

Interleukin-1 System Gene Polymorphisms Are Associated with Fat Mass in Young Men

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Context: There is growing evidence for interactions between the regulation of body fat and the immune system. Studies of knockout mice indicate that IL-1 has an antiobesity effect.

Objective: The objective of the study was to investigate our hypothesis that common polymorphisms of the IL-1 system, which are associated with IL-1 activity, also are associated with fat mass.

Design, Setting, and Study Subjects: The Gothenburg Osteoporosis and Obesity Determinants (GOOD) study is a population-based cross-sectional study of 18- to 20-yr-old men (n = 1068), mostly Caucasian, from the Gothenburg area (Sweden). Three different polymorphisms, IL-1 β +3953 C/T, IL-1 β -31 T/C, and IL-1 receptor antagonist (IL-1RN) variable number tandem repeat of 86 bp, were investigated in relation to body fat mass.

Main Outcome Measure: The main outcome measures were genotype distributions and their association with body fat mass

in different compartments, measured with dual-energy x-ray absorptiometry.

Results: Carriers of the T variant (CT and TT) of the +3953 C to T (F_T = 0.25) IL-1 β gene polymorphism had significantly lower total fat mass (P = 0.013) and also significantly reduced arm, leg, and trunk fat, compared with CC individuals. IL-1RN*2 carriers with two repeats of the IL-1RN variable number tandem repeat polymorphism had increased total fat (P = 0.036), serum leptin, and fat of trunk and arm as well as serum levels of IL-1RN and IL-1RN production *ex vivo*. The IL-1 β -31 polymorphism did not correlate with the fat measurements.

Conclusions: The IL-1 system, recently shown to affect fat mass in experimental animals, contains gene polymorphisms that are associated with fat mass in young men. (*J Clin Endocrinol Metab* 91: 2749–2754, 2006)

THERE IS GROWING evidence of interactions between the regulation of body fat and the immune system. The cytokine IL-6 suppresses body fat mass and enhances energy expenditure in both mice and men (1–3). Another cytokine, IL-1 β , also exerts proinflammatory effects, and there is some overlap between the effects of IL-1 and IL-6 on immune functions. We and others have recently found indications that the IL-1 system, like IL-6, influences body fat mass. Mice with depleted IL-1 signaling due to knockout of the gene coding for the biologically active IL-1 receptor I (IL-1RI) develop obesity (4). Conversely, mice with enhanced IL-1 activity due to IL-1 receptor antagonist (IL-1RN) gene knockout are lean and resistant to diet-induced obesity (5).

The IL-1 system has several components, including two agonists, IL-1 β and the less potent IL-1 α . The biological effects are exerted via the IL-1RI. The binding of IL-1 to IL-1RI can be inhibited by the endogenous receptor antagonist IL-

1RN. The effect of IL-1 can also be inhibited by binding a second type of IL-1 receptor, IL-1RII. This receptor acts as a decoy and prevents IL-1 from binding IL-1RI. A delicate balance between IL-1 and IL-1RN is of importance for regulation of immune function (6, 7).

There are some common polymorphisms that appear to be associated with differences in the activity of the IL-1 system. The C to T single nucleotide polymorphism (SNP) at nucleotide +3953 from the transcription start of the IL-1 β gene seems to be functional because it has been associated with increased production of IL-1 β *in vitro*, worsened rheumatoid arthritis, enhanced inflammatory serum parameters, and decreased risk of certain infections (8–11). The T to C SNP at nucleotide –31 from the transcription start of the IL-1 β gene has also been associated with changes in biological parameters *in vivo* (12). The IL-1RN gene contains a polymorphic region in the second intron, which has an 86-bp variable number tandem repeat (VNTR). The presence of two repeats, IL-1RN*2, in this polymorphism has been reported to be associated with serum IL-1RN levels, production of IL-1 β *in vitro*, and occurrence of inflammatory diseases *in vivo* (7, 12–14).

In the present study, we aimed to investigate the association between genetic differences in the IL-1 system and the regulation of body fat. Therefore, common and functional polymorphisms of the IL-1 system were examined in association with several measures of fat mass in a homogenous

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Abbreviations: BMI, Body mass index; CV, coefficient of variation; DXA, dual-energy x-ray absorptiometry; GOOD, Gothenburg Osteoporosis and Obesity Determinants study; IL-1RI, IL-1 receptor I; IL-1RN, IL-1 receptor antagonist; LD, linkage disequilibrium; LPS, lipopolysaccharide; SNP, single nucleotide polymorphism; VNTR, variable number tandem repeat.

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cohort of young Swedish men. To further investigate the IL-1 system, we also measured serum levels of IL-1RN as well as IL-1RN and IL-1 β levels in cell culture medium from lipopolysaccharide (LPS)-stimulated leukocytes.

Subjects and Methods

Subjects

The present study was conducted on subjects from the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study. The subjects were 18- to 20-yr-old men ($n = 1068$), mostly Caucasian (98%), from the Gothenburg area (Sweden) who were randomly selected from national population registers (15). The GOOD study was approved by the Ethics Committee at Göteborg University. Written and oral informed consent was obtained from all study participants.

Measurement of body composition

Lean tissue mass and fat masses for total body, arm, leg, and trunk were determined by using dual-energy x-ray absorptiometry (DXA; Lunar Prodigy DXA, GE Lunar Corp., Madison, WI). The coefficient of variation (CV) values for the DXA measurements was 1.8% for lean tissue mass and 3.4% for fat measures.

Blood chemistry

Serum was obtained from whole blood using standard procedures, frozen without delay, and stored at -70°C . Leptin was analyzed in serum samples (that had not undergone additional freeze-thaw cycles) using a commercially available kit (active human leptin ELISA, Diagnostic Systems Laboratories Inc., Webster, TX) with a detection limit of 0.05 ng/ml. Intra- and interassay CVs were 6.2 and 5.3%, respectively. IL-1RN levels in serum were measured with Fluorokine MAP human IL-1ra kit (R&D, Minneapolis, MN) with a detection limit of 27.6 pg/ml and interassay CV of 14%.

Isolation of leukocytes and stimulation of cytokine release

This experiment was performed in 148 individuals drawn at random from the GOOD study. Mononuclear cells from blood collected in heparinized tubes were prepared by centrifugation on Ficoll-Hypaque (Lymphoprep; Nycomed Pharam a/s, Oslo, Norway) at $827 \times g$ for 10 min at room temperature. A total of 2×10^6 cells in a volume of 2 ml were put in the wells of a microtiter plate (Nunc; Nunc a/s, Roskilde, Denmark). LPS (1 $\mu\text{g}/\text{ml}$; Sigma, St. Louis, MO) was used as a stimulator. Wells without any stimulator were used as negative controls. The cells were then incubated in RPMI 1640 supplemented with 5×10^{-5} M 2-mercaptoethanol, 10% human AB-serum (Sera-Lab, Sussex, UK), and gentamicin (final concentration 100 U/ml; Schering-Plough International, Kenilworth, NJ) in 5% CO_2 at 37°C for 47 h. The supernatants were collected and frozen for later cytokine analyses.

IL-1 β was measured with Bio-Plex human cytokine assay (Bio-Rad Laboratories AB, Sundbyberg, Sweden). Beads coated with capture antibodies (5000 beads/well) were incubated with premixed standards or sample supernatants (50 μl) in 96-well filter plates. Plates were shaken for 30 sec at high speed (1000 rpm) and then incubated at room temperature for 30 min at low speed (300 rpm). After incubation, detection antibodies (1 $\mu\text{g}/\text{ml}$) were added and plates shaken and incubated as before. After washing using a vacuum device (Millipore Corp., Billerica, MA), streptavidin-phycoerythrin (2 $\mu\text{g}/\text{ml}$) was added to the wells, and the plates were incubated for 10 min at room temperature with shaking. After washing, the beads were resuspended in 120 μl Bio-Plex assay buffer and read by the Bio-Plex assay reader (Bio-Rad). Data were analyzed with the Bio-Plex Manager software (version 2.0; Bio-Rad).

Genomic DNA isolation

Genomic DNA was prepared from blood, using the PUREGENE DNA isolation kit (Gentra Systems Inc., Minneapolis, MN), diluted, and stored on 96-well plates at -20°C .

IL-1 β +3953 and IL-1 β -31 genotyping

The +3953 C/T and -31 T/C SNPs were genotyped using the MassARRAY technology platform (Sequenom, San Diego, CA) (16). MassARRAY Assay Design 2.0 software was used to design primers for amplification and extension. The forward and reverse amplification primers for +3953 C/T were 5'-ACGTTGGATGCAGTTCAGTGATCG-TACAGG and 5'-ACGTTGGATGGTCTCCACATTTCAGAACC, respectively. The forward and reverse amplification primers for -31 T/C were 5'-ACGTTGGATGTTCTCAGCCTCTACTTCTG and 5'-ACGT-TGGATGCCTCGAAGAGGTTTGGTATC, respectively. The extension primers for IL-1 β +3953 and -31 were 5'-CATTTTCAGAACCTATCT-TCTT and 5'-CTCCCTCGCTGTTTTTAT, respectively. The terminator mix for +3953 and -31 included ddA/C/GTP (dideoxyribonucleotide triphosphates) and ddA/C/TTP, respectively. The genotype analysis was performed according to the manufacturer's manual (Sequenom TYPHER, version 3.1) at SweGene Profiling Polygenic Diseases in Malmö, Sweden.

IL-1RN VNTR genotyping

The genotyping was performed at SweGene Profiling Polygenic Diseases. The PCR primers were labeled with FAM and HEX (DNA Technology, Aarhus, Denmark) and tailed according to Brownstein *et al.* (17). The forward and reverse primers were 5'-CTCAGCAACACTCTCTAT and 5'-GTTTCTTCTCTGGTCTGCAGGTAA, respectively. The PCRs were performed in a total volume of 6 μl containing 4 ng of template DNA, 10 mM Tris-HCl, 50 mM KCl (pH 8.3) (GeneAmp 10 \times buffer; Perkin-Elmer, Boston, MA), 0.25 U AmpliTaq Gold DNA polymerase, 2.5 mM MgCl_2 , 0.25 mM deoxynucleotide triphosphates, and 0.33 μM of each primer. Amplifications were performed using GeneAmp 9700 machines (Applied Biosystems, Foster City, CA) with dual-384 heads as follows: 95 $^{\circ}\text{C}$ for 9 min, 35 cycles of 95 $^{\circ}\text{C}$ for 1 min, 59 $^{\circ}\text{C}$ for 1 min, 72 $^{\circ}\text{C}$ for 2 min, and finally 72 $^{\circ}\text{C}$ for 10 min. The PCR products were separated on an ABI 3730 capillary machine using LIZ or ROX as size standard. Raw data were converted to genotype data using GeneMapper (version 3.0, Applied Biosystems).

Statistical analysis

Data analysis was performed with SPSS (version 13.0.1; SPSS Inc., Chicago, IL) and the genetic analysis package GAP (version 1.0–3) in R (version 2.1.1.). Existence of Hardy-Weinberg equilibrium was tested with χ^2 analysis. Because the T variant of the IL-1 β +3953 polymorphism has been reported to be dominant, we primarily pooled the CT and TT individuals for investigation of body composition, as done by several others (9–11). Moreover, the TT genotype constituted only 6.4% of the population in the present study. A potential dose effect of the T-allele was then investigated by comparing the CT and TT genotypes. The IL-1RN 86-bp VNTR polymorphism was also pooled into carriers or noncarriers of IL-1RN*2 for investigation of body composition, as done by others (13, 14, 18). Association between fat mass data and polymorphisms or serum IL-1RN was analyzed by multiple linear regression with age, present physical activity, lean tissue mass, and height as covariates. Haplotype association analyses using the function hap.score in R were performed using the same covariates as above. Measures of linkage disequilibrium were computed using the software PyPop (19). Multiallelic D' was used when applicable. Logarithmic transformations of all fat and cytokine variables were performed to obtain normally distributed variables. Beta values are standardized. $P < 0.05$ was considered significant.

TABLE 1. Characteristics of the study population

Variables	Mean \pm SD ($n = 1068$)
Age (yr)	18.9 \pm 0.6
Total fat (kg)	13.4 \pm 8.0
Total lean tissue (kg)	57.4 \pm 6.2
Serum leptin (ng/ml)	7.7 \pm 8.5
BMI (kg/m^2)	22.4 \pm 3.2
Trunk fat (kg)	6.8 \pm 4.3
Arm fat (kg)	0.56 \pm 0.41
Leg fat (kg)	2.5 \pm 1.4

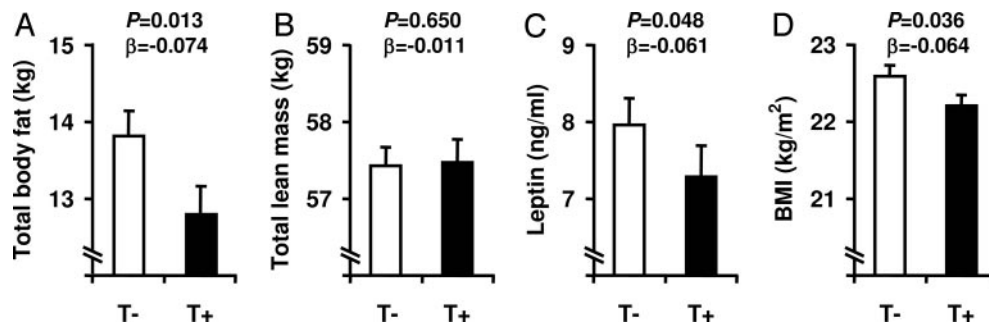


FIG. 1. Whole-body measures of fat in relation to the IL-1 β +3953 polymorphism. Total body fat (A), total lean tissue (B), leptin (C), and BMI (D) are shown. Data are mean \pm SE. Statistical significance and beta were calculated on log-transformed response by linear regression including age, present physical activity, height, and lean tissue. Note that lean tissue is not included as a covariate in panel B and that neither lean tissue nor height is included as a covariate in panel D. Also note the discontinued y-axis.

Results

General characteristics of the study population consisting of a homogenous group of well-characterized young men are shown in Table 1. To investigate whether the C to T +3953 polymorphism of the IL-1 β gene is affecting body fat, we analyzed this polymorphism in relation to fat mass determined by DXA in this cohort. Allele frequencies for C and T in the +3953 IL-1 β polymorphism were 0.75 and 0.25, respectively. The CC, CT, and TT genotypes were present in 591 (56.8%), 382 (36.7%), and 67 (6.4%) subjects, respectively. χ^2 Analysis showed no deviation from Hardy-Weinberg equilibrium ($P = 0.6$, $n = 1040$). Multiple linear regression analysis of different fat parameters in relation to the IL-1 β +3953 polymorphism showed that carriers of the T variant had significantly lower total body fat mass. The surrogate parameters body mass index (BMI) and serum leptin were also significantly decreased in T carriers. In contrast, there was no association at all between the polymorphism and total lean tissue mass (Fig. 1). Univariate analysis of total body fat also showed a significant association with the IL-1 β +3953 polymorphism (beta = -0.071 , $P = 0.023$). Additionally, the decrease in fat mass was also observed in regional measures of fat in trunk, leg, and arm (Fig. 2), whereas lean tissue mass in the same regions was not changed (data not shown). No significant differences between the CT and TT genotypes were found in any of the fat variables, indicating a lack of dose effect of the T allele. There was no association between the IL-1 β gene polymorphism and any of the covariates.

Allele frequencies for C and T in the -31 IL-1 β SNP were 0.35 and 0.65, respectively. The CC, CT, and TT genotypes were present in 130 (12.7%), 448 (43.8%), and 445 (43.5%)

subjects, respectively. The χ^2 analysis showed no deviation from Hardy-Weinberg equilibrium ($P = 0.300$, $n = 1023$). This polymorphism did not correlate with total fat mass [CC = 13.3 ± 8.4 , CT = 13.3 ± 7.3 , TT = 13.3 ± 8.3 , beta = -0.030 , $P = 0.332$, data are mean (picograms per milliliter) \pm SD] or any other of the investigated fat parameters (data not shown).

The IL-1RN*2 allele was present in 437 of 955 individuals. The allele frequencies for the most common alleles, IL-1RN*1 and IL-1RN*2, were 0.72 and 0.27, respectively. The combined frequency for the other alleles was less than 0.02. There was a slight deviation from Hardy-Weinberg equilibrium ($P = 0.042$, $n = 955$). Total body fat, leptin, and trunk and arm fat were significantly increased, whereas total lean tissue was decreased in IL-1RN*2 carriers (Table 2). Univariate analysis of total body fat showed no significant association with the IL-1RN*2 polymorphism (beta = -0.046 , $P = 0.16$). No covariates other than lean tissue were associated with the IL-1RN 86-bp VNTR polymorphism (data not shown).

Studies of linkage disequilibrium (LD) showed that IL-1 β +3953 and IL-1RN are in LD ($D' = 0.52$, $P < 0.0001$) and that the IL-1 β +3953 C allele is associated with the IL-1RN*2 allele. Additionally, there is a moderate association between this haplotype and increased total body fat ($P = 0.031$). The frequency of this IL-1 β +3953 C/IL-1RN*2 haplotype was 0.24. There were also LD between IL-1 β -31 and IL-1 β +3953 ($D' = 0.69$, $P < 0.0001$) and some LD between IL-1 β -31 and IL-1RN ($D' = 0.39$, $P < 0.0001$). However, IL-1 β -31 itself did, as previously mentioned, not correlate with any obesity parameters and was therefore not investigated further.

Serum IL-1RN levels showed a significant positive dose-dependent association with the number of IL-1RN*2 alleles.

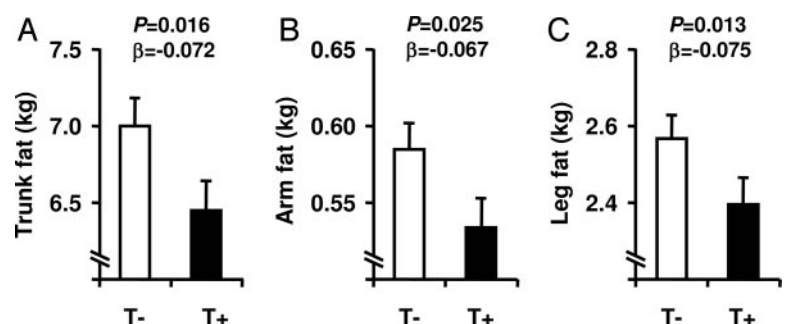


FIG. 2. Regional measures of fat in relation to the IL-1 β +3953 polymorphism. Trunk fat (A), arm fat (B), and leg fat (C) are shown. Data are mean \pm SE. Statistical significance and beta were calculated on log-transformed response by linear regression including age, present physical activity, height, and lean tissue. Note the discontinued y-axis.

TABLE 2. IL-1RN 86-bp repeat polymorphism in relation to fat measures

Variables	IL-1RN*2-/-	IL-1RN*2+/- and +/+	Beta	P
Total fat (kg)	13.0 ± 7.8	13.7 ± 8.1	0.066	0.036
Total lean tissue (kg) ^a	57.8 ± 6.1	57.1 ± 6.3	-0.050	0.048
Serum leptin (ng/ml)	7.6 ± 9.0	7.9 ± 8.2	0.070	0.028
BMI (kg/m ²) ^{a,b}	22.4 ± 3.2	22.4 ± 3.3	0.001	0.972
Trunk fat (kg)	6.5 ± 4.3	6.9 ± 4.4	0.068	0.028
Arm fat (kg)	0.54 ± 0.40	0.58 ± 0.42	0.075	0.017
Leg fat (kg)	2.4 ± 1.4	2.5 ± 1.4	0.056	0.075

Data are mean ± SD. *P* and beta are calculated on log-transformed response by linear regression. The genotypes were recoded as the following: IL-1RN*2-/- = 0 and IL-1RN*2+/- and +/+ = 1. Covariates are age, height, present physical activity (hours per week), and lean tissue mass.

^a Lean tissue mass is not included as a covariate.

^b Height is not included as a covariate.

There was no correlation between serum IL-1RN levels and the two IL-1 β polymorphisms (Table 3). Hurme and Santtila (14) reported that the association between the IL-1RN polymorphism and serum IL-1RN levels was drastically accentuated in individuals lacking IL-1 β +3953T, but we observed no such association (data not shown). The serum levels of IL-1RN had a clear-cut positive association with total body fat (beta = 0.216, *P* < 0.001).

The release of IL-1 β and IL-1RN into the cell culture medium from primary human leukocyte cultures was measured after treatment with LPS. The levels of IL-1RN in the medium were positively associated with the number of IL-1RN*2 alleles [IL-1RN*2-/- = 6131 ± 2599, IL-1RN*2+/- = 6993 ± 3037, IL-1RN*2+/+ = 8316 ± 4228, beta = 0.187, *P* = 0.030, data are mean (picograms per milliliter) ± SD] in a similar way as the serum levels. The levels of IL-1 β were not associated with any of the polymorphisms investigated (data not shown).

Discussion

In the present study, we investigated associations between genetic variations in the IL-1 system and DXA determined fat mass in a homogenous and well-characterized cohort of 18- to 20-yr-old men in the GOOD study (15). The results indicate a subtle but clearly significant association between fat mass in several compartments and polymorphisms of the IL-1 system, *i.e.* the +3953 C to T SNP of the IL-1 β gene and the 86-bp VNTR polymorphism of the IL-1RN gene. The results of several studies, as well as our own results (discussed

TABLE 3. Serum levels of IL-1RN in relation to IL-1 system polymorphisms

Gene	Genotype	IL-1RN (pg/ml)	Beta	P
IL-1 β +3953 C/T	CC	127 ± 77	0.033	0.298
	CT	136 ± 96		
	TT	128 ± 58		
IL-1 β -31	CC	140 ± 119	-0.03	0.344
	CT	129 ± 78		
	TT	130 ± 77		
IL-1RN	IL-1RN*2-/-	125 ± 63	0.091	0.005
	IL-1RN*2+/-	134 ± 90		
	IL-1RN*2+/+	155 ± 152		

Data are mean ± SD. *P* and beta are calculated on log-transformed response by linear regression. The genotypes were recoded as the following: CC = 0, CT = 1, TT = 2 for IL-1 β +3953 and -31; IL-1RN*2-/- = 0, IL-1RN*2+/- = 1, and IL-1RN*2+/+ = 2.

below), indicate that both of these polymorphisms are functional, affecting production of IL-1 β and IL-1RN, inflammation levels, and susceptibility to infections (7–11, 13, 14). Therefore, the present results suggest a link between body fat mass and genetically determined changes in the function of the IL-1 system in humans.

Iwakura and co-workers (5) found that increased IL-1 activity due to IL-1RN knockout results in resistance to obesity and subsequently increased leanness. Moreover, we recently found that IL-1RI knockout mice develop mature onset obesity (4). Taken together, these two studies indicate that IL-1RI activation can affect body fat *in vivo* over a wide dose range in experimental animals. Therefore, we hypothesized that functional polymorphisms of the IL-1 system can affect body fat accumulation in humans. The present findings support this hypothesis.

The possible mechanism for the fat-reducing effect of IL-1 could involve mediation of the effects of leptin at the hypothalamic level as indicated by studies by Rothwell and co-workers (20). IL-1 β is expressed in the hypothalamus, and the levels are enhanced by leptin and reduced by fasting (20, 21). In humans, it has been shown by Meier *et al.* (22) that the plasma levels of IL-1RN are enhanced in obese individuals. Based on their own findings and those of Rothwell and co-workers, these authors suggested that decreased IL-1 activity due to elevated IL-1RN production could contribute to leptin resistance in obese individuals (22). It remains to be investigated whether the IL-1 β +3953 or IL-1RN VNTR polymorphisms affect leptin sensitivity in humans.

Confirming earlier reports (22), we found a clear-cut positive correlation between IL-1RN levels and fat mass. The IL-1RN in serum of humans is probably to a large extent produced by adipose tissue, mainly by adipocytes but also by stromal cells (23, 24). It is possible that some of the IL-1RN in the stromal cells is produced by macrophages that accumulate in adipose tissue of obese individuals (25, 26). Although the major hypothesis is that IL-1 suppresses fat tissue mass via a hypothalamic effect (see above), it is also possible that IL-1 suppresses obesity via direct effects in the adipose tissue. IL-1 β has been reported to inhibit adipocyte differentiation from preadipocytes and also to decrease the lipid content in mature adipocytes (27). Moreover, IL-1 may increase leptin secretion from preadipocytes and seems essential for inflammation-induced release of leptin from adipose tissue (27, 28). IL-1 may also decrease the release of the

antidiabetic hormone adiponectin (29), possibly due to decreased adipocyte differentiation (27).

The results of the present study and another (30) report indicate that BMI is decreased in IL-1 β +3953 T carriers. BMI is a nonspecific measure that is influenced by fat mass, lean tissue mass, and even height (31), and during certain pathophysiological conditions, IL-1 may decrease nonfat mass (32). However, by use of DXA, we could show that IL-1 β +3953 T carriers did not have different lean tissue mass. In contrast, the fat mass was clearly decreased in several compartments. Based on our DXA data, it might be assumed that the differences in BMI in the Korean population also are mainly due to changes in body fat. Therefore, it could be concluded that the IL-1 β +3953 T carriers have changed body composition, probably including decreased fat mass, in two very different populations: Korean women aged 18–47 yr and males, mostly Caucasian, aged 18–20 yr. This suggests that the association between IL-1 and body fat regulation in humans is robust and not substantially affected by ethnicity, gender, or age. Finally, the fact that IL-1 β +3953 T is associated with both decreased fat mass and increased IL-1 activity (8) fits well with the finding that IL-1RI stimulation suppresses fat mass in mice (4).

This study shows a significant association between the IL-1RN*2 allele and obesity variables, such as total fat and serum leptin levels. Um *et al.* (18) reported that IL-1RN*2 carriers of a Korean population tended to have a higher risk of being obese, although this effect was not significant. A possible reason for the significance in our study but not the Korean study may be that our study group was three to four times larger. Alternatively, there may be gender, age, or ethnic differences.

We found that the IL-1RN gene variant IL-1RN*2, in addition to its association with obesity, was associated with a slight but clearly significant increase in circulating IL-1RN levels, in line with an earlier report in a smaller material (14). Moreover, the IL-1RN*2 variant was associated with increased IL-1RN production *in vitro*. However, we could not confirm the results of an earlier study on a material one third the size of ours (13) that this gene variant is associated with increased IL-1 β production. Based on the data from our study, we hypothesize that the IL-1RN*2 gene variant is associated with increased IL-1RN production, which in turn decreases IL-1 bioactivity and thereby contributes to obesity. This assumption mirrors the hypothesis that IL-1 β +3953T+ is associated with increased IL-1 bioactivity and thereby contributes to decreased obesity. In the present study, there was a LD resulting in an association between the IL-1 β +3953C and the IL-1RN*2 alleles in line with an earlier study (14). This haplotype was associated with obesity, as would be expected from our hypothesis. Thus, there is some evidence that the IL-1 system genes and their protein products can affect obesity. However, the increased adipose tissue in obese individuals may itself cause increased production of IL-1RN (22–24). More studies are needed to investigate these complex interactions.

Earlier results from our group show that another proinflammatory cytokine with overlapping effects to those of IL-1 β , IL-6, can influence fat mass. IL-6-deficient mice develop mature-onset obesity (1), and the weaker variant of a

functional polymorphism of the IL-6 promoter is associated with increased fat mass and decreased energy expenditure in humans (2, 3). In mice, the interactions between the effects of IL-1 and IL-6 on body fat have been described in a recent report (33). Finally, knockout mice that lack granulocyte macrophage-colony stimulating factor display a phenotype very similar to IL-6 knockout and IL-1RI knockout mice with a moderate degree of mature-onset obesity (34). All these cytokines seem to act in the central nervous system, possibly the hypothalamus (1, 21, 34, 35). Therefore, an emerging pattern seems to be that several different cytokines are regulating body fat via central effects in the absence of clinical inflammation in experimental animals and possibly also in humans.

In conclusion, this study on a cohort of more than 1000 young men shows that polymorphisms of the IL-1 system are associated with measures of fat mass in humans. This opens the possibility that the IL-1 system is linked to the development of obesity and obesity-related morbidities in humans.

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