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Polymorphisms rs1800587 and rs1143634 of the Interleukin-1α Gene are associated with the Progression of Carotid Atherosclerosis in Caucasians with Type 2 Diabetes Mellitus

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Abstract

Background: Our study was designed to test the possible association between either polymorphisms T889C (rs1800587) or C3954T (rs1143634) of the interleukin-1 alpha (IL-1α) gene with subclinical markers of carotid atherosclerosis in patients with type 2 diabetes mellitus (T2DM). Moreover, the effect of both polymorphisms on progression of carotid atherosclerosis in 3.8-year follow-up was studied.

Patients and methods: 595 subjects with T2DM were enrolled in the prospective study. Subclinical markers of carotid atherosclerosis were assessed with ultrasound at the time of recruitment and after 3.8-years. Genotyping of two polymorphisms (rs1800587, rs1143634) was performed with real-time PCR System.

Results: The comparison of atherosclerosis parameters was performed with regard to different genotypes of IL-1α rs1800587 and rs1143634 polymorphisms upon enrolment. Multiple linear regression analysis revealed the association of IL-1α rs1143634 on total plaque thickness progression in a 3.8 year follow up.

Conclusions: An association between either the IL-1α rs1800587 or rs1143634 and total plaque thickness at the time of recruitment was reported. Additionally, we demonstrated the effect of the IL-1α rs1143634 on total plaque thickness progression in the 3.8-year follow-up in patients with T2DM.

Keywords: Carotid atherosclerosis; Genetic polymorphism; Interleukin-1 α; Cross-sectional study

Introduction

Atherosclerosis of carotid arteries is a complex multifactorial disorder that is thought to result from interactions between an individual's genetic background and lifetime exposure to various environmental factors.

It is generally accepted that beside cholesterol accumulation chronic inflammatory is very much involved in the development of atherosclerosis, and inflammation is considered to play a crucial role in the initiation of atherosclerotic process [1-3]. A genetic variability of the inflammatory genes is considered to affect the development of atherosclerosis via interaction with conventional risk factors [4].

Interleukin-1 (IL-1) is an important inflammatory mediator that has been reported to promote the production of inflammatory markers, such as fibrinogen and C-reactive protein, which are involved in the development of atherosclerosis [5-8]. Moreover, high levels of IL-1a mRNA transcripts were demonstrated in atherosclerotic plaques from human patients [9].

The IL-1 gene family exists in two forms, IL-1 and IL-1, which are produced by lymphocytes or monocytes, and one antagonistic cytokine, IL-1 receptor antagonist (IL-1Ra) [10]. The variability of the IL-1α gene has been reported to be associated with either ischemic stroke or coronary heart disease in few studies, but the results are inconsistent [7,8,11-14].

Despite several data demonstrating a relationship between the variability of the IL-1α gene and cardiovascular disorders [7,8,11-14], there is no study evaluating relationship between the tested polymorphisms (rs1800587 and rs1143634) and progression of subclinical markers of carotid atherosclerosis in subjects with T2DM. Therefore, the exact role of rs1800587 and rs1143634 on the development and progression of carotid atherosclerosis remains to be fully elucidated.

Aim of the Study

Our study was designed to test the possible association between either the rs1800587 or the rs1143634 of the IL-1α gene with subclinical markers of carotid atherosclerosis (carotid intima media thickness [CIMT], number of affected segments of carotid arteries, the

sum of plaques thickness, the presence of carotid plaques, and the presence of unstable carotid plaques) in patients with type 2 diabetes mellitus (T2DM).

Moreover, the effect of both polymorphisms on progression of carotid atherosclerosis in 3.8-year follow-up was studied.

Materials and Methods

Patients

In this prospective study approved by the Slovene Medical Ethics Committee, 595 consecutive subjects with T2DM, admitted to the diabetes outpatient clinics of the General Hospitals Murska Sobota, and Slovenj Gradec, Slovenia, and from the outpatient department Medicor, Ljubljana were enrolled.

Patients were classified as having T2DM according to the current report of the American Diabetes Association [15]. Exclusion criteria were: homozygous familial hypercholesterolemia or a history of cardiovascular event (i.e. acute coronary syndrome or a cerebrovascular stroke). Clinical data were obtained from medical records and detailed questionnaires, as previously described [16].

Ultrasonographic analysis

Ultrasonographic analysis was performed by two experienced radiologists according to the same protocol. They were blinded for the participant's clinical status. The CIMT, plaque thickness, were measured, and plaques presence/types were evaluated on both sides, as previously described [16].

Subjects with T2DM were prospectively followed, and after 3.8 ± 0.5 years control ultrasound examination of carotid arteries was performed on 426 out of 595 subjects (71.6%) with T2DM. The annual CIMT progression rate, the increase in total plaque thickness and the number of sites with plaques were evaluated to appreciate the progression of carotid atherosclerosis.

Biochemical analyses and genotyping

Standard biochemical analyses were performed in the hospital's accredited lab after a 12-hour fasting period: lipid status, fasting blood glucose and glycated haemoglobin (HbA1c), and hs-CRP.

After the genomic DNA was extracted with FlexiGene DNA isolation kit (Qiagen GmbH, Hilden, Germany) polymorphisms T889C (rs1800587) of IL-1α gene and C3954T (rs1143634) of IL-1β gene were genotyped with KASP genotyping chemistry in UK (LGC Genomics Ltd, Unit 1-2 Trident Industrial Estate, Hoddesdon, Herts, United Kingdom) according standard protocol.

Thermal cycling conditions for KASP chemistry: hot-start activation 94°C for 15 minutes, 10 cycles: 94°C for 20 seconds, 61-55°C for 60 seconds (dropping 0.6°C per cycle), 26 cycles: 94°C for 20 seconds, 55°C for 60 seconds.

Statistical analysis

Data were expresseticald as frequencies (percentages) or as means ± standard deviation (SD), when normally distributed, and as median (interquartile range) when asymmetrically distributed.

Continuous clinical data were compared by unpaired Student's t-test or analysis of variance or the Kruskal-Wallis H-test when asymmetrically distributed. Hardy–Weinberg equilibrium was confirmed using the χ^2 test. Moreover, multivariable linear regression analysis was performed, as described previously to determine the association of the tested polymorphisms with the CIMT/annual progression of CIMT and change in number of sites with plaque/total plaque thickness.

A p-value of <0.05 was considered to be statistically significant. Statistical analysis was performed using the SPSS program for Windows 2000 version 19 (SPSS Inc. Chicago, Illinois, USA).

Results

Baseline characteristics (demographic and clinical) of patients with T2DM are demonstrated in Table 1. Almost two third of patients with T2DM were men, and two third of patients were on statin therapy (Table 1). Both polymorphisms (rs1800587 and rs11436348) were demonstrated to be associated with total plaque thickness upon enrolment, whereas they were not associated with either the presence of plaque or presence of unstable plaque (Tables 2 and 3).

The comparison of atherosclerotic markers was performed with regard to different genotypes of IL-1α rs1800587 and rs11436348 upon enrolment and during follow-up (Tables 2-4).

	Subjects with T2DM n=595
Age (years)	62.39 ± 9.61
Male sex (%)	338 (56.8)
Diabetes duration (years)	11.25 ± 7.88
Cigarette smoking (%)	53 (8.91)
Waist circumference (cm)	108.65 ± 12.88
BMI (kg/m^2)	31.00 ± 4.74
SBP (mm Hg)	147.1 ± 19.80
DBP (mm Hg)	85.78 ± 11.60
Fasting glucose (mmol/l)	8.04 ± 2.57
HbA1c (%)	7.89 ± 3.56
Total cholesterol (mmol/l)	4.70 ± 1.18
HDL cholesterol (mmol/l)	1.20 ± 0.35
LDL cholesterol (mmol/l)	2.63 ± 0.94
Triglycerides (mmol/l)	1.9 (1.2-2.7)
hs - CRP (mg/l)	3.5 ± 1.18
CIMT (μm)	1013 ± 208
Number of sites with plaque	2.52 ± 1.63
Total plaque thickness (mm)	7.89 ± 3.51

BMI-Body Mass Index; SBP-Systolic Blood Pressure; DBP-Diastolic Blood Pressure; Hba1c-Glycated Haemoglobin; Hs-CRP-High Sensitivity C-Reactive Protein; CIMT-Carotid Intima Media Thickness.

Table 1: Baseline characteristics (clinical, ultrasonographical) of subjects with T2DM.

rs1800587		CC	CT	TT	P
CIMT (μm)		1022 ± 197	1010 ± 215	1009 ± 214	0.85
Number of sites with plaque		2.57 ± 1.68	2.43 ± 1.55	2.40 ± 1.39	0.7
Total plaque thickness (mm)		8.56 ± 3.66	7.23 ± 3.12	6.70 ± 3.09	0.04
Presence of plaques	+	278 (85.0)	192 (83.5)	28 (73.7)	0.19
	-	49 (15.0)	38 (16.5)	10 (26.3)	
Presence of unstable plaques	+	163 (58.6)	103 (53.6)	16 (57.1)	0.56
	-	115 (41.4)	89 (46.4)	12 (42.9)	
rs1143634		CC	CT	TT	p
CIMT (μm)		1017 ± 210	1010 ± 205	995 ± 212	0.92
Number of sites with plaque		2.62 ± 1.63	2.30 ± 1.63	2.17 ± 1.51	0.13
Total plaque thickness (mm)		8.40 ± 3.78	7.94 ± 3.29	6.69 ± 2.89	0.02
Presence of plaques	+	315 (84.9)	162 (82.2)	21 (77.8)	0.49
	-	56 (15.1)	35 (17.8)	6 (22.2)	
Presence of unstable plaques	+	186 (59.0)	88 (54.3)	12 (57.1)	0.61
	-	129 (41.0)	74 (45.7)	9 (42.9)	

Table 2: Ultrasonographic markers of carotid atherosclerosis due to rs1800587 and rs1143634 genotypes in patients with T2DM at the time of recruitment.

In our group of patients with T2DM 8.9% of them were cigarette smokers. The genotype distribution in patients with T2DM was in Hardy-Weinberg equilibrium for the IL-1α gene [rs1800587: T2DM genotype frequencies: TT genotype 6.4%, TC genotype 38.7%, CC genotype 54.9%; χ^2=0.08; p=0.77; rs1143634: T2DM genotype frequencies: TT genotype 4.5%, TC genotype 33.1%, CC genotype 62.4%; χ^2=0.02; p=0.89).

The comparison of atherosclerosis parameters was performed with regard to different genotypes of IL-1α rs1800587 and rs11436348 polymorphisms upon enrolment. Multiple linear regression analysis found the association between the rs1143634 TT genotype and total plaque thickness progression in a 3.8 year follow-up (Table 5).

Discussion

In the present study we reported that both polymorphisms of the IL-1α (rs1800587 and rs11436348) were associated with total plaque thickness upon enrolment in subjects with T2DM. Our findings are in accordance with the results of the retrospective association study reported on Chinese population [14].

Zhang and co-workers reported that the TT genotype of the rs1800587 was associated with ischemic stroke due to large artery atherosclerosis in the Han population in Northern China [14]. Contrary to our study, few retrospective studies [7,8,14] did not demonstrate a significant effect of the gene variability of the IL-1α on either ischemic stroke or coronary heart disease. Zee and co-workers failed to demonstrate an important effect of the gene variability of the IL-1α on athero-thrombotic disorders in a prospective cohort of 14916 initially healthy American men (incident myocardial infarction, incident ischemic stroke) [13]. They, however, reported a modest association of rs1143623 of the IL-1β with reduced risk of incident myocardial infarction.

	Presence of plaque		Presence of unstable plaque	
rs1800587	OR (95% CI)	p	OR (95% CI)	P
Hypertension (0=no; 1=yes)	2.36 (0.82-4.67)	0.11	1.25 (0.37-1.95)	0.93
Systolic blood pressure (mmHg)	1.07 (0.97-1.12)	0.34	1.16 (0.79-1.38)	0.41
LDL cholesterol (mmol/L)	1.26 (0.79-1.98)	0.31	1.12 (0.77-1.63)	0.56
HDL cholesterol (mmol/L)	0.14 (0.04-0.52)	0.003	0.27 (0.07-1.06)	0.06
Triglycerides (mmol/L)	1.17 (0.61-1.32)	0.07	1.12 (0.85-1.29)	0.26
Hba1c (%)	1.08 (0.60-1.24)	0.16	1.12 (0.86-1.46)	0.39
CT*	0.89 (0.16-3.22)	0.21	0.93 (0.25-1.34)	0.09
TT*	0.74 (0.20-2.89)	0.89	0.98 (0.43-1.66)	0.58
rs1143634				
Hypertension (0=no; 1=yes)	2.47 (0.91-4.55)	0.07	1.89 (0.74-3.29)	0.84
Systolic blood pressure (mmHg)	1.05 (0.96-1.18)	0.16	1.03 (0.96-1.23)	0.28
LDL cholesterol (mmol/L)	1.26 (0.79-1.97)	0.33	1.34 (0.71-1.89)	0.86
HDL cholesterol (mmol/L)	0.15 (0.04-0.55)	0.004	0.26 (0.07-1.08)	0.06
Triglycerides (mmol/L)	1.37 (0.53-1.96)	0.07	1.60 (0.77-2.42)	0.19
Hba1c (%)	1.38 (0.63-1.86)	0.22	1.09 (0.83-1.44)	0.55
CT**	0.79 (0.22-1.49)	0.64	0.48 (0.19-0.87)	0.22
TT**	0.58 (0.18-1.27)	0.92	0.29 (0.16-0.68)	0.56

All the models were adjusted for age, gender, smoking and statin treatment. *Reference group were homozygotes for the allele C (rs1800587). **Reference group were homozygotes for the allele C (rs1143634).

Table 3: Association of the presence of plaques/unstable plaques with rs1800587 and rs1143634 genotypes in patients with T2DM at the time of recruitment.

rs1800587	**CC**	**CT**	**TT**	**P**
CIMT progression rate (μm/year)	20.17 (13.90-30.65)	18.68 (14.25-26.08)	16.34 (10.95-22.28)	0.23
Δ number of sites with plaque	2.0 (1.0-3.0)	2.0 (1.0-3.0)	2.0 (1.0-2.25)	0.93
Δ total plaque thickness (mm)	6.30 (3.17-8.50)	5.85 (4.30-9.30)	4.35 (2.80-7.22)	0.38
rs1143634	CC	CT	TT	P
CIMT progression rate (μm/year)	20.67 (13.53-28.25)	20.33 (10.53-25.54)	18.62 (12.34-25.72)	0.77
Δ number of sites with plaque	2.0 (1.0-3.0)	2.0 (0.75-3.0)	1.5 (1.0-2.75)	0.94
Δ total plaque thickness (mm)	5.08 (2.38-7.18)	4.83 (3.30-7.24)	4.64 (3.48-6.88)	0.36
CIMT- carotid intima-media thickness.				

Table 4: Ultrasonographic markers of carotid atherosclerosis progression due to rs1800587 and rs1143634 genotypes in patients with T2DM.

	CIMT progression rate		**Δ Number of sites with plaque**		**Δ Total plaque thickness**	
rs1800587	B	p	β	p	β	P
Hypertension (0=no; 1=yes)	1.03	0.86	1.206	0.71	1.092	0.56
Systolic blood pressure (mmHg)	0.048	0.79	0.051	0.39	0.05	0.76
LDL cholesterol (mmol/L)	0.284	0.56	0.559	0.25	0.45	0.72
HDL cholesterol (mmol/L)	-0.204	0.19	-0.253	0.76	-0.223	0.11
Triglycerides (mmol/L)	0.278	0.09	0.646	0.37	0.118	0.41
Hba1c (%)	1.132	0.32	1.082	0.12	1.175	0.16
CT*	-0.117	0.79	0.337	0.51	-0.144	0.09
TT*	-0.211	0.18	0.332	0.42	-0.225	0.29
rs1143634	B	p	β	p	B	P
Hypertension (0=no; 1=yes)	1.075	0.65	1.26	0.73	1.057	0.7
Systolic blood pressure (mmHg)	0.11	0.51	0.032	0.96	0.044	0.77
LDL cholesterol (mmol/L)	0.255	0.69	0.653	0.29	0.113	0.37
HDL cholesterol (mmol/L)	-0.196	0.24	-0.127	0.91	-0.288	0.06
Triglycerides (mmol/L)	0.237	0.14	0.619	0.41	0.116	0.4
Hba1c (%)	0.14	0.33	0.168	0.28	0.16	0.19
CT**	-0.037	0.84	-0.033	0.61	-0.127	0.69
TT**	-0.06	0.67	-0.064	0.65	-0.229	0.04
All the models were adjusted for age, gender, smoking, statin treatment and baseline value of dependent variable. *Reference group were homozygotes for the allele C (rs1800587). **Reference group were homozygotes for the allele C (rs1143634).						

Table 5: Association of the rs1800587 and rs1143634 genotypes with ultrasonographic markers of carotid atherosclerosis progression in patients with T2DM.

We think that the reported differences in the results of association studies might be due to the fact that different populations and different cohorts of enrolled subjects represent different genetic and environmental background with complex interaction between them. Moreover, multiple linear regression analysis revealed the effect of IL-1α rs1143634 on total plaque thickness progression in a 3.8 year follow up in Caucasians with T2DM.

Our study is the first to report a relationship between the tested polymorphisms (rs1800587 and rs1143634) and progression of subclinical markers of carotid atherosclerosis in subjects in subjects

with T2DM. According to the findings of our study, the exact role of rs1800587 and rs1143634 on the development and progression of carotid atherosclerosis can only be speculated. There are several possible mechanisms of action of IL-1 on the development of atherosclerosis. Gene variability of the IL-1α may exert its affect through the expression (plasma level of inflammatory cytokines) or through other intermediate phenotypes (i.e. obesity and increased body-mass index) [1,10,18,19]. Um and co-workers demonstrated that the polymorphism of rs1800587 affected the transcriptional activity of IL-1a in pre-adipocyte 3T3-L1 cells [10]. Finally, rs1800587 was reported to be associated with few chronic inflammatory diseases including rheumatoid arthritis, Alzheimer disease, and periodontitis [20-22], and chronic inflammation plays a key role in establishing the atherosclerotic lesion [1-3].

The strength of our cross-sectional prospective study is the community-based sample of Caucasians with T2DM, the meticulous evaluation of subclinical markers of carotid atherosclerosis, a rather large cohort of Caucasians with T2DM, and prospective nature of the study. According to calculations the study was appropriately powered to detect differences in subclinical markers of carotid atherosclerosis.

A limitation of the study might be the number of participants involved in the study; however, the study was appropriately powered to detect differences in subclinical markers of carotid atherosclerosis in this cohort of subjects. Another limitation of the study might be the fact that we did not investigate serum levels of IL-1 at the beginning of the study and during follow-up.

To conclude, in our study we reported the effect of both polymorphisms of the IL-1α (rs1800587 and rs11436348) on total plaque thickness upon enrolment in Caucasians with T2DM. Moreover, we demonstrated the effect of IL-1α rs1143634 on total plaque thickness progression in a 3.8 year follow up in Caucasians with T2DM. Our findings indicate that both polymorphisms of the IL-1α gene may have at least a modest effect in the development of carotid atherosclerosis in subjects with T2DM.

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