T-786C single nucleotide polymorphism of the endothelial nitric oxide synthase gene as a risk factor for endothelial dysfunction in polymyalgia rheumatica

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Abstract

Objective

We investigated the association of the T-786C single nucleotide polymorphism (SNP) of the endothelial nitric oxide synthase gene (NOS3), which is characterised by reduced expression of the enzyme in response to shear stress or interleukin-10 stimulation and significantly associated with coronary heart disease or rheumatoid arthritis, with the occurrence of isolated polymyalgia rheumatica.

Methods

A cohort of 78 patients who had presented at a rheumatological specialist practice in Heidelberg was tested for the T-786C SNP by means of restriction fragment length polymorphism analysis, and the result was compared with the data of a control cohort (n=2061) compiled from the genotyping of umbilical cord arteries from newborns. Patients were tested for an association with the genotype and their clinical characteristics obtained at the time of the initial presentation and during the first year of treatment.

Results

The T-786C SNP of the NOS3 *gene was significantly associated with isolated PMR (p=0.0009; OR 2.475). The C-allele frequency in patients with PMR was higher than in patients with rheumatoid arthritis, who also showed a significant association with the T-786C SNP (PMR 0.481 vs. 0.422 RA). A significant association with clinical features of the patients could not be detected.*

Conclusion

The T-786C SNP of the NOS3 *gene, which predisposes towards the development of endothelial dysfunction, is significantly associated with polymyalgia rheumatica manifesting itself without any clinically detectable vascular involvement.*

Key words

polymyalgia rheumatica, nitric oxide synthase type III, genetic pre-disposition, single nucleotide polymorphism, genotype, endothelial dysfunction, risk factor

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Introduction

Since endothelial dysfunction is the initial step setting off a chronic inflammatory process in the vessel wall that leads to atherosclerotic lesion formation, it has been suggested that this pathological condition is linked to rheumatic diseases as well. The pathogenic as well as prognostic relevance of a vascular involvement has been discussed in particular for polymyalgia rheumatica (PMR), a frequent, mainly clinically defined complex disorder characterised by a multitude of inflammatory syndromes (1, 2). Up to 20 percent of patients with PMR suffer from a subclinical giant cell arteritis (GCA), while about half of those afflicted with GCA develop PMR (3, 4). GCA is a vasculitis of large vessels with a segmental and focal inflammation of arteries, which is typically characterised by an infiltration with multinucleated giant cells, T-cells and macrophages, together with intimal hyperplasia and a fragmented internal elastic lamina (5).

As was shown by ^{18}F-fluorodeoxyglucose-positron emission tomography in patients with isolated PMR, inflammatory activity was not only prominent in the shoulder, hip and processi spinosi, but in up to one third of the patients a subclinical vasculitis could be detected in the thoracic and abdominal aorta, and especially in the subclavian artery (6). The detection of a coexisting GCA in patients with PMR is of particular relevance, since it is associated with severe vascular complications such as closure of the arteria centralis retinae resulting in sudden blindness, ischaemic insults to the CNS, and a high incidence of aortic aneurysms.

To date, there is no parameter that allows the differentiation between patients with clinically isolated PMR who carry a high or low risk for vascular complications, respectively. As was already shown for a variety of other chronic inflammatory diseases such as rheumatoid arthritis (RA), psoriatic arthritis and ankylosing spondyloarthritis, evaluation of a large PMR patient population revealed a significantly increased risk for cardiovascular disorders as well (7, 8). Although these data clearly indicate a link between inflammatory diseases and an increased cardiovascular morbidity, it remains unclear how, if at all, endothelial dysfunction as the initial defect leading to vascular inflammation, and as such an early indicator for cardiovascular complications, also predisposes or contributes to inflammatory responses in other organ systems, and to PMR in particular.

Endothelial dysfunction may be triggered by a number of risk factors, including smoking, hypertension and aging (9-11). Nitric oxide (NO) deficiency as a main characteristic of endothelial dysfunction can also be caused by genetic defects. One well documented example is the T-786C single nucleotide polymorphism (SNP) in the promoter of the endothelial NO synthase (NOS3)-encoding gene. Homozygosity for the C-variant is significantly associated with coronary heart disease just like the (chronic) inflammatory diseases RA and psoriasis (19%, 19.1% and 18%, respectively, vs. 12% in the control group) (12-14). Both *in vitro* and *ex vivo* human endothelial cells homozygous for the C-variant exhibit a markedly reduced maintenance of NOS3 expression by shear stress, which normally ensures a physiological level of NO production. Human umbilical vein endothelial cells from donors with the genotype CC also fail to up-regulate NOS3 expression following stimulation with interleukin-10 (IL-10), and to suppress production of the pro-inflammatory interleukin 12 (IL-12) after stimulation with CD40 ligand (12, 13).

In this study, it was our aim to show that the T-786C SNP of the human *NOS3* gene is associated with isolated PMR. As an easily detectable genetic predisposition affecting inflammatory and cardiovascular diseases alike, this gene variant may thus in the future help to identify patients afflicted with PMR who carry an increased risk for vascular complications and cardiovascular comorbidities.

Methods

DNA samples and medical data of 78 PMR patients who presented at a rheumatological specialist practice in Heidelberg between January 2003 and August 2008 were evaluated.

They were initially diagnosed according to the criteria of Healey *et al*. but were re-examined retrospectively to assess their meeting the Provisional Classification Criteria for PMR of 2012 at the time of diagnosis and after one year (15, 16). Patients matching the ACR criteria for GCA were excluded (17). Genotyped umbilical cords from clinics in Heidelberg (n=2061) served as a normal cohort for comparison and statistical analysis of the distribution of alleles C and T within the patient groups *versus* the total population of northern Baden. All materials were collected after informed consent of the patient or the parents and all investigations were approved by the Ethics Committee of the Medical Faculty Heidelberg. Zygosity for the two variants of the T-786C polymorphism of the *NOS3* gene was determined by polymerase chain reaction-based restriction fragment length polymorphism analysis of genomic DNA isolated from blood or umbilical cord arteries (13). Allele frequencies found for DNA samples of both the control cohort and the patient collective met the Hardy-Weinberg Equilibrium according to the Pearson chi-square test with Yates continuity correction (18).

The chi-square approximation with Yates correction and a two-sided *p*-value was used to calculate the odds ratios, and an unpaired two-tailed Student's *t*-test was used to detect differences within the study cohorts with $p<0.05$ considered statistically significant.

Table I. Allele frequencies of the T-786C SNP of the *NOS3* gene in patients with polymyalgia rheumatica (PMR) *vs*. control group (data from hospitals in Heidelberg).

	PMR (n=78)	control (n=2061)	OR	*p*-value
C/C	20 (25.6%)	252 (12.2%)	2.4754	0.0009
C/T	35 (44.9%)	935 (45.4%)	0.9802	1.0000
T/T	23 (29.5%)	874 (42.4%)	0.5679	0.0314
C-allele	75 (48.1%)	1439 (34.9%)	1.7264	0.0010
T-allele	81 (51.9%)	2683 (65.1%)	0.5792	0.0010

Chi-square approximation with Yates correction and two-sided *p*-value. OR: odds ratio.

Table II. Genotype distribution of the T-786C-polymorphism in 78 patients with polymyalgia rheumatica regarding baseline demographics and clinical characteristics.

Genotype (number of patients)	group; number (%)* C/C (n=20)	C/T (n=35)	T/T (n=23)
Women	12 (60)	22 (61.1)	16 (70.8)
Age at diagnosis (mean ± SD, years)	67.3 ± 6.9	66.3 ± 6.4	65.3 ± 6.4
ESR (mean ± SD, mm/h)	‡27.0 ± 23.7	43.1 ± 28.6	37.8 ± 21.7
CRP (mean ± SD, mg/l)	29.9 ± 49.5	26.1 ± 28.8	14.6 ± 13.2
Steroid responsiveness	20 (100)	34 (97.1)	23 (100)
Shoulder pain	20 (100)	34 (97.1)	23 (100)
Pelvic girdle pain	13 (65)	26 (74.3)	17 (73.9)
Synovitis	2 (10)	5 (14.3)	4 (17.4)
Morning stiffness >45 min	20 (100)	35 (100)	23 (100)

*If not stated otherwise.
Patients with C/C-genotype *vs*. patients with C/T or T/T genotype: ‡C/C *vs*. C/T, $p=0.0377$. Unpaired two-tailed Student's *t*-test. ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; SD: standard deviation.

Results

The allele frequencies of the controls did not differ from a genotyping of umbilical cords previously conducted in the area of Göttingen in Lower Saxony (13).

A highly significant association between the homozygous C-variant in patients with PMR as compared to the control group could be demonstrated (25.6% *vs*. 12.2%), the same applied to the distribution of the C-allele (48.1% *vs*. 34.9%) (Table I). In patients with PMR, the C-allele proportion of 0.481 was even higher than in patients with RA (0.422) (12).

On grounds of the cohort size, no significant difference between the genotypes could be detected when considering the demographic data and clinical characteristics except for the erythrocyte sedimentation rate, which was significantly different between C/C genotype and C/T genotype patients with PMR (Table II).

The patients examined in our study had an isolated PMR and were not routinely examined angiologically. Patients who had clinical symptoms indicative of giant cell arteritis (GCA), such as headache symptoms, blurred vision, claudication while chewing, were examined angiologically with Doppler sonography. These patients, however, were not analysed in this study, as an accompanying GCA could not be excluded.

Eleven of the 78 patients in this cohort had a synovitis, which in all cases manifested itself in the metacarpophalangeal joints, in some cases also in the wrist and the metatarsophalangeal joints. One of these 11 patients showed the oedematous swelling of the dorsum of the hand typical for the remitting seronegative symmetrical synovitis with pitting oedema (RS3PE) syndrome.

Discussion

Inflammatory rheumatic diseases just like arteriosclerosis are multi-factorial diseases in which it is only the sum of various external and internal stimuli against the individual's genetic background that eventually leads to their clinical manifestation. In this complex interplay, the endothelial cells are on the front line, as the effects of inflammatory mediators, immune cells, antibodies and external trigger factors merge in this boundary layer between blood and tissue and are processed differently, depending on the genotype and hence phenotype of the endothelial cell. Although endothelial dysfunction is mainly defined by a decreased NO-dependent vasodilation, it stands for an inadequate endothelial activation in general which, inter alia, causes an insufficient control of the local and spatial extent and intensity of the inflammatory response. Endothelial dysfunction has been established in a number of chronic inflammatory diseases, especially RA (19, 20).

Also on the level of modifiable risk factors, overlaps between chronic in-

flammatory diseases and atherosclerosis have already been proven. Thus, classical risk factors of coronary heart disease such as smoking and dyslipidaemia increase the incidence of RA and favour the progression of the disease (21, 22). Also in case of psoriasis there is an association between smoking and the incidence of the disease (23, 24). Furthermore, classical risk factors enhance the rate of ischaemic complications in patients with GCA (25). Besides the history of collected classic risk factors there is no screening parameter in the clinical routine available so far that allows an evaluation of the individual cardiovascular risks of patients with chronic inflammatory diseases or risk for vascular manifestations especially in patients with PMR.

NOS3 is the key basal NO producer of the endothelium and, under physiological conditions, regulated especially on the level of expression and activity by changes in wall shear stress (26). Particularly in an inflammatory milieu, IL-10 also seems to be particularly important for the maintenance of NOS3 expression (12, 27). The majority of potentially damaging mediators such as oxidised low-density lipoproteins or tumour necrosis factor α may have an influence on the bioavailability of NO by causing a decrease in NOS3 expression or an increase in superoxide anion production (26). Interestingly, affected endothelial cells try to compensate the reduced IL-10 and shear stress sensitivity of the homozygous C-variant of the *NOS3* gene by genotype-specific changes in gene expression. Thus homozygous C-genotype endothelial cells try to stabilise their NO bioavailability by a shear stress-dependent increase in manganese superoxide dismutase expression, mediated by disinhibition of the NO-inhibited expression of the transcription factor EGR-1, which results in an enhanced degradation of superoxide anions that would otherwise inactivate NO. Gene dose effects do not appear to be particularly strong; therefore no significant difference between the T/T and C/T genotype could be determined (28). By an already genetically reduced NO bioavailability, the T-786C SNP of the *NOS3* gene could lay the foundation for an increased vulnerability of the vascular endothelium which cannot be adequately compensated by part of the population, taking into account other risk factors and despite existent compensatory mechanisms, and thus causes the manifestation of endothelial dysfunction.

The association of the T-786C SNP of the *NOS3* gene with RA, psoriasis and now PMR shows that a genetic predisposition to endothelial dysfunction may be a risk factor for the development of chronic inflammatory diseases (12, 14). The attempt to also prove an association between this polymorphism and GCA failed some 10 years ago, a circumstance that in our opinion was based on the relatively high prevalence of homozygous C-allele carriers (19.0%) and the C-allele frequency (0.440) within the rather small control group (n=98) (29). In a comparatively small cohort of the general population of northern Spain (30), a much lower prevalence (14.0%) of homozygous carriers of the C-allele was found likewise in larger Caucasian cohorts in the United Kingdom (14.5%) (31), Italy (13.0%) (32) and Canada (15.0%) (33). In our even larger control group (n=2061) of the general population of northern Baden in Germany, prevalence of the C/C genotype was even lower at 12.2%.

The question remains to what extent the C/C genotype of the *NOS3* gene in patients with PMR defines a subpopulation of patients that has an increased vascular risk or suffers from a subclinical GCA at the same time. In the retrospective analysis of the patient data of our PMR cohort no consistent data could be collected that would prove the presence of cardiovascular comorbidities or the development of cardiovascular complications. If further studies confirm an association of the T-786C SNP of the *NOS3* gene with cardiovascular complications and vascular manifestations in the patient populations of RA and PMR, it is conceivable that this marker would permit a specific risk stratification and thus an adapted intense diagnosis and treatment regimen for these chronic inflammatory diseases.

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