Brief Genetics Report

Interleukin-1 Receptor Antagonist Genotype Is Associated With Coronary Atherosclerosis in Patients With Type 2 Diabetes

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Recently, inflammation has received considerable attention in the pathogenesis of both type 2 diabetes and atherosclerosis. The interleukin-1 receptor antagonist (IL-1ra) is a major modulator of the interleukin-1 proinflammatory pathway. We studied the relationship between a variable number tandem repeat (VNTR) polymorphism in intron 2 of the IL-1ra gene (IL1RN) and coronary artery disease (CAD) in patients with and without type 2 diabetes, following 787 consecutive patients admitted for suspected CAD. According to the current criteria of the American Diabetes Association, 250 patients had type 2 diabetes. In this group of patients, allele 2 carriers (n = 108) had an increased prevalence of CAD compared with noncarriers (85.2 vs. 73.2%), a difference that remained significant in a multivariate logistic regression model (odds ratio 2.2, 95% CI 1.1–4.3, P = 0.02). No association of CAD with allele 2 carrier status was present among nondiabetic patients (n = 537). Enzyme-linked immunosorbent assays showed decreased baseline plasma levels of IL-1ra in patients with type 2 diabetes, which may in part explain the role of the IL1RN VNTR in these patients. *Diabetes* 51:3582–3585, 2002

Type 2 diabetes is associated with an increased risk of cardiovascular disease. A series of metabolic risk factors have been proposed to account for the accelerated development of atherosclerosis in patients with this disease. In addition, in recent years, chronic inflammation has received increasing attention as an important pathophysiological mechanism in both type 2 diabetes and atherosclerosis. Therefore, genetic variability in factors involved in the regulation of inflammation may have a particular impact on the progression of atherosclerosis in patients with type 2 diabetes.

The interleukin-1 (IL-1) pathway is a central mediator of inflammatory reactions. Increased activation of the IL-1 proinflammatory axis has been reported in diabetes, and IL-1 is also involved in atherogenesis by a multitude of mechanisms. The IL-1 gene cluster contains the genes encoding the proinflammatory cytokines IL-1α and -1β and the anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra). Among the polymorphic sites in this region, a variable number tandem repeat (VNTR) in intron 2 of the IL-1ra gene (IL-1RN) appears to be of particular clinical significance. Five alleles, comprising between two and six repeats of an 86-bp sequence, are known. The four-repeat (allele 1 [IL1RN*1]) and two-repeat (allele 2 [IL1RN*2]) variants are the most common, whereas the other alleles occur with a total frequency of <5%. Allele 2 has been reported to be associated with more severe clinical outcome in several inflammatory diseases, including systemic lupus erythematosus (1), rheumatoid arthritis, and ulcerative colitis (2). An increased frequency of allele 2 has also been described in diabetes patients with nephropathy (3). However, conflicting results have been reported regarding the association of this polymorphism with coronary artery disease (CAD) (4,5).

The aim of the present study was to assess the association of the IL1RN genotype with the presence of CAD in diabetic and nondiabetic patients. A total of 787 consecutive patients admitted to the department of cardiology and evaluated for CAD were included, and the patients were classified as having type 2 diabetes based on medical history and on fasting plasma glucose (FPG) according to the current American Diabetes Association (ADA) criteria (see RESEARCH DESIGN AND METHODS). In the diabetes group (n = 250), IL1RN*2 carriers had a significantly higher prevalence of CAD than noncarriers (85.2 vs. 73.2%; P = 0.02). No significant difference in the prevalence of other atherosclerosis risk factors (age, sex, BMI, hypertension, hyperlipidemia, fasting plasma glucose [FPG], or HbA_{1c} levels) was present between IL1RN*2 carriers and noncarriers within the diabetes group (Table 1).

Univariate comparison of type 2 diabetes patients with (n = 196) and without CAD (n = 54) showed that hypertension (51.9 vs. 32.8%; P = 0.01), hyperlipidemia

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CAD, coronary artery disease; ELISA, enzyme-linked immunosorbent assay; FPG, fasting plasma glucose; IL-1, interleukin-1; IL-1ra, IL-1 receptor antagonist; IL1RN, IL-1ra gene; IQR, interquartile range; OR, odds ratio; VNTR, variable number tandem repeat.

TABLE 1
Prevalence of CAD and cardiovascular risk factors among IL1RN*2 carriers and noncarriers in the diabetes group

	IL1RN*2 carriers	IL1RN*2 noncarriers	*P*
n	108	142	—
CAD (%)	85.2	73.2	0.02
Age (years)	64 (56–73)	65.5 (57–73)	0.6
Men (%)	66.7	65.5	0.8
BMI (kg/m^2)	27.1 (24.3–29.5)	27.4 (24.4–30.1)	0.5
Arterial hypertension (%)	63.0	63.1	1.0
Hyperlipidemia (%)	71.3	67.4	0.5
FPG (mmol/l)	8.3 (7.2–10.1)	7.7 (6.8–9.6)	0.1
HbA_{1c} (%)	6.7 (6.2–8.4)	7.0 (6.4–8.0)	0.5

Data are *n* or continuous data presented as median (IQR). Percentages were calculated for dichotomous variables. The Mann-Whitney *U* test was used for comparison of continuous data, and the χ^2 test was applied to compare proportions.

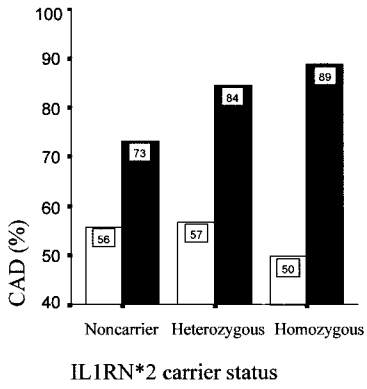

FIG. 1. Gene dosage-dependent association of IL1RN*2 with the prevalence of CAD in patients with type 2 diabetes (■) ($P = 0.02$, χ^2 test for trend) and the absence of such an association in patients without diabetes (□). The numbers given represent the prevalence of CAD in each subgroup in percent.

(73.8 vs. 51.9%; $P = 0.002$), and smoking (36.2 vs. 16.7%; $P = 0.006$) were significantly associated with CAD in this collective. No significant difference was present between the two groups with regard to age, sex, BMI, FPG, or HbA_{1c}. The IL1RN*2 carrier frequencies were 46.9% in the CAD group and 29.6% in the non-CAD group. To assess an independent association of IL1RN*2 with CAD in the diabetes group, a multivariate binary logistic regression model adjusted for age, sex, hypertension, hyperlipidemia, BMI, and smoking was calculated. The IL1RN*2 carrier status remained significantly associated with CAD in this model (Table 2).

As Fig. 1 suggests, the IL1RN*2 association with the risk of CAD in patients with type 2 diabetes is gene dosage dependent: the CAD prevalence is highest in allele 2 homozygotes (89%), and decreases progressively with heterozygosity (84%) and the absence of allele 2 (73%) ($P = 0.02$, χ^2 test for trend).

In the nondiabetic group ($n = 537$), no association of the IL1RN*2 carrier status with an increased prevalence of CAD was found (data not shown). The results were confirmed in the subgroup of patients who underwent coronary angiography ($n = 607$; see RESEARCH DESIGN AND METHODS) (data not shown). Because oral glucose tolerance testing was not performed in this study, the diagnosis of type 2 diabetes might have been missed in some patients. Therefore, we recalculated the entire statistical analysis using $HbA_{1c} \geq 6.5\%$ instead of FPG as a criterion for the diagnosis of diabetes (6). This yielded essentially identical results (data not shown).

To get some insight into the mechanism underlying the association of IL1RN*2 with CAD and the restriction of this association to patients with type 2 diabetes, we measured IL-1β and IL-1ra baseline plasma levels in representative samples of diabetic and nondiabetic patients by enzyme-linked immunosorbent assay (ELISA). In agreement with previous reports, IL-1β and IL-1ra levels were higher in IL1RN*2 carriers than in noncarriers (Fig. 2), although the observed difference was not statistically significant. Most interestingly, however, patients with type 2 diabetes had significantly lower IL-1ra plasma concentrations than nondiabetic patients (median [interquartile range]: 89 [61–159] vs. 140 [113–306] pg/ml; $P = 0.001$). IL-1β levels were similar in both groups.

IL-1 is involved in the pathogenesis of atherosclerosis by a variety of mechanisms, including endothelial activation with expression of leukocyte adhesion molecules, increased gene expression of clotting factors and inhibitors of fibrinolysis, induction of chemokines, and increased proliferation of vascular smooth muscle cells. We observed a gene dosage-dependent increase of CAD prevalence in type 2 diabetes patients carrying the IL1RN*2 allele. ELISA assays showed consistently higher levels of both IL-1β and IL-1ra in IL1RN*2 carriers than in noncarriers, although the differences were not statistically significant. Our results were consistent with those from in vitro studies that showed increased release of IL-1β by activated monocytes from IL1RN*2 carriers. This has been proposed to be the primary effect of IL-1RN*2 and the reason for the association of this allele with more severe inflammation (7). The induction of IL-1ra by IL-1β is an important counterregulatory mechanism and may at least partially account for the increased IL-1ra levels in IL-1RN*2 carriers (7,8). The significantly decreased IL-1ra concentrations in diabetic patients suggest a deficiency of this regulatory mechanism, which may be particularly pronounced in IL1RN*2 carriers and could explain the higher incidence of CAD. In addition, in agreement with reports in the literature (9–11), our type 2 diabetic patients had significantly higher levels of inflammation markers C-reactive protein and fibrinogen than nondiabetic patients (data not shown). This overall increase in systemic inflammation associated with type 2 diabetes may also enhance the proatherogenic effect of IL1RN*2 in diabetic patients.

Francis et al. (4) reported an association of IL1RN*2 with single-vessel, but not multiple-vessel, CAD. We could

TABLE 2
Multivariate logistic regression analyses assessing the independent association of IL1RN*2 and the presence of CAD in patients with type 2 diabetes

Detection of IL1RN*2	OR	95% CI	*P*
By univariate logistic regression	2.1	1.1–4.0	0.02
Model adjusted for age and sex	2.1	1.1–4.1	0.02
Model adjusted for age, sex, hypertension, hyperlipidemia, BMI, and smoking	2.2	1.1–4.3	0.02

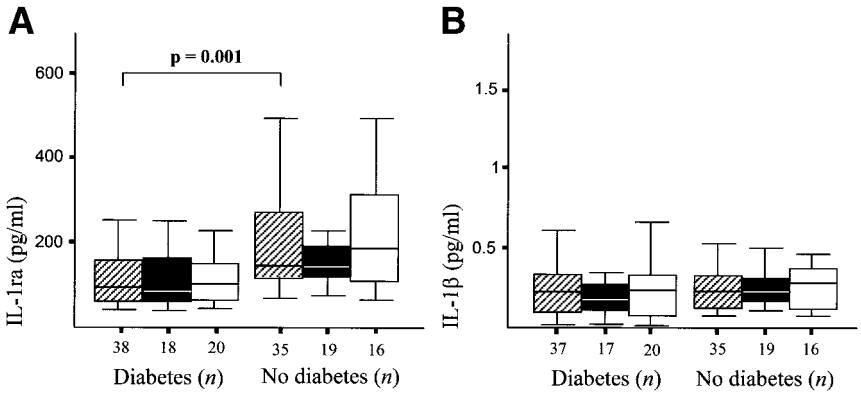

FIG. 2. IL-1ra (*A*) and IL-1β (*B*) baseline plasma levels in representative samples of diabetic and nondiabetic patients. IL1RN*2 carriers (□) had higher levels of IL-1β and IL-1ra than noncarriers (■). The difference was not statistically significant. Patients with type 2 diabetes had significantly lower IL-1ra concentrations than nondiabetic patients (P = 0.001, Mann-Whitney U test). ▨, carriers and noncarriers.

not reproduce these findings in the 607 patients who were evaluated by coronary angiography in our study, possibly because of ethnic differences or different selection criteria of the patients for angiography. It may also be noteworthy that in one of the two cohorts studied by Francis et al., the association of IL1RN*2 with single-vessel CAD lost statistical significance when patients with diabetes and patients with hypertension were excluded. In a recent report by Zee et al. (5), no association of IL1RN*2 with myocardial infarction was found. It would be interesting to know the association of IL1RN*2 with CAD in the subgroups of diabetic individuals in these studies.

In addition to the association reported here with an increased prevalence of CAD, the presence of allele 2 of the IL-1ra gene VNTR has been described as a risk factor for diabetic nephropathy (3). Therefore, the IL1RN VNTR genotype may provide a useful genetic marker for risk estimation of patients with type 2 diabetes. Recently, high-dosage α-tocopherol supplementation has been shown to reduce monocyte activity in type 2 diabetes patients and decrease IL-1β secretion (12). If the clinical associations of the IL1RN genotype can be confirmed in independent studies, such therapeutic strategies may be especially indicated for IL1RN*2 carriers. Therefore, we would like to propose extending our study to large groups of patients, of various ethnic background if possible.

RESEARCH DESIGN AND METHODS

Subjects. The study group was comprised of 787 consecutive patients who were admitted to the Department of Cardiology, University of Vienna for evaluation of chest pain or suspected CAD. The majority of the patients (n = 607) underwent coronary angiography; the remaining 180 were evaluated by ergometry and/or myocardial perfusion scintigraphy. CAD was defined as angiographically proven stenosis with >30% narrowing of a main coronary artery, or positive ergometry and myocardial perfusion scintigraphy. Patients were classified as having type 2 diabetes based on medical history and a fasting plasma glucose ≥7.0 mmol/l, according to the current ADA criteria for the diagnosis and classification of diabetes (13). Arterial hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or a known history of hypertension and treatment with antihypertensive medication. Hyperlipidemia was defined as either hypercholesterolemia, with baseline cholesterol levels >5.17 mmol/l (200 mg/dl) or serum LDL levels >3.36 mmol/l (130 mg/dl), or hypertriglyceridemia >2.05 mmol/l (180 mg/dl) after overnight fasting.

IL-1ra gene intron 2 variable number tandem repeat polymorphism screening. The IL-1ra gene intron 2 VNTR polymorphism was analyzed as previously described (14) under the following PCR conditions: denaturation at 94°C for 5 min, followed by 37 cycles at 94°C for 30 s, 48°C for 30 s, 72°C for 30 s, and a final extension step at 72°C for 7 min. The reaction was performed in 50-μl volumes containing ~50 ng DNA in a Mastercycler gradient thermocycler (Eppendorf). Electrophoresis was carried out using Novex 6% polyacrylamide gels (Invitrogen). The gels were stained with Sybr Green (Molecular Probes).

IL-1ra and IL-1β ELISAs. Plasma levels of IL-1ra were determined with the IL-1ra Cytoscreen ELISA Kit (BioSource), according to the manufacturer's instructions. For IL-1β, the Quantikine HS High Sensitivity ELISA Kit (R&D Systems) was used.

Statistical analysis. Continuous data are presented as median and interquartile range (IQR). Percentages were calculated for dichotomous variables. The Mann-Whitney U test was used for univariate comparison of continuous data, and the χ^2 test was applied to compare proportions. Multivariate logistic regression was used to assess the independent association of the IL-1ra allele status with the presence of CAD in the type 2 diabetes group and to adjust for confounding factors. The final model was adjusted for the following established CAD risk factors: age, sex, hypertension, hyperlipidemia, BMI, and smoking status. Results of the logistic regression model were expressed as odds ratio (OR) and 95% CI. All P values were evaluated in a two-sided model, and $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the SPSS 10.0 software package (SPSS, Chicago, IL).

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