The interleukin-6 –174 G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men

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Aims Inflammation is a key component of coronary heart disease, and genes coding for cytokines are candidates for predisposing to coronary heart disease risk. We have examined the effect of two polymorphisms (−174G>C and −572G>C) in the promoter of the interleukin-6 (IL-6) gene on risk of coronary heart disease, and on intermediate risk traits including fibrinogen and systolic blood pressure, in 2751 middle-aged healthy U.K. men.

Results The −174C allele (frequency 0·43, 95% CI 0·42–0·44) was not associated with significant effects on fibrinogen levels, but was associated with a significantly (P=0·007) higher systolic blood pressure (mean mmHg (95% CI): GG=135·5 (134·3–136·7); GC=137·9 (136·9–138·9); CC=138·0 (136·3–139·8)). This effect was of similar magnitude in smokers and non-smokers, and was greater in men in the top two tertiles of body mass index (>24·86 kg . m⁻²) than in those in the bottom tertile. Compared to those with the genotype GG, men carrying the −174C allele had a relative risk of coronary heart disease of 1·54 (95% CI 1·0–2·23, P=0·048) and this effect was greatest in smokers (compared to GG non-smokers, RR 2·66, CI 1·64–4·32). These effects remained statistically significant after adjusting for classical risk factors including blood pressure (P=0·04). The −572C allele (frequency 0·05, 0·04–0·06) was not associated with a significant effect on blood pressure, fibrinogen or relative risk of coronary heart disease. In a subset of the genotyped men (n=494), carriers of the −174C allele had higher levels of C-reactive protein than non-carriers.

Conclusions These data confirm the importance of the inflammatory system in the development of coronary heart disease. They suggest that, at least in part, the effect of the IL-6 −174G>C polymorphism on blood pressure is likely to be operating through inflammatory mechanisms, but the genotype effect on coronary heart disease risk is largely unexplained by its effect on blood pressure. The molecular mechanisms whereby genetically determined differences in plasma levels of IL-6 are having these effects remain to be determined.

Key Words: Interleukin-6, polymorphisms, systolic blood pressure, inflammation, prospective study, smoking.

Introduction

Inflammation is a key component of atherosclerotic disease, and genes coding for inflammatory cytokines are therefore candidates for predisposition to risk of coronary heart disease. Interleukin-6 (IL-6) is involved in inflammation, bone metabolism, immunity, reproduction, neural development and haematopoiesis[3,11], and in particular, it is a major regulator of the synthesis of acute phase reactants by the liver[1,2]. The source of IL-6 is generally assumed to be macrophages activated by infection, or undergoing inflammatory activation in the vessel wall, with additional contributions from fibroblasts and endothelial cells. However, increased IL-6 production may occur in the absence of infections, and its role in coronary heart disease may be more central than that of C-reactive protein or fibrinogen, both of which are elevated in patients with coronary heart
Adipose tissue is another major source of IL-6, accounting for up to 30% of total circulating concentrations of IL-6 in healthy subjects\(^\text{[a]–[c]}\). IL-6 mRNA and protein have been demonstrated in adipose tissue explants and adipocyte cell lines\(^\text{[d, e]}\). This may be of direct clinical relevance to the aetiology of heart disease in obese subjects. Relationships between both C-reactive protein and IL-6 with markers of endothelial dysfunction and features of the insulin resistance syndrome—dyslipidaemia, hypertension, and impaired fibrinolysis\(^\text{[f, g]}\), as well as with coronary heart disease\(^\text{[h, i]}\) and unstable angina\(^\text{[99]}\).

We have reported a common G>C polymorphism 174 base pairs upstream from the start of the transcription of the IL-6 gene\(^\text{[10]}\). This variation effects promoter function in vitro, and is associated with differences in plasma IL-6 levels in a small group of healthy subjects\(^\text{[10]}\). The role of the −174G>C polymorphism in determining the pathogenesis of atherosclerotic disease has recently been explored in subjects taking part in the U.K. Small Aneurysm Trial\(^\text{[11]}\). The results showed an association between the −174C allele and higher plasma levels of IL-6 and that subjects with the C allele had a higher mortality over 5 years follow-up\(^\text{[12]}\). Using a single strand conformation polymorphism technique we have now identified a G>C polymorphism at position −572. In this paper we have investigated the effects of the −174 and −572 IL-6 polymorphisms on several intermediate risk factors for coronary heart disease, and risk of clinical coronary heart disease events, in healthy U.K. men. Given that adipose tissue contributes a substantial amount of the total circulating IL-6, we also examined the interaction between IL-6 genotype and measures of obesity with respect to risk factors.

Materials and methods

Subjects

3052 healthy men aged 50 to 61 years, registered with nine general medical practices, were recruited for prospective surveillance as described\(^\text{[13, 14]}\). Briefly, all were free of a history of unstable angina, myocardial infarction or evidence of a silent infarction, coronary surgery, aspirin or anticoagulant therapy, cerebrovascular disease, malignancy (except skin cancer other than melanoma), or any condition precluding informed consent. The final response rate was 77% (total eligible 3984). Each participant attended non-fasting, having been instructed to avoid heavy meals before examination and to refrain from smoking or vigorous exercise from midnight beforehand. Each answered a questionnaire for smoking habit\(^\text{[15]}\). A standard 12-lead electrocardiogram (ECG) was recorded and coded according to Minnesota criteria\(^\text{[16]}\) (42 men with changes indicative of myocardial infarction (codes 1, 12, 1, to 1,2,7 or 1,2,8 plus 5,1 or 5,2) were excluded from the study).

To date there have been over 22 000 person-years of follow-up in individuals who have been genotyped. Survivors have been recalled annually for interview and blood lipid concentrations were measured at baseline and yearly for 6 years. A routine ECG was repeated at the sixth examination. Coronary heart disease events taken as end points were sudden coronary death, fatal (sudden or not) and non-fatal myocardial infarction, based on WHO criteria\(^\text{[17]}\), plus coronary artery surgery and Q wave changes indicative of silent myocardial infarction on the follow-up ECG (in which case the time to event was assumed to have been mid-way between the baseline and follow-up records). Clinical information for each event was assembled by enquiries through the participating practices, hospitals attended and, for fatal events, coroners’ offices. This was collated and submitted to an independent assessor who assigned qualifying events to the appropriate category. Height (m) was measured on a stadiometer and weight (kg) on a balance scale to calculate body mass index (kg.m\(^{-2}\)). Blood pressure was recorded twice with a random zero mercury sphygmomanometer after the subject had been seated for 5 min and averaged for statistical analysis.

Biochemical measures

A 5 ml sample of venous blood was taken by the Vacutainer technique (Becton Dickinson, Cowley, Oxford, U.K.) into a glass tube. Serum was transferred to plastic screw-cap vials (Nunc) and stored at −40 °C pending analysis. Cholesterol and triglyceride concentrations were determined by automated enzyme procedures with reagents from Sigma (Poole, Dorset, U.K.) and Wako Chemicals (Alpha Laboratories, Eastleigh, U.K.) respectively. In a subset of the NHIS-II men (all from the North Mymms and Parkstone practices plus a randomly selected group from the entire cohort) C-reactive protein was measured using the C-reactive protein ELISA HS kit from Kordia (U.K.), with an inter-assay CV of 9-7% and an intra assay CV of 8.5%.

DNA extraction SSCP analysis and genotyping

DNA was extracted by the salting-out method\(^\text{[18]}\). SSCP analysis was carried out as described\(^\text{[10]}\), using oligo primer pairs in the introns to give coverage of the entire coding exons plus 15–20 bases of the intron–exon junctions (primer sequences available from the authors on request). Sequencing of detected polymorphisms were carried out as described\(^\text{[10]}\). Genotyping for the −174G/C polymorphism was carried out by polymerase chain reaction and NlaIII digestion as previously reported\(^\text{[10]}\). Genotyping for the −573 G/C polymorphism was carried out by polymerase chain reaction (forward primer GAGACGCCTTGAAGTACTGC; reverse primer: GAGTTCCTGTGACTCCATCGCAG), followed by
Statistical methods

All statistical analyses were carried out using STATA (Intercooled Stata 6.0). Only samples from white Caucasian individuals were included in the analysis. Observed and expected (from Hardy–Weinberg proportions) genotype distributions were compared using a chi-square table, and the extent of allelic association (delta) was estimated as described[10]. Concentrations of serum triglyceride, fibrinogen, C-reactive protein, systolic and diastolic blood pressure and body mass index were log transformed. Transformations were necessary to ensure that assumptions required for the statistical analysis were not invalidated. In order to present results in a more familiar form, for those variables requiring log transformations, the means are the antilog of the mean of log transformed values, and standard deviations given are approximate.

Differences in baseline characteristics between cases and controls were assessed using t-tests for continuous variables and chi-squared tests for binary variables. Non-parametric tests were also considered as appropriate. The associations between genotype and plasma levels of selected key traits were tested by ANCOVA, using log transformed data where appropriate. The associations of genotype and smoking status with coronary heart disease risk were assessed by Cox proportional hazards models and results are presented as hazard ratios with 95% confidence intervals (CI). Interaction terms were included to test for differences in the smoking effect by genotype. Data for never- and ex-smokers were combined for analysis. In comparing current smokers to non-smokers in this way, the estimates of risk of coronary heart disease due to smoking would be conservative.

In NPHSII, age, body mass index, baseline levels of plasma cholesterol, fibrinogen, systolic blood pressure and smoking were statistically independent determinants of risk of coronary heart disease[14], and risk estimates were adjusted for these factors. Kaplan–Meier plots were used to compare risk graphically in subgroups. Correlations considered were between appropriately transformed variables. Throughout a P-value of <0·05 was taken as statistically significant. Power calculations were carried out using the 'Epi Info 6.0' package (sample size for case-control) using a=0·05 and 1–β=0·80.

Results

Identification of IL-6 gene variants

Using SSCP analysis all coding exons plus intron-exon junctions were screened for common polymorphisms in DNA from 45 NPHS men selected at random from the whole group. Only a 'wobble position' polymorphism in codon F201 (TTT>TTC) was identified in 2/45 subjects. Since this was unlikely to be of functional significance it was not studied further. Screening of the promoter region up to −1180 bp from the start of transcription revealed four sequence changes; −628C>A in 1/94 subjects (which was not pursued further because of low frequency), −598G>A, −572G>C and the previously described −174G>C polymorphism[10]. For −598G>A, allele frequency was 0·43 (0·36–0·50) in 99 subjects previously described[10]. This was almost identical to that for the −174G>C polymorphism with strong allelic association between the two (Delta=0·04, P=0·0005), and for this reason this polymorphism was not pursued further.

Genotypes and risk factors

Genotyping was carried out for the −174G>C and −572G>C polymorphisms in the entire NPHS-II sample, and the baseline characteristics for the genotyped men with or without an event are presented in Table 1. Compared with the event-free group, men with a coronary heart disease event had a significantly higher mean body mass index and systolic blood pressure and higher concentrations of plasma fibrinogen, cholesterol and triglycerides. In those who had an event, 40·1% were smokers as compared with 27·5% of those who remained free of an event.

Genotype distributions and allele frequencies are also shown in Table 1. For both polymorphisms genotype distributions in the non-event group were as expected for a sample in Hardy–Weinberg equilibrium. The frequency of the −174C allele was 0·43 (0·42–0·44), and for the −572C allele was 0·050 (0·04–0·06). For the −174G>C polymorphism the genotype distribution in the cases was significantly different from Hardy–Weinberg proportions (P=0·01), and from the frequency distribution in the non-event group (P=0·046), although the frequency of the −174C allele (0·45, 0·40–0·51) was not different from the non-event group. Compared to the men with the genotype GG, the relative risk for coronary heart disease for GC heterozygotes was 1·54 (1·06–2·22), while for CC homozygotes it was 1·11 (0·67–1·83). After adjustment for classical risk factors including systolic blood pressure these estimates remained substantially unaltered (respectively 1·55, 1·06–2·25, and 1·07, 0·65–1·77). Event rates are presented in Fig. 1 as a Kaplan–Meier survival plot, demonstrating a reduced survival rate in men with the genotype GC as compared to the GG group, while the rate in the CC group was similar to the GG subjects.
When subjects were divided into never and ex-smokers combined and current smokers there was no significant evidence for genotype–smoking interaction with respect to risk (P=0.44). However, by inspection of the risk estimates presented in Fig. 2 it was apparent that the higher risk in men with the genotype GC was confined mostly to the smokers (compared to GG non-smokers, RR 2.66 CI 1.64–4.32). The −572G>C polymorphism was not associated with a significant effect on coronary heart disease risk (compared to GG, hazard ratio for GC+CC=1.33, 0.83–2.15).

**Genotype and risk factor levels**

Analysis of baseline fibrinogen and systolic and diastolic blood pressure by IL-6 genotypes is shown in Table 2. It can be seen that the −174G>C polymorphism was not associated with a significant effect on fibrinogen concentration compared to GG (hazard ratio for GC+CC=1.33, 0.83–2.15). However, the −573G>C polymorphism was associated with a significant increase in fibrinogen concentration compared to GG (hazard ratio for GC+CC=1.33, 0.83–2.15).
associated with a significant effect on baseline systolic blood pressure (P=0.007) and diastolic (P=0.005) blood pressure, with the CC individuals having a mean systolic blood pressure approximately 2·5 mmHg higher than the GG individuals. This polymorphism was not associated with a significant effect on body mass index, fibrinogen, C-reactive protein levels or any other measured trait. The −572G>C polymorphism was not associated with a significant effect on blood pressure, C-reactive protein or fibrinogen levels.

To extend the analysis of the effect of the −174G>C polymorphism on blood pressure, data are presented in Fig. 3 showing the effect of genotype on systolic blood pressure and plasma fibrinogen in men who are current smokers compared to never and ex-smokers combined. It can be seen that the raising effect on blood pressure associated with the −174C allele was of similar magnitude in both groups (genotype–smoking interaction P=0.74), although the effect was only statistically significant in the (larger) group of non-smokers (P=0.02). As expected, fibrinogen levels were significantly higher in current smokers compared to non-smokers, but there was no evidence of an effect of −174C genotype on levels in either group. The relationship between genotype and systolic blood pressure in men in thirds of the distribution of body mass index is presented in Fig. 4. The raising effect associated with the −174C allele was smaller in men in the lowest tertile of body mass index (GG+CC 1·4 mmHg higher than GG, P=0·29) than in men in the middle and top tertile (2·9 mmHg, P=0·05

Table 2  Mean (+SE) for fibrinogen, systolic and diastolic blood pressure and C-reactive protein in NPHS men

<table>
<thead>
<tr>
<th>Traits (95% CI)</th>
<th>−174 G/C</th>
<th>−572 G/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (g l⁻¹)</td>
<td>2·71 (2·67–2·74)</td>
<td>2·71 (2·69–2·74)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>153·5 (134·3–136·7)</td>
<td>137·9 (136·9–138·9)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>82·8 (82·1–83·6)</td>
<td>84·7 (83·6–84·8)</td>
</tr>
<tr>
<td>C-reactive protein (mg l⁻¹)</td>
<td>1·14 (0·96–1·36)</td>
<td>1·26 (1·1–1·44)</td>
</tr>
</tbody>
</table>

P values from ANCOVA after adjustment for age, smoking habit and body mass index. *P value for linear trend=0·13.
and 2.6 mmHg, $P=0.05$, respectively), although the interaction was not statistically significant ($P>0.4$). A similar effect by body mass index on diastolic blood pressure was also observed (not shown). Blood pressure data were available for most men from several annual visits, and the effect of genotype on systolic blood pressure was maintained throughout follow-up, with a relatively constant difference in blood pressure between the genotypes over time (not shown).

## C-reactive protein, blood pressure and genotype

Plasma levels of C-reactive protein were available in 564 men and the correlation between C-reactive protein and other coronary heart disease risk factors is shown in Table 3. As can be seen, C-reactive protein showed a significant correlation with body mass index, fibrinogen, triglyceride and systolic blood pressure, a weaker correlation with age and diastolic blood pressure, and no correlation with cholesterol. Mean C-reactive protein levels were significantly higher ($P=<0.00005$) in current-smokers (mean 1.78 mg/l, 95% CI 1.50–2.11) compared to never and ex-smokers combined (mean 1.07 mg/l, 95% CI 0.97–1.19). Differences between never- and ex-smokers were non-significant ($P=0.09$). In the group of men where C-reactive protein data were available, those with the $−174C$ allele had significantly higher systolic blood pressure ($P=0.02$) as shown in Figure 3, and although C-reactive protein levels were higher in the C allele group these differences were not statistically significant ($P$ value for linear trend across $−174$ genotypes=$0.13$). Although there was significant
evidence of a relationship between C-reactive protein and systolic blood pressure, \( P = 0.01 \), after adjusting for age, body mass index, smoking and practice, this effect was no longer statistically significant \( P = 0.17 \). However, after adjusting for C-reactive protein there remained significant evidence of an association between systolic blood pressure and genotype \( P = 0.04 \).

### Discussion

The study presented here was based on the hypothesis that variation in the promoter region of the IL-6 gene, if of functional consequence, would influence IL-6 gene transcription and thus affect plasma or tissue levels of IL-6 which would in turn influence both plasma levels of key coronary heart disease risk factors and thus coronary heart disease risk itself. Two variants were studied, the \(-174\text{G}>\text{C}\) change previously identified by us\[^{10}\] and a newly identified site \(-572\text{G}>\text{C}\), which was also recently reported by others\[^{20}\]. For the \(-572\text{G}>\text{C}\) polymorphism no association was observed with levels of any trait or risk. The low frequency of the \(-572\text{C}\) allele means that the sample only has adequate power to detect a 1.9-fold higher risk in carriers, and although it cannot be ruled out that this polymorphism may have an effect on risk in some situations, the data imply that this polymorphism has negligible or extremely modest effects on determining plasma IL-6 levels and risk in healthy middle aged men. This is supported by the reported lack of major impact on promoter strength in vitro at least in the HeLa cells used\[^{20}\].

The major novel finding of this study was the association between IL-6 \(-174\text{G}>\text{C}\) genotype and blood pressure. In these healthy men the \(-174\text{C}\) allele was associated with an approximate difference of 2.4–2.5 mmHg in mean systolic blood pressure between the common \(-174\text{G}\) allele homozygotes and subjects carrying one or more \(-174\text{C}\) allele, who altogether represent 68% of the population. Although the effect is modest it appears statistically robust, being seen in smokers and non-smokers, in men in all thirds of body mass index and being maintained over 5 years of follow-up. Elevated systolic blood pressure is an accepted risk

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### Table 3

<table>
<thead>
<tr>
<th>Correlation between C-reactive protein and coronary heart disease risk factors in 564 NPHS-II men</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>CRP</td>
</tr>
<tr>
<td>( P ) value</td>
</tr>
</tbody>
</table>

**CRP=C-reactive protein.**

*Correlations relate to log transformed data.*

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### Table 4

**Mean (± SE) C-reactive protein and systolic blood pressure in men with different \(-174\text{G}>\text{C}\) genotype**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>C-reactive protein (mg L(^{-1}))</th>
<th>Systolic blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>1·40 (1·958, 3·157) [170]</td>
<td>135·5 (134·3, 136·7) [865]</td>
</tr>
<tr>
<td>GC+CC</td>
<td>1·300 (1·155, 1·464) [324]</td>
<td>137·9 (137·0, 138·8) [1853]</td>
</tr>
<tr>
<td>( P ) value</td>
<td>0·21</td>
<td>0·0017</td>
</tr>
</tbody>
</table>

*Analysis on log transformed data. SE are approximate.

†Genotype and C-reactive protein only available on 494 men.
The higher risk observed in carriers of the interval estimates for the CC and GC groups overlap. Compared to the GG men this would equate to approximately a 5% higher risk of myocardial infarction in those homozygous for the C allele.

The second finding of this study was the overall 1.5-fold higher risk of a coronary heart disease event in men carrying the −174C allele compared to GG homozygotes. This effect was seen most strongly in smoking men who had a risk of 2.7-fold compared to GG non-smokers, and remained statistically significant after adjustment for other baseline measured risk factors including age, body mass index and lipid and fibrinogen levels. In particular, the effect was not markedly diminished by adjustment for baseline systolic blood pressure, indicating that only part of the genotype effect on risk is likely to be operating through its effect on blood pressure. The effect on risk must be interpreted with caution. The number of events studied in this sample of healthy men is relatively small (n=162) and the confidence interval on the risk estimate is large.

Calculations indicate that the study has the power to detect a relative risk of 1.6 in carriers of the −174C allele at conventional levels of statistical significance and of 2.2 in smokers who represent 28% of the sample. Thus the estimates of risk observed are as expected for a study of this size. The estimate of risk in individuals homozygous for the −174C allele was smaller than that for GC men, and thus the pattern of risk observed is not that expected for a co-dominant model, which would be predicted if risk was operating through a simple geno-
type effect on levels of IL-6. However the CC men are the smallest group of the study and the confidence interval estimates for the CC and GC groups overlap. The higher risk observed in carriers of the −174C allele is in accord with the higher coronary mortality observed in subjects in the Small Aneurysm Trial[12], and thus these data confirm the involvement of the −174C allele as a risk factor for cardiovascular disease.

IL-6 −174G>C and effect on transcription

The data obtained in this sample of men appear to be contradictory to our earlier small study of 102 healthy men and women[10], where GG homozygotes had circulating IL-6 concentrations approximately twice as high as those homozygous for the −174C allele. Using a reporter gene assay system and transient transfection into Hela cells to determine promoter strength, we also reported that compared to the −174G allele the −174C allele showed lower basal expression and much poorer expression following stimulation with LPS or IL-1[10]. Further work from our laboratory has confirmed these effects in Hela cells (unpublished), and using different promoter fragments and constructs, a report has recently appeared confirming that variation in the IL-6 promoter is of functional significance, although finding largest effects in an epithelial-like cell line and not in Hela cells[20].

The discrepancy between the lower promoter activity in vitro of the −174C allele and the association seen in these healthy men with higher levels of C-reactive protein in vivo may be explained by several possible mechanisms. There may be differential allele expression in different tissues so that, for example, in Hela cells the −174C allele is a poor promoter but in adipose tissue is a better promoter than the −174G allele. Experiments are in progress to test promoter strength in different cell lines. IL-6 gene expression is under complex regulation, with many factors known to switch on and off transcription such as glucocorticoids, IL-1 and oestrogen (17-beta oestradiol)[22-24].

IL-6 itself also switches off its own transcription through a well described homeostatic mechanism[25]. It is therefore likely that the simple determination of reporter gene expression using only part of the IL-6 upstream regulatory sequence, and in a cell line which may not have all the necessary cellular receptors to respond appropriately, will at best be an approximation of the in vivo functional effects of a promoter variant. Thus for example in vivo the −174C allele may have a lower transcription peak after stimulation but may show a slower decline to baseline than the G allele. This would result in higher chronic levels of IL-6 in −174C allele carriers compared to GG subjects, but higher levels in GG subjects in response to acute inflammation. The data recently published supports this idea to some extent[20] and further experiments in vitro to examine this possibility are in progress.

It is worthy of note, however, that although the precise molecular mechanisms of the promoter variants reported here is unclear, they cannot be acting as markers for functional variants altering amino acids of the protein, since no variants were detected in the coding region of the gene. Similarly, although much of the introns in the gene were not studied, all intron-exon junctions were examined and no variants found, making it unlikely that common variation causing splicing differences exist as an explanation for the IL-6 genotype effects seen here. It is, however, possible that other unidentified variants may exist in regions further upstream of the start of transcription that were not studied here.

IL-6 −174G>C mechanism of effect on blood pressure

As discussed above, most probably this variant directly influences gene transcription and thus determines either basal levels of IL-6 production or levels produced in response to an inflammatory stimulation. In support of this, in subjects in the Small Aneurysm Trial the −174C allele was strongly associated with higher plasma levels of IL-6[12]. In the current study, C-reactive protein levels were only available on a subsample of the men, and since the effect associated with genotype was not
statistically significant the data provide only suggestive evidence that the \(-174C\) allele is associated with higher levels of C-reactive protein. However in this subsample \(-174G>C\) genotype was associated with a statistically significant effect on blood pressure even after adjustment for C-reactive protein levels, implying that the effect of genotype on blood pressure is not explained simply by systemic levels of inflammatory markers. This raises the possibility that local and not systemic production of IL-6 may be the key factor in determining the blood pressure effect. Several tissues and cell types are known to secrete IL-6 including monocytes and macrophages, T- and B-cells, fibroblasts, bone marrow stromal cells, endothelial cells, hepatocytes, smooth muscle cells and adipose tissue\(^{[1,7–9]}\). Of these, adipose tissue appears to be a major contributor, accounting for up to 30% of plasma levels\(^{[7]}\). The finding that the raising effect associated with the \(-174C\) allele was smaller and nonsignificant in lean men compared to men in the middle and top tertile of body mass index suggests the idea that increasing amounts of adipose tissue will lead to higher plasma levels of IL-6 and thus systemic inflammatory processes, and that this occurs in a genotype-specific manner. The suggestive association between IL-6 genotype and C-reactive protein levels but not fibrinogen levels may be because C-reactive protein gene expression is more sensitive to small changes in IL-6 than is fibrinogen gene expression. The C-reactive protein promoter contains both type-1 and type-2 IL-6 response elements\(^{[23]}\) while the fibrinogen promoters only contain type-2 elements\(^{[24]}\), and this may be the molecular explanation of this difference in genotype effect.

Given that the \(-174C\) allele appears to be associated with higher plasma levels of IL-6 in aneurysmal patients\(^{[23]}\) and of C-reactive protein (this study), there are a number of possible mechanisms by which IL-6 could affect blood pressure. IL-6 has been well characterised as an acute inducer of fibrinogen and other proteins of the acute phase response, and fibrinogen is a major determinant of blood viscosity\(^{[26]}\). Both blood viscosity and fibrinogen are positively correlated with blood pressure\(^{[27]}\). However, this is unlikely to be the mechanism by which the IL-6 polymorphism is influencing blood pressure in NPHS-II as it was not significantly associated with fibrinogen levels in this sample. A second mechanism would be via effects on angiotensinogen expression. IL-6 has been reported to induce angiotensinogen expression\(^{[25,29]}\) thus leading to higher concentrations of angiotensin II which is a potent vasoconstrictor. Levels of plasma angiotensinogen have been positively correlated with blood pressure in subjects with a family history of hypertension\(^{[40]}\), and polymorphisms in the angiotensinogen gene have been reported to affect blood pressure in some, but not all, studies\(^{[31–34]}\). In the NPHS-II men levels of angiotensinogen were not available to test this possibility directly. A third possible mechanism would be through the reported effect of IL-6 on increased vessel wall collagen synthesis\(^{[35,36]}\) and to reduce its degradation\(^{[37,38]}\). It is therefore possible that, over time, high levels of IL-6 (either systemic or locally at the site of atherosclerosis) would affect vascular compliance and therefore blood pressure.

**IL-6 \(-174G>C\) mechanism of effect on coronary heart disease risk**

In these middle-aged men, atherosclerosis is already likely to be well established, with factors determining more rapid progression of plaques and increased plaque rupture being more important in determining coronary heart disease events than the development of new lesions in initially healthy regions of vessels. High levels of IL-6 could promote plaque growth and rupture by increasing expression of several key genes. Immunohistochemistry of the human arterial atherosclerotic wall has shown IL-6 mRNA expression localized to cellular and extracellular deposits in the connective tissue matrix, with the fibrous plaque having statistically significantly higher level of IL-6 than the intima and media\(^{[39]}\). Studies in apoE knockout mice, who develop atherosclerotic plaques in the aorta, also show that elevated levels of IL-6 mRNA predominate in the plaque area compared to normal mice\(^{[39]}\). In addition, the upregulation of inhibitors of matrix metallo-proteinases by IL-6\(^{[20]}\) may shift the balance in favour of matrix deposition leading to the progression of atherosclerosis and plaque rupture. Smoking is known to damage endothelial cells, to induce macrophage recruitment in the lungs and to be associated with increased plasma levels of inflammatory markers such as C-reactive protein and fibrinogen\(^{[26–27]}\). If the IL-6 \(-174C\) allele shows a greater response over time to the inflammatory effects of smoking it may result in smokers having higher tissue levels of IL-6 compared to GG subjects.

Although the blood pressure and coronary heart disease risk effects associated with the \(-174G>C\) genotype need to be confirmed, the data support the role of the inflammatory system in the development of coronary heart disease. The observations made here refer only to male subjects and since IL-6 gene expression is down regulated by \(17-\beta\) oestradiol in oestrogen sensitive tissues such as stromal cells\(^{[24]}\) there may be an additional negative feedback loop affecting circulating IL-6 in women. Further epidemiological, physiological and cellular studies are required to examine the molecular mechanisms of these effects.

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