W amino acid change, as a susceptibility factor for various autoimmune diseases such as type I diabetes mellitus, systemic lupus erythematosus, rheumatoid arthritis and Hashimoto's thyroiditis [1–4]. The function of PTPN22 and the effect of R620W have been characterized recently [1,5]. PTPN22 codes for a protein tyrosine phosphatase, LYP, which is involved in the regulation of T cell receptor (TCR) signalling [6]. The 620W allele (+ 1858T) of PTPN22, known to be associated with autoimmunity, is a gain-of-function form of the enzyme inhibiting more strongly TCR-mediated signals [5]. It has thus been suggested that the increased risk of autoimmunity associated with this allele is due to failure to delete autoreactive T cells during intrathymic selection [5].

Substantial evidence speaks for the importance of inflammation in the pathogenesis of atherosclerosis (reviewed in [7–9]), and there is also increasing evidence that both adaptive and innate immunity are involved in the regulation of atherosclerosis [8,10]. The atheroma plaque contains T lymphocytes, most of which are of the proinflammatory Th1 type, and it is generally held that these cells are proatherogenic [9]. The antigen specificity of these T cells is largely unknown, but at least a fraction of them are autoreactive, recognizing oxidized (oxLDL) or modified LDL molecules [9]. However, these LDL-specific T cells are not necessarily proatherogenic: in several animal models immunization with oxLDL has been observed to lead to protection against the development of atherosclerosis [11]. In addition to the

recognition of the role of inflammation in the development of atherosclerosis, the risk of accelerated and precocious atherosclerosis in various autoimmune diseases is currently also well established [12,13].

We hypothesized that analysis of the genetic polymorphism of the PTPN22 enzyme associated with an increased risk of autoimmunity could also prove informative as to the possible role of autoimmunity in the pathogenesis of atherosclerosis. We therefore analysed the PTPN22 + 1858 genotype and carrier frequencies in 2268 young adults in whom carotid artery intima-media thickness (IMT), a presymptomatic predictor of atherosclerosis, as well as traditional risk factors for atherosclerosis, had been measured as part of a population-based cohort study.

Subjects and methods

Subjects

The Cardiovascular Risk in Young Finns Study is a population-based prospective multi-centre cohort study of atherosclerosis risk factors in children and adolescents from five university hospital cities and their rural surroundings in Finland. The first cross-sectional survey was conducted in 1980 and follow-up studies were conducted in 1983, 1986 and 2001. The current study comprised the 2268 subjects (1248 female, 1020 male) who were re-examined in 2001, when the subjects had reached the age of 24–39 years [14].

The data on cardiovascular risk variables in the cohort in 2001 [levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, body mass index (BMI), waist circumference, waist-to-hip ratio, systolic and diastolic blood pressure, smoking habits, oral contraceptive use] had been recorded by methods described previously [14]. Plasma high sensitive C-reactive protein (CRP) concentrations had been analysed by latex turbidometric immunoassay (Wako Chemicals GmbH, Düseldorf, Germany). Carotid IMT measurements had been made in 2001, as described elsewhere [14].

PTPN22 + 1858 genotyping

DNA specimens from whole blood samples were prepared using a commercially available kit (Qiagen Inc., Hilden, Germany) in 2001. Genotyping of +1858 C/T (R620W) SNP of the PTPN22 gene (GenBank SNP Database rs number 2476601) was performed with *Taq*Man 5′ nuclease assay for polymerase chain reaction (PCR) and allelic discrimination using the ABI Prism 7900HT Sequence Detector System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Primers and probes were designed by Applied Biosystems. The forward primer was 5′-CCAGCTTCCTCAACCACAATAAATG-3′ and the reverse primer was 5′-CAACTGCTCCAAGGATAGATGATGA-3′. The *Taq*Man minor groove binder (MGB) probe sequences were 5′-TCAGGTGTCCATACAGG-3′ and 5′-TCAGGTGTC CGTACAGG-3′. The probes were labelled with the fluorescent dyes VIC® and FAM™, respectively. The allele distributions followed the Hardy–Weinberg equilibrium.

Statistical analysis

Values for CRP and triglycerides were transformed logarithmically prior to statistical analyses due to their skewed distributions. Smoking was regarded as a dichotomous variable (current smokers and ex-smokers *versus* never-smokers). Comparison of continuous variables was performed by Student's *t*-test. Multivariate backward linear regression analysis was made to establish the independent effect of PTPN22 polymorphism on IMT in males. Findings were considered statistically significant at $P < 0{\cdot}05$. Statistical analyses were performed with SPSS 13·0 for Windows.

Ethical approval

The study was approved by local ethics committees and all subjects participating gave written informed consent.

Results

The data on various risk factors for atherosclerosis and the genotype and carrier frequencies of the PTPN22 + 1858 C/T in 2268 young adults from the Cardiovascular Risk in Young Finns Study are presented in Table 1. The data on the cardiovascular risk factors grouped by PTPN22 + 1858 allele T carrier status in female and male adults are presented in Tables 2 and 3, respectively. As IMT was significantly higher in male subjects carrying the PTPN22 + 1858 allele T compared with non-carriers, a linear regression model was constructed to analyse the independent effect of the PTPN22 polymorphism on IMT. The results of the multivariate linear regression analysis are shown in Table 4. Age, sex, PTPN22 genetics, BMI, systolic blood pressure and smoking habits had a significant effect on IMT in this model. When a similar model was constructed in females and males separately, PTPN22 T allele carrier status affected IMT only in males (data not shown). PTPN22 genetics did not affect IMT in females when data on oral contraceptive use were added to the multivariate model (data not shown).

Discussion

The data in this report demonstrate that polymorphism of PTPN22 + 1858 C/T, a gene associated with autoimmune diseases, has an effect on IMT and on risk factors for atherosclerosis in young adults. There is a strong female preponderance in most autoimmune diseases, and in some previous studies [15–17] the autoimmune disease associations of PTPN22 have also been gender-specific. Therefore, we analysed the data separately in females and in males. In females,

Table 1. Risk factors for atherosclerosis and genotype and carrier frequencies of the PTPN22 + 1858 C/T in 2268 young (24–39 years) adults.

Variable	Mean ± s.d.
Age, years	31·4 ± 5·0
Sex	
Females	1248
Males	1020
HDL, mmol/l	1·29 ± 0·32, *n* = 2266
LDL, mmol/l	3·27 ± 0·86, *n* = 2266
Triglyceride, mmol/l	1·34 ± 0·85
BMI, kg/m^2	25·0 ± 4·23, *n* = 2187
Waist circumference, cm	84·1 ± 12·3, *n* = 2228
Waist-to-hip ratio	0·84 ± 0·08, *n* = 2226
Systolic blood pressure, mmHg	122 ± 14, *n* = 2235
Diastolic blood pressure, mmHg	73 ± 9, *n* = 2235
CRP, mg/l	1·93 ± 3·95
IMT, mm	0·58 ± 0·09, *n* = 2242
Current or ex-smokers (%)	1238 (54·6%)
PTPN22 + 1858 genotypes	
CC	1621 (71·5%)
C/T	584 (25·7%)
TT	63 (2·8%)
PTPN22 + 1858 T allele carriers	647 (28·5%)

HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; BMI = body mass index; CRP = C-reactive protein; IMT = common carotid artery intima-media thickness. Values are mean ± s.d. unless indicated otherwise.

carriage of the T allele of PTPN22 + 1858 was associated with several risk factors for atherosclerosis (BMI, waist circumference, waist-to-hip ratio, serum concentrations of CRP and triglycerides), while the association with IMT, a surrogate marker of atherosclerosis, was weak. In contrast, in males carriage of this allele was associated significantly with IMT, but associations with other phenotypes analysed were negligible.

There was, however, also a non-significant trend towards an association between PTPN22 gene polymorphism and IMT in univariate analysis in females. Premenopausal women develop clinical manifestations of atherosclerosis slower than men, and as PTPN polymorphism associated significantly with several risk factors of atherosclerosis in these young female adults the possibility cannot be excluded that this polymorphism would affect IMT in females later in life.

As PTPN22 allele T was associated with IMT in males without affecting the classical risk factors of atherosclerosis (LDL cholesterol level was even lower in T allele carriers of the PTPN22 gene in males), it seems that this autoimmune gene is an independent predictor of atherosclerosis in males. This finding was confirmed in a multivariate model.

The biological effect of allele T of PTPN22 + 1858 could be either a non-specific inflammatory response against a variety of autoantigens in the body or an autoimmune reaction against certain antigens specific for the pathogenesis of atherosclerosis. The latter possibility would indicate that atherosclerosis resembles a 'normal' autoimmune disease and, additionally, that the genetics related to autoimmunity favours the development of atherosclerosis. In males, the association of the T allele of PTPN22 + 1858 with IMT was statistically significant, and it remained so in a linear regression model where the classical risk factors for atherosclerosis were included. This implies that the T allele-associated autoimmunity in males would be specific for the antigens related to the atherosclerotic process and would therefore not be explained merely by a non-specific inflammatory response.

However, as the PTPN22 T allele in females was associated with several cardiovascular risk factors without affecting IMT, it is tempting to speculate that in females the T allele-associated changes would be due to a non-specific

Table 2. Comparison of risk factors for atherosclerosis in carriers and non-carriers of the T allele of PTPN22 + 1858 of 1248 young female (24–39 years) adults.

	PTPN22 + 1858		
Variable	Allele T carriers (*n* = 363)	Allele T non-carriers (*n* = 885)	*P*-value
Age, years	32·1 ± 4·8	31·5 ± 5·0	0·075
IMT, mm	0·58 ± 0·09, *n* = 361	0·57 ± 0·08, *n* = 873	0·076
HDL, mmol/l	1·38 ± 0·32	1·40 ± 0·30	0·270
LDL, mmol/l	3·15 ± 0·77	3·16 ± 0·79	0·765
Triglyceride	1·23 ± 0·62	1·17 ± 0·71	0·037
BMI, kg/m^2	24·8 ± 4·7, *n* = 348	24·0 ± 4·2, *n* = 847	0·006
Waist circumference, cm	80·9 ± 11·9, *n* = 353	78·8 ± 11·1, *n* = 861	0·004
Waist-to-hip ratio	0·80 ± 0·07, *n* = 353	0·79 ± 0·06, *n* = 859	0·039
Systolic blood pressure, mmHg	117 ± 13, *n* = 359	116 ± 12, *n* = 872	0·289
Diastolic blood pressure, mmHg	72 ± 9, *n* = 359	72 ± 9, *n* = 872	0·481
CRP	2·46 ± 4·52	2·20 ± 4·33	0·010

IMT = common carotid artery intima-media thickness; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; BMI = body mass index. The concentrations of triglyceride and C-reactive protein were transformed logarithmically before statistical analysis. Values are mean ± s.d. unless indicated otherwise.

Table 3. Comparison of risk factors for atherosclerosis in carriers and non-carriers of the T allele of PTPN22 + 1858 of 1020 young male (24–39 years) adults.

	PTPN22 + 1858		
Variable	Allele T carriers $n = 284$	Allele T non-carriers $n = 736$	*P*-value
Age, years	31·8 ± 4·8	31·7 ± 5·1	0·700
IMT, mm	0·61 ± 0·10, $n = 283$	0·59 + 0·10, $n = 725$	0·010
HDL, mmol/l	1·16 ± 0·28, $n = 282$	1·16 ± 0·28	0·669
LDL, mmol/l	3·31 ± 0·89, $n = 282$	3·46 ± 0·93	0·026
Triglyceride	1·48 ± 1·03	1·55 ± 0·98	0·078
BMI, kg/m^2	25·9 ± 3·7, $n = 276$	25·8 ± 3·9, $n = 716$	0·723
Waist circumference, cm	90·0 ± 10·5, $n = 283$	90·0 ± 10·9, $n = 731$	0·702
Waist-to-hip ratio	0·90 ± 0·06, $n = 283$	0·90 ± 0·06, $n = 731$	0·958
Systolic blood pressure, mmHg	129 ± 13, $n = 281$	129 ± 14, $n = 723$	0·963
Diastolic blood pressure, mmHg	75 ± 8, $n = 281$	75 ± 9, $n = 723$	0·462
CRP	1·94 ± 4·16	1·27 ± 2·11	0·054

IMT = common carotid artery intima-media thickness; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; BMI = body mass index. The concentrations of triglyceride and C-reactive protein were logarithmically transformed before statistical analysis. Values are mean ± s.d. unless indicated otherwise.

inflammatory reaction caused by generally increased autoreactivity in the whole body. It is, however, noteworthy that the traits associated here with the PTPN22 T allele in females belong to a cluster of metabolic syndrome risk factors and, consequently, atherosclerosis [18], thus adding PTPN22 to the list of genes known to regulate this cluster.

Signs of early atherosclerosis have been found in rheumatoid arthritis, systemic lupus erythematosus (SLE) and Sjögren's syndrome [12,19], i.e. in several autoimmune diseases for which PTPN22 gene polymorphism has been suggested to be a susceptibility factor [2,3,20]. Here we found the PTPN22 polymorphism to be associated significantly with early atherosclerosis or risk factors for it in a large population-based group of young Finnish adults. On the basis of this finding it would appear that the genetics linked to autoimmunity also enhances the development of atherosclerosis. In patient groups with autoimmune diseases such as rheumatoid arthritis, SLE and Sjögren's syndrome, it would be of interest to establish whether PTPN22 gene polymorphism confers an increased risk of atherosclerosis in these autoimmune diseases in addition to having an effect on susceptibility to these diseases. If so, PTPN2 polymorphism would link autoimmune diseases and atherosclerosis to each other in both directions.

Table 4. Multivariate backward linear regression model of the relationships between the risk factors for atherosclerosis and IMT (mm) in 2268 young (24–39 years) adults.

Risk variable	B ± SE	*P*-value
Age	0·005 ± 0·000	< 0·0001
Sex	0·010 ± 0·004	0·020
PTPN22 T allele carrier status	0·010 ± 0·004	0·013
BMI	0·003 ± 0·000	< 0·0001
Systolic blood pressure	0·000 ± 0·000	0·002
Current smoker or ex-smoker/ never smoker	0·010 ± 0·004	0·008

IMT = common carotid artery intima-media thickness; BMI = body mass index. Variables in the backward linear regression model: age, sex, PTPN22 allele T carrier status, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, logarithmically transformed concentration of triglyceride, body mass index, systolic blood pressure, diastolic blood pressure, logarithmically transformed concentration of C-reactive protein, smoking habits (current smoker or ex-smoker/never smoker).

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