

# Association between the Interleukin-6 Promoter Polymorphism –174G/C and Serum Lipoprotein(a) Concentrations in Humans

Heiner K. Berthold<sup>1,2\*</sup>, Matthias Laudes<sup>3,4</sup>, Wilhelm Krone<sup>3</sup>, Ioanna Gouni-Berthold<sup>3</sup>

**1** Research Group on Geriatrics, Evangelical Geriatrics Center Berlin (EGZB), Charité University Medicine Berlin, Berlin, Germany, **2** Lipid Clinic at the Interdisciplinary Metabolism Center, Campus Virchow Clinic, Charité University Medicine Berlin, Berlin, Germany, **3** Center of Endocrinology, Diabetes, and Preventive Medicine, University of Cologne, Cologne, Germany, **4** Department of Internal Medicine I and Cluster of Excellence in Inflammation at Interfaces, University of Kiel, Kiel, Germany

## Abstract

**Background:** Lipoprotein(a) [Lp(a)] is an independent risk factor for cardiovascular disease. The interleukin-6 (IL-6) receptor antagonist tocilizumab has been shown to lower serum Lp(a) concentrations. We investigated whether the IL-6 single nucleotide polymorphism –174G/C is associated with baseline serum Lp(a) concentrations.

**Methodology/Principal Findings:** We divided 2321 subjects from the Lipid Analytic Cologne (LIANCO) cohort into 2 groups, the ones with substantially elevated Lp(a), defined as concentrations  $\geq 60$  mg/dl ( $n = 510$ ), and the ones with Lp(a)  $< 60$  mg/dl ( $n = 1811$ ). The association with the genotypes GG (33.7%), GC (50.75%) and CC (15.55%) was investigated. The GC and the CC genotype were associated with a significantly increased odds ratio of having substantially elevated Lp(a) concentrations (OR = 1.3, 95% CI 1.04 to 1.63,  $P = 0.02$  and OR = 1.44, 95% CI 1.06 to 1.93,  $P = 0.018$ ). These associations remained significant after adjusting for age, sex, smoking behavior, body mass index, serum lipoproteins, hypertension and diabetes. Of these covariates, only LDL cholesterol was significantly and independently associated with elevated Lp(a) concentrations.

**Conclusions/Significance:** The IL-6 single nucleotide polymorphism –174G/C is associated with increased odds of having elevated Lp(a). Whether this association plays a role in the Lp(a)-lowering effects of IL-6 receptor antagonists remains to be established.

**Citation:** Berthold HK, Laudes M, Krone W, Gouni-Berthold I (2011) Association between the Interleukin-6 Promoter Polymorphism –174G/C and Serum Lipoprotein(a) Concentrations in Humans. PLoS ONE 6(9): e24719. doi:10.1371/journal.pone.0024719

**Editor:** Nicholas John Timpson, University of Bristol, United Kingdom

**Received:** May 3, 2011; **Accepted:** August 17, 2011; **Published:** September 14, 2011

**Copyright:** © 2011 Berthold et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** The study was sponsored in part by the Wilhelm Doerenkamp Foundation (Cologne, Germany). No additional external funding was received for this study. Lipid Analytic Cologne (LIANCO) was supported by Bayer Vital GmbH (Leverkusen, Germany). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist. Lipid Analytic Cologne (LIANCO) was supported by Bayer Vital GmbH (Leverkusen, Germany). This does not alter the authors' adherence to all the PLoS ONE policies on sharing data and materials.

\* E-mail: heiner.berthold@charite.de

## Introduction

Lipoprotein(a) [Lp(a)] has been considered a cardiovascular risk factor for many years [1]. Together with recent genetic findings and based on robust and specific associations between elevated Lp(a) concentrations and increased cardiovascular disease risk, it is now accepted that Lp(a) is *causally* related to premature cardiovascular disease [2]. A European Atherosclerosis Society Consensus Panel recommends screening for elevated Lp(a) for all patients at intermediate or high cardiovascular risk [2]. Moreover, the panel points to the need for randomized controlled intervention trials with selective reduction in plasma Lp(a) concentrations to reduce cardiovascular disease in both primary and secondary prevention settings, as they are urgently required to define more precisely whom to treat and to what target levels [2].

There is no generally accepted drug treatment for Lp(a) lowering, although some drugs have been described to be effective [3]. Among these are niacin [4], the cholesteryl ester transfer protein (CETP) inhibitor anacetrapib [5], the thyroid hormone

analogue eprotirome [6], the apoB synthesis inhibitor mipomersen [7], and hormone replacement therapy or raloxifene, among others [3]. However, no specific therapy primarily targeting Lp(a) is available and no study until now has shown that selectively decreasing Lp(a) will decrease cardiovascular disease risk.

A recent study from our group revealed that the humanized monoclonal antibody against the interleukin-6 (IL-6) receptor, tocilizumab, decreased Lp(a) concentrations in humans [8]. Tocilizumab is approved for the treatment of rheumatoid arthritis and inhibition of IL-6 signaling seems to have multiple metabolic effects, *e.g.* decreasing insulin resistance. Variations caused by IL-6 promoter polymorphisms are associated with diabetes mellitus, obesity and several features of the metabolic syndrome [9]. The IL-6 gene single nucleotide polymorphism (SNP) –174G/C of the 5' flanking region influences plasma levels of IL-6 [10–12]. Based on our discovery that the IL-6 antibody tocilizumab decreases Lp(a), we proceeded to investigate whether the presence of this SNP is associated with baseline Lp(a) concentrations in humans.

We were able to confirm our hypothesis that the SNP –174G/C predisposes to elevated Lp(a) levels.

## Methods

### Study design and subjects

The subjects were obtained from the LIANCO database (*Lipid Analytic Cologne*) [13,14]. In brief, LIANCO was designed to assess the relationship between genetic mutations, serum lipoproteins, other biochemical parameters, and clinical data on atherosclerotic disease, hypertension and diabetes. Approval of the study protocol was obtained from the ethics committee of the medical faculty of the University of Cologne (<http://www.medfak.uni-koeln.de/179.html>).

Written informed consent was obtained from all subjects. The ethics committee approved the LIANCO study but did not specifically approve the current retrospective study. However, the current study is fully covered by the ethics committee's vote. The study was performed doing a reanalysis of existing LIANCO data, *i.e.* there were no *de novo* experiments on the subjects. Institutional Review Board (IRB) approval for this study was obtained prior to creating the LIANCO registry.

Between spring 1999 and March 2002 a total of about 5000 patients were recruited in the Cologne (Germany) area by hospitals and office-based physicians. Patients' data were recorded, their lipoproteins analyzed and samples frozen for DNA extraction.

Lp(a) was measured using an immunoturbidimetric assay [15] using a COBAS INTEGRA 400 analyser (Roche Diagnostics, Mannheim, Germany). The intra-assay coefficient of variation was 0.7–2.3% and the inter-assay coefficient of variation was 2.9–3.3%.

Lp(a) measurements were available in 3904 subjects and determination of the –174G/C SNP in 2490 subjects. A total of 2321 subjects participated in the current study, the ones in whom both measurements were available. Lp(a) concentrations were similar in the total ( $n = 3904$ ) and in the participating ( $n = 2321$ ) cohort (median [interquartile range] 19.0 [45.0] or 18.0 [42.0] mg/dl, respectively). Genotype frequencies were also similar between the total ( $n = 2490$ ) and the participating ( $n = 2321$ ) cohort (GG, GC and CC 33.49%, 50.88% and 15.62% or 33.49%, 50.87% and 15.64%, respectively).

Atherosclerotic disease was defined as the presence and/or history of  $\geq 1$  of the following: history of stroke, transient ischemic attack, prolonged ischemic neurological deficit, coronary heart disease (CHD), and peripheral vascular disease (PAD). CHD was defined as the presence of  $\geq 1$  of the following: angiographic evidence of CHD, myocardial infarction, angina pectoris, coronary bypass surgery or positive stress test. Hypertension was defined as known or newly diagnosed hypertension according to current national guidelines, *i.e.* systolic blood pressure values  $>140$  mmHg or diastolic values  $>90$  mmHg. If hypertension was controlled by medication, patients were still considered hypertensive, irrespective of their blood pressure readings.

### Detection of the –174G/C polymorphism

Genomic DNA was prepared from peripheral blood using standard techniques. The –174G/C SNP was detected by PCR followed by restriction fragment length polymorphism as previously described [16]. In short, a 198 bp fragment of the IL-6 gene was amplified. Forward and reverse primer sequences were 5'-TGACTTCAGCTTTACTCTTTGT-3' and 5'-CTGATTG-GAAACCTTATTAGG-3', respectively. Each PCR cycle consisted of denaturation for 60 seconds at 94°C, annealing for

95 seconds at 55°C, and extension for 60 seconds at 72°C, followed by a final extension at 72°C for nine minutes. PCR products were digested with *Sfa*NI (Fermentas, St. Leon-Rot, Germany) and separated by electrophoresis in 3% agarose. The presence of a single 198 bp band corresponds to the CC genotype; bands at 140 and 58 bp correspond to the GG genotype and the presence of three bands corresponds to the GC genotype.

The PCR products were automatically sequenced (ABI Prism Genetic Analyzer model 310, Applied Biosystems, Foster City, CA). The sequence of both strands was determined.

Quality checks to ensure correctness of the genotypes were carried out by independent rating of the results by two investigators. Discrepancies were resolved by either reaching consensus or re-genotyping.

### Statistical analysis

Statistical analysis was carried out using Stata Version 11 (StataCorp LP, College Station, TX). Statistical significance was defined as  $P < 0.05$  and all tests were performed 2-sided. Descriptive statistics are given, unless otherwise indicated, as mean  $\pm$  SD. Comparison of mean values was performed by unpaired Student's *t*-test. Chi square *P* values were calculated to test for differences in allele and genotype frequencies and in categorical variables. Allele frequencies were estimated by gene counting.

Two-way analysis of variance was used to establish the influence of categorical variables on continuous parameters. A logistic modeling approach was used to investigate parameters that may be associated with the presence of hypertension, diabetes, or atherosclerotic disease. Multiple logistic regression was used to adjust for the effects of sex, age, body mass index (BMI), and smoking. Lp(a) and triglyceride values were log-transformed before statistical analysis. Original rather than log-transformed values are presented in the results for clarity.

## Results

### Study cohort and biochemical analysis

The characteristics of the 2321 study subjects are presented in **Table 1**. There were 1811 subjects with Lp(a)  $<60$  mg/dl and 510 subjects with Lp(a)  $\geq 60$  mg/dl. The level of Lp(a)  $\geq 60$  mg/dl was chosen as a cut-off based on the significantly increased risk for CHD above this level, as observed in several recent studies [17–19]. There were no differences between the groups in sex distribution, age, smoking behavior, blood pressure or the presence of hypertension. BMI was lower in the group with higher Lp(a). Total cholesterol, LDL and HDL cholesterol concentrations were higher while triglycerides were lower in this group. There were no differences in lipid-lowering medication use. The rate of atherosclerotic disease was significantly higher in the high Lp(a) group (28 *vs.* 18%), while the prevalence of diabetes was lower (13 *vs.* 18%). Of the 2321 study subjects, 687 (29.6%) had Lp(a) concentrations  $<10$  mg/dl, 781 (33.6%) had Lp(a)  $\geq 10$  and  $<30$  mg/dl, 343 (14.8%) had Lp(a)  $\geq 30$  and  $<60$  mg/dl, 267 (11.4%) had Lp(a)  $\geq 60$  and  $<100$  mg/dl and 243 (10.5%) had Lp(a)  $\geq 100$  mg/dl.

### Genotyping of the –174G/C polymorphism in the study cohort

The allele frequencies of the G and C allele in the whole study population were 59.1% G and 40.9% C, respectively, whereas the matching genotype frequencies were 33.7% GG, 50.75% GC and 15.55% CC. The data are shown in **Table 2**. Genotype frequencies in the total study population were marginally

**Table 1.** Main characteristics of the study population.

Parameter	Total subjects	Subjects with Lp(a) <60 mg/dl	Subjects with Lp(a) ≥60 mg/dl	P value (Lp(a) <60 vs. ≥60 mg/dl)
Sample size	2321	1811 (78.0%)	510 (22.0%)	
Male	1248 (53.8%)	977 (53.9%)	271 (53.1%)	0.75*
Age (years)	56.9±12.4	56.9±12.3	57.0±12.6	0.96**
Smoking	Never	1634 (70.4%)	1269 (70.1%)	365 (71.6%)
	Former	198 (8.5%)	156 (8.6%)	42 (8.2%)
	Current	488 (21.0%)	385 (21.3%)	103 (20.2%)
Body mass index (kg/m <sup>2</sup> )	27.0±4.4	27.2±4.5	26.4±4.1	0.0003**
Atherosclerotic disease	473 (20.4%)	331 (18.3%)	142 (27.8%)	<0.0001*
Total cholesterol (mg/dl)	257±63	255±63	262±61	0.022**
LDL cholesterol (mg/dl)	162±50	160±50	168±49	0.003**
HDL cholesterol (mg/dl)	59±17	56±17	59±17	0.0003**
Triglycerides (mg/dl)		204±226	210±236	184±184
	Median (IQR)	149 (129)	153 (131)	135 (117)
Lipoprotein(a) (mg/dl)		38±51	17±15	113±61
	Median (IQR)	19 (46)	13 (19)	96 (63)
Lipid lowering medication	229 (9.9%)	181 (10.0%)	48 (9.4%)	0.19*
Systolic blood pressure (mmHg)	137±19	137±19	136±20	0.74**
Diastolic blood pressure (mmHg)	83±10	83±10	82±10	0.60**
Hypertension	1139 (49.0%)	888 (49.0%)	251 (49.2%)	0.94*
Diabetes mellitus	394 (17.0%)	329 (18.2%)	65 (12.7%)	0.004*

Quantitative variables are presented as mean ± standard deviation and median and interquartile range (IQR) where appropriate; counts are given as *n* and percent. \*Chi-square P value. \*\*Unpaired Student's *t*-test (the data of Lp(a) and triglycerides were log-transformed before analysis). doi:10.1371/journal.pone.0024719.t001

departing from Hardy-Weinberg equilibrium (chi square  $P = 0.017$ ), while the two groups considered separately were in agreement with HWE (low Lp(a) group chi square  $P = 0.08$ , high Lp(a) group chi square  $P = 0.06$ ).

**Influence of the –174G/C SNP on Lp(a) concentrations**

In the total cohort, there were differences in Lp(a) concentrations depending on genotype. The mean ± SD concentrations were 35.2±46.9 mg/dl, 37.9±51.2 mg/dl, and 40.3±55.3 mg/dl for the GG, GC, and CC genotype, respectively. The corresponding geometric means (95% CI) were 15.9 (14.4 to 17.5) mg/dl, 16.9 (15.6 to 18.3) mg/dl, and 18.5 (15.9 to 21.4) mg/dl, respectively ( $P$  for trend 0.042). We analyzed the effects of the –174G/C SNP on

Lp(a) concentration using logistic regression analysis. The GC and CC genotypes were associated with a higher odds ratio for the prevalence of elevated Lp(a), defined as concentrations ≥60 mg/dl. For GC the odds ratio was 1.30 (95% CI 1.04 to 1.63,  $P = 0.02$ ) and for CC the odds ratio was 1.44 (95% CI 1.06 to 1.93,  $P = 0.018$ ). When taken together, the C-carriers had an odds ratio of 1.33 (95% CI 1.08 to 1.65,  $P = 0.009$ ) compared to the GG wildtype.

**Influence of the –174G/C SNP on other parameters**

Using analysis of variance we investigated whether the polymorphism influenced other continuous parameters. For simplicity, we divided the subjects in the ones having the GG wildtype (reference group) and the ones carrying the C-allele. The polymorphism was

**Table 2.** Genotype distribution of the –174G/C SNP between subjects with lower (<60 mg/dl) or higher (≥60 mg/dl) Lp(a) levels.

Subjects	GG	GC	CC	Chi square P value
Total cohort (n = 2321)	782 (33.7%)	1178 (50.75%)	361 (15.55%)	0.024
Lp(a) <60 mg/dl (n = 1811)	635 (35.1%)	905 (50.0%)	271 (14.9%)	
Lp(a) ≥60 mg/dl (n = 510)	147 (28.8)	273 (53.5%)	90 (17.6%)	
	<b>GG</b>	<b>C-carriers</b>		
Total cohort (n = 2321)	782 (33.7%)	1539 (66.3%)		0.008
Lp(a) <60 mg/dl (n = 1811)	635 (35.1%)	1176 (64.9%)		
Lp(a) ≥60 mg/dl (n = 510)	147 (28.8%)	363 (71.2%)		

doi:10.1371/journal.pone.0024719.t002

not associated with age ( $P = 0.98$ ), BMI ( $P = 0.14$ ), total cholesterol ( $P = 0.92$ ), LDL cholesterol ( $P = 0.63$ ), HDL cholesterol ( $P = 0.44$ ), systolic or diastolic blood pressure ( $P = 0.19$  or  $0.36$ , respectively). It was significantly associated with triglycerides ( $P = 0.04$ ), in that C-carriers had slightly higher triglyceride levels.

Using contingency tables and chi square statistics we investigated the association between the polymorphism and categorical data. There was no association with the presence of atherosclerosis ( $P = 0.62$ ), diabetes ( $P = 0.61$ ), or hypertension ( $P = 0.17$ ).

### Multiple logistic regression analysis

In multiple logistic regression analysis using the presence of high Lp(a) concentrations as the dependent and age, sex, smoking, BMI, lipid concentrations, hypertension and diabetes as covariates we found that the polymorphism remained independently associated with high Lp(a) concentrations (odds ratio of the C-carriers 1.35, 95% CI 1.08 to 1.69,  $P = 0.009$ ). The only significant covariate was LDL that also exerted an independent significant influence on Lp(a) concentrations.

### Discussion

The main finding of this study is that the common –174G/C polymorphism of the IL-6 gene is significantly associated with the risk of elevated Lp(a) concentrations ( $\geq 60$  mg/dl).

Low grade inflammation has been shown to be important in the pathogenesis of atherosclerosis in cell lines, animal models and humans [20]. Of the many cytokines examined in this context, IL-6 exhibits a strong association not only with cardiovascular mortality itself [21] but also with cardiovascular risk factors like type 2 diabetes [22,23], hypertension [24] and lipid abnormalities [25].

IL-6 is a pro-inflammatory cytokine with a molecular weight of 26 kD. The receptor of this signaling protein consists of two subunits, gp130 and IL-6 receptor-alpha (IL-6-R- $\alpha$ ), and is linked to the *Janus kinase* (JAK) – *signal transducer and activator of transcription* (STAT) signaling cascade [26]. Interestingly, in addition to this classical signaling pathway, IL-6 can also act via the so called “trans-signaling”, whereby IL-6 binds to a soluble IL-6-R- $\alpha$ , thus activating cells only expressing the gp130 protein at the cell surface [27]. Furthermore, it has been shown that the *suppressor of cytokine signaling* (SOCS)-3, a protein known to impair insulin signaling, is induced by the JAK-STAT signaling pathway, suggesting a molecular mechanism by which IL-6 exerts metabolic effects [28].

A common SNP in the human IL-6 gene, –174G/C, has been associated with altered serum levels of this pro-inflammatory cytokine in humans, with carriers of the C-allele exhibiting higher serum levels of IL-6 in some [29] but not all [30] studies. However, whether the –174G/C polymorphism is associated with IL-6 concentrations is unclear. A number of studies examining this association produced widely discrepant results with some studies showing an association [29,31–34], while many did not [30,35–37]. Also, carriers of the C-allele show significantly higher C-reactive protein (CRP) levels compared to carriers of the G-allele [38], which is of interest since IL-6 is one of the major determining factors of CRP levels in humans. In agreement with the association studies on IL-6 serum levels and cardiovascular disease mentioned above, carriers of the C-allele have been shown to have a higher risk for coronary heart disease [39] and stroke [40] and also show a higher prevalence of risk factors like hypertension [41] and type 2 diabetes [42]. Moreover, in a cohort of 285 nonagenarians, the frequency of the “protective” G-allele was significantly associated with longevity, indicating a clinical significance of this common IL-6 polymorphism [43].

Using the humanized IL-6 receptor antibody tocilizumab we recently demonstrated an approximately 30% reduction of serum

Lp(a) levels due to inhibition of IL-6 signaling in humans [8]. The higher frequency of the C-allele at the –174 locus in the IL-6 gene in human subjects with elevated Lp(a) levels reported here is in complete agreement with these findings, since the C-allele at this locus is associated with higher IL-6 serum levels [29]. Furthermore, an earlier study suggested regulation of Lp(a) serum levels by *acute phase response* (APR), for which IL-6 is crucial [44]. Taken together, these findings suggest that IL-6 is a major determining factor of serum Lp(a) levels in human subjects.

Despite evidence from previous reports [42], we did not find in our cohort a significant association of the –174G/C polymorphism with type 2 diabetes. In this respect it is important to note that our cohort was not collected to examine genetic influence on diabetes development but to identify target genes involved in severe dyslipidemia. Therefore, the study cohort does not reflect a representative population of type 2 diabetic subjects. Furthermore, it should be mentioned that an independent association of the –174G/C polymorphism with type 2 diabetes risk was found in several reports, but not in the largest of such studies performed in Denmark in 2005 [9]. Therefore, the potential role of the –174G/C SNP in the pathogenesis of type 2 diabetes remains controversial. Moreover, we found no association of the SNP with BMI, which is in agreement with previous reports [45].

Besides influencing Lp(a) levels, we also found significantly higher serum triglyceride concentrations in carriers of the C-allele at the –174 locus of the human IL-6 gene. This is of interest, since studies in rodents suggest that IL-6 treatment increased hepatic triglyceride secretion without decreasing the clearance of triglyceride-rich lipoproteins [46]. Furthermore, apolipoprotein B, which is important for the formation of very-low-density lipoprotein (VLDL) particles, has been found to be secreted from hepatocytes in response to IL-6 treatment [47]. Moreover, our findings are in agreement to those of Henningsson et al., who showed that male carriers of the C allele displayed elevated serum triglycerides [48]. We also observed that Lp(a) levels are inversely associated with the risk of type 2 diabetes, an interesting finding recently also reported by the group of Ridker et al. [49]. Moreover, we observed that Lp(a) concentrations are associated with LDL-, total- and HDL-cholesterol concentrations, in accordance to previous reports [50,51]. The inverse association between triglyceride levels and BMI with Lp(a) levels found in the present study is in agreement with the findings of Chien et al. [52], although the association of Lp(a) levels with BMI remains controversial [53].

Taken together, the positive association of the C-allele at the –174 locus of the human IL-6 gene with elevated Lp(a) concentrations together with the Lp(a)-decreasing effects of the IL-6 antagonist tocilizumab strongly suggests that Lp(a) serum levels are regulated by IL-6 in humans. Since presently the number of drugs to treat elevated Lp(a) levels in patients with cardiovascular diseases is limited [54], these findings may open an interesting field for biomedical research in order to develop novel pharmacological strategies for affected patients.

### Acknowledgments

We thank Nadine Spennath and Doris Vollmar for their excellent technical assistance.

### Author Contributions

Conceived and designed the experiments: HKB WK IG-B. Performed the experiments: HKB IG-B. Analyzed the data: HKB ML IG-B. Contributed reagents/materials/analysis tools: WK IG-B. Wrote the paper: HKB ML WK IG-B.

## References

- Utermann G (2001) Lipoprotein(a). In: Scriver C, Beaudet A, Sly W, Valle D, eds. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill Professional. pp 2753–2787.
- Nordestgaard BG, Chapman MJ, Ray K, Boren J, Andreotti F, et al. (2010) Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur Heart J* 31: 2844–2853. [10.1093/eurheartj/ehq386](https://doi.org/10.1093/eurheartj/ehq386) [pii];10.1093/eurheartj/ehq386 [doi].
- Joshi PH, Krivitsky E, Qian Z, Vazquez G, Voros S, et al. (2010) Do We Know When and How to Lower Lipoprotein(a)? *Curr Treat Options Cardiovasc Med* 12: 396–407. [10.1007/s11936-010-0077-6](https://doi.org/10.1007/s11936-010-0077-6) [doi].
- Carlson LA, Hamsten A, Asplund A (1989) Pronounced lowering of serum levels of lipoprotein Lp(a) in hyperlipidaemic subjects treated with nicotinic acid. *J Intern Med* 226: 271–276.
- Cannon CP, Shah S, Dansky HM, Davidson M, Brinton EA, et al. (2010) Safety of anacetrapib in patients with or at high risk for coronary heart disease. *N Engl J Med* 363: 2406–2415. [10.1056/NEJMoa1009744](https://doi.org/10.1056/NEJMoa1009744) [doi].
- Ladenson PW, Kristensen JD, Ridgway EC, Olsson AG, Carlsson B, et al. (2010) Use of the thyroid hormone analogue eprotromone in statin-treated dyslipidemia. *N Engl J Med* 362: 906–916. [10.1056/NEJMoa0905633](https://doi.org/10.1056/NEJMoa0905633) [doi].
- Raal FJ, Santos RD, Blom DJ, Marais AD, Charng MJ, et al. (2010) Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: a randomised, double-blind, placebo-controlled trial. *Lancet* 375: 998–1006. [S0140-6736\(10\)60284-X](https://doi.org/10.1016/S0140-6736(10)60284-X) [pii];10.1016/S0140-6736(10)60284-X [doi].
- Schultz O, Oberhauser F, Saech J, Rubbert-Roth A, Hahn M, et al. (2010) Effects of inhibition of interleukin-6 signalling on insulin sensitivity and lipoprotein (a) levels in human subjects with rheumatoid diseases. *PLoS One* 5: e14328. [10.1371/journal.pone.0014328](https://doi.org/10.1371/journal.pone.0014328) [doi].
- Hamid YH, Rose CS, Urhammer SA, Glumer C, Nolsoe R, et al. (2005) Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. *Diabetologia* 48: 251–260. [10.1007/s00125-004-1623-0](https://doi.org/10.1007/s00125-004-1623-0) [doi].
- Yeh KY, Li YY, Hsieh LL, Chen JR, Tang RP (2010) The –174 G/C polymorphism in interleukin-6 (IL-6) promoter region is associated with serum IL-6 and carcinoembryonic antigen levels in patients with colorectal cancers in Taiwan. *J Clin Immunol* 30: 53–59. [10.1007/s10875-009-9324-6](https://doi.org/10.1007/s10875-009-9324-6) [doi].
- Pereira DS, Garcia DM, Narciso FM, Santos ML, Dias JM, et al. (2011) Effects of 174 G/C polymorphism in the promoter region of the interleukin-6 gene on plasma IL-6 levels and muscle strength in elderly women. *Braz J Med Biol Res* 44: 123–129. [S01000-0079X2010007500152](https://doi.org/10.1007/s01000-0079X2010007500152) [pii].
- Olivieri F, Bonafe M, Cavallone L, Giovagnetti S, Marchegiani F, et al. (2002) The –174 C/G locus affects *in vitro/in vivo* IL-6 production during aging. *Exp Gerontol* 37: 309–314. [S0531556501001978](https://doi.org/10.1016/S0531556501001978) [pii].
- Gouni-Berthold I, Giannakidou E, Muller-Wieland D, Faust M, Kotzka J, et al. (2005) Peroxisome proliferator-activated receptor-gamma2 Pro12Ala and endothelial nitric oxide synthase-4a/b gene polymorphisms are not associated with hypertension in diabetes mellitus type 2. *J Hypertens* 23: 301–308.
- Gouni-Berthold I, Giannakidou E, Faust M, Berthold HK, Krone W (2005) The peroxisome proliferator-activated receptor delta +294T/C polymorphism in relation to lipoprotein metabolism in patients with diabetes mellitus type 2 and in non-diabetic controls. *Atherosclerosis* 183: 336–341. [S0021-9150\(05\)00225-X](https://doi.org/10.1016/j.atherosclerosis.2005.03.016) [pii];10.1016/j.atherosclerosis.2005.03.016 [doi].
- Siekmeier R, März W, Scharnagl H, Nauck M, Mayer H, et al. (1996) Bestimmung von Lipoprotein(a): Vergleich eines neuen latexverstärkten immunoturbidimetrischen Assay mit einem immunoradiometrischen Assay. *Journal of Laboratory Medicine* 20: 294–298.
- Libra M, Signorelli SS, Bevelacqua Y, Navolanic PM, Bevelacqua V, et al. (2006) Analysis of G(–174)C IL-6 polymorphism and plasma concentrations of inflammatory markers in patients with type 2 diabetes and peripheral arterial disease. *J Clin Pathol* 59: 211–215. [59/2/211](https://doi.org/10.1136/jcp.2004.025452) [pii];10.1136/jcp.2004.025452 [doi].
- Kamstrup PR, Tybjaerg-Hansen A, Steffensen R, Nordestgaard BG (2009) Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. *JAMA* 301: 2331–2339. [301/22/2331](https://doi.org/10.1001/jama.2009.801) [pii];10.1001/jama.2009.801 [doi].
- Clarke R, Peden JF, Hopewell JC, Kyriakou T, Goel A, et al. (2009) Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *N Engl J Med* 361: 2518–2528. [361/26/2518](https://doi.org/10.1056/NEJMoa0902604) [pii];10.1056/NEJMoa0902604 [doi].
- Erqou S, Kaptoge S, Perry PL, Di AE, Thompson A, et al. (2009) Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *JAMA* 302: 412–423. [302/4/412](https://doi.org/10.1001/jama.2009.1063) [pii];10.1001/jama.2009.1063 [doi].
- Libby P, Ridker PM, Hansson GK (2009) Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol* 54: 2129–2138. [S0735-1097\(09\)03006-X](https://doi.org/10.1016/j.jacc.2009.09.009) [pii];10.1016/j.jacc.2009.09.009 [doi].
- Tan J, Hua Q, Li J, Fan Z (2009) Prognostic value of interleukin-6 during a 3-year follow-up in patients with acute ST-segment elevation myocardial infarction. *Heart Vessels* 24: 329–334. [10.1007/s00380-008-1128-8](https://doi.org/10.1007/s00380-008-1128-8) [doi].
- Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, et al. (2003) Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* 52: 812–817.
- Huth C, Heid IM, Vollmert C, Gieger C, Grallert H, et al. (2006) IL6 gene promoter polymorphisms and type 2 diabetes: joint analysis of individual participants' data from 21 studies. *Diabetes* 55: 2915–2921. [55/10/2915](https://doi.org/10.2337/db06-0600) [pii];10.2337/db06-0600 [doi].
- Kim KI, Lee JH, Chang HJ, Cho YS, Youn TJ, et al. (2008) Association between blood pressure variability and inflammatory marker in hypertensive patients. *Circ J* 72: 293–298. [JST/JSTAGE/circj/72.293](https://doi.org/10.1253/circj.72.293) [pii].
- Jonkers IJ, Mohrschlatt MF, Westendorp RG, van der Laarse A, Smelt AH (2002) Severe hypertriglyceridemia with insulin resistance is associated with systemic inflammation: reversal with bezafibrate therapy in a randomized controlled trial. *Am J Med* 112: 275–280. [S0002934301011238](https://doi.org/10.1056/NEJMoa011238) [pii].
- Kamimura D, Ishihara K, Hirano T (2003) IL-6 signal transduction and its physiological roles: the signal orchestration model. *Rev Physiol Biochem Pharmacol* 149: 1–38. [10.1007/s10254-003-0012-2](https://doi.org/10.1007/s10254-003-0012-2) [doi].
- Rose-John S, Scheller J, Elson G, Jones SA (2006) Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. *J Leukoc Biol* 80: 227–236. [jlb.1105674](https://doi.org/10.1189/jlb.1105674) [pii];10.1189/jlb.1105674 [doi].
- Rieusset J, Bouzakri K, Chevillotte E, Ricard N, Jacquet D, et al. (2004) Suppressor of cytokine signaling 3 expression and insulin resistance in skeletal muscle of obese and type 2 diabetic patients. *Diabetes* 53: 2232–2241. [53/9/2232](https://doi.org/10.23232-2241) [pii].
- Wypasek E, Undas A, Sniezek-Maciejewska M, Kapelak B, Plicner D, et al. (2010) The increased plasma C-reactive protein and interleukin-6 levels in patients undergoing coronary artery bypass grafting surgery are associated with the interleukin-6-174G>C gene polymorphism. *Ann Clin Biochem* 47: 343–349. [47/4/343](https://doi.org/10.1258/acb.2010.090305) [pii];10.1258/acb.2010.090305 [doi].
- Fernandez-Real JM, Broch M, Vendrell J, Richart C, Ricart W (2000) Interleukin-6 gene polymorphism and lipid abnormalities in healthy subjects. *J Clin Endocrinol Metab* 85: 1334–1339.
- Haddy N, Sass C, Maumus S, Marie B, Droesch S, et al. (2005) Biological variations, genetic polymorphisms and familial resemblance of TNF-alpha and IL-6 concentrations: STANISLAS cohort. *Eur J Hum Genet* 13: 109–117. [10.1038/sj.ejhg.5201294](https://doi.org/10.1038/sj.ejhg.5201294) [doi].
- Oberbach A, Lehmann S, Kirsch K, Krist J, Sonnabend M, et al. (2008) Long-term exercise training decreases interleukin-6 (IL-6) serum levels in subjects with impaired glucose tolerance: effect of the –174G/C variant in IL-6 gene. *Eur J Endocrinol* 159: 129–136. [EJE-08-0220](https://doi.org/10.1530/EJE-08-0220) [pii];10.1530/EJE-08-0220 [doi].
- Jones KG, Brull DJ, Brown LC, Sian M, Greenhalgh RM, et al. (2001) Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. *Circulation* 103: 2260–2265.
- Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, et al. (1998) The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 102: 1369–1376. [10.1172/JCI2629](https://doi.org/10.1172/JCI2629) [doi].
- Sanders J, Hawe E, Brull DJ, Hubbard C, Lowe GD, et al. (2009) Higher IL-6 levels but not IL-6 –174G>C or –572G>C genotype are associated with post-operative complication following coronary artery bypass graft (CABG) surgery. *Atherosclerosis* 204: 196–201. [S0021-9150\(08\)00615-1](https://doi.org/10.1016/j.atherosclerosis.2008.08.032) [pii];10.1016/j.atherosclerosis.2008.08.032 [doi].
- Margaglione M, Bossone A, Cappucci G, Colaizzo D, Grandone E, et al. (2001) The effect of the interleukin-6 c/g-174 polymorphism and circulating interleukin-6 on fibrinogen plasma levels. *Haematologica* 86: 199–204.
- Rauramaa R, Vaisanen SB, Luong LA, Schmidt-Trucksass A, Penttila IM, et al. (2000) Stromelysin-1 and interleukin-6 gene promoter polymorphisms are determinants of asymptomatic carotid artery atherosclerosis. *Arterioscler Thromb Vasc Biol* 20: 2657–2662.
- Vickers MA, Green FR, Terry C, Mayosi BM, Julier C, et al. (2002) Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein. *Cardiovasc Res* 53: 1029–1034. [S000863630100534X](https://doi.org/10.1056/NEJMoa01100534X) [pii].
- Berg KK, Madsen HO, Garred P, Wiseth R, Gunnæs S, et al. (2009) The additive contribution from inflammatory genetic markers on the severity of cardiovascular disease. *Scand J Immunol* 69: 36–42. [SJ12187](https://doi.org/10.1111/j.1365-3083.2008.02187.x) [pii];10.1111/j.1365-3083.2008.02187.x [doi].
- Revilla M, Obach V, Cervera A, Davalos A, Castillo J, et al. (2002) A –174G/C polymorphism of the interleukin-6 gene in patients with lacunar infarction. *Neurosci Lett* 324: 29–32. [S0304394002001696](https://doi.org/10.1016/S0304394002001696) [pii].
- Losito A, Kalidas K, Santoni S, Jeffery S (2003) Association of interleukin-6 –174G/C promoter polymorphism with hypertension and left ventricular hypertrophy in dialysis patients. *Kidney Int* 64: 616–622. [kid119](https://doi.org/10.1046/j.1523-1755.2003.00119.x) [pii];10.1046/j.1523-1755.2003.00119.x [doi].
- Kristiansen OP, Mandrup-Poulsen T (2005) Interleukin-6 and diabetes: the good, the bad, or the indifferent? *Diabetes* 54 Suppl 2: S114–S124. [54/suppl\\_2/S114](https://doi.org/10.23232-2241) [pii].
- Hurme M, Lehtimäki T, Jylha M, Karhunen PJ, Hervonen A (2005) Interleukin-6 –174G/C polymorphism and longevity: a follow-up study. *Mech Ageing Dev* 126: 417–418. [S0047-6374\(04\)00255-6](https://doi.org/10.1016/j.mad.2004.10.001) [pii];10.1016/j.mad.2004.10.001 [doi].
- Yun KA, Lee W, Min WK, Chun S, Lee YW, et al. (2004) Discrepancy of interleukin-6 levels between end-stage renal disease patients and patients with

- acute-phase response with increased lipoprotein(a) concentrations. *Scand J Clin Lab Invest* 64: 223–228.
45. Qi L, Zhang C, van Dam RM, Hu FB (2007) Interleukin-6 genetic variability and adiposity: associations in two prospective cohorts and systematic review in 26,944 individuals. *J Clin Endocrinol Metab* 92: 3618–3625. [jc.2007-0877 \[pii\];10.1210/jc.2007-0877 \[doi\]](#).
  46. Nonogaki K, Fuller GM, Fuentes NL, Moser AH, Staprans I, et al. (1995) Interleukin-6 stimulates hepatic triglyceride secretion in rats. *Endocrinology* 136: 2143–2149.
  47. Sparks JD, Cianci J, Jokinen J, Chen LS, Sparks CE (2010) Interleukin-6 mediates hepatic hypersecretion of apolipoprotein B. *Am J Physiol Gastrointest Liver Physiol* 299: G980–G989. [ajpgi.00080.2010 \[pii\];10.1152/ajpgi.00080.2010 \[doi\]](#).
  48. Henningson S, Hakansson A, Westberg L, Baghaei F, Rosmond R, et al. (2006) Interleukin-6 gene polymorphism –174G/C influences plasma lipid levels in women. *Obesity (Silver Spring)* 14: 1868–1873. [14/11/1868 \[pii\];10.1038/oby.2006.216 \[doi\]](#).
  49. Mora S, Kamstrup PR, Rifai N, Nordestgaard BG, Buring JE, et al. (2010) Lipoprotein(a) and risk of type 2 diabetes. *Clin Chem* 56: 1252–1260. [clinchem.2010.146779 \[pii\];10.1373/clinchem.2010.146779 \[doi\]](#).
  50. Boyer H, de Gennes JL, Truffert J, Chatellier G, Dairou F, et al. (1990) Lp(a) levels in different types of dyslipidemia in the French population. *Atherosclerosis* 85: 61–69.
  51. Candido AP, Ferreira S, Lima AA, de Carvalho Nicolato RL, de Freitas SN, et al. (2007) Lipoprotein(a) as a risk factor associated with ischemic heart disease: Ouro Preto Study. *Atherosclerosis* 191: 454–459. [S0021-9150\(06\)00240-1 \[pii\];10.1016/j.atherosclerosis.2006.04.031 \[doi\]](#).
  52. Chien KL, Lee YT, Sung FC, Su TC, Hsu HC, et al. (1999) Lipoprotein (a) level in the population in Taiwan: relationship to sociodemographic and atherosclerotic risk factors. *Atherosclerosis* 143: 267–273. [S0021915098002986 \[pii\]](#).
  53. Mora S, Lee IM, Buring JE, Ridker PM (2006) Association of physical activity and body mass index with novel and traditional cardiovascular biomarkers in women. *JAMA* 295: 1412–1419. [295/12/1412 \[pii\];10.1001/jama.295.12.1412 \[doi\]](#).
  54. Gouni-Berthold I, Berthold HK (2011) Lipoprotein(a): Current Perspectives. *Curr Vasc Pharmacol;BSP/CVP/E-Pub/0000160 [pii]*.