CLINICAL STUDY

Chemerin is associated with markers of inflammation and components of the metabolic syndrome but does not predict coronary atherosclerosis

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Abstract

Objectives: Chemerin is a recently discovered adipokine that regulates adipocyte differentiation and modulates chemotaxis and activation of dendritic cells and macrophages. Given the convergence of adipocyte and macrophage function, chemerin may provide an interesting link between obesity, inflammation and atherosclerosis in humans. We sought to examine the relationship of i) chemerin and markers of inflammation, ii) chemerin and components of the metabolic syndrome, and iii) chemerin and coronary atherosclerotic plaque burden and morphology.

Design: Serum chemerin levels were determined in 303 patients with stable typical or atypical chest pain who underwent dual-source multi-slice CT-angiography to exclude coronary artery stenosis. Atherosclerotic plaques were classified as calcified, mixed, or non-calcified.

Results: Chemerin levels were highly correlated with high sensitivity C-reactive protein ($r=0.44$, $P<0.0001$), interleukin-6 ($r=0.18$, $P=0.002$), tumor necrosis factor- α ($r=0.24$, $P<0.0001$), resistin ($r=0.28$, $P<0.0001$), and leptin ($r=0.36$, $P<0.0001$) concentrations. Furthermore, chemerin was associated with components of the metabolic syndrome including body mass index ($r=0.23$, $P=0.0002$), triglycerides ($r=0.29$, $P<0.0001$), HDL-cholesterol ($r=-0.18$, $P=0.003$), and hypertension ($P<0.0001$). In bivariate analysis, chemerin levels were weakly correlated with coronary plaque burden ($r=0.16$, $P=0.006$) and the number of non-calcified plaques ($r=0.14$, $P=0.02$). These associations, however, were lost after adjusting for established cardiovascular risk factors (odds ratio, OR 1.17, 95% confidence interval (CI) 0.97–1.41, $P=0.11$ for coronary plaque burden; OR 1.06, 95% CI 0.96–1.17, $P=0.22$ for non-calcified plaques).

Conclusions: Chemerin is strongly associated with markers of inflammation and components of the metabolic syndrome. However, chemerin does not predict coronary atherosclerosis.

European Journal of Endocrinology 161 339–344

Introduction

Obesity and atherosclerosis are increasingly viewed as inflammatory states. Biomarkers that integrate metabolic and inflammatory signals may be attractive candidates for assessing risk of atherosclerotic cardiovascular disease (1). Chemerin is a recently discovered chemokine (2) highly expressed in liver and white adipose tissue (3, 4) that modulates chemotaxis and activation of dendritic cells and macrophages through distinct G protein-coupled receptors such as CMKLR1, GPR1, and CCRL2 (5–7). Upon secretion, enzymatic proteolysis of pro-chemerin can result in both activating and inhibitory peptides. These molecules with opposing activities are generated by different classes of proteases (8). Serine proteases released by granulocytes upon

degranulation were shown to cleave the C-terminal extremity of pro-chemerin and to release its chemotactic potential (9). Cysteine proteases derived from activated macrophages cleave chemerin to generate potent anti-inflammatory products that inhibit the production of pro-inflammatory mediators such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and IL-6 while inducing the expression of anti-inflammatory cytokines such as transforming growth factor- β and IL-10 (5). In addition to its immunomodulatory effects, chemerin was reported to be associated with components of the metabolic syndrome including body mass index (BMI), plasma triglyceride (TG) levels, and hypertension (3). Furthermore, chemerin was shown to regulate adipocyte differentiation in an autocrine/paracrine manner via the CMKLR1 receptor, to modulate the expression of

adipocyte genes involved in glucose and lipid homeostasis such as glucose transporter-4, fatty acid synthase, adiponectin, and leptin (4), and to enhance insulin signaling in 3T3-L1 adipocytes (10). Given the convergence of adipocyte and macrophage function, chemerin may provide an interesting link between obesity, inflammation, and atherosclerosis in humans. To examine the relationship of chemerin and markers of inflammation, components of the metabolic syndrome, and coronary atherosclerosis, we determined serum chemerin levels in 303 patients with stable typical or atypical chest pain who underwent dual-source CT (DSCT)-coronary angiography to exclude coronary artery stenosis.

Materials and methods

Ascertainment of subjects

A total of 303 consecutive (relating to the order in which they attended the clinic) patients who underwent DSCT-coronary angiography for exclusion of coronary artery stenosis due to stable typical or atypical chest pain were recruited during 20 consecutive months from March 2006 to October 2007 as previously described (11). All patients were of Caucasian origin. After providing informed written consent, study subjects were asked to complete a brief questionnaire and have blood drawn. The study protocol was approved by the Ethics Committee of the Ludwig-Maximilians-University Munich, Germany. Baseline characteristics of the study cohort (Table 1) have in part been previously reported (11).

Dual-source multi-slice CT-coronary angiography

CT-coronary angiography and image analyses were performed as previously described (11, 12). Briefly, for the analysis of coronary plaque morphology, all reconstructed data sets were evaluated at different ECG-phases for diagnostic image quality and the optimal data set was then chosen. The DSCT datasets were evaluated by two independent investigators blinded to serum chemerin levels using a dedicated cardiac workstation (Siemens, Leonardo Circulation). Atherosclerotic plaques were classified as calcified, mixed, or non-calcified. Calcified plaques were defined as lesions with a HU value above 130. Non-calcified plaques were defined as structures clearly assignable to the vessel wall (in at least two views) with densities less than the lumen contrast. Plaques with < 50% calcified plaque area were classified as mixed. The coronary tree was segmented according to the suggestions of the American Heart Association (AHA) into a 15 segment model. Each segment was further divided into a proximal and a distal segment. Each segment was

then classified as containing either calcified, non-calcified, mixed, or no plaque. Based on the number of diseased segments, a plaque score was calculated. Adequate image quality for evaluation of coronary plaques was obtained in 281 out of 303 patients.

The same images as for the analysis of atherosclerotic plaques were used to determine pericardial adipose tissue (PAT) volume. PAT volume was measured in milliliters using the volume analysis software tool of the Siemens Leonardo Circulation workstation. PAT volume was determined as previously described (11, 12). We defined PAT as the adipose tissue surrounding the myocardium. The upper cut-off point in the axial slices was the bifurcation of the pulmonary artery. Adequate image quality for evaluation of PAT volume was obtained in 287 out of 303 patients.

Laboratory procedures

Blood samples were stored at -70°C until analysis. Serum levels of adiponectin, chemerin, leptin, and resistin were determined with commercially available ELISAs (R&D, Wiesbaden, Germany). Both, the monoclonal capture and the polyclonal detection antibody used for the chemerin ELISA were raised against residues 21–157 of recombinant human chemerin. Therefore, the assay used in this study measures prochemerin, chemerin, and likely some of the proteolytically processed short forms. Unfortunately, there is no information available regarding the affinity of these antibodies to the different chemerin forms. Intra- and inter-assay coefficients of variance for the chemerin ELISA were 2.7 and 9.3% respectively.

Plasma LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), and TG were measured by routine enzymatic methods. Determination of high sensitivity C-reactive protein (hsCRP), IL-6, and TNF- α levels was performed at the Department of Clinical Chemistry (Campus Grosshadern, University of Munich, Germany).

Statistical analysis

Statistical analyses were performed by R 2.8.1 (Vienna, Austria) software. Data are reported as *n* or median (interquartile range). Spearman correlation and Wilcoxon two-sample test were used in the bivariate analysis of chemerin with other variables. A generalized linear regression model was used to assess the association of chemerin serum levels with atherosclerotic plaque burden, number of calcified plaques, mixed plaques, or non-calcified plaques adjusted for age, sex, BMI, diabetes, hypertension, family history of coronary artery disease (CAD), smoking, LDL-C, HDL-C, TG, hsCRP, IL-6, TNF- α , leptin, resistin, adiponectin, PAT volume, and medication – possible confounders of chemerin levels. All tests were two-tailed with a 0.05 type I error rate.

Table 1 Baseline characteristics and CT-angiographic findings of the study population.

Characteristics	
Age (years)	63 (55–70)
Sex (m/f)	202/101
Body mass index (kg/m ²)	26.2 (24.1–29.0)
Hypertension ^a	54%
Diabetes mellitus ^a	7%
Smoker ^b	15%
Family history of CAD ^b	28%
Laboratory profile	
LDL-cholesterol (mg/dl)	122 (95–149)
HDL-cholesterol (mg/dl)	52 (44–59)
Triglycerides (mg/dl)	143 (106–207)
High-sensitivity CRP (mg/dl)	0.23 (0.05–0.54)
IL-6 (pg/ml) ^c	2.0 (2.0–3.16)
TNF- α (pg/ml)	6.3 (4.5–7.9)
Resistin (ng/ml)	11.5 (9.4–14.3)
Leptin (ng/ml)	6.9 (3.6–16.1)
Adiponectin (μ g/ml)	5.1 (3.3–7.8)
Chemerin (ng/ml)	108 (88–133)
Medical treatment ^d	
Statin	120
Acetylsalicylic acid, clopidogrel or marcumar	170
Betablocker	173
ACE-I or ARB	138
Diuretics	87
Insulin or OAD	16
CT data	
Pericardial adipose tissue volume (ml) ^e	190 (132–259)
Number of coronary artery plaques (total) ^f	3 (1–6) (range 0–26)
Number of calcified plaques (154 patients)	1 (0–3) (range 0–22)
Number of mixed plaques (102 patients)	0 (0–1) (range 0–10)
Number of non-calcified plaques (126 patients)	0 (0–2) (range 0–9)

Values are presented as *n* or median (interquartile range).

^aHistory of diabetes and hypertension is known in 273 patients. All diabetic subjects were type 2 diabetics. Diagnosis of hypertension was based on patient history.

^bHistory of smoking and family history of CAD are known in 272 patients.

^cDetection limit of the IL-6 assay was 2 pg/ml.

^dMedication is known in 258 patients.

^eAdequate image quality for evaluation of PAT volume was obtained in 287 patients.

^fAdequate image quality for evaluation of coronary plaques was obtained in 281 patients. A total of 60 patients had no coronary plaques (21%).

Results

Chemerin is associated with markers of inflammation and components of the metabolic syndrome

Chemerin was strongly correlated with all markers of inflammation including hsCRP ($r=0.44$, $P<0.0001$), IL-6 ($r=0.18$, $P=0.002$), TNF- α ($r=0.24$, $P<0.0001$), resistin ($r=0.28$, $P<0.0001$), and leptin ($r=0.36$, $P<0.0001$; Table 2). The association of chemerin with hsCRP persisted after adjusting for anti-inflammatory drugs such as acetylsalicylic acid and statins ($P<0.0001$).

Chemerin was positively correlated with BMI ($r=0.23$, $P=0.0002$) and PAT volume ($r=0.20$, $P=0.0007$) defined as the volume of adipose tissue surrounding the myocardium with an upper cut-off point determined by the bifurcation of the pulmonary artery, and TG ($r=0.29$, $P<0.0001$), yet negatively correlated with HDL-C ($r=-0.18$, $P=0.003$).

The association of chemerin with TG remained significant after adjusting for BMI ($P<0.0001$) or lipid lowering therapy ($P<0.0001$). Chemerin did not correlate with LDL-C ($r=0.05$, $P=0.43$) and adiponectin ($r=-0.09$, $P=0.15$).

Among demographic characteristics, hypertension ($P<0.0001$) was associated with higher chemerin levels. This association persisted after adjusting for anti-hypertensive agents ($P=0.009$). No association was seen with diabetes mellitus ($P=0.15$), smoking ($P=0.23$), and family history of CAD ($P=0.96$).

Chemerin does not predict coronary atherosclerotic burden or plaque morphology

In bivariate analysis, chemerin levels weakly correlated with coronary plaque burden ($r=0.16$, $P=0.006$) and the number of non-calcified plaques ($r=0.14$, $P=0.02$; Table 2). After adjusting for established cardiovascular risk factors, these associations were lost (odds ratio,

Table 2 Correlations and associations between chemerin and makers of inflammation, components of the metabolic syndrome, and coronary atherosclerosis.

Characteristics		P
Age	0.22	0.0002
Sex		0.0003
Male	102 (84–126)	
Female	121 (95–140)	
Body mass index (kg/m ²)	0.23	0.0002
Actual hypertension		<0.0001
Yes	122 (99–148)	
No	100 (81–120)	
Diabetes mellitus		0.15
Yes	127 (90–145)	
No	108 (89–132)	
Actual smoker		0.23
Yes	109 (93–144)	
No	108 (88–132)	
Family history of CAD		0.96
Yes	111 (90–133)	
No	109 (88–134)	
LDL-cholesterol (mg/dl)	0.05	0.43
HDL-cholesterol (mg/dl)	−0.18	0.003
Triglycerides (mg/dl)	0.29	<0.0001
High-sensitivity CRP (mg/dl)	0.44	<0.0001
IL-6 (pg/ml)	0.18	0.002
TNF- α (pg/ml)	0.24	<0.0001
Resistin (ng/ml)	0.28	<0.0001
Leptin (ng/ml)	0.36	<0.0001
Adiponectin (μ g/ml)	−0.09	0.15
Pericardial adipose tissue (ml)	0.20	0.0007
Total number of	0.16	0.006
coronary artery plaques		
Number of calcified plaques	0.11	0.06
Number of mixed plaques	0.05	0.37
Number of non-calcified plaques	0.14	0.02

Values are presented as correlation coefficient or median (interquartile range).

OR 1.17, 95% CI 0.97–1.41, $P=0.11$ for coronary plaque burden (Table 3); OR 1.06, 95% CI 0.96–1.17, $P=0.22$ for non-calcified plaques). Further adjustment for markers of inflammation, PAT volume and medication did not alter the results.

No association was seen between chemerin and calcified, or mixed plaques in either bivariate (Table 2) or multivariate analyses (data not shown).

Discussion

Our study was aimed to address the relationship of chemerin and inflammation, components of the metabolic syndrome and atherosclerosis. Increased levels of TNF- α , IL-6, CRP, leptin, and resistin have been linked to obesity and atherosclerotic cardiovascular disease (13–18). In the present study, we found chemerin to strongly correlate with all of these markers of inflammation. Furthermore, chemerin was correlated with BMI, ectopic adipose tissue (PAT), TG, low HDL-C and hypertension, components of the metabolic syndrome trait cluster. Considering the convergence of inflammation and the metabolic syndrome in the pathogenesis of atherosclerotic cardiovascular disease, we expected chemerin to correlate with coronary atherosclerosis. However, in a multivariate model adjusted for established cardiovascular risk factors chemerin was not associated with coronary atherosclerotic plaque burden or morphology.

Our study has several strengths and limitations. To our knowledge, this is the first study to examine the association of chemerin and systemic markers of inflammation as well as atherosclerotic plaque burden and morphology in a well characterized cohort of subjects with stable chest pain. Furthermore, our study supports previous data (3) demonstrating that chemerin is associated with components of the metabolic syndrome.

Chemerin was shown to have both pro- and anti-inflammatory properties depending on the modality of enzymatic cleavage by different classes of proteases (8). Our study cannot address whether positive correlations of chemerin with several markers of inflammation

Table 3 Multivariate association of chemerin levels with coronary atherosclerotic plaque burden.

	Coronary atherosclerotic plaque burden		
	OR	95% CI	P
Model 1 ^a	1.25	1.06–1.48	0.008
Model 2 ^b	1.17	0.97–1.41	0.11
Model 3 ^c	1.21	0.98–1.49	0.08
Model 4 ^d	1.19	0.95–1.49	0.14
Model 5 ^e	1.08	0.85–1.38	0.53
Model 6 ^f	1.09	0.85–1.40	0.50

Odds ratio (OR) and corresponding 95% CI for the association of having coronary atherosclerotic plaques for a 10 ng/ml increase in serum chemerin levels.

^aAdjusted for age, sex, and BMI.

^bAdjusted for age, sex, BMI, hypertension, diabetes mellitus, smoking, family history of CAD, LDL-C, HDL-C, and TG.

^cAdjusted for age, sex, BMI, hypertension, diabetes mellitus, smoking, family history of CAD, LDL-C, HDL-C, TG, and hsCRP levels.

^dAdjusted for age, sex, BMI, hypertension, diabetes mellitus, smoking, family history of CAD, LDL-C, HDL-C, TG, hsCRP, IL-6, TNF- α , leptin, resistin, and adiponectin levels.

^eAdjusted for age, sex, BMI, hypertension, diabetes mellitus, smoking, family history of CAD, LDL-C, HDL-C, TG, hsCRP, IL-6, TNF- α , leptin, resistin, adiponectin levels, and PAT volume.

^fAdjusted for age, sex, BMI, hypertension, diabetes mellitus, smoking, family history of CAD, LDL-C, HDL-C, TG, hsCRP, IL-6, TNF- α , leptin, resistin, adiponectin levels, PAT volume, and medication.

indicate a pro-inflammatory role of chemerin in patients with known cardiovascular risk factors and/or coronary artery disease. It is also conceivable that chemerin may be up-regulated in states of inflammation such as obesity, metabolic syndrome, and atherosclerosis to dampen inflammatory processes and to improve metabolic regulation. Assessment of chemerin cleavage products will be crucial to better define the role of chemerin *in vivo*. Unfortunately, the chemerin ELISA used in the present study measures prochemerin, chemerin, and likely some of the proteolytically processed short forms. Since the full-length isoform of chemerin was reported to have significantly lower bioactivity compared with its proteolytic peptides (8) the overall significance for total chemerin assessment is limited. Lack of association between total chemerin and coronary atherosclerosis in our cross-sectional study suggests that total chemerin levels do not reflect atherosclerotic plaque burden. However, it does not rule out the possibility that distinct chemerin forms may play a role in the pathogenesis of atherosclerosis. Insufficient statistical power may be another potential explanation for the lack of association between total chemerin and coronary atherosclerosis. Experiments in animal models of atherosclerosis are needed to examine the impact of chemerin on atherogenesis.

Our study population comprised patients with stable typical or atypical chest pain. It remains to be determined whether chemerin levels may be associated with acute coronary syndrome, e.g. as a marker of plaque rupture and thrombosis.

Chemerin was reported to improve insulin sensitivity in 3T3-L1 adipocytes *in vitro* (10). However, in humans no association of chemerin and measures of insulin resistance or diabetes were found in the only study examining this issue so far (3). Unfortunately, in the present study, parameters of insulin resistance were not available in the non-diabetic subjects, and diabetic patients were underrepresented in our cohort (7% of patients). Therefore, our study cannot address the association of chemerin and insulin resistance or diabetes. Furthermore, the association of chemerin and atherosclerosis in insulin resistant and/or diabetic subjects remains to be determined.

Goralski *et al.* (4) reported that transduction of adipocytes with adenoviral vectors expressing shRNA for chemerin resulted in reduced adiponectin secretion into adipocyte media. Given the effect of chemerin on adiponectin expression *in vitro* and the association of adiponectin (11) and chemerin with BMI and PAT volume *in vivo*, we expected chemerin to be inversely correlated with serum adiponectin levels. The lack of association may be due to the determination of total chemerin rather than specific chemerin forms by our ELISA. Furthermore, assessment of high molecular weight adiponectin rather than total adiponectin may be more appropriate to examine the association of chemerin and adiponectin.

Our study population consisted of Caucasian subjects. Therefore, findings may not be generalizable to other ethnicities.

Finally, we cannot rule out the possibility that recruitment of 303 consecutive subjects (relating to the order in which they attended the clinic) may have resulted in an ascertainment bias due to selection of subjects living in proximity to our hospital. However, since both Departments of Cardiology and Radiology are well known for their expertise in cardiac imaging, patients were referred to our University hospital from both colleagues in proximity to the clinic and colleagues in the greater Munich area.

In conclusion, we demonstrate that chemerin is strongly associated with markers of inflammation and components of the metabolic syndrome. Chemerin, however, does not predict coronary atherosclerotic plaque burden or morphology.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Acknowledgements

We are indebted to Elisabeth Fleischer-Brielmaier, Inge Biller-Friedmann, and Kerstin Henze for expert technical assistance.

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Received 8 May 2009

Accepted 1 June 2009