CLINICAL STUDY

Gene variants of monocyte chemoattractant protein 1 and components of metabolic syndrome in KORA S4, Augsburg

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Abstract

Objective: Monocyte chemoattractant protein 1 (MCP-1) has been suggested to be involved in the development of several components of metabolic syndrome (MetS). The present study investigated the association of nine MCP-1 single nucleotide polymorphisms (SNPs) with MetS, type 2 diabetes mellitus and metabolic risk factors.

Subjects and methods: The population-based study sample comprised 1630 subjects aged 55–74 years from KORA S4 (Cooperative Health Research in the Region of Augsburg Survey 4). Genotyping was carried out by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) analysis of allele-dependent primer extension products.

Results: The MCP-1 SNP *c.-3813C>T* exhibited trends for differences between the genotype groups in triglycerides, 2-h glucose and uric acid ($P=0.0084$, 0.014 , 0.027). Other trends were observed for *c.-928G>C* associated with height and fasting glucose ($P=0.0024$, 0.033), for *c.105T>C* with height and leukocytes ($P=0.0095$, 0.047), for *c.*65C>T* and *c.*3879C>T* with MCP-1 levels (both $P=0.012$) and for *c.-2138A>T* with interleukin-6 levels. After correction for multiple testing, none of the analysed SNPs, except *c.-928G>C* in men showed a significant association with MetS, T2DM or other analysed parameters. Haplotype MCP-1*1 and *c.-928G>C* in men ($P=0.0002$, 0.0004) were significantly associated with an increase in height.

Conclusions: This is the first study to investigate the associations of MCP-1 SNPs with MetS. We found trends for several components of MetS. These parameters were hyperlipidaemia, fasting and 2-h glucose, and uric acid. A new finding is that MCP-1*1 haplotype is associated with height. Further investigation in larger populations is needed to clarify the involvement of MCP-1 in MetS.

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Introduction

Monocyte chemoattractant protein 1 (MCP-1) is a member of the CC chemokine family (1). The corresponding gene (*MCP-1* or *CCL2*) is mainly expressed by adipocytes, endothelial cells, macrophages and osteocytes (2–4). MCP-1 expression is stimulated among others by tumour necrosis factor α (TNF α), interleukin-6 (IL-6) and IL-1 β and is suppressed by IL-10 (2, 5). MCP-1 itself influences the expression of IL-6, β_1 -integrins and lipoprotein lipase (6–8). MCP-1 actions are mediated by chemokine (C–C motif) receptor 2 (CCR2) (6). MCP-1 seems to play an important role in several of the clustering risk factors of metabolic syndrome (MetS) as well as in the pathogenesis of MetS itself (9). MetS and its risk factors are highly heritable (10, 11). It is characterized by visceral obesity,

atherogenic dyslipidaemia, hyperglycaemia, hypertension, a proinflammatory state and hyperuricaemia (12, 13). MCP-1 has typical proinflammatory properties, like promoting the arrest and transmigration of monocytes (14, 15). Additionally, MCP-1 is involved in adipocyte metabolism (2, 7). The association of high MCP-1 levels with obesity is clear in mice, but uncertain in humans (7, 16–18). It was further shown that increased MCP-1 levels are related to insulin resistance and type 2 diabetes mellitus (T2DM; 9, 17). In addition, MCP-1 is involved in foam cell differentiation and progression of atherosclerosis (19, 20).

The association of the single nucleotide polymorphism (SNP) $-2578A>G$ with higher MCP-1 levels has been investigated in several association studies, although the findings remain controversial (18, 20–23). Rovin *et al.* demonstrated the functionality

of this SNP by showing upregulation of IL-1β-induced MCP-1 gene expression (24). Two association studies for −2578A>G and T2DM showed controversial results (18, 22). There are very few other association studies assessing metabolic parameters or MCP-1 SNPs. Until now, no study has addressed the potential association of MCP-1 SNPs with MetS.

The present study evaluates whether SNPs of the MCP-1 gene are associated with MetS, according to the International Diabetes Federation (IDF) definition, and its related traits, including T2DM. We therefore conducted an association analysis of the whole gene based on an elderly population-based study sample from KORA S4 (Cooperative Health Research in the Region of Augsburg Survey 4), Germany.

Subjects and methods

Study population

KORA S4 (formerly known as S2000) is a population-based study of adults performed in southern Germany, which contains a rather homogeneous population (25, 26). This survey was conducted under the same conditions as the previous three surveys within the WHO MONICA Augsburg project (25). In KORA S4, 1653 subjects were included in the 55–74 years age group. The following number of individuals were excluded for different analyses: MetS (23 individuals with type 1 diabetes, autoantibodies to glutamic acid decarboxylase or diabetes onset in the context of pancreatitis), T2DM (same as MetS + 168 non-fasting individuals that were not characterized for diabetic status), quantitative parameters (same as T2DM + 231 type 2 diabetes patients). An oral glucose tolerance test (OGTT) was performed in 1353 participants due to an exclusion of 131 subjects with known diabetes, and 169 dropouts as a result of non-fasting, technical problems, vomiting during OGTT and missing 2-h glucose (27). Body weight was measured in light clothing to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Waist circumference was measured at maximum abdominal girth to the nearest 0.1 cm. Blood pressure (BP) was measured in a sitting position from the right arm thrice, after 15-min rest periods, using an automatic device (OMRON HEM 705-CP). The mean of the second and third measurement was used for analysis.

Blood glucose was assessed using a hexokinase method (Gluco-quant, Roche Diagnostics). High density lipoprotein (HDL) cholesterol was measured using the phosphotungstic acid method (Boehringer Mannheim). Triglycerides were assessed with the Boehringer GPO-PAP assay. Serum IL-6 and MCP-1 levels were measured by ELISA, as described elsewhere (28, 29). Population stratification for KORA S4 was excluded by two genomic control studies. Steffens *et al.* compared 210 SNPs in

three German populations (including 730 participants of KORA S4) and detected maximal inflation factor λ=1.779 between KORA and the most distant population (30). From these 210 SNPs, Winkelmann *et al.* compared 79 between 550 KORA subjects and 367 controls from all over Germany (λ=1.01) (31).

Definition of metabolic syndrome

MetS was defined according to the IDF for Europid persons by the presence of central obesity (waist circumference >94 cm in men, >80 cm in women) and two out of four additional factors (32). These factors are (i) raised triglyceride levels (≥150 mg/dl) or specific treatment for this lipid abnormality, (ii) reduced HDL cholesterol (<40 mg/dl in men, <50 mg/dl; in women) or treatment for this abnormality, (iii) raised blood pressure (systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg) or treatment of previously diagnosed hypertension, (iv) raised fasting plasma glucose (≥100 mg/dl) or previously diagnosed T2DM.

Genotyping

For the MCP-1 gene, all available tagging SNPs from HapMap (September 2005), as well as SNPs that already showed associations, were chosen for genotyping. In addition, one SNP was added per exon, intron and ±5 kb. In the 3' region of the gene, two SNPs were additionally genotyped. Genomic DNA of KORA participants was extracted from blood leukocytes using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA), according to the manufacturer's recommendation. Genotyping for the MCP-1 SNPs c.-3813C>T, c.-2138A>T, c.-928G>C, c.76+334C>T, c.77–109C>G, c.105T>C, c.194+25C>T, c.*65C>T and c.*3879C>T (Table 1) was carried out by means of matrix-assisted laser desorption ionization time of flight (MALDI-TOF) analysis of allele-dependent primer extension products as described elsewhere (33).

Table 1 Overview of genotyped SNPs, their localization, synonyms and aliases.

SNP	Localization	Synonym	Aliases
rs1860188	5'UTR	c.-3813C>T	
rs1024611	5'UTR	c.-2581A>G	–2578A>G, –2518A>G
rs1024610	5'UTR	c.-2138A>T	–2136A/T
rs3760396	5'UTR	c.-928G>C	–927C/G
rs2857657	Intron 1	c.77–109C>G	+764G>C
rs13306748	Intron 1	c.76+334C>T	
rs4586	Exon 2	c.105T>C	
rs3917888	Intron 2	c.194+25C>T	
rs13900	3'UTR	c.*65C>T	
rs991804	3'UTR	c.*3879C>T	

SNP synonyms were named to reflect the distance in nucleotides from the translational start point in NM_002982.3 of the Reference Sequence database (<http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=56119169>); UTR, untranslated region.

Statistical analysis

Violation of the Hardy–Weinberg equilibrium (HWE) was tested by Fisher's exact test. Quantitative parameters that were components of the IDF definition of MetS, such as waist circumference, triglycerides, HDL cholesterol, systolic and diastolic BP and type 2 diabetes were analysed. Additional parameters related to risk factors of MetS were investigated and are shown in Table 2. For parameters consisting of several components, individual components were analysed separately. For example, body mass index (BMI) consists of height and weight, which were analysed separately. Quantitative traits that are normally distributed on the original or logarithmic scale were analysed by model-free linear regression. Traits that were not normally distributed were analysed by the Kruskal–Wallis test. For each quantitative trait and SNP, a global *P* value was calculated based on the hypothesis that there were no differences between the genotype groups. In case of significant differences between genders in the characteristics of the study population and a trend towards differences between the genotype groups, a global *F*-test was performed separately for men and women. For analysis of BP, subjects with medication against hypertension were generally excluded. Associations of genotypes with IDF-defined MetS or T2DM were assessed by logistic regression. A Bonferroni correction was used to adjust significance level. The number of SNPs, for which correction was needed, was calculated using SNP Spectral Decomposition (34). *P* values <0.0004 were considered to be significant as a result of the correction for 25 traits and 5 effective SNPs, and *P* values <0.05 were considered as a trend for an association. In case of a trend in two SNPs for the same quantitative parameter, haplotype analysis was carried out with R (V. 2.3.1. including haplo.stats package) using the haplo.glm procedure. This procedure performs an iterative two-step expectation maximization, with the posterior probabilities of pairs of haplotypes per subject used as weights to update the regression coefficients, and the regression coefficients used to update posterior probabilities (35). SNPs were selected for haplotype analysis when $D' > 0.95$ and $r^2 < 0.80$. Linkage disequilibrium (LD) calculation was performed with JLIN (<http://www.genepi.com.au/projects/jlin>). An r^2 value >0.8 between two SNPs was considered to be a strong LD. All other analyses were carried out using SAS (V. 9.1, Cary, NC, USA).

Results

Characteristics of the study population

Characteristics of the study population are presented separately for men and women in Table 3. Height, weight, waist-to-hip ratio, waist circumference, triglycerides, serum uric acid, systolic and diastolic BP, fasting plasma glucose, leucocyte count and IL-6 levels were significantly higher in men than in women. In contrast, women had

significantly higher values for body fat, hip circumference, HDL cholesterol, HbA_{1c} and adiponectin levels. After log-transformation triglycerides, adiponectin, MCP-1 and fasting insulin levels were approximately normally distributed. HOMA-IR and IL-6 levels were not normally distributed.

Genetic analyses

Two SNPs, $c.76+334C>T$ and $c.194+25C>T$, were monomorphic in the KORA S4 population and thus were not considered further in the manuscript. The genotyping success rates of the seven analysed SNPs ranged from 95.2 to 98.9%. The discordance rate was <1%. All seven SNPs were in HWE (Table 4). SNPs $c.*3879C>T$ and $c.*65C>T$ showed strong LD ($r^2=0.996$) and, additionally, both showed complete correlation ($r^2=1$) with $c.-2581A>G$. A strong LD was also observed between $c.-2138A>T$ and $c.77-109C>G$ ($r^2=0.981$). In six of the seven MCP-1 SNPs, trends towards differences in some of the analysed parameters between the genotype groups were observed using the global *F*-test. After correction for multiple testing, none of the analysed SNPs showed a significant association with MetS, T2DM or other analysed parameters. However, $c.-928G>C$ in men and haplotype analysis showed a significant association with height (Tables 2 and 5).

Metabolic syndrome and parameters from IDF definition

None of the analysed MCP-1 SNPs were significantly associated with the presence of IDF-defined MetS (Table 2). No association was found between the seven MCP-1 SNPs and T2DM, even after excluding 200 subjects that were taking lipid-lowering drugs (data not shown). However, $c.-3813C>T$ and $c.-928G>C$ showed trends, for differences in triglyceride levels and fasting glucose respectively, between the genotype groups ($P=0.0084$, 0.033; Table 2).

Parameters related to risk factors of metabolic syndrome

Within anthropometric parameters, height, analysed as a component of BMI, showed trends towards differences between the genotype groups in $c.105T>C$ and $c.-928G>C$ ($P=0.0095$, 0.0024; Table 2). In men, a significant association of $c.-928G>C$ with height was observed ($P=0.0004$). In the following haplotype analysis, a significant association was observed, as carriers of MCP-1*1 were 1.3 cm taller than carriers of the control haplotype ($P=0.0002$). Haplotype MCP-1*4 also showed a trend for 1 cm taller carriers (Table 5). No trends were observed for parameters related to hyperlipidaemia. For $c.-3813C>T$, 2-h glucose, a parameter related to hyperglycaemia, was

Table 2 Global *P* values for all single nucleotide polymorphisms and parameters from nominal analysis.

		<i>c.-3813C>T</i>		<i>c.2138A>T</i>		<i>c.-928G>C</i>		<i>c.77-109C>G</i>		<i>c.105T>C</i>		<i>c.*65C>T</i>		<i>c.*3879C>T</i>	
MCP-1	Parameter	<i>n</i>	<i>P</i>	<i>n</i>	<i>P</i>	<i>n</i>	<i>P</i>	<i>n</i>	<i>P</i>	<i>n</i>	<i>P</i>	<i>n</i>	<i>P</i>	<i>n</i>	<i>P</i>
<i>Metabolic syndrome</i> [†] Parameter from IDF definition		c:665/ m:841	0.12	c:683/ m:874	0.59	c:682/ m:872	0.97	c:683/ m:872	0.46	c:667/ m:833	0.44	c:677/ m:866	0.71	c:687/ m:873	0.64
	Waist circumference	1167	0.59	1211	0.79	1210	0.97	1209	0.76	1166	0.35	1198	0.70	1214	0.75
	Triglycerides ^a	1153	0.0084	1196	0.41	1195	0.34	1194	0.43	1151	0.052	1183	0.34	1199	0.41
	HDL cholesterol ^a	1163	0.44	1206	0.20	1205	0.79	1204	0.19	1161	0.069	1193	0.59	1209	0.71
	Sys. blood pressure ^a	770	0.27	791	0.34	790	0.20	790	0.34	764	0.80	782	0.079	794	0.068
	Dia. blood pressure ^a	770	0.27	791	0.70	790	0.64	790	0.62	764	0.29	782	0.11	794	0.10
	T2DM [†]	c:1163/ d:222	0.23	c:1207/ d:227	0.46	c:1206/ d:225	0.089	c:1205/ d:227	0.46	c:1163/ d:214	0.83	c:1194/ d:226	0.93	c:1210/ d:227	0.67
	Fasting glucose ^a	1165	0.088	1208	0.27	1207	0.033	1206	0.19	1163	0.30	1195	0.94	1211	0.91
<i>Parameters related to the metabolic syndrome</i>															
Parameters related to obesity	BMI	1165	0.68	1208	0.51	1207	0.88	1206	0.45	1163	0.26	1195	0.83	1211	0.86
	Weight	1165	0.96	1208	0.59	1207	0.57	1206	0.53	1163	0.35	1195	0.39	1211	0.37
	Height	1167	0.14	1211	0.075	1210	0.0024	1209	0.087	1166	0.0095	1198	0.098	1214	0.063
	Waist-to-hip ratio	1167	0.087	1211	0.65	1210	0.37	1209	0.68	1166	0.63	1198	0.28	1214	0.34
	Hip circumference	1166	0.75	1210	0.73	1209	0.67	1208	0.67	1165	0.065	1197	0.53	1213	0.52
	Body fat	1157	0.49	1200	0.87	1199	0.63	1198	0.75	1156	0.064	1187	0.87	1203	0.80
Parameters related to hyperglycaemia	Adiponectin ^a	1154	0.14	1197	0.78	1196	0.14	1195	0.75	1153	0.10	1184	0.73	1200	0.84
	LDL cholesterol ^a	1162	0.96	1205	0.82	1204	0.25	1203	0.81	1160	0.77	1192	0.83	1208	0.69
	Total cholesterol ^a	1164	0.92	1207	0.61	1206	0.63	1205	0.60	1162	0.84	1194	0.58	1210	0.56
	2 h glucose ^a	1165	0.014	1208	0.56	1207	0.46	1206	0.56	1163	0.68	1195	0.47	1211	0.47
	HbA _{1c} ^a	1164	0.38	1207	0.97	1206	0.71	1205	0.96	1162	0.88	1194	0.67	1210	0.82
	Insulin ^a	1161	0.93	1204	0.74	1203	0.26	1202	0.79	1159	0.82	1191	0.73	1207	0.81
Proinflammatory parameters	HOMA-IR*	1164	0.99	1208	0.98	1207	0.44	1206	0.98	1163	0.78	1195	0.91	1211	0.88
	Leucocyte count ^a	1165	0.71	1208	0.37	1207	0.97	1206	0.36	1163	0.047	1195	0.47	1211	0.53
	MCP-1 ^a	454	0.78	479	0.38	478	0.32	477	0.39	453	0.93	476	0.012	480	0.012
	IL-6*	1161	0.19	1205	0.044	1204	0.47	1203	0.080	1160	0.77	1192	0.82	1208	0.84
	Uric acid ^a	1165	0.027	1208	0.43	1207	0.086	1206	0.44	1163	0.34	1195	0.18	1211	0.29

P, global *P* value calculated in the model free model; *n*, analysed subjects; c, analysed controls; m, analysed subjects with metabolic syndrome; d, analysed type 2 diabetes mellitus cases; sys, systolic; dia, diastolic; all variables are adjusted for sex and age, ^aBMI; ^{*}*P* values from Kruskal–Wallis test; [†]*P* values from logistic regression; *P* values below 0.05 are grey shaded.

Table 3 Characteristics of non-diabetic survey participants aged 55–74 years.

Parameters	N total (m/w)	Men	Women
Age (years)	1231 (624/607)	64.0±5.6	63.8±5.4
Body mass index (kg/m ²)	1227 (621/606)	28.0±3.5	28.4±4.7
Height (cm) ^a	1230 (623/607)	172±6.4	159±6.1
Weight (kg) ^a	1227 (621/606)	82.7±11.2	71.9±12.0
Body fat (%) ^a	1219 (618/601)	32.9±4.1	40.3±5.0
Waist-to-hip ratio ^a	1230 (623/607)	0.96±0.05	0.84±0.06
Waist circumference (cm) ^a	1230 (558/586)	99.9±9.3	89.9±10.9
Hip circumference (cm) ^a	1229 (623/606)	105±6.5	107±9.4
Uric acid (mg/dl) ^a	1231 (624/607)	6.3±1.3	4.9±1.1
HDL cholesterol (mmol/l) ^a	1229 (622/606)	1.4±0.4	1.7±0.4
LDL cholesterol (mmol/l)	1228 (622/584)	3.9±1.0	4.1±1.0
Total cholesterol (mmol/l) ^a	1230 (624/606)	6.2±1.1	6.5±1.1
Fasting glucose (mg/dl) ^a	1231 (624/607)	101±9.5	96.9±9.2
2-h glucose (mg/dl)	1231 (624/607)	115±33.1	115±30.0
Systolic blood pressure (mmHg) ^a	1227 (621/606)	139±18.8	130±19.0
Diastolic blood pressure (mmHg) ^a	1227 (621/606)	81.8±10.3	77.7±9.8
Leukocytes (×10 ⁻³ /μl) ^a	1230 (624/606)	6.2±1.7	5.9±1.3
HbA _{1c} (%)	1229 (623/606)	5.55±0.35	5.62±0.35
HOMA-IR	1224 (621/603)	2.5 (1.6/3.7)	2.3 (1.6/3.4)
Fasting insulin (mU/l)	1224 (621/603)	9.9 (6.9/12.3)	9.6 (6.9/13.8)
Triglycerides (mg/dl) ^a	1219 (617/602)	120 (82.0/165)	104 (81.0/143)
Interleukin-6 (pg/ml) ^a	1221 (618/603)	2.0 (1.1/3.4)	1.7 (1.0/2.8)
Adiponectin (μg/ml) ^a	1217 (618/599)	7.5 (5.3/9.9)	11.5 (8.7/15.0)
MCP-1 (pg/ml)	488 (266/222)	316 (220/421)	299 (224/403)

Data are presented as means ± s.d. or medians with 25th and 75th percentiles (in parentheses) for not normally distributed parameters. m, men; w, women.

^a*P* < 0.05 for sex differences in age-adjusted linear regression analysis.

different between the genotype groups, although not significantly (*P* = 0.014). Most trends for differences between the genotype groups were observed within proinflammatory parameters. The highly correlated SNPs *c.*65C > T* and *c.*3879C > T* exhibited differences in MCP-1 levels (both *P* = 0.012). Genotype groups of *c.105T > C* and *c.-2138A > T* had different leucocyte counts and IL-6 levels respectively (*P* = 0.047, 0.044). A difference in uric acid was observed between the genotype groups for *c.-3813C > T* (*P* = 0.027; Table 2).

Discussion

In the present study based on 1630 KORA S4 participants, we found that MCP-1 SNPs exhibit trends

for association with triglyceride levels, fasting and 2-h glucose, height, leucocyte count, uric acid, MCP-1 and IL-6 levels. Significant associations were observed for only *c.-928G > C* in men and haplotype analysis with height.

Metabolic syndrome and parameters from IDF definition

Metabolic syndrome The concept of MetS as a distinct disorder is controversially discussed, but clarifying the pathway will contribute to discussion and may enhance the development of new medications (12, 36). Until now, studies assessing MetS and MCP-1 levels have been rare, yielded controversial results and have only been

Table 4 Analysed single nucleotide polymorphisms (SNPs) with allele and genotype frequencies, genotyping success rates, number of genotyped subjects and *P* values of test for Hardy–Weinberg equilibrium (HWE).

SNP	Allele frequency		Genotype frequency			Call rate (%)	n	P HWE
	Major allele	Minor allele	Homozygote wild-type	Heterozygote	Homozygote minor			
<i>c.-3813C > T</i>	C: 0.853	T: 0.148	CC: 0.725	CT: 0.255	TT: 0.020	95.8	1569	0.560
<i>c.-2138A > T</i>	A: 0.800	T: 0.200	AA: 0.641	AT: 0.318	TT: 0.041	98.9	1620	0.731
<i>c.-928G > C</i>	G: 0.777	C: 0.223	GG: 0.599	GC: 0.356	CC: 0.045	98.7	1617	0.360
<i>c.76+334C > T</i>				Monomorphic				
<i>c.77–109C > G</i>	C: 0.801	G: 0.199	CC: 0.643	GC: 0.316	GG: 0.041	98.7	1616	0.740
<i>c.105T > C</i>	T: 0.622	C: 0.378	TT: 0.390	TC: 0.464	CC: 0.146	95.2	1559	0.683
<i>c.194+25C > T</i>				Monomorphic				
<i>c.*65C > T</i>	C: 0.720	T: 0.281	CC: 0.516	CT: 0.407	TT: 0.077	97.9	1604	0.681
<i>c.*3879C > T</i>	C: 0.719	T: 0.282	CC: 0.515	CT: 0.407	TT: 0.078	98.9	1620	0.857

Table 5 Haplotype analysis for height.

Haplotype	Freq	c.-3813C>T	c.-928G>C	c.77-109C>G	c.105T>C	c.*3879C>T	β	P
MCP-1*1	0.22	C	C	C	T	C	1.30	0.00020
MCP-1*2	0.09	C	G	C	C	C	0.19	0.71
MCP-1*3	0.21	C	G	G	T	C	-0.099	0.78
MCP-1*4	0.15	T	G	C	T	C	1.01	0.010
Rare	0.05	*	*	*	*	*	0.23	0.72
Reference	0.29	C	G	C	C	T		

P values below 0.05 are grey shaded; P values below 0.0004 are grey shaded and printed in bold; freq, frequency.

conducted on rather small populations (<350 individuals each) (37–39). The association between MCP-1 SNPs and MetS has not yet been investigated, but Kanda *et al.* recently suggested that MCP-1 may play an important role in the pathogenesis of MetS (9). In this study, neither an association nor a trend was observed for MetS. This suggests that MCP-1 SNPs indirectly influence MetS, as trends for associations with risk factors of MetS were found.

Triglyceride levels Epidemiological studies showed that higher MCP-1 levels were associated with higher triglycerides in healthy populations (17, 20, 40, 41). In small studies with <160 participants suffering from peripheral arterial disease and systemic lupus erythematosus, this association was also observed, although elevated MCP-1 levels may have been correlated with lipid abnormalities (42–44). Herder *et al.* found a significant positive association between systemic MCP-1 and elevated triglyceride levels in 722 subjects of KORA S4, a subgroup of our study population (28). Our results indicate that MCP-1 may influence triglyceride levels. Other cytokines, like TNF α and IL-1, have been previously shown to be involved in the regulation of serum triglyceride levels (45, 46).

Fasting glucose Association analysis for MCP-1 SNPs with fasting glucose has also not been previously reported. Epidemiological studies revealed controversial results for a correlation of fasting glucose and MCP-1 levels in type 1 and 2 diabetic patients. This controversy might be due to the influence of blood glucose on MCP-1 production in several cell types and differential glycaemic control in diabetic patients (47, 48). In this study, a trend for differences in fasting glucose was found for the different genotypes of c.-928G>C. This finding is supported by the observation that MCP-1 interferes with insulin signalling, which leads to a reduction in glucose uptake by adipocytes (7).

Type 2 diabetes Several epidemiologic studies have been conducted to discover whether MCP-1 levels are associated with T2DM, although findings were

inconsistent (17, 18, 20–22). It was recently suggested that this inconsistency results from the confounding effect of cardiovascular and cerebrovascular conditions. Considering this fact, Herder *et al.* reported that MCP-1 levels were associated with risk for incident T2DM (17). Zietz *et al.* showed in a genetic study that 426 subjects with T2DM had significantly higher MCP-1 levels, but they were not associated with the SNP -2578A>G. This lack of association might be due to co-medication with angiotensin converting enzyme inhibitors and lipid-lowering drugs, which can influence MCP-1 levels (18). In this work, no association was observed for any analysed SNP in the MCP-1 gene with T2DM, even when subjects taking lipid-lowering drugs were excluded from the analysis. This lack of association might be a power problem, as the analysed sample only included 254 T2DM cases. Larger association studies or a meta-analysis may be needed to exclude an influence of MCP-1 SNPs on T2DM.

Parameters related to risk factors of metabolic syndrome

Height Height was included in the analysis as a component of the BMI obesity parameter. Until now, no study has investigated an association of MCP-1 levels or MCP-1 SNPs with height. One recent study revealed an association of height with an IL-6 SNP. Grallert *et al.* suggested that IL-6 and other related cytokines exert an influence on osteoclast and osteoblast development and function (49). Rahimi *et al.* further reported that the MCP-1 protein is involved in osteoclast recruitment and development in mice (4). In this study, a trend was observed for c.-928G>C, c.105T>C and MCP-1*4 with height. Since height showed significant gender differences in the characteristics of the study population, it was further analysed separately for men and women. A significant association was observed in men, which implicated that the trend in the entire group was caused by men. Furthermore, haplotype analysis showed a statistically significant increase in height for carriers of MCP-1*1, which includes minor alleles of the two SNPs showing trends and c.*3879C>T (Table 5).

Accumulating evidence suggests that the receptor activator NF κ B ligand (RANKL), among others, induces the MCP-1 protein production, leading to differentiation and higher activity of osteoclasts, which could lead to increased bone resorption (4, 50). Furthermore, Evans *et al.* showed that osteoclast activity exerts an influence on long bone length (51).

Two-hour glucose Epidemiological studies on MCP-1 and 2-h glucose are rare. Only two studies have been conducted and showed that there is no association of MCP-1 levels with 2-h glucose in a population-based approach or in patients with massive weight loss by bariatric surgery (28, 52). Similar to fasting glucose, no study has investigated the influence of MCP-1 SNPs on 2-h glucose. For *c.-3813C>T*, a trend for higher 2-h glucose was observed between the genotype groups. This trend is also supported by the observation that MCP-1 reduces insulin-stimulated glucose uptake in adipocytes.

Leucocyte count Only $-2578A>G$ was analysed for associations with leukocytes or subpopulations. In two studies of 550 and 150 participants respectively, no association of this SNP with higher leucocyte counts was observed (53, 54). In this analysis, the two SNPs in strong LD with $-2578A>G$ showed no trend for differences in leukocytes between the genotype groups. However *c.105T>C* genotypes exhibited differences in leukocytes. This finding needs to be replicated, as there is no literature concerning an association of this SNP with serum leukocytes.

MCP-1 serum levels An association of SNP $-2578A>G$ with MCP-1 serum levels was investigated by several studies (18, 20–23). Although functionality of this SNP has been previously demonstrated, the results of the association studies remain contradictory (24). The SNPs *c.*65C>T* and *c.*3879C>T* exhibited trends for different MCP-1 levels between the genotype groups. These SNPs were in strong LD with $-2578A>G$, so our study did find previously demonstrated associations, even though MCP-1 levels were only measured in subjects with IGT and matched controls within each group comprising about 240 subjects. Glucose tolerance status did not correlate with MCP-1 levels (28).

IL-6 levels A single study investigated the association of the MCP-1 SNP $-2578A>G$ with IL-6 levels. Zietz *et al.* detected a negative correlation ($P=0.025$) (18). This was not observed for the two SNPs analysed, which were in strong LD with $-2578A>G$. The SNP *c.-2138A>T* exhibited a trend for differences between the genotype groups, although the strongly correlated

c.77–109C>G did not. Since MCP-1 is involved in IL-6 expression, an association seems conceivable (8), but further replication is necessary.

Uric acid Nakagawa *et al.* suggested uric acid to be related to MetS by inhibiting endothelial dysfunction (13). Therefore, uric acid was included in the present analysis. SNP *c.-3813C>T* showed a trend for differences in serum uric acid between the genotype groups. There are no previous studies on MCP-1 or MCP-1 SNPs and uric acid. Thus, our finding must be replicated.

Strengths and limitations of the study The elderly KORA S4 study population is excellently suited for a candidate gene approach in the field of MetS genetics as it is suitably phenotyped for metabolic parameters, such as fasting triglycerides, fasting plasma glucose or HOMA-IR. To our knowledge, this survey, comprising 1630 subjects aged 55–74 years, is the largest population for MCP-1 SNPs providing an OGTT, which is of great relevance when investigating associations with T2DM, MetS or related parameters. In addition, we covered the whole MCP-1 gene, from 5 kb upstream to 5 kb downstream, in our analysis.

One limitation was that MCP-1 levels were only measured in about one third of the study participants, so the power for analysing this parameter was low. Furthermore, all results generated in subjects from 55 to 74 years cannot be transferred to other age groups.

Conclusion

In the present study, we analysed SNPs of the whole MCP-1 gene in a large population-based sample and revealed trends for differences between the genotype groups for several parameters of MetS. We provide the first suggestion of a potential association of MCP-1 SNPs with triglyceride levels and fasting glucose, two components of the IDF MetS definition. We propose to evaluate additional parameters for its relation to MCP-1: 2-h glucose, serum leukocytes, IL-6 levels and uric acid. Since *c.-3813C>T* exhibited trends for triglyceride levels, 2-h glucose and uric acid, it is a target SNP for further investigation. Another new finding was the significant association of *c.-928G>C* in men and MCP-1*1 with height. Although these new findings have to be replicated first, we conclude that MCP-1 may be an interesting gene for further investigation in MetS research.

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