CLINICAL STUDY

The association of interleukin-18 genotype and serum levels with metabolic risk factors for cardiovascular disease

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Abstract

Objective: Circulating levels of interleukin (IL)-18 are associated with the metabolic syndrome and risk for the development of cardiovascular disease (CVD). This study investigated the association between the circulating IL-18 levels and the −137 G/C polymorphism within the IL-18 gene with metabolic risk factors for CVD in normal-weight and obese black South African women.

Methods: Blood pressure (BP), body composition (dual-energy X-ray absorptiometer), visceral adiposity (computerized tomography), as well as fasting glucose, insulin, lipid profile, IL-18 levels, and IL-18 genotype were measured in 104 normal-weight (body mass index (BMI) ≤25 kg/m²) and 124 obese (BMI ≥ 30 kg/m²) black South African women.

Results: Subjects with a GC genotype (23%) had a greater mean arterial pressure (MAP, 90.6 ± 11.1 vs 85.5 ± 10.3 mmHg, P < 0.001) than the subjects with the GG genotype. Serum IL-18 levels were not associated with IL-18 genotype (P = 0.985); however, they significantly correlated with percentage of body fat (r = 0.25, P < 0.001), visceral adiposity (r = 0.32, P < 0.001), MAP (r = 0.22, P = 0.001), HOMA-IR (r = 0.33, P < 0.001), fasting insulin (r = 0.25, P < 0.001), triglyceride (r = 0.16, P < 0.05), and high-density lipoprotein-cholesterol (r = −0.14, P < 0.05) levels, after adjusting for age and body fatness.

Conclusions: We show for the first time that the GC genotype of the IL-18 −137 G/C polymorphism and the circulating IL-18 levels are independently associated with raised BP. Moreover, fasting IL-18 levels are associated with the other metabolic risk factors for CVD in normal-weight and obese black South African women.

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Introduction

Obesity is an increasing health problem among urbanized black South African women (1) and according to the South African Demographic and Health Survey, black women have a high prevalence of overweight and obesity (58%) when compared with black men (26%) and white women (49%) (2). Moreover, black South African women have a relatively high prevalence of central obesity (43.4%), as measured by a waist circumference > 88 cm (2, 3). Central obesity is associated with increased risk for noncommunicable diseases in these women (3), including type 2 diabetes mellitus (T2DM), hypertension, and cardiovascular disease (CVD) (2).

Obesity is not only an independent CVD risk factor (4), but also increases the incidence of other risk factors, notably high blood pressure (BP), dyslipidemia, and insulin resistance (5–7). The clustering of these metabolic risk factors, known as the metabolic syndrome, further increases the risk for CVD (8). A chronic subclinical inflammatory state is present in obesity and obesity-related disorders (9–11) and links to insulin resistance (12) and future risk of vascular disease (13).

Key mediators of this link may be pro-inflammatory cytokines. This is highlighted by studies that have measured circulating interleukin (IL)-18 levels, an important mediator of the innate immune response (14). High circulating levels of IL-18 are found in patients with acute coronary syndromes (15), and have been identified as a strong predictor of cardiovascular death (16), possibly via effects on atherosclerotic plaque destabilization (15). Moreover, IL-18 administration to mice accelerates atherosclerotic lesion development and increases plaque size (17). Conversely, IL-18 binding protein, a natural antagonist of IL-18, decreases inflammatory cell infiltration and generates a stable plaque phenotype (18). More recently, Hung et al. (19) found that serum IL-18 levels correlated with metabolic risk factors, including body mass index (BMI), waist circumference, fasting triglyceride (TG), high-density
lipoprotein-cholesterol (HDL-C), glucose, and insulin levels as well as systolic and diastolic BP in a Caucasian population.

A potentially functional G/C polymorphism within the promoter region of the IL-18 gene at position −137 has been identified (20). The C allele of this polymorphism is associated with various diseases, including type 1 diabetes (21), atopic eczema (22), and allergic rhinitis (23). Recently, a haplotype-based analysis revealed that variants of the IL-18 gene influenced circulating IL-18 levels and clinical outcome in patients with cardiac disease, suggesting that IL-18 is involved in the development of atherosclerosis and its complications (24). However, a putative link between the IL-18 gene and serum IL-18 levels as well as systolic and diastolic BP in a Caucasian population.

Therefore, the aim of this study was to examine the association between the −137 G/C polymorphism within the IL-18 gene and serum IL-18 levels with metabolic risk factors for CVD in normal-weight and obese black South African women.

Methods

Subjects

The study population consisted of a convenience sample of 104 normal-weight (BMI < 25 kg/m²) and 124 obese (BMI ≥ 30 kg/m²) premenopausal urban black South African women. Subjects were recruited from church groups, community centers, and universities, and included in the study if they were i) aged 18 to 45 years; ii) not taking medication for diabetes, hypertension, human immunodeficiency virus/acquired immune deficiency syndrome, or any other metabolic disorders; iii) not pregnant or lactating; and iv) of black South African ancestry. The Health Sciences Research Ethics Committee of the University of Cape Town gave the study ethics approval. Written informed consent was obtained from all subjects.

Testing procedures

Participants arrived at the laboratory in the morning after an overnight fast (10–12 h). Basic anthropometric measurements including weight (in light clothing), height, waist (at level of the umbilicus), and hip (largest gluteal area) circumference were taken. Body composition (fat and fat-free soft tissue) was measured using dual-energy X-ray absorptiometer (DXA: Hologic QDR 4500 Discovery-W, software version 4.40, Hologic Inc., Bedford, MA, USA) according to standard procedures in 198 of the subjects. In vivo precision (coefficients of variation) was determined for fat-free tissue mass (0.7%) and fat mass (1.67%) by measuring 30 individuals twice on the same day with repositioning. Body fat distribution was measured using a single slice computerized tomography (CT) scan at the level of the L4-L5 lumbar vertebrae in 171 of the subjects. Visceral adipose tissue (VAT), deep subcutaneous adipose tissue (SAT), and superficial SAT respectively) areas were quantified as described previously (25).

After at least 5 min of seated rest, BP was measured three times at 1-min intervals using an appropriately-sized cuff and an automated BP monitor (Omron 711, Omron Health Care, Hamburg, Germany). An average of the last two readings was used in the analysis.

Fasting blood samples were drawn from the antecubital vein for the determination of plasma glucose, and serum insulin. IL-18, total cholesterol (TC), HDL-C, LDL-cholesterol (LDL-C), TG, and free fatty acid (FFA) concentrations for DNA extractions.

Biochemical analysis

Fasting plasma glucose concentrations were determined using the glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA). Plasma insulin levels were determined by a microparticle enzyme immunoassay (MEIA: AxSym Insulin Kit, Abbot). Serum FFA concentrations were analyzed using a commercial kit (FFA Half-micro test; Roche). Blood lipids were analyzed using the Roche modular autoanalyzer and enzymatic colorimetric assays were used to analyze TC, TG, and HDL-C concentrations. The LDL-C concentrations were determined using the Friedewald formula (26). Serum IL-18 concentrations were analyzed using a commercially available immunoassay kit (Biosource Human IL-18 ELISA kit, Minneapolis, MN, USA).

DNA extraction and genotype analysis

After DNA was extracted from the venous blood samples (5 ml) using the method of Lahiri & Nurnberger (27), the −137 G/C polymorphism (rs187238) within the promoter of the IL-18 gene was genotyped in 215 of the samples using PCR-SSP in a final volume of 50 μl as previously described (20). The PCR fragments were resolved on 2% Agarose gels and stained with ethidium bromide for visualization under u.v. light.

Insulin sensitivity

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin levels (28).

Metabolic syndrome

Diagnostic criteria for the metabolic syndrome were based on the definition by the International Diabetes Federation (29). Metabolic syndrome was defined by a waist circumference > 80 cm along with any two of the following four factors: circulating TG levels.
> 1.7 mmol/l, HDL-C of <1.29 mmol/l, systolic BP > 130 mmHg or diastolic BP > 85 mmHg, and a fasting plasma glucose concentration > 5.6 mmol/l (29).

### Statistical analysis

In order to explore the metabolic effects of extreme ranges in BMI, while maintaining a unimodal distribution for all other parameters (weight, percentage of body fat, waist, VAT, SAT, BP, HOMA-IR, lipid profile, and IL-18 levels), overweight women (BMI, 25–30 kg/m²) were excluded from the study. Data are presented as medians and ranges. Non-normally distributed parameters were logarithmically transformed for parametric analysis. Analysis of covariance (ANCOVA), adjusting for age, was used to compare means for the lean and obese women. Wilcoxon Mann–Whitney nonparametric tests were used to compare medians between individuals with the different genotypes.χ² tests were used to compare genotype frequencies. Coefficients of correlation were calculated using Spearman’s nonparametric method. Multiple linear regression analyses were used to compare means of variables between genotypes, while adjusting for age, body fatness (percentage of fat), circulating IL-18 levels, and VAT. Similar models were used to assess the relationship between the specific variable and circulating IL-18 levels, while adjusting for age, body fatness (percentage of fat), IL-18 genotype, and VAT. Metabolic score is a Poisson variable and was modeled as a linear function of IL-18 level, body fatness, and age. Data were analyzed with the STATISTICA version 7 (StatSoft Inc., Tulsa, OK, USA) statistical program. Hardy–Weinberg equilibrium was tested using the Genepop web version 3.4 program (http://wbiomed.curtin.edu.au/genepop/).

### Results

#### Subject characteristics

The majority of the subjects reported being of Xhosa ancestry (N=175, 76%), with the other 24% being either of Zulu (N=4), Tswana (N=3), Sotho (N=5) or mixed (N=41) South African tribal ancestry. The normal-weight and obese subjects had similar heights (Table 1). The obese subjects were significantly older than the normal-weight subjects and as a result of this, all subsequent analyses included age as a covariate. As expected, all measures of obesity and fat distribution were significantly different between the normal-weight and obese subjects (Table 1).

Metabolic parameters for the normal-weight and obese subjects are shown in Table 2. Obese subjects had higher mean diastolic BP, but not mean systolic BP. The obese subjects also had higher fasting glucose concentrations, higher fasting plasma insulin and HOMA-IR levels, higher TG levels, but lower HDL-C concentrations than the normal-weight subjects. Serum IL-18 levels were higher in obese subjects.

#### IL-18 genotype and circulating IL-8 levels

Of the 215 subjects genotyped for the IL-18 −137 G/C polymorphism, 165 (77%) had the GG genotype and 50 (23%) had the GC genotype. None of the subjects were homozygous for the C-allele. The genotype distribution between normal-weight and obese subjects was not different (normal-weight: 78 GG, 79.6%; 20 GC, 20.4%; Obese: 87 GG, 74.4%; 30 GC, 25.6%; χ²=0.82, P=0.37). The IL-18 genotype distributions of the normal-weight (P=0.59) and obese (P=0.20) women included in this study were in Hardy–Weinberg equilibrium. Moreover, similar genotype distributions were obtained when the normal-weight and obese subjects of only Xhosa ancestry (n=170) were compared (χ²=1.39; P=0.24), indicating that there was no population stratification in this sample. Circulating IL-18 levels were not associated with the IL-18 genotype (means: GC: 243 pg/ml versus GG: 240 pg/ml; P=0.985).

When comparing the subjects according to IL-18 genotype (Table 3), age, height, weight, body fatness, and fat distribution did not differ between subjects with

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject characteristics of normal-weight and obese black South African women.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal-weight</strong></td>
<td><strong>Obese</strong></td>
</tr>
<tr>
<td><strong>N</strong></td>
<td><strong>Median (range)</strong></td>
</tr>
<tr>
<td>Age (years)</td>
<td>104</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>104</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>104</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>104</td>
</tr>
<tr>
<td>Fat (kg)</td>
<td>97</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>97</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>104</td>
</tr>
<tr>
<td>WHR</td>
<td>104</td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td>73</td>
</tr>
<tr>
<td>SAT (cm²)</td>
<td>73</td>
</tr>
</tbody>
</table>

Values are expressed as medians with ranges in parenthesis. P value adjusted for age. BMI, body mass index; WHR, waist–hip ratio; VAT, visceral adipose tissue area; SAT, s.c. adipose tissue area.
a GG and GC genotype (Table 3). However, fasting plasma IL-18 levels correlated with age, all body size measurements including body fatness, as well as VAT and SAT (Table 3). With the exception of BMI ($P = 0.04$), waist ($P = 0.03$), and SAT ($P = 0.02$), similar IL-18 genotype associations were obtained only when the subjects with both parents of Xhosa ancestry were included in the analysis (results not shown).

BP was closely associated with fasting plasma IL-18 levels, as well as IL-18 genotype, even after adjusting for total body fatness and age. Subjects with a GC genotype had a higher systolic and diastolic BP than the subjects with the GG genotype (Table 4). When VAT was entered into the models, the relationship between IL-18 genotype and systolic BP remained significant ($P = 0.047$), but was slightly weakened for diastolic BP ($P = 0.08$). Serum IL-18 levels remained independently associated with both systolic and diastolic BP ($P = 0.018$ and $P = 0.002$ respectively).

Fasting lipid profiles, plasma glucose, insulin levels and HOMA-IR did not differ between subjects with the GG and GC genotypes (Table 4). In contrast, serum IL-18 levels correlated with TG, HDL-C, fasting glucose and insulin levels, and HOMA-IR (Table 4). The association between serum IL-18 levels and fasting insulin levels and the inverse relationship with HOMA-IR was independent of genotype, total body fatness, age, and visceral obesity (Table 4).

**Metabolic syndrome score**

Twenty seven (12%) of the subjects presented with three or more risk factors, including central obesity, and were therefore classified as having the metabolic syndrome. The percentage of all subjects presenting with each trait of the metabolic syndrome is summarized in Table 5.

Fasting serum IL-18 levels increased with increasing number of metabolic syndrome parameters (metabolic syndrome score).
Table 4 Metabolic outcomes for genotypes of the −137 G/C polymorphism within the interleukin (IL-18) gene in black South African women.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GC genotype</th>
<th>Genotype</th>
<th>Serum IL-18 (pg/ml)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N Median (range)</td>
<td>N Median (range)</td>
<td>Unadjusted (N=215)</td>
<td>Adjusted (N=215)</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>165 107.5 (80.5–160.5)</td>
<td>50 111.5 (87–170)</td>
<td>0.007</td>
<td>0.028</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>165 72.5 (47.5–103.5)</td>
<td>50 76.5 (53.5–99.5)</td>
<td>0.009</td>
<td>0.046</td>
</tr>
<tr>
<td>Mean arterial BP (mmHg)</td>
<td>165 84.8 (60.3–122.5)</td>
<td>50 88.6 (64.7–123.0)</td>
<td>0.004</td>
<td>0.028</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>160 4.4 (2.9–7.6)</td>
<td>48 4.5 (3.2–5.4)</td>
<td>0.734</td>
<td>0.667</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>163 7.9 (2.4–38.7)</td>
<td>50 9.2 (3.6–45.6)</td>
<td>0.522</td>
<td>0.206</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>160 0.87 (0.26–11.11)</td>
<td>48 1 (0.39–5.95)</td>
<td>0.905</td>
<td>0.085</td>
</tr>
<tr>
<td>Lipid profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>162 0.6 (0.1–2.3)</td>
<td>49 0.7 (0.3–1.8)</td>
<td>0.236</td>
<td>0.247</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>162 3.9 (0.4–7.7)</td>
<td>49 4 (2.5–6.8)</td>
<td>0.22</td>
<td>0.195</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>162 1.3 (0.1–2.5)</td>
<td>49 1.3 (0.4–2.4)</td>
<td>0.563</td>
<td>0.563</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>162 2.2 (0.3–5.1)</td>
<td>49 2.4 (0.8–4.8)</td>
<td>0.441</td>
<td>0.239</td>
</tr>
</tbody>
</table>

Values are expressed as medians with ranges in parenthesis. Metabolic syndrome parameters defined as waist circumference ≥ 80 cm; TG, triglyceride ≥ 1.7 mmol/l; HDL, high-density lipoprotein (HDL) ≥ 1.3 mmol/l; BP, systolic blood pressure ≥ 135 mmHg or diastolic blood pressure ≥ 85 mmHg; glucose ≥ 5.6 mmol/l.

Discussion

The main findings of this study were that the GC genotype of the −137 G/C polymorphism within the IL-18 gene and increasing circulating IL-18 levels were associated with elevated BP in a sample of black South African women. Moreover, circulating IL-18 levels were associated with measures of insulin resistance (HOMA-IR) and increasing metabolic syndrome score, after adjusting for genotype, age, body fatness, and visceral obesity.

In the present study we found that the IL-18 −137 G/C polymorphism was associated with systolic and diastolic BP. IL-18 genotype, circulating IL-18 levels, and age were all significantly associated with systolic BP, accounting for 22.6% of the variability. It has been suggested that the IL-18 −137 G/C polymorphism may be functional (20), as the C allele of this polymorphism has been previously associated with other diseases such as type 1 diabetes (21), atopic eczema (22), and allergic rhinitis (23). However, this is the first study to show a genetic association between the IL-18 −137 G/C polymorphism and BP.

Recently, Tiret et al. (24) examined the genetic variability of four functionally important genes in the IL-18 system in relation to circulating IL-18 levels and...
cardiovascular mortality. Their analysis of other polymorphisms (G-887T, C-105T, S35S, A+183G, and T+533C) within the IL-18 gene did not show an association between any of the individual SNPs and IL-18 levels or cardiovascular outcome. However, a haplotype (GCAGT) of these polymorphisms was associated with circulating IL-18 levels and clinical outcome in patients with cardiac disease, but the association with cardiovascular risk was not independent of circulating IL-18 levels and explained <2% of the IL-18 interindividual variability. Although both the –137 G/C polymorphism and baseline IL-18 levels were associated with individual metabolic risk factors, there was no association between the –137G/C polymorphism and circulating IL-18 levels in this study. The IL-18 –137 G/C promoter polymorphism has been found to have clear promoter activity when stimulated with PMA and ionomycin, yet nonsignificant differences in IL-18 expression were observed between alleles (20), suggesting that the functional relevance of this polymorphism warrants further investigation.

Although the mechanisms are unclear, both endothelial cells and smooth muscle cells express the IL-18 receptor, which show enhanced expression in response to other inflammatory cytokines namely, IL-1β, tumor necrosis factor α (TNFα), IL-6, and IFN-γ (30), and the potent vasoconstrictor, angiotensin II (31). Stimulation of endothelial and smooth muscle by IL-18 produces biologically active IFN-γ, as well as IL-1β, IL-6, and TNF-α (32–34). The potential for these cytokines to be reciprocally induced by IL-18 signaling suggests a positive feedback loop in the vasculature, which might contribute to dysregulation in the inflammatory response (30). This is of particular relevance to BP regulation as high concentrations of TNF-α in the vascular lumen inhibit nitric oxide synthase activation and nitric oxide production (35), leaving unopposed vasoconstriction of endothelin-1 (36). Alternatively, or in addition, IL-18 may exert its effects on BP via the RAS system, as IL-6, IL-1, TNF-α, and IFN-γ have been shown to up-regulate angiotensin gene expression (37).

Within the South African context, black hypertensive patients respond poorly to angiotensin-converting enzyme inhibitors, unless combined with a diuretic (38), suggesting that the latter is unlikely to be the predominant mechanism by which IL-18 is involved in BP modulation in this sample. However, these same patients respond well to hydralazine, a directly acting arteriolar vasodilator (38). Interestingly, hydralazine has been shown to significantly decrease IL-18 levels in preeclamptic women when compared with controls (39); however, the exact mechanism whereby this drug causes vascular smooth muscle relaxation is not known (40).

Although the distribution of the –137 G allele in our study cohort was lower than in other populations (White Americans: GG = 51.8%, GC = 40.0%, CC = 8.2%; African-Americans: GG = 59.1%, GC = 36.3%, CC = 4.7%) (41), it is unlikely that this could account for our unique findings. Notably, this discrepancy is similar to that observed between Zulus and African-Americans when comparing the distribution of the 592 C/A polymorphism within the IL-10 gene (42). Moreover, there was no indication of population stratification in this sample as we found similar genotype distributions and associations when only the subjects of Xhosa ancestry (76% of sample) were analyzed.

Circulating IL-18 levels correlated with all parameters of the metabolic syndrome in our study, and mean serum IL-18 levels rose progressively with increasing number of metabolic risk factors. Similarly, Hung et al. (19) showed an association between the serum IL-18 levels and the metabolic syndrome in a representative sample of 910 subjects aged 27–77 years (19). Other studies examining individual risk factors for CVD have also found associations between increasing circulating IL-18 levels and individual CVD risk factors such as central adiposity (43, 44), hyperglycemia (45), and insulin resistance (43, 46).

The associations between circulating IL-18 levels and features of the metabolic syndrome in our study population were perhaps surprising given the relatively young age (~27 years) and health status of the subjects. Only 27 (12%) of the subjects presented with the metabolic syndrome, with <5% of the subjects having TG or blood glucose levels above the cut-points for the metabolic syndrome. In contrast, 45% of the women had low HDL-C levels, which most likely represents an artifact of low TC levels in the black population (47–49). A posteriori analysis using TC/HDL ratio > 4.4 as diagnostic criteria for the metabolic syndrome (50, 51) revealed that only 6% (n=14) of the subjects were at risk. Nonetheless, IL-18 appears to represent an early marker of disease risk. Indeed, a prospective study in a population-based cohort of middle-aged men and women (MONICA/KORA Augsburg Study, 1984–2002) showed an association between circulating IL-18 levels and risk of developing T2DM, which was independent of other known risk factors for type 2 diabetes and other inflammatory markers (52). Therefore, the
pro-inflammatory cytokine IL-18, released from adipose tissue, might provide a link between metabolic risk factors, T2DM and CVD.

Conclusion

In the current study we show that increased circulating IL-18 levels were associated with measures of insulin resistance, raised BP, and increasing metabolic syndrome score, after adjusting for age, body fatness, and visceral obesity in a population of black South African women. This study therefore strengthens the putative role of IL-18 as an inflammatory mediator of obesity-related CVD. Further research is required to verify the association between the −137 G/C polymorphism within the IL-18 gene and BP in a higher risk population.

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