

# Association of Interleukin-1 gene polymorphisms with central obesity and metabolic syndrome in a coronary heart disease population

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**Abstract** The objective of this study was to determine whether single nucleotide polymorphisms (SNPs) in the Interleukin-1 (*IL-1*) gene family are associated with central obesity and metabolic syndrome in a coronary heart disease population. The *IL-1 $\alpha$*  C-889T (rs1800587) and *IL-1 $\beta$*  +3954 (rs1143634) SNPs were studied in a Western Australian coronary heart disease (CHD) population ( $N = 556$ ). Subjects who were *TT* homozygous at either SNP had larger waist circumference (*IL-1 $\alpha$* : 1.8 cm greater,  $P = 0.04$ ; *IL-1 $\beta$* : 4 cm greater,  $P = 0.0004$ ) compared with major allele homozygotes. Individuals with two copies of the *IL-1 $\alpha$ :IL-1 $\beta$*  *T:T* haplotype had greater waist circumference (4.7 cm greater,  $P = 0.0001$ ) compared to other haplotypes. There was a significant interaction between the *IL-1 $\beta$*  SNP and BMI level on waist circumference ( $P = 0.01$ ). When the cohort was stratified by median BMI, *TT* carriers for *IL-1 $\beta$*  with above median BMI had greater waist circumference (6.1 cm greater,  $P = 0.007$ ) compared to baseline carriers, whilst no significant association was seen in the below median group.

Similarly, when the cohort was stratified by median fibrinogen level (*IL-1 $\alpha$*  interaction  $P = 0.01$ ; *IL-1 $\beta$*  interaction  $P = 0.04$ ), *TT* carriers for both SNPs in the above median fibrinogen group had greater waist circumference (*IL-1 $\alpha$*  2.7 cm greater,  $P = 0.007$ ; *IL-1 $\beta$*  3.3 cm greater,  $P = 0.003$ ) compared with major allele homozygotes. This association was not seen in the below median group. Also, we found a trend of increased metabolic syndrome for *IL-1 $\beta$*  *TT* homozygotes ( $P = 0.07$ ). In conclusion, our findings suggest that in a CHD population *IL-1* gene polymorphisms may be involved in increased central obesity, and the genetic influences are more evident among patients who have a higher level of obesity or inflammatory markers.

## Introduction

Obesity is a significant global health problem, which has been associated with increased risk of metabolic disorders,

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such as type 2 diabetes, and development of cardiovascular diseases. It has been suggested that obesity corresponds to a sub-clinical inflammatory condition, which can lead to development of insulin resistance and the cluster of cardiovascular risk factors associated with the metabolic syndrome through the over production of pro-inflammatory factors (Alexandraki et al. 2006; Garcia et al. 2006; Qatanani and Lazar 2007; Thalmann and Meier 2007). Whilst the aetiology of the metabolic syndrome is still not fully characterised, predisposing factors include obesity, insulin resistance, a pro-inflammatory state and genetics (Lorenzo et al. 2006). Despite the increasing prevalence of metabolic syndrome worldwide, few studies to date have attempted to identify genetic factors directly related to this condition (Miller et al. 2007).

The Interleukin 1 (*IL-1*) pathway is a key mediator of inflammatory reactions. The *IL-1* gene family consists of three structurally related polypeptides. These are Interleukin-1 $\alpha$  (IL-1 $\alpha$ ), Interleukin-1 $\beta$  (IL-1 $\beta$ ) and Interleukin-1 receptor antagonist (IL-1Ra). Both IL-1 $\alpha$  and IL-1 $\beta$  are pro-inflammatory cytokines with similar biological activities (Dinarello 1996). IL-1Ra is an anti-inflammatory cytokine that mediates an inhibitory effect on IL-1 $\alpha$  and IL-1 $\beta$  by binding to the IL-1 receptor, which in turn prevents IL-1 from binding and initiating a signal transduction. IL-1 has been demonstrated to have an influence on fat mass, fat metabolism and body mass, with IL-1 receptor knockout mice developing obesity (Garcia et al. 2006), and IL-1Ra knockout mice being leaner and resistant to diet-induced obesity (Somm et al. 2006). IL-1 has also been implicated in inducing insulin resistance (Chida et al. 2006; Jager et al. 2007). On the basis of this, polymorphisms within the IL-1 pathway may be potentially key genetic regulators of the IL-1 system and its subsequent effects on inflammation, obesity and metabolic disorders.

In this study we investigated the association of *IL-1 $\alpha$*  rs1800587 (-889 C>T) and *IL-1 $\beta$*  rs1143634 (+3954 C>T) single nucleotide polymorphisms (SNPs) with metabolic syndrome and its biological components. In the literature, both of these SNPs have been demonstrated to have a functional effect on the production of their respective proteins (Dinarello 1996; Pociot et al. 1992), in addition to being widely studied in a range of inflammation related disorders, including dermatitis (de Jongh et al. 2008) and lung cancer (Engels et al. 2007). In particular, we examined the relationship with waist circumference, as a measure of central obesity and as a central component of the metabolic syndrome. We examined these polymorphisms in subjects from a large and well-characterised Western Australian cohort of coronary heart disease (CHD) patients, who as expected had a high risk profile in terms of their metabolic and cardiovascular risk factors and increased levels of inflammatory markers (McCaskie et al. 2006). Identifying

associations between these candidate inflammatory genes and obesity-related conditions may have important implications in terms of identifying the role of IL-1 in the pathway of these conditions. Also, we wanted to test if the effects of these polymorphisms on central obesity and metabolic syndrome were influenced by the underlying obesity and pro-inflammatory states.

## Methods

### Subjects

Study participants were from the carotid ultrasound in patients with ischaemic heart disease (CUPID) study (McCaskie et al. 2006). The CUPID cohort consisted of 556 subjects aged between 26 and 60 years. Inclusion criteria for CUPID were subjects aged 60 years or less (at time of angiography) and angiographically proven coronary artery disease. All subjects had a past history of angina, unstable angina or myocardial infarction, angiographically demonstrated coronary artery disease with at least one vessel with >50% stenosis, and all were medically stable at the time of data collection. Self-administered questionnaires, anthropomorphic measurements, lipids and blood pressures were recorded as previously described (McCaskie et al. 2006). One person who had six standard deviations from the mean waist circumference was excluded.

The CUPID study was approved by the Ethics Committee of the University of Western Australia and the Sir Charles Gairdner Hospital Research Institutional Ethics Committee. All study participants gave written informed consent.

Metabolic syndrome was defined using the latest National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATPIII) standard (Grundey et al. 2005). Presence of metabolic syndrome was defined as the presence of at least three of the following indicators: central obesity, waist circumference of  $\geq 102$  cm in men and  $\geq 88$  cm in women; elevated fasting glucose,  $\geq 100$  mg/dL (5.6 mmol/L), or treatment for elevated glucose; hypertension, systolic blood pressure (SBP)  $\geq 130$  mm Hg or diastolic blood pressure (DBP)  $\geq 85$  mm, or drug treatment for hypertension; low HDL cholesterol,  $< 40$  mg/dL (1.03 mmol/L) in men and  $< 50$  mg/dL (1.29 mmol/L) in women, or treatment for reduced HDL; and hypertriglyceridemia,  $\geq 150$  mg/dL (1.7 mmol/L), or drug treatment for elevated triglyceride (TG) level.

### Laboratory measurements

Measurements of HDL, LDL and TG levels were obtained from fasting venous blood samples. Fasting plasma insulin

and glucose levels were measured as described previously (McCaskie et al. 2006; McQuillan et al. 1999).

### Genotyping

Genomic DNA was extracted from leucocytes using the salt, phenol/chloroform method. The *IL-1 $\alpha$*  SNP (rs1800587) was genotyped using restriction fragment length polymorphism (RFLP) and the PCR product was cut with *NcoI*. Primers for the *IL-1 $\alpha$*  polymorphism were 5'-AAGCTTGTTCTACCACCTGAACTAGGC-3' and 5'-TTACATATGAGCCTTCCATG-3'. The *IL-1 $\beta$*  SNP (rs1143634) was genotyped using mutation-specific PCR (msPCR). Two allele-specific forward primers 5'-TGCTCCACATTCAGAACCTATCTTCGTC-3' and 5'-TTTTATACCTAACAAACATGGTCTCCACATTCAGAACCTATCTTCGTT-3', and the reverse primer 5'-CTTGTTGCTCCATATCCTGTCCCTGGAGG-3' were used to amplify the region containing the polymorphism. The PCR product was run out on 3% agarose gel.

### Statistical analysis

The primary binary outcome analysed was the presence of metabolic syndrome as defined by the NCEP ATP III criteria. The secondary, quantitative, metabolic-related variables analysed were the five biological factors used to define NCEP metabolic syndrome, namely plasma levels of glucose, HDL, SBP, TG and waist circumference. Natural log transformations were applied to insulin, glucose and TG levels to normalise skewed distributions.

The principal explanatory variables were *IL-1 $\alpha$*  rs1800587 and *IL-1 $\beta$*  rs1143634 polymorphisms. The *IL-1 $\alpha$*  and *IL-1 $\beta$*  SNPs were coded into three classes and analysed categorically with reference to the most common homozygous genotype. The SNPs were analysed initially as a codominant factor, and explored subsequently under dominant, recessive or additive models given a nominally significant result. Each SNP was tested for departure from Hardy–Weinberg equilibrium (HWE) using JLIN (Carter et al. 2006).

Linear and logistic regressions were used to model the effects of multiple covariates on quantitative and binary outcomes. Independent predictors of each outcome of interest were determined by stepwise variable selection procedures. SimHap (McCaskie et al. 2007) was used to perform all genotypic and haplotypic association analyses. SimHap resolves all possible haplotype configurations for each individual with unknown phase (and the posterior probability of each) and uses simulation to incorporate these probabilities to correctly deal with the uncertainty associated with imputed haplotypes. As with SNP analyses, haplotypes were initially analysed as codominant

factors, relative to the most commonly occurring haplotype. Haplotypes were further explored individually (relative to all other haplotypes combined) under dominant, recessive or additive models where necessary, based on the trend of the beta-coefficients under a codominant model. Phenotype–genotype interactions were explored with SimHap, using a codominant genetic model adjusted for conventional confounders. Statistical significance was defined at the nominal 5% level. Data management and other statistical tests were conducted using *R* (Ihaka and Gentleman 1996).

## Results

### Genotypic distribution and linkage disequilibrium

The genotype frequencies for *IL-1 $\alpha$*  rs1800587 [minor allele frequency (MAF) = 0.29] and *IL-1 $\beta$*  rs1143634 (MAF = 0.21) are listed in Table 1. Pairwise linkage disequilibrium (LD) analysis showed a strong correlation between the polymorphisms ( $D' = 0.82$ ,  $r^2 = 0.46$ ,  $P < 0.0001$ ). The genotype frequencies of these SNPs were consistent with HWE ( $P = 0.83$ ,  $P = 0.99$  for *IL-1 $\alpha$* , *IL-1 $\beta$* , respectively).

### Population characteristics

Clinical characteristics of the CUPID population are detailed in Table 1, shown by *IL-1 $\alpha$*  rs1800587 and *IL-1 $\beta$*  rs1143634 genotype and overall. The cohort consisted primarily of males, the majority of whom were on lipid lowering and blood pressure lowering medication. NCEP defined metabolic syndrome prevalence reflects the elevated cardiovascular and metabolic risk factors in this population. In terms of inflammatory profile, there were significantly elevated mean CRP levels for *IL-1 $\beta$*  *TT* individuals compared with major allele carriers, even though statin cholesterol therapy (which may reduce CRP levels) occurred equally between groups. Levels of fibrinogen, a robust marker of inflammation, were significantly higher for both *IL-1 $\alpha$*  and *IL-1 $\beta$*  *TT* carriers compared with major allele carriers. This suggests that *TT* individuals for either SNP may possess a more pro-inflammatory profile.

### Association of *IL-1* polymorphisms with NCEP ATP III metabolic syndrome and waist circumference

Table 2 shows the association between *IL-1* polymorphisms and NCEP metabolic syndrome and waist circumference adjusted for conventional confounders. In addition, results of subgroup analyses are provided (detailed below). Analysis of *IL-1 $\alpha$*  rs1800587 in the whole cohort under a codominant

**Table 1** Description of the study population characteristics

Variable	<i>IL-1<math>\alpha</math></i>			<i>IL-1<math>\beta</math></i>			Overall (555)
	CC (283)	CT (225)	TT (41)	CC (348)	CT (183)	TT (23)	
Males (%) ( <i>n</i> )	87 (246)	88 (199)	86 (35)	86 (300)	90 (164)	83 (19)	87 (484)
Physician-diagnosed type 2 diabetes (%) ( <i>n</i> )	18 (50)	13 (29)	15 (6)	16 (57)	15 (27)	9 (2)	16 (87)
Physician-diagnosed hypertension (%) ( <i>n</i> )	46 (131)	48 (107)	63 (26)	47 (162)	50 (91)	57 (13)	50 (266)
Cholesterol-lowering medication (%) ( <i>n</i> )	69 (194)	65 (146)	61 (25)	68 (235)	66 (120)	61 (14)	67 (370)
Blood pressure lowering medication (%) ( <i>n</i> )	52 (147)	52 (117)	63 (26)	66 (187)	49 (90)	65 (15)	53 (292)
NCEP ATPIII metabolic syndrome (%) ( <i>n</i> )	41 (117)	36 (81)	44 (18)	41 (142)	36 (66)	48 (11)	39 (219)
Obesity (BMI $\geq$ 30) (%) ( <i>n</i> )	26 (74)	31 (69)	29 (12)	26 (90)	35 (64)	22 (5)	29 (159)
Waist circumference (cm)	95.0 (11)	94.9 (11)	96.5 (11)	94.6 (11)	95.9 (10)	97.0 (12)	95.1 (11)
Age (years)	49.7 (5.2)	49.6 (4.8)	50.1 (5.2)	49.5 (5.3)	49.8 (4.9)	51.7 (3.9)	49.7 (5.1)
Body mass index (BMI) (kg/m <sup>2</sup> )	28.3 (4.3)	28.2 (4.0)	28.4 (3.7)	28.2 (4.2)	28.5 (4.1)	28.1 (2.9)	28.3 (4.1)
Systolic blood pressure (SBP) (mmHg)	126 (17)	126 (16)	126 (17)	126 (16)	126 (17)	125 (16)	126 (16)
HDL cholesterol (mmol/L)	1.09 (0.3)	1.13 (0.3)	1.06 (0.3)	1.09 (0.3)	1.13 (0.3)	1.13 (0.3)	1.11 (0.3)
Log TG (mmol/L) <sup>a</sup>	0.58 (0.5)*	0.44 (0.5)*	0.65 (0.4)*	0.55 (0.5)	0.47 (0.5)	0.69 (0.5)	0.53 (0.5)
Log glucose (mmol/L) <sup>a</sup>	1.66 (0.3)	1.61 (0.3)	1.65 (0.3)	1.65 (0.3)	1.61 (0.3)	1.64 (0.3)	1.64 (0.3)
Log insulin (pmol/L) <sup>a</sup>	4.00 (0.6)	3.94 (0.7)	3.96 (0.6)	3.99 (0.6)	3.97 (0.6)	3.76 (0.5)	3.97 (0.6)
CRP	2.17 (2.9)	1.92 (2.7)	2.88 (2.5)	2.08 (2.9) <sup>†</sup>	2.01 (2.5) <sup>†</sup>	3.62 (2.5) <sup>†</sup>	2.10 (2.8)
Fibrinogen	3.22 (1.0)*	3.10 (0.9)*	3.48 (1.1)*	3.17 (1.0) <sup>†</sup>	3.11 (0.9) <sup>†</sup>	4.01 (1.2) <sup>†</sup>	3.18 (1.0)

Coefficients shown are mean (standard deviation) for continuous traits and % (*N*) for dichotomous traits

<sup>a</sup> Coefficients shown are geometric mean (geometric standard deviation) for natural logarithm transformed continuous traits

\*  $P < 0.05$  for between genotype variation for *IL-1 $\alpha$*

<sup>†</sup>  $P < 0.05$  for between genotype variation for *IL-1 $\beta$*

model revealed no significant association (global  $P = 0.32$ ) with metabolic syndrome. When we examined *IL-1 $\beta$*  rs1143634 under a codominant model we found a marginally non-significant association (global  $P = 0.07$ ) with metabolic syndrome for *CT* and *TT* carriers relative to major allele homozygotes. Examining this result further using a recessive model, the result remained non-significant with *TT* carriers sharing a trend to greater risk (OR = 2.57, 95% CI = 0.91–7.23,  $P = 0.07$ ) of metabolic syndrome compared with major allele carriers.

Examining the *IL-1 $\beta$*  rs1143634 polymorphism in the complete cohort we found a significant association (global  $P = 0.002$ ) between waist circumference under a codominant model, with coefficients suggesting a recessive model. Under this model the association strengthened, with minor allele *TT* homozygotes having significantly larger mean waist circumference (4 cm greater,  $P = 0.0004$ ) compared with major (*C*) allele carriers (results shown in Table 2). Whilst we found no significant association (global  $P = 0.11$ ) between waist circumference and *IL-1 $\alpha$*  rs1800587 under a codominant model, the coefficients suggested fitting a recessive model was appropriate. Analysis under this model revealed *TT* carriers had significantly larger mean waist circumference (1.8 cm greater,  $P = 0.04$ ) compared with major allele carriers (results shown in Table 2).

No significant results were found for any of the other biological factors with metabolic syndrome for either SNP (results not shown). Although a significant association was observed between *IL-1 $\alpha$*  rs1800587 and TG levels ( $P = 0.01$ ), the effect was in opposite directions for *CT* versus *TT* carriers, suggesting this result may be spurious.

Subgroup analysis of BMI on the association between *IL-1 $\alpha$*  and *IL-1 $\beta$*  and waist circumference

We conducted further subgroup analysis after finding a statistical interaction between BMI and the *IL-1 $\beta$*  SNP on waist circumference ( $P = 0.01$ ). When we stratified the CUPID group by their median BMI of 27.76 Kg/m<sup>2</sup>, we found that *IL-1 $\beta$*  *TT* carriers had significantly elevated waist circumference (6.1 cm greater,  $P = 0.007$ ) compared to major allele carriers in the above median BMI group. No significant BMI and *IL-1 $\alpha$*  SNP interaction was found ( $P = 0.12$ ), although when stratified by median BMI, *IL-1 $\alpha$*  *TT* carriers in the above median BMI group had marginally non-significantly greater waist circumference ( $P = 0.07$ ) compared to major allele carriers. There was no significant association of either SNP with metabolic syndrome in the above median BMI group, and no significant associations with waist circumference or metabolic syndrome in the below median BMI group.

**Table 2** Association of *IL-1* polymorphisms with waist circumference and NCEP ATPIII defined metabolic syndrome in all of CUPID and stratified by median BMI

	All CUPID			Below median BMI group			Above median BMI group		
	Genotype (%) (n)	Waist (cm) <sup>a</sup>	Metabolic syndrome	Genotype (%) (n)	Waist (cm) <sup>b</sup>	Metabolic syndrome	Genotype (%) (n)	Waist (cm) <sup>a</sup>	Metabolic syndrome
<i>IL-1α</i>									
CC	51.5 (283)	93.9 (93.4, 94.3)	1.00	53.1 (147)	87.1 (86.0, 88.1)	1.00	50.0 (136)	101.4 (100.4, 102.5)	1.00
CT	41.0 (225)	93.9 (93.4, 94.3)	0.81 (0.51, 1.31)	40.4 (112)	87.9 (86.7, 89.1)	0.71 (0.33, 1.53)	41.5 (113)	101.4 (100.4, 102.5)	0.84 (0.47, 1.53)
TT	7.5 (41)	95.7 (94.1, 97.4)	1.57 (0.66, 3.74)	6.5 (18)	86.4 (83.2, 89.6)	1.01 (0.20, 5.10)	8.5 (23)	104.8 (101.2, 108.3)	1.77 (0.60, 5.24)
Global P		0.04	0.32		0.46	0.67		0.07	0.40
<i>IL-1β</i>									
CC	62.8 (348)	93.9 (93.5, 94.4)	1.00	64.4 (179)	87.1 (86.1-88.0)	1.00	61.2 (169)	101.3 (100.3, 102.4)	1.00
CT	33.0 (183)	93.9 (93.5, 94.4)	0.69 (0.42, 1.14)	32.0 (89)	88.1 (86.8, 89.4)	0.38 (0.15, 0.93)	34.1 (94)	101.3 (100.3, 102.4)	1.03 (0.56, 1.89)
TT	4.2 (23)	97.9 (95.7, 100.0)	2.27 (0.79, 6.48)	3.6 (10)	86.8 (82.2, 91.4)	1.10 (0.12, 10.0)	4.7 (13)	107.4 (103.1, 111.7)	2.82 (0.77, 10.27)
Global P		0.0004	0.07		0.46	0.08		0.007	0.26

Coefficients shown are OR and 95% CI for NCEP ATPIII defined metabolic syndrome, and mean and 95% CI for waist circumference

Metabolic syndrome was adjusted for BMI, insulin level; waist circumference was adjusted for sex, insulin level, BMI, SBP

<sup>a</sup> Recessive effect modelled, i.e. reference group includes both CC and CT genotypes

<sup>b</sup> Codominant effect modelled, i.e. CT and TT genotype groups versus CC genotype reference group

### Association of *IL-1α:IL-1β* haplotypes with waist circumference

To further investigate the significant associations between TT carriers of *IL-1α* rs1800587 and *IL-1β* rs1143634 with increased waist circumference, we conducted haplotype analysis. We initially examined a global haplotype effect, comparing the influence of each of the possible haplotypes with the baseline most common C:C haplotype under a codominant model. The frequencies of the *IL-1α:IL-1β* haplotypes were: C:C (0.68), T:T (0.18), T:C (0.11) and C:T (0.03). The initial global haplotype test revealed a significant association with waist circumference (global  $P = 0.007$ ), with the T:T haplotype driving the association. We conducted analysis of the T:T haplotype under a codominant model, with the results detailed in Table 3. The results of this analysis suggested that a recessive genetic model was more appropriate. Under this model, we found that carriers of two copies of the *IL-1α:IL-1β* T:T haplotype had significantly greater waist circumference (5.7 cm greater,  $P = 0.0001$ ) compared with individuals with zero or one copy (as shown in Table 3).

### Subgroup analysis of fibrinogen levels on the association between *IL-1α* and *IL-1β* and waist circumference

As previous studies have suggested IL-1 may act differently under inflammatory conditions from those in a non-inflamed state, we conducted further analysis of the waist circumference findings using fibrinogen levels. Given the use of statins within the cohort and their lowering effect on CRP levels, we used fibrinogen rather than CRP as the marker of inflammation. After finding a statistical interaction between *IL-1α* and *IL-1β* and fibrinogen levels on the waist circumference finding (*IL-1α* interaction  $P = 0.004$ ;

**Table 3** Association of *IL-1α:IL-1β* haplotypes with waist circumference

<i>IL-1α:IL-1β</i> haplotype	Waist (cm)
0 copies T:T	93.9 (93.4, 94.4)
1 copy T:T	93.9 (93.1, 94.7)
2 copies T:T	98.6 (96.3, 101.0)
Global P	0.0005
<i>IL-1α:IL-1β</i> haplotype	Waist (cm) <sup>a</sup>
0 or 1 copies T:T	93.9 (93.5, 94.3)
2 copies T:T	98.6 (96.3, 101.0)
P value	0.0001

Coefficients shown are mean and 95% CI for waist circumference

Waist circumference was adjusted for sex, insulin level, BMI, SBP

<sup>a</sup> Recessive effect modelled, i.e. reference group includes individuals with 0 or 1 copies of the T:T haplotype

*IL-1 $\beta$*  interaction = 0.04), the cohort was divided by median fibrinogen level (3.0 g/L). The results of this analysis are shown in Table 4. In the above median fibrinogen group, the *TT* individuals for either SNP had a significantly greater waist circumference (*IL-1 $\alpha$*  2.7 cm greater,  $P = 0.007$ ; *IL-1 $\beta$*  3.3 cm greater,  $P = 0.003$ ) than major allele carriers. Whilst the results in the below median fibrinogen group for *IL-1 $\beta$*  suggested a possible recessive genetic effect, the result under a recessive model was not significant ( $P = 0.15$ ).

## Discussion

We examined the association between the two most well characterised, examined and functional SNPs within the Interleukin-1 system, *IL-1 $\alpha$*  rs1800587 and *IL-1 $\beta$*  rs1143634 SNPs, and the occurrence of metabolic syndrome and its biological components, in particular central obesity, in a CHD population. We found that *TT* homozygotes for either SNP had significantly higher mean waist circumference compared with major allele carriers. Individuals carrying two copies of the *IL-1 $\alpha$ :IL-1 $\beta$*  *T:T* haplotype likewise had significantly higher mean waist circumference. A novel finding was that the effect of the *IL-1* SNPs on waist circumference (as a measure of visceral fat mass) appeared to be modulated by the underlying state of obesity and inflammatory condition. The *TT* carriers for *IL-1 $\beta$*  in the above median BMI group had significantly greater waist circumference compared to baseline carriers, whereas no effect on waist circumference was found for *TT* carriers in the below median BMI group. Similarly, *TT* carriers for

either SNP in the above median fibrinogen group had significantly greater waist circumference, but no significant association was found in the below median fibrinogen group. For metabolic syndrome, we found a trend to increased risk of metabolic syndrome for *IL-1 $\beta$*  *TT* homozygotes. No significant associations were found with other biological components of the metabolic syndrome.

*IL-1 $\beta$*  is one of the major cytokines involved in the pathogenesis of type 2 diabetes (Alexandraki et al. 2006), and studies such as those by Salmenniemi et al. (2004) have demonstrated a significant increase in *IL-1 $\beta$*  levels associated with NCEP defined metabolic syndrome. In vitro studies have shown the *IL-1 $\beta$*  rs1143634 *TT* genotype up-regulates production of *IL-1 $\beta$*  levels (Pociot et al. 1992). The SNP is located in exon five and is a synonymous change (Phe105Phe). A recent study by Shen et al. (2007) examined NCEP defined metabolic syndrome in participants from a lipid lowering trial, and found a different *IL-1 $\beta$*  SNP was significantly associated with metabolic syndrome, though their finding for rs1143634 was not significant. Whilst less is known of the biological role of *IL-1 $\alpha$* , the C-889T *IL-1 $\alpha$*  rs1800587 polymorphism is located in the promoter region of *IL-1 $\alpha$* , and carriers of the minor *T* allele have been shown to have increased transcriptional activity of the gene (Dominici et al. 2002). We were unable to find a significant association between *IL-1* SNPs and metabolic syndrome in this study, although we observed *TT* carriers for the *IL-1 $\beta$*  SNP exhibited a trend of increased risk of metabolic syndrome, compared with major allele carriers.

In this study, homozygous *TT* carriers (for either SNP) had a significantly greater mean waist circumference (4 cm increase with *IL-1 $\beta$* , 1.8 cm increase with *IL-1 $\alpha$* ). We found a significant interaction effect between BMI on waist circumference. Stratifying by BMI revealed that the effect of the *IL-1 $\beta$*  *TT* genotype on waist circumference was greater in the above median BMI group (a 6.1 cm increase) compared with the overall cohort (4 cm). Recent work by Shen et al. (2007) supports the hypothesis of *IL-1* gene and environmental interactions with the demonstration that environmental conditions (polyunsaturated fatty acids) modulate the genetic effect of *IL-1* on metabolic syndrome.

There is strong evidence that *IL-1* is involved in body fat mass, though the direction of effect has been contradictory. Strandberg et al. (2006) found that BMI was decreased in *IL-1 $\beta$*  *T* allele carriers in a large study of young, healthy males (mean BMI 22.4; mean age 19). Um et al. (2004) found a similar result in Taiwanese females, though the frequency of the *T* allele is only 5%, compared to about 22% in Caucasians. Similarly, animal studies have demonstrated that increased *IL-1* activity through knocking out *IL-1Ra* results in decreased fat mass, and that decreased *IL-1* activity through knocking out the *IL-1* receptor leads to mature onset obesity (Garcia et al. 2006). Our results suggest the

**Table 4** Association of *IL-1* polymorphisms and waist circumference, stratified by median fibrinogen level

	Below median fibrinogen group	Above median fibrinogen group
	Waist (cm)	Waist (cm) <sup>a</sup>
<i>IL-1<math>\alpha</math></i>		
<i>CC</i>	93.1 (92.2, 93.9)	94.7 (94.1, 95.3)
<i>CT</i>	93.7 (92.8, 94.6)	94.7 (94.1, 95.3)
<i>TT</i>	92.9 (89.7, 95.9)	97.4 (95.5, 99.2)
Global <i>P</i>	0.57	0.007
<i>IL-1<math>\beta</math></i>		
<i>CC</i>	93.2 (92.4, 93.9)	94.7 (94.1, 95.3)
<i>CT</i>	93.7 (92.7, 94.8)	94.7 (94.1, 95.3)
<i>TT</i>	98.4 (91.5, 105.4)	98.0 (95.9, 100.1)
Global <i>P</i>	0.25	0.003

Coefficients shown are mean and 95% CI for waist circumference

Waist circumference was adjusted for sex, insulin level, BMI, SBP

<sup>a</sup> Recessive effect modelled, i.e. reference group includes both *CC* and *CT* genotypes

opposite effect to that seen in these studies. In support of our findings, Di Renzo et al. (2007) has demonstrated higher levels of IL-1 $\alpha$  and IL-1 $\beta$  in normal-BMI obese subjects (defined by fat mass >30% body weight and BMI <25). Similarly, Juge-Aubry et al. (2004) showed significantly upregulated IL-1 $\beta$  in visceral white adipose tissue (WAT), and speculated that the increased ratio of IL-1Ra:IL-1 $\beta$  in WAT may contribute to an increase in body fat and weight gain. Leptin has been suggested as a possible mechanism for this, with elevated IL-1 $\beta$  levels demonstrated to inhibit leptin release and gene expression (Bruun et al. 2002).

Univariate analysis indicated that the *TT* homozygous genotype, compared to the major allele, was associated with higher levels of inflammatory markers, CRP and fibrinogen (Table 1). We hypothesised that if obesity in general is already a pro-inflammatory state, then further heightened inflammation via IL-1 pathways may lead to greater central obesity. Consistent with this, we found a significant interaction effect between *IL-1* SNPs and fibrinogen levels (as a marker of inflammatory status unaffected by statin therapy) on waist circumference. When the CUPID subjects were stratified by median fibrinogen level, we found homozygous *TT* carriers (for either SNP) in the high fibrinogen group had significantly greater waist circumference (3.3 cm increase with *IL-1 $\beta$* , 2.7 cm increase with *IL-1 $\alpha$* ). We found no significant association in the below median fibrinogen group, suggesting that the effect of these polymorphisms on central obesity is related to inflammatory factors. This likewise supports our above median BMI finding, as obesity is characterised by a state of inflammation.

#### Study limitations

Our findings for waist circumference were statistically significant for *TT* genotype individuals, which, however, represented 8 and 4% of the population for *IL-1 $\alpha$*  and *IL-1 $\beta$* , respectively. It is also not possible to determine a causal association from a cross-sectional study. We acknowledge that inclusion of IL-1 cytokine levels or examination of additional IL-1 SNPs may further help to clarify our findings. Given the complex nature of the delicate balance between the pro-inflammatory and anti-inflammatory IL-1 cytokines, further studies of IL-1 are needed to help elucidate the mechanisms by which the examined polymorphisms regulate the role of Interleukins in inflammatory processes.

In summary, our findings suggest that in a CHD population, *IL-1* gene polymorphisms may be involved in increased central obesity, and the genetic influences are more evident among patients who have a high level of obesity or inflammatory markers. Whilst our findings do

not address potential causal relationships, our results add support to the key role which IL-1 plays in inflammatory pathways, and subsequent development of obesity and metabolic disorders.

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