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ORIGINAL ARTICLE

Joint analysis of individual participants' data from 17 studies on the association of the *IL6* variant -174G > C with circulating glucose levels, interleukin-6 levels, and body mass index

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Abstract

Background. Several studies have investigated associations between the -174G > C single nucleotide polymorphism (rs1800795) of the *IL6* gene and phenotypes related to type 2 diabetes mellitus (T2DM) but presented inconsistent results. *Aims.* This joint analysis aimed to clarify whether *IL6* -174G > C was associated with glucose and circulating interleukin-6 concentrations as well as body mass index (BMI).

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Methods. Individual-level data from all studies of the *IL6*-T2DM consortium on Caucasian subjects with available BMI were collected. As study-specific estimates did not show heterogeneity ($P > 0.1$), they were combined by using the inverse-variance fixed-effect model.
Results. The main analysis included 9440, 7398, 24,117, or 5659 non-diabetic and manifest T2DM subjects for fasting glucose, 2-hour glucose, BMI, or circulating interleukin-6 levels, respectively. *IL6* -174 C-allele carriers had significantly lower fasting glucose (-0.091 mmol/L, $P = 0.014$). There was no evidence for association between *IL6* -174G > C and BMI or interleukin-6 levels, except in some subgroups.
Conclusions. Our data suggest that C-allele carriers of the *IL6* -174G > C polymorphism have lower fasting glucose levels on average, which substantiates previous findings of decreased T2DM risk of these subjects.

Key words: *Blood glucose, body mass index, diabetes mellitus type 2, genes, inflammation mediators, interleukin-6, intermediate phenotype, meta-analysis, molecular epidemiology, single nucleotide polymorphism*

Key messages

- Several studies have investigated associations between the -174G > C polymorphism (rs1800795) of the *IL6* gene but presented inconsistent results. For all analyzed phenotypes, our joint analysis represents the largest study on individual-level data conducted to date to address the role of *IL6* -174G > C.
- We were able to reveal lower fasting glucose levels in carriers of the *IL6* -174 C-allele. There was no evidence for association between *IL6* -174G > C and BMI or circulating interleukin-6 levels, except in some subgroups.

Abbreviations

BMI	body mass index
CHD	coronary heart disease
HWE	Hardy-Weinberg equilibrium
IL-6	interleukin-6
IL6	interleukin-6 gene
OR	odds ratio
T2DM	type 2 diabetes mellitus
2-h	2-hour
95% CI	95% confidence interval

Introduction

Type 2 diabetes mellitus (T2DM) is a public health problem of pandemic proportions. Conservative estimates indicate that there are 171 million people in the world with symptomatic or asymptomatic diabetes mellitus and that this global prevalence will double by 2030 to 366 million people (1). The spread of the disease is also alarming in Europe, even though Caucasians have a low to moderate prevalence of T2DM compared with most other ethnic groups worldwide (2).

Type 2 diabetes mellitus is a multifactorial disease. While a lot is known about environmental risk factors for T2DM, identification of the genetic etiology of T2DM has proven to be challenging (3). An interesting candidate variant for T2DM is the -174G > C polymorphism (rs1800795) of the *IL6* gene that codes for the cytokine interleukin-6 (IL-6). Interleukin-6 exerts pleiotropic biological functions in the regulation of the acute-phase reaction and immune responses, and is associated with T2DM and related diseases (4–7). The promoter polymorphism *IL6* -174G > C, which has been shown to affect *IL6* promoter activity (8,9), was reported to be associated with circulating IL-6 levels (8,10–14), T2DM (15), insulin resistance (16), obesity (17–20), coronary heart disease (CHD) (21), cholesterol levels (6), and metabolic syndrome (22). However, association estimates pointed in inconclusive directions, while other studies of these traits did not find any association.

At the start of our project, meta-analyses on association between *IL6* -174G > C and two different outcomes had been reported: 1) Sie et al. did not find statistically significant evidence for an association with CHD in 19,798 individuals (23). Having large heterogeneity between association estimates, Qi et al. did not detect a statistically significant association with T2DM in 17,452 individuals of mainly published studies (24). We established an international *IL6*-T2DM consortium on individual-level published and unpublished data and showed an almost 9% reduced T2DM risk for C-allele carriers in 20,976 individuals (25). However, it was not clear yet whether the *IL6* -174G > C polymorphism impacts IL-6 levels which together with the T2DM association would support a causal role of IL-6 levels in T2DM. Moreover, studies on genetic association with obesity pointed in a direction that was opposite to the T2DM association (17,18). Finally, analyzing the dichotomous trait T2DM is a substantial restriction of information, which can be overcome by analyzing the quantitative trait circulating plasma or serum glucose in the fasting state or after an oral

glucose load. This is of special importance because the T2DM finding was borderline statistically significant ($P=0.037$) (25), and a consistent glucose association would greatly underscore this finding.

The objective of the present study was to clarify the epidemiological evidence for the association of *IL6* -174G>C polymorphism with circulating glucose levels, body mass index (BMI), and IL-6 levels as intermediate phenotypes on the way to T2DM. We used by far the largest individual-level data set on this polymorphism in relation to quantitative phenotypes to date. It is important to note that earlier studies detected associations between -174G>C and IL-6 levels or obesity mostly in the presence of an inflammatory stimulus (10,11) or in subjects having a chronic subclinical (19), or an acute (12,14) inflammatory state. The inclusion of T2DM studies in our joint analysis was essential to investigate whether the impact of *IL6* -174G>C indeed differed by the subclinical inflammatory state observed in type 2 diabetic individuals, as opposed to healthy subjects.

Subjects and methods

All studies of the *IL6*-T2DM consortium on Caucasian subjects with available BMI were included in this joint analysis of quantitative traits. Information on the inclusion criteria, the search strategy and recruitment of the studies, data cleaning, and genotyping methods is provided in the online appendix.

Definition of analyzed samples and data collection

Because the majority of included studies were cross-sectional population-based or T2DM case-control studies, our main analysis focused on a combined sample of non-diabetic and prevalent T2DM subjects. We included the baseline examination of the two longitudinal cohort studies excluding subjects that developed T2DM during follow-up for this main analysis. All analyses were confined to Caucasian adults who were at least 18 years old with data on the genotype of the *IL6* -174G>C polymorphism, age, sex, BMI, and T2DM status. When known, type 1 diabetic individuals were excluded. In family studies, only the sibling generation was used. Data on circulating fasting and 2-hour plasma or serum glucose (measured two hours after consumption of 75 g glucose) and plasma or serum IL-6 levels were collected where available.

All participating studies have been conducted according to the principles expressed in the Declaration of Helsinki. Individual studies had either written informed consent for all subjects for genetic analyses or approval from their institutional review committee for genetic analyses. Further details on design of individual studies and use in this joint analysis are presented in the online appendix Table A-II.

Statistical analyses

Study-specific β-coefficients for the association between *IL6* -174G>C and the quantitative traits were estimated by linear regression, using SAS PROC GLM for studies with unrelated individuals and SAS PROC MIXED for family studies. All analyses were adjusted for age and sex. Primary analyses were performed including all subjects (full data analyses) additionally adjusting for T2DM status when analyzing BMI or IL-6 levels. Secondary analyses were conducted separately for the non-diabetic and the prevalent T2DM subjects (T2DM status-specific analyses), additionally adjusting for BMI when analyzing fasting, 2-hour glucose, or IL-6 levels. If a study featured less than 50 participants for a specific analysis, this study was not included in the joint analysis. Studies with fasting glucose only on either T2DM cases or controls (EDSC, EPIC-POTSDAM_nCC-T2DM, and MONICA-S3) were excluded from the full data glucose analysis to assure a full range of glucose levels.

IL6 -174G>C genotypes were analyzed model-free, comparing CC- or GC-genotype with the wild-type GG, and additionally by applying a dominant genetic model for the C-allele, which was the model reported previously for the T2DM association (25). Heterogeneity between study-specific β-coefficients was tested by the chi-square-based Q-statistic, and its impact was quantified by I^2 (26). As the heterogeneity between study-specific β-coefficients was non-significant in all analyses ($P>0.10$), the β-coefficients were combined using the inverse-variance fixed-effect model (27). As recommended, summary association estimates of all studies with the genotype frequencies of non-diabetic subjects being in Hardy-Weinberg equilibrium (HWE) were reported as main results (28).

A conservative Bonferroni-corrected significance level of 0.017 ($=0.05/3$) was applied to account for the testing of the three phenotypes (glucose, BMI, and IL-6) in the primary (full data) analyses. Note that the phenotypes fasting and 2-hour postprandial glucose levels were highly correlated and thus not counted as two independent phenotypes here. In the secondary analyses, the significance level was further corrected for the two investigated subgroups yielding a significance level of 0.008 (0.05/6). More detailed information on statistical procedures is given in the online appendix.

Analysis in incident T2DM patients

In an additional exploratory analysis, we combined the linear regression estimates of baseline data from the incident T2DM cases of the two cohort studies to obtain estimates of the *IL6* -174G > C association with BMI and IL-6 levels (glucose not available) among future T2DM subjects before they became cases ('prediabetic subjects').

Results

Study recruitment

Seventeen studies with 25,635 participants met the study and subject inclusion criteria and were included in the joint analyses (see Table I for an overview). Online appendix Table A-III shows for which outcome the respective study qualified for analysis; online appendix Table A-IV presents the number of participants per study included in the analyses of the respective quantitative trait. All 17 studies with 25,635 subjects were analyzed for the outcome BMI. Association analyses with BMI as main outcome had been unpublished in 12 studies at the time of study recruitment (called 'unpublished for BMI' in the following). Eight studies with 10,725 participants were analyzed for fasting glucose, seven of them unpublished for this trait; seven studies with 8399 participants were analyzed for 2-hour glucose (all unpublished). For the outcome circulating IL-6 levels, data from seven studies with 5659 participants were analyzed, six of them unpublished for IL-6 levels.

Study-specific statistics

Detailed characteristics of included studies and participants are summarized in online appendix Table A-V. All studies had been conducted in European populations. Genotype frequencies of non-diabetic subjects were in HWE for all studies, except for the BOTNIA and the TGN study, which were thus excluded from the main analyses. This left 9440, 7398, 24,117, and 5659 subjects for the analysis of fasting glucose, 2-hour glucose, BMI, and circulating IL-6, respectively (see online appendix Table A-IV). The *IL6* -174 C-allele frequency of non-diabetic subjects with genotype frequencies in HWE ranged from 41.1% (95% CI = 38.3–43.8) in the KORA-MIFAM study to 55.0% (95% CI = 51.4–58.6) in the FUSION 1 study.

IL6 -174G > C and circulating glucose

The outcome fasting glucose was investigated as full data main analysis for the seven studies where the genotype frequencies of non-diabetic

Table I. Characteristics of included studies.

Study[a]	Full study name	Country[b]	*n* T2DM/non-diabetic subjects[c]
BOTNIA	Botnia Study	SF	731/557
CAPPP	Captopril Prevention Project	S	42/424
DANISH	Danish Study	DK	1212/4399
EDSC	Ealing Diabetes Study of Coagulation	UK	299/0
EPIC-POTSDAM	European Prospective Investigation into Cancer and Nutrition Potsdam (EPIC-Potsdam)	D	0/348
FUSION 1	The Finland-United States Investigation of NIDDM Genetics, 1st sampling wave	SF	508/367
FUSION 2	The Finland-United States Investigation of NIDDM Genetics, 2nd sampling wave	SF	437/201
GIRONA	Girona Genetics of Diabetes Study	E	42/123
KORA-MIFAM	KORA MI Family Study	D	95/881
KORA-S4	KORA Survey S4	D	225/1190
KORA-T2DMFAM	KORA T2DM Family Study	D	776/513
MONICA/KORA-BASE	MONICA/KORA Case Cohort Study S123 (MONICA/KORA-S123)	D	101/1744
MONICA-S3	MONICA/KORA Survey S3	D	151/3551
NPHS II	Second Northwick Park Heart Study	UK	0/2652
RMIFAM	Regensburg MI Family Study	D	662/2614
TGN	Tarraco Study	E	166/64
UDACS	University College Diabetes and Cardiovascular Study	UK	560/0

[a]Abbreviated study name used in the present publication.
[b]Country of recruitment: D = Germany, DK = Denmark, E = Spain, SF = Finland, S = Sweden, UK = United Kingdom.
[c]Number of type 2 diabetic/non-diabetic subjects included in analyses of the outcome BMI.
MI = myocardial infarction; NIDDM = non-insulin-dependent diabetes mellitus.

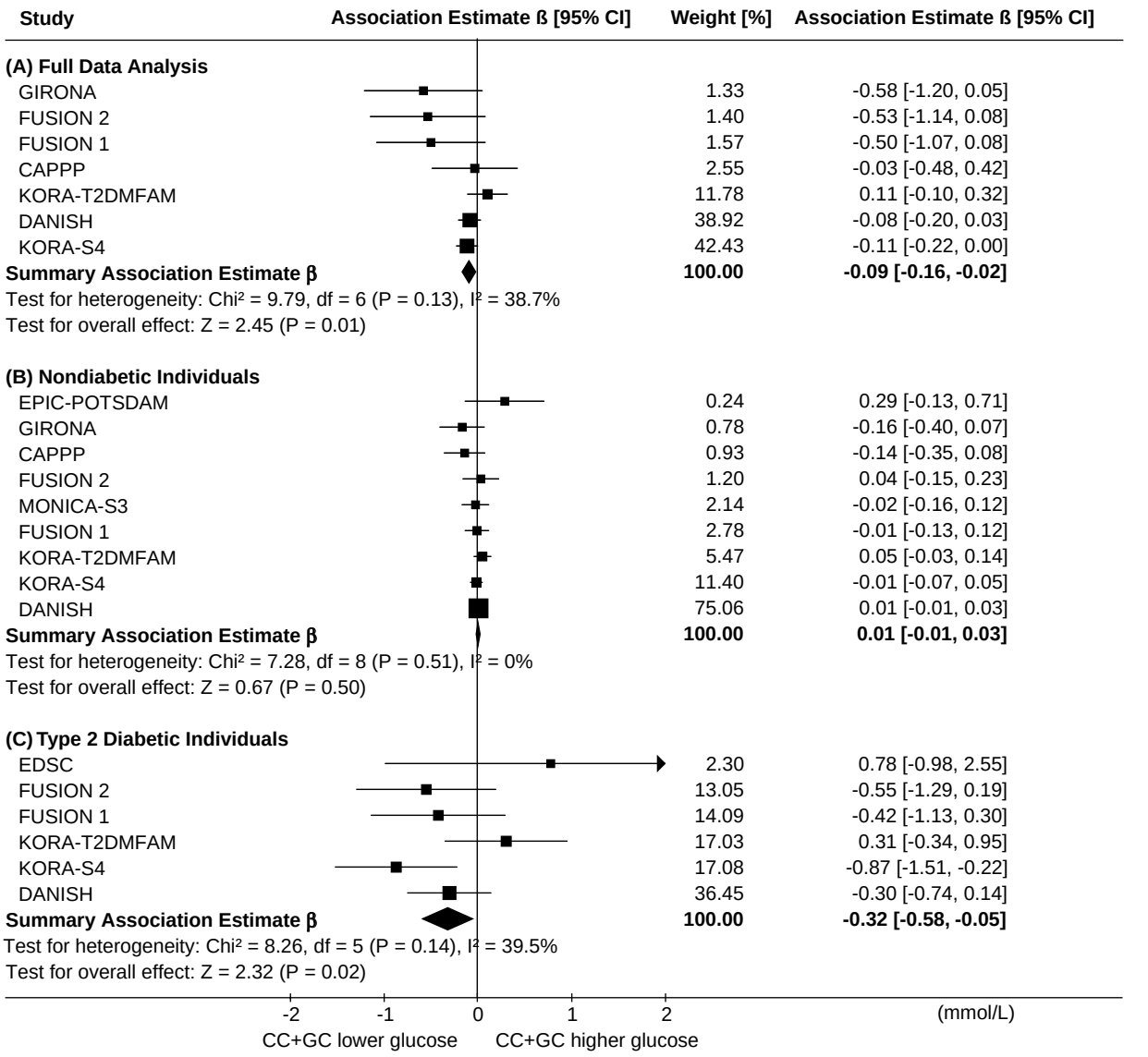

Figure 1. Forest plot, illustrating the study-specific β-coefficients with 95% CIs for the association between *IL6* -174G>C, dominant model for the C-allele, and fasting glucose in (A) the full data analysis (combined group of non-diabetic and prevalent type 2 diabetes mellitus (T2DM) subjects), (B) only in non-diabetic subjects, and (C) only in prevalent T2DM subjects, adjusted for age, sex, and body mass index (BMI). Additionally, the summary fixed-effect ß-coefficient is shown. I^2 measures the impact of inconsistency across studies and can range between 0% and 100%.

subjects were in HWE, including 9440 subjects. Figure 1 shows the study-specific and the summary β-coefficients, using a dominant model for the C-allele. Most studies exhibited a reduction of fasting glucose levels, though not significant in each study separately. The summary estimate provided a statistically significant decrease of −0.091 mmol/L (95% CI = (−0.163)–(−0.018), $P = 0.014$), corresponding to a decrease of about 1.5% for subjects with GC- or CC-genotypes compared to GG. T2DM status-specific analyses showed a more pronounced reduction of −0.317 mmol/L (95% CI = (−0.584)–(−0.049), $P = 0.020$) among T2DM subjects.

Table II provides model-free summary estimates, indicating that the dominant genetic model was consistent with the data.

The full data main analysis of 2-hour glucose included six studies with 7398 subjects. There was no statistically significant association between *IL6* -174G>C and 2-hour glucose (C-allele dominant model for full data analysis: β = −0.075 mmol/L, 95% CI = (−0.201)–(0.051), $P = 0.243$) (Table II).

IL6 -174G>C and body mass index

The full data main analysis of the outcome BMI included 15 studies with 24,117 subjects. Figure 2

Table II. Summary results of association between *IL6* -174G > C and fasting glucose, 2-h glucose, body mass index (BMI), or interleukin-6 (IL-6) levels. The fixed-effect results are presented for the full data analysis[a] and for analyses stratified for type 2 diabetes status (T2DM).

	Full data analysis[a]					Non-diabetic subjects					Type 2 diabetic subjects				
	n		ß-coefficient (*P*-value)[b]			*n*		ß-coefficient (*P*-value)[b]			*n*		ß-coefficient (*P*-value)[b]		
Outcome	Studies	Subjects	CC vs GG	GC vs GG	Dominant[c]	Studies	Subjects	CC vs GG	GC vs GG	Dominant[b]	Studies	Subjects	CC vs GG	GC vs GG	Dominant[b]
Fasting glucose (mmol/L)	7	9440	−0.081 (0.108)	−0.099 (0.011)	**−0.091 (0.014)**	9	7420	0.007 (0.626)	0.007 (0.523)	0.007 (0.501)	6	2412	−0.322 (0.076)	−0.354 (0.013)	**−0.317 (0.020)**
2-hour glucose (mmol/L)	6	7398	−0.127 (0.127)	−0.053 (0.441)	−0.075 (0.243)	6	6731	−0.022 (0.592)	−0.003 (0.920)	−0.009 (0.775)	4	618	−0.043 (0.927)	−0.114 (0.760)	−0.135 (0.703)
BMI (kg/m^2)	15	24117	0.097 (0.197)	0.086 (0.151)	**0.088 (0.120)**	13	19007	0.057 (0.478)	0.080 (0.213)	0.071 (0.242)	11	5026	0.216 (0.255)	0.154 (0.321)	0.176 (0.226)
Circulating IL-6[d]	7	5659	0.034 (0.292)	0.018 (0.467)	0.024 (0.304)	6	4621	0.001 (0.969)	0.022 (0.441)	0.018 (0.504)	4	966	**0.159 (0.044)**	0.004 (0.938)	0.042 (0.415)

[a]Analysis of non-diabetic and prevalent T2DM subjects together. The sum of the number of non-diabetic and T2DM subjects in stratified analyses does not yield the number of subjects in the full data analysis, because stratified analyses were performed in individual studies only if there were at least 50 non-diabetic or T2DM subjects available.
[b]Summary estimates from generalized linear models adjusted for age and sex (for BMI and IL-6 levels: additionally adjusted for T2DM status; for glucose and IL-6 levels: additionally adjusted for BMI). Main findings are printed in bold.
[c]Dominant model comparing CC- and GC-subjects versus GG.
[d]IL-6 ß-coefficients were computed and are displayed on the logarithm of the original scale (pg/mL).

shows the study-specific and the summary β-coefficients applying the dominant model for the C-allele. There was no statistically significant evidence for an association between *IL6* -174G > C and BMI in the full data (β = 0.088, 95% CI = (−0.023)–(0.200), $P = 0.120$) or the T2DM status-specific analyses ($P > 0.10$) (Table II).

IL6 -174G > C and circulating interleukin-6 levels

There was no evidence in the full data joint analysis including 5659 subjects of seven studies for an association between *IL6* -174G > C and circulating IL-6 levels (Table II and Figure 3). Likewise, there was no statistically significant IL-6 level association among the 4621 non-diabetic subjects of six studies or among the 966 prevalent T2DM subjects of four studies.

Analysis of BMI and IL-6 levels in incident T2DM cases before they became cases

Among the 641 incident T2DM subjects before they became type 2 diabetic, there was no association with BMI ($P > 0.10$). Regarding circulating IL-6, the *IL6* -174G > C showed no association in the dominant model, but higher levels for the homozygous CC-genotype ($\beta_{CCvsGG} = 0.266$, 95% CI = 0.085–0.448, $P = 0.004$) compared to the GG-genotype.

Sensitivity analyses

Regarding fasting glucose, 2-hour glucose, or IL-6 levels, there was no evidence for publication bias: the Egger's regression test was non-significant ($P > 0.10$), and excluding published studies would not change the main findings (online appendix Table A-VI). Regarding BMI, there was some evidence for publication bias. In the five published studies with 10,704 subjects, the *IL6* -174 C-allele was statistically significantly associated with higher BMI (β = 0.190, 95% CI = 0.023–0.358). In contrast, there was no association in the ten unpublished studies with 13,413 subjects (β = 0.007, 95% CI = (−0.142)–(0.157)). Moreover, the funnel plot (online appendix Figure A-1) and the Egger's regression test ($P = 0.06$) showed some evidence for publication bias of the summary BMI estimate when including all 15 studies, but none when restricting to the ten unpublished studies ($P = 0.27$).

Including studies with genotype HWE violation (online appendix Table A-VII) or omitting adjustment for BMI or T2DM status (data not shown) would not change our main findings. Furthermore, there were no large differences between association

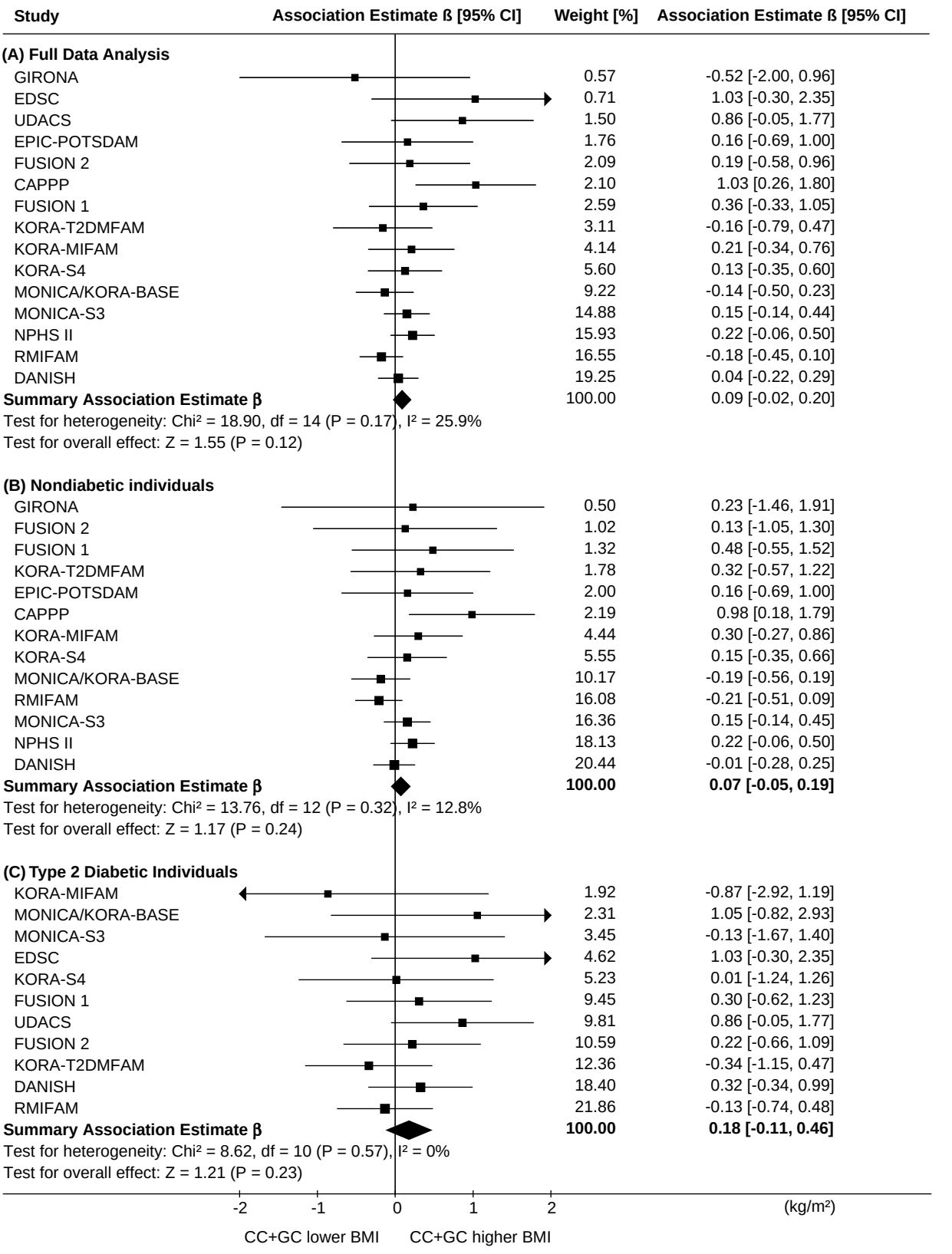

Figure 2. Forest plot, illustrating the study-specific ß-coefficients with 95% CIs for the association between *IL6* -174G>C, dominant model for the C-allele, and body mass index (BMI) of (A) the full data analysis, (B) only non-diabetic subjects, and (C) only prevalent type 2 diabetes mellitus (T2DM) subjects, adjusted for age, sex, and T2DM status (A). Please refer to Figure 1 legend for more details.

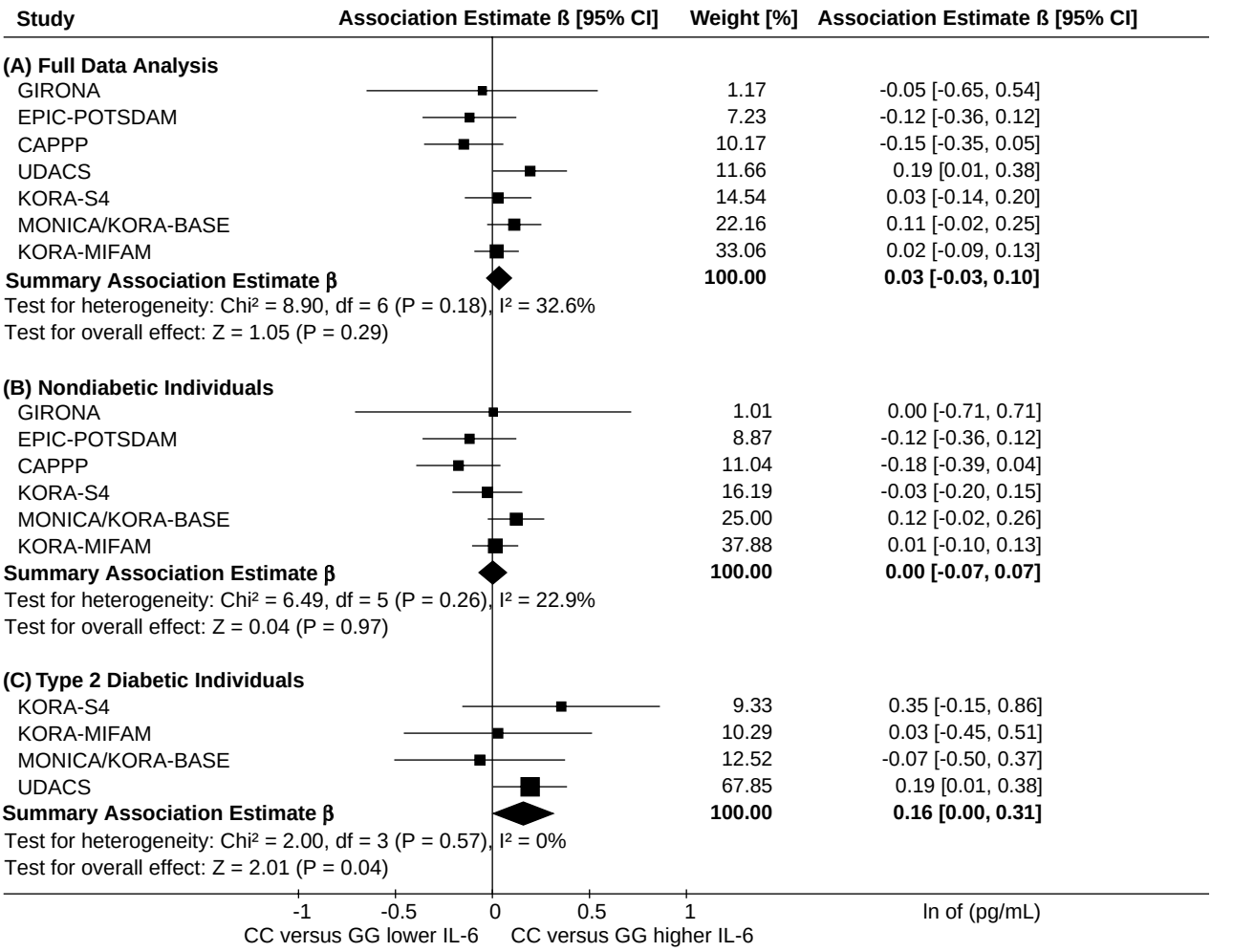

Figure 3. Forest plot, illustrating the study-specific ß-coefficients with 95% CIs for the association between *IL6* -174 CC versus GG and natural logarithm of circulating interleukin-6 (IL-6) levels of (A) the full data analysis, (B) only non-diabetic subjects, and (C) only prevalent type 2 diabetes mellitus (T2DM) subjects, adjusted for age, sex, body mass index (BMI), and T2DM status (A). Please refer to Figure 1 legend for more details.

estimates of men and women in sex-stratified analyses (data not shown).

Sensitivity analyses using ln-transformed fasting glucose levels, ln-transformed BMI levels, or age- and sex-preadjusted standard *z*-scores (all outcomes) to obtain phenotype distributions which are closer to normal yielded results which were very similar to the main analyses. Likewise, exclusion of outliers (fasting glucose <2.78 or >20.00 mmol/L, full data analysis: $n=47$; BMI <16.0 or >50.0 kg/m^2, full data analysis: $n=28$; IL-6 levels <0.20 or >20.0 pg/mL, full data analysis: $n=250$, prevalent T2DM subjects: $n=30$) did not change the main results, except for a stronger association between IL-6 levels (natural logarithm) and the *IL6* -174 CC- versus GG-genotype ($\beta=0.226$, 95% CI: 0.098–0.355, $P=0.0006$). Pooling the individual data rather than using a meta-analysis approach yielded very similar results for all analyses. Interestingly, a subgroup analysis with exclusion of the two studies (DANISH and RMI-FAM) with self-reported BMI data increased the BMI association estimate (C-allele dominant model: $\beta=0.172$ kg/m^2, 95% CI: 0.033–0.312, $P=0.015$).

Discussion

Major findings

In this joint analysis of individual-level data from 9440 study participants, C-allele carriers of the *IL6* -174G > C polymorphism had lower fasting glucose levels (-0.091 mmol/L, $P=0.014$) independently of BMI. No association was found with BMI in 24,117 subjects or with IL-6 levels in 5659 subjects. The estimates were robust against exclusion of outliers, various transformations of variables including use of

standardized *z*-scores, or when applying different meta-analysis methodology.

IL6 -174G>C and circulating glucose

The present analysis results of the *IL6* -174G>C with fasting glucose was in line with our previous joint analysis on T2DM, which had shown that individuals carrying the *IL6* -174 C-allele had 9% lower odds for T2DM compared to individuals with the GG-genotype ($P=0.037$) (25). We also confirmed the dominant genetic model. Our previous T2DM analysis had included a larger number of subjects; when restricting to the seven studies with fasting glucose data, a T2DM summary odds ratio (OR) of 0.89 (95% CI = 0.78–1.02) with a non-significant *P*-value of 0.09 would have been yielded, which impressively demonstrates the gain from additional information by using glucose as a quantitative variable.

The recent finding of an association between the *IL6* -174 C-allele and T2DM (OR C-allele dominant model = 0.75 (95% CI = 0.57–0.98)) in the French D.E.S.I.R. study including 307 T2DM cases and 2919 normoglycemic controls (29) adds strength to our hypothesis that *IL6* -174G>C truly has an impact on individual T2DM risk.

IL6 -174G>C and body mass index

Several lines of evidence suggest that the cytokine IL-6 plays a role in the regulation of body composition, probably by acting in a catabolic manner (30–32). To date, association between *IL6* -174G>C and obesity has been investigated by several comparably small studies with inconsistent results (17,18,20,21,33). Qi et al. recently conducted a large meta-analysis of mainly published studies including 26,944 subjects and found statistically significant heterogeneity between study-specific association estimates. Applying a random effects model in consequence, there was no evidence for an association between *IL6* -174G>C and BMI (34). Our joint analysis of 24,117 subjects also did not find an association between *IL6* -174G>C and BMI and added to the previous meta-analysis by sole inclusion of individual-level data which enabled application of completely standardized analysis methods resulting in low between-study heterogeneity and by inclusion of mostly unpublished studies (56% of the analyzed study participants). There was only a small study overlap of 6631 subjects (27%) between the present joint analysis and the Qi et al. meta-analysis. Our power to detect a BMI difference of 0.2 kg/m^2 or 0.3 kg/m^2 between *IL6* -174 C-allele carriers and non-carriers was more than 80% or nearly 100%, respectively, given the type 1 error probability of 0.050/3 = 0.017, a GG-genotype frequency of 31.2%, and a BMI standard deviation of 4.2 kg/m^2.

IL6 -174G>C and circulating interleukin-6 levels

The current literature on the association between the *IL6* -174G>C and circulating IL-6 levels is inconclusive. Several studies, with the largest including 641–1526 Caucasians (23,24,35–37), did not find evidence for association. However, other studies with the largest being the recently published Cardiovascular Health Study (CHS) with 4714 elderly Caucasians (72 years median age) and a high prevalence of T2DM (28%) showed borderline significantly higher IL-6 levels for CC-genotype subjects compared to GG ($P=0.04$) (38). In our joint analysis of 5659 Caucasians there was no evidence for association between *IL6* -174G>C and circulating IL-6 levels including non-diabetic and manifest T2DM subjects. Reasons for our non-finding of an effect as compared to the CHS study might be a smaller power in a joint analysis compared to a single study, an effect only present in special risk groups, or a true non-existence of the effect. Our exploratory analysis of increased IL-6 levels with the CC-genotype in the 641 incident T2DM subjects would be in line with the CHS study. In summary, our joint analysis was not able to resolve the puzzle whether and how IL-6 levels mediate the association between *IL6* -174G>C and T2DM by pin-pointing a direct association between the polymorphism and IL-6 levels. Thus, even larger studies optimally with a prospective design might be warranted.

Strengths and limitations of this joint analysis

The large number of subjects analyzed in our investigation was a definite strength. For all analyzed phenotypes, our study represents the largest study on individual-level data conducted to date to address the role of *IL6* -174G>C. Thus, we were able to reveal small associations, which nevertheless are of importance due to the high prevalence of the genetic variant in the general population (41%–55%) and their potential to help understand the pathogenesis of this severe disease. Our non-finding of an association with BMI in this highly powered study may also be of great importance to solve to some extent the puzzle from contrary reports.

Furthermore, it was a distinctive strength of our joint analysis that it was based on individual participants' data allowing for standardized data cleaning and analysis, to which the low heterogeneity among study-specific estimates may be attributed.

Finally, our analysis included many unpublished studies, which guards against the greatest threats of meta-analyses, the publication or selective reporting bias. Stratifying for published or unpublished studies depicted a potential of some bias for the published association studies of *IL6* -174G > C and BMI.

It may be considered a limitation that all T2DM status-specific analyses exhibited truncated glucose distributions, which consequently also affected the correlated BMI and IL-6 level distributions. In addition, no data were available on antidiabetic medication for T2DM subjects for the glucose analyses or on physical activity before blood extraction for the circulating IL-6 level analyses. This might have decreased the precision of the estimates but is unlikely to have caused false-positive findings.

Conclusion

This joint analysis represents by far the largest individual participants' data analysis to date on the association of the *IL6* promoter polymorphism -174G > C with quantitative phenotypes relevant for T2DM. Our data indicate that C-allele carriers of the widely debated *IL6* -174G > C polymorphism have lower fasting glucose levels on average, which substantiates previous findings of decreased T2DM risk of these subjects. Therefore, our study suggests *IL6* as a T2DM gene. No statistically significant association was found for quantitative BMI or IL-6 concentrations. In general, we demonstrated that a consortium-based approach involving individual participants' data and standardized analysis is well suited to investigate genetic variants with small effects.

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References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–53.
2. Kempf K, Rathmann W, Herder C. Impaired glucose regulation and type 2 diabetes in children and adolescents. Diabetes Metab Res Rev. 2008 Jun 13 (Epub ahead of print).
3. Das SK, Elbein SC. The genetic basis of type 2 diabetes. Cellscience. 2006;2:100–31.
4. Carey AL, Bruce CR, Sacchetti M, Anderson MJ, Olsen DB, Saltin B, et al. Interleukin-6 and tumor necrosis factor-alpha are not increased in patients with Type 2 diabetes: evidence that plasma interleukin-6 is related to fat mass and not insulin responsiveness. Diabetologia. 2004;47:1029–37.
5. Andreozzi F, Laratta E, Cardellini M, Marini MA, Lauro R, Hribal ML, et al. Plasma interleukin-6 levels are independently associated with insulin secretion in a cohort of Italian-Caucasian nondiabetic subjects. Diabetes. 2006;55:2021–4.
6. Henningsson S, Hakansson A, Westberg L, Baghaei F, Rosmond R, Holm G, et al. Interleukin-6 gene polymorphism -174G/C influences plasma lipid levels in women. Obesity. 2006;14:1868–73.
7. Thorand B, Baumert J, Kolb H, Meisinger C, Chambless L, Koenig W, et al. Sex Differences in the prediction of type 2 diabetes by inflammatory markers: Results from the MONICA/KORA Augsburg case-cohort study, 1984–2002. Diabetes Care. 2007;30:854–60.
8. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, et al. 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. 1998;102:1369–76.
9. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem. 2000;275:18138–44.
10. Brull DJ, Montgomery HE, Sanders J, Dhamrait S, Luong L, Rumley A, et al. Interleukin-6 gene -174g > c and -572g > c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arterioscler Thromb Vasc Biol. 2001;21:1458–63.
11. Bennermo M, Held C, Stemme S, Ericsson CG, Silveira A, Green F, et al. Genetic predisposition of the interleukin-6 response to inflammation: implications for a variety of major diseases? Clin Chem. 2004;50:2136–40.
12. Acalovschi D, Wiest T, Hartmann M, Farahmi M, Mansmann U, Auffarth GU, et al. Multiple levels of regulation of the interleukin-6 system in stroke. Stroke. 2003;34:1864–9.
13. Gaudino M, Andreotti F, Zamparelli R, Di Castelnuovo A, Nasso G, Burzotta F, et al. The -174G/C interleukin-6 polymorphism influences postoperative interleukin-6 levels and postoperative atrial fibrillation. Is atrial fibrillation an inflammatory complication? Circulation. 2003;108 Suppl 1:II195–9.

14. Kilpinen S, Hulkkonen J, Wang XY, Hurme M. The promoter polymorphism of the interleukin-6 gene regulates interleukin-6 production in neonates but not in adults. Eur Cytokine Netw. 2001;12:62–8.
15. Vozarova B, Fernandez-Real JM, Knowler WC, Gallart L, Hanson RL, Gruber JD, et al. The interleukin-6 (−174) G/C promoter polymorphism is associated with type-2 diabetes mellitus in Native Americans and Caucasians. Hum Genet. 2003;112:409–13.
16. Fernandez-Real JM, Broch M, Vendrell J, Gutierrez C, Casamitjana R, Pugeat M, et al. Interleukin-6 gene polymorphism and insulin sensitivity. Diabetes. 2000;49:517–20.
17. Berthier MT, Paradis AM, Tchernof A, Bergeron J, Prud'homme D, Despres JP, et al. The interleukin 6-174G/C polymorphism is associated with indices of obesity in men. J Hum Genet. 2003;48:14–19.
18. Klipstein-Grobusch K, Möhlig M, Spranger J, Hoffmann K, Rodrigues FU, Sharma AM, et al. Interleukin-6 g.-174G > C promoter polymorphism is associated with obesity in the EPIC-Potsdam Study. Obes Res. 2006;14:14–18.
19. Walch K, Grimm C, Zeillinger R, Huber JC, Nagele F, Hefler LA. A common interleukin-6 gene promoter polymorphism influences the clinical characteristics of women with polycystic ovary syndrome. Fertil Steril. 2004;81:1638–41.
20. Grallert H, Huth C, Kolz M, Meisinger C, Herder C, Strassburger K, et al. IL-6 promoter polymorphisms and quantitative traits related to the metabolic syndrome in KORA S4. Exp Gerontol. 2006;41:737–45.
21. Georges JL, Loukaci V, Poirier O, Evans A, Luc G, Arveiler D, et al. Interleukin-6 gene polymorphisms and susceptibility to myocardial infarction: the ECTIM study. Etude Cas-Temoin de l'Infarctus du Myocarde. J Mol Med. 2001;79:300–5.
22. Hamid YH, Rose CS, Urhammer SA, Glumer C, Nolsoe R, Kristiansen OP, et al. Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. Diabetologia. 2005;48:251–60.
23. Sie MP, Sayed–Tabatabaei FA, Oei HH, Uitterlinden AG, Pols HA, Hofman A, et al. Interleukin 6 -174 g/c promoter polymorphism and risk of coronary heart disease: results from the Rotterdam study and a meta-analysis. Arterioscler Thromb Vasc Biol. 2006;26:212–7.
24. Qi L, van Dam RM, Meigs JB, Manson JE, Hunter D, Hu FB. Genetic variation in IL6 gene and type 2 diabetes: tagging-SNP haplotype analysis in large-scale case-control study and meta-analysis. Hum Mol Genet. 2006;15:1914–20.
25. Huth C, Heid IM, Vollmert C, Gieger C, Grallert H, Wolford JK, et al. IL6 gene promoter polymorphisms and type 2 diabetes: joint analysis of individual participants' data from 21 studies. Diabetes. 2006;55:2915–21.
26. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21:1539–58.
27. Normand SL. Meta-analysis: formulating, evaluating, combining, and reporting. Stat Med. 1999;18:321–59.
28. Thakkinstian A, McElduff P, D'Este C, Duffy D, Attia J. A method for meta-analysis of molecular association studies. Stat Med. 2004;24:1291–306.
29. Vaxillaire M, Veslot J, Dina C, Proenca C, Cauchi S, Charpentier G, et al. Impact of common type 2 diabetes risk polymorphisms in the DESIR prospective study. Diabetes. 2008;57:244–54.
30. Wallenius V, Wallenius K, Ahren B, Rudling M, Carlsten H, Dickson SL, et al. Interleukin-6-deficient mice develop mature-onset obesity. Nat Med. 2002;8:75–9.
31. van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, et al. Interleukin-6 stimulates lipolysis and fat oxidation in humans. J Clin Endocrinol Metab. 2003;88:3005–10.
32. Lyngso D, Simonsen L, Bulow J. Metabolic effects of interleukin-6 in human splanchnic and adipose tissue. J Physiol. 2002;543:379–86.
33. Barbieri M, Rizzo MR, Papa M, Acampora R, De Angelis L, Olivieri F, et al. Role of interaction between variants in the PPARG and interleukin-6 genes on obesity related metabolic risk factors. Exp Gerontol. 2005;40:599–604.
34. Qi L, Zhang C, van Dam RM, Hu FB. Interleukin-6 genetic variability and adiposity: associations in two prospective cohorts and systematic review in 26,944 individuals. J Clin Endocrinol Metab. 2007;92:3618–25.
35. Bennet AM, Prince JA, Fei GZ, Lyrenas L, Huang Y, Wiman B, et al. Interleukin-6 serum levels and genotypes influence the risk for myocardial infarction. Atherosclerosis. 2003;171:359–67.
36. Herbert A, Liu C, Karamohamed S, Liu J, Manning A, Fox CS, et al. BMI modifies associations of IL-6 genotypes with insulin resistance: the Framingham Study. Obesity. 2006;14:1454–61.
37. van Oijen M, Arp PP, de Jong FJ, Hofman A, Koudstaal PJ, Uitterlinden AG, et al. Polymorphisms in the interleukin 6 and transforming growth factor beta1 gene and risk of dementia. The Rotterdam Study. Neurosci Lett. 2006;402:113–7.
38. Walston JD, Fallin MD, Cushman M, Lange L, Psaty B, Jenny N, et al. IL-6 gene variation is associated with IL-6 and C-reactive protein levels but not cardiovascular outcomes in the Cardiovascular Health Study. Hum Genet. 2007;122:485–94.

Appendix

Research design and methods

Study inclusion criteria, search strategy, and study recruitment

All available published and unpublished studies fulfilling the following criteria were recruited for the *IL6*-T2DM consortium: 1) association study conducted in humans, 2) polymorphic genotype data for *IL6* -174G>C, 3) T2DM cases and non-diabetic controls, 4) published before September 2005 or unpublished, 5) availability of individual participants' data (IPD). Studies were excluded if the control group consisted only of individuals with pre-diabetes (one study) (1), or if ethnic admixture of unrelated study subjects was reported in the original publication (Pima Indian case-control study, reported in (2)).

Published studies were identified in the PubMed database using the following search terms: (IL-6 OR IL6 OR interleukin-6) AND (diabetes OR T2DM OR NIDDM) AND (gene OR genes OR genet* OR polymorphism* OR allele*). To further extend the search, the reference lists from all identified original studies and review articles on this topic were examined. Unpublished studies were recruited by a call for participation at the symposium 'Immunogenetic Contribution to Type 2 Diabetes and Parameters of the Metabolic Syndrome', which was held in September 2004 at the 40th Annual Meeting of the European Association for the Study of Diabetes, and by personally contacting investigators in the field.

Data cleaning, phenotyping, and genotyping methods

The study center at the Helmholtz Zentrum München checked all incoming data for plausibility and for consistency with information provided by the investigators or the published article. Plausible and corrected data were converted into a standard format and incorporated into a central database.

Body mass index was calculated as weight (kg) divided by squared height (m^2) from measured anthropometric data, except for the RMIFAM and DANISH studies where self-reported data were used. An overview on methods used to quantify circulating interleukin-6 (IL-6) levels is presented in Table A-I.

A questionnaire was sent to all principal investigators to collect data on genotyping methods and quality. This information is also summarized in Table A-I. As *IL6* -174G>C is a G/C-polymorphism, and allele G is complementary to allele C, the genotyping sequences and strands, assessed via the questionnaire, were compared with a reference to confirm that the allele labeling was performed consistently across all studies.

Further details on the statistical analyses

Statistical analyses were performed using SAS software version 9.1 (Cary, NC, USA). *IL6* -174G>C allele and genotype frequencies were estimated, accounting for the correlation in family data by use of an exchangeable structure in a generalized estimating equations approach (SAS PROC GENMOD). Hardy-Weinberg equilibrium (HWE) was tested in non-diabetic subjects (SAS PROC ALLELE); for family studies only one randomly drawn subject per family was included. In order to approximate a normal distribution, circulating IL-6 was logarithmically transformed.

Publication bias was investigated by visual inspection of funnel plots and by the Egger's regression test (3). Funnel and forest plots were prepared using Review Manager software version 4.2 (Cochrane Collaboration, Copenhagen, DK).

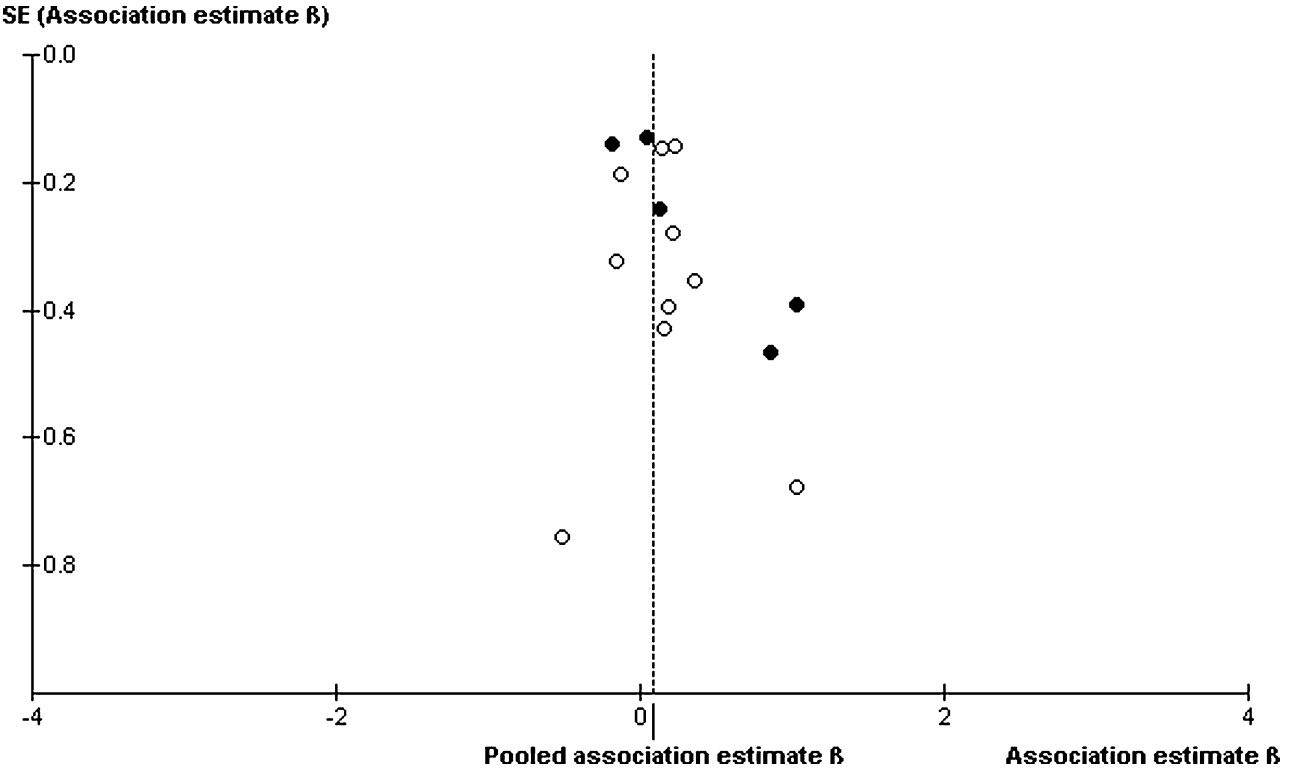

Figure A-1. (online appendix). Funnel plot for the association between *IL6* -174G >C and body mass index (BMI). For each study, the ß-coefficient of the dominant model for the C-allele, adjusted for age, sex, and type 2 diabetes status, is plotted against its standard error as a measure of study precision. All studies with *IL6* -174G >C genotypes of non-diabetic individuals in Hardy-Weinberg equilibrium (HWE) are included. Black circles represent BMI published studies, white circles represent BMI unpublished studies. The vertical line marks the pooled ß-coefficient (0.09 kg/m^2).

Table A-I. (online appendix). Methods used for quantifying circulating interleukin (IL)-6 levels and genotyping *IL6* -174G >C (rs1800795 according to http://www.ncbi.nlm.nih.gov).

Study	IL-6 level quantification	Genotyping method	Call rate (%)[a]
Botnia Study	n.a.	Allelic discrimination assay-by-design on ABI 7900 (Applied Biosystems)	100
Captopril Prevention Project	Sandwich ELISA, R&D Systems, Abingdon, UK	Dynamic allele specific hybridization (DASH)	100
Danish Study	n.a.	Chip-based MALDI-TOF MS (MassArray, Sequenom)	96
Ealing Diabetes Study of Coagulation	n.a.	Nla III RFLP, MADGE	97
European Prospective Investigation into Cancer and Nutrition Potsdam	Sandwich ELISA, R&D Systems, Abingdon, UK	SNuPE, MegaBACE 1000	100
The Finland-United States Investigation of NIDDM Genetics	n.a.	Illumina GoldenGate	98
Girona Genetics of Diabetes Study	Solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay, DPC DIPESA S.A., Madrid, Spain	SfaNI RFLP	99
KORA MI Family Study	Sandwich ELISA, R&D Systems, Abingdon, UK	Hsp92II RFLP, PAGE	97
KORA Survey S4	Sandwich ELISA, CLB, Amsterdam, Netherlands	Chip-based MALDI-TOF MS (MassArray, Sequenom)	97
KORA T2DM Family Study	n.a.	Chip-based MALDI-TOF MS (MassArray, Sequenom)	98
MONICA/KORA Case Cohort Study S123	Sandwich ELISA, CLB, Amsterdam, Netherlands	Chip-based MALDI-TOF MS (MassArray, Sequenom)	99
MONICA/KORA Survey S3	n.a.	Chip-based MALDI-TOF MS (MassArray, Sequenom)	99
Regensburg MI Family Study	n.a.	Hsp92II RFLP, PAGE	97
Second Northwick Park Heart Study	n.a.	PCR by MADGE, NIaIII RFLP	99
Tarraco Study	n.a.	SfaNI RFLP	99
University College Diabetes and Cardiovascular Study	Sandwich ELISA, R&D Systems, Abingdon, UK	PCR by MADGE, NIaIII RFLP	99

[a]Successfully genotyped individuals in percent of all subjects intended for genotyping.
n.a. =not applicable; NIDDM =non-insulin-dependent diabetes mellitus; MI =myocardial infarction.

Table A-II. (online appendix). Description of included studies.

Study name (official abbreviation)	Study description[a]
Botnia Study	The Botnia Study began in 1990 as a family-based study aiming to identify genes increasing susceptibility to T2DM. Type 2 diabetic subjects from the area of five health care centers in the Botnia region of Western Finland were invited to participate together with their family members. For the purpose of this joint analysis, unrelated individuals were genotyped for *IL6* -174G > C. According to a priori defined criteria, one type 2 diabetic individual per family was selected. Non-diabetic subjects comprise cases' spouses and unrelated individuals, all being 35 years or older.
Captopril Prevention Project (CAPPP)	CAPPP is a prospective randomized clinical trial conducted in Sweden and Finland during the 1990s. Patients aged 25–66 years, with a measured diastolic blood pressure of 100 mmHg or more on two occasions, were recruited at health centers and randomly assigned to captopril or conventional antihypertensive treatment. Exclusion criteria were secondary hypertension, serum creatinine concentration of more than 150 μmol/L, and disorders that required treatment with ß-blockers. Cases had T2DM at base-line or were diagnosed during the follow-up. This joint analysis includes a substudy of the Swedish part of CAPPP, which has been genotyped for *IL6* -174G > C. This substudy comprises all patients that got myocardial infarction (MI), plus two control subjects without MI per patient, matched with respect to gender, age, and smoking. Further details: (4).
Danish Study	The DANISH case-control study of T2DM involves all 4568 subjects with normal glucose tolerance (NGT) from the Inter99 cohort and 1389 unrelated type 2 diabetic patients recruited from the outpatient clinic at Steno Diabetes Center, Copenhagen and the Research Center for Prevention and Health through the Inter99 study. The Inter99 cohort is a population-based randomized non-pharmacological intervention study for prevention of cardiovascular disease done at the Research Center for Prevention and Health involving 6514 Caucasian subjects (6164 with data from an oral glucose tolerance test). Further details: (5).
Ealing Diabetes Study of Coagulation (EDSC)	The type 2 diabetic individuals of the EDSC study were recruited consecutively from the Ealing Hospital diabetes clinic in London, UK. Patients completed a questionnaire with details of age, ethnicity, smoking habit, fasting status, duration of diabetes, and other clinical details. Blood was collected for plasma and DNA analysis. Several further parameters, such as BMI, were measured. Type 2 diabetic individuals ($n = 927$) comprised primarily three ethnic groups: Indian Asian, $n = 503$; UK white, $n = 331$; black Afro-Caribbean, $n = 93$. Further details: (6). To ensure comparability with the other Caucasian studies, only the white subjects were included in this joint analysis.
European Prospective Investigation into Cancer and Nutrition Potsdam (EPIC-Potsdam)	A nested case-control study was designed within the European Prospective Investigation into Cancer and Nutrition Potsdam cohort (EPIC-POTSDAM_nCC-T2DM), which is part of the European multicenter, population-based EPIC-study including 27,548 subjects from the area around Potsdam, Germany (women aged 35–65 years and men aged 40–65 years). Base-line examination and blood sampling were conducted between 1994 and 1998. Data presented in this joint analysis are based on the first follow-up questionnaires sent to the study participants on average 2.3 years after base-line examination. Further details: (7). To ensure comparability with the other cross-sectional studies, only the non-diabetic control subjects were included in this joint analysis. Cases were free of T2DM at base-line and developed their incident T2DM during the follow-up. Analyses of their data are presented separately.

Table A.II (*Continued*)

Study name (official abbreviation)	Study description[a]
The Finland-United States Investigation of NIDDM Genetics (FUSION)	The index probands in the FUSION study were identified primarily from the National Hospital Discharge Registry (NHDR), which includes records since 1970 of all hospitalized patients with diabetes, and from previous studies carried out by the National Public Health Institute in Finland. From the NHDR, all patients who were hospitalized with a diagnosis of T2DM in Finland during 1987–1993 were identified in the first wave of sampling (FUSION 1). In the second wave of sampling (FUSION 2), patients hospitalized with T2DM during 1994–1995 were identified. Potential families for FUSION 2 also included some identified during FUSION 1 but not invited to participate at that time due to distance from the study clinics. An index proband with his family was eligible for participation in the FUSION study if 1) the proband or another affected sibling was diagnosed with T2DM between 35 and 60 years of age, 2) there was no history of type 1 diabetes in first-degree relatives, 3) the proband had one or more living full siblings diagnosed with T2DM at any age, and 4) at least one parent was apparently non-diabetic, with preference given to families with living parents or parents who had lived a long life without known diabetes. Further details on FUSION 1: (8); on FUSION 2: (9). Participants of FUSION 1 and FUSION 2 were analyzed separately. 'FUSION 1' in this joint analysis comprises one type 2 diabetic individual from each FUSION 1 family, and non-diabetic spouses of type 2 diabetic FUSION participants, as well as elderly subjects that were all born in 1925 and were normal glucose tolerant by oral glucose tolerance tests (OGTTs) at both ages 65 and 70. 'FUSION 2' comprises the sibling generation of the FUSION 2 sampling wave.
Girona Genetics of Diabetes Study	The type 2 diabetic patients of the Girona Genetics of Diabetes Study were consecutively recruited subjects from the diabetes clinics at the Hospital of Girona, Spain. The non-diabetic subjects are unrelated healthy Caucasian middle-aged subjects recruited from the general population. Further details: (2).
KORA Studies in chronological order	KORA (Cooperative Health Research in the Region Augsburg) is a regional research platform in the German city of Augsburg and the two adjacent counties, for population-based studies, subsequent follow-up studies, and family studies in the fields of epidemiology, health economics, and health care research. KORA was established in 1996 to expand the WHO (World Health Organization) MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) project in Augsburg. In the framework of MONICA, three independent cross-sectional population-representative surveys were conducted in 1984/85 (S1), 1989/90 (S2), and 1994/95 (S3), and a population-based acute myocardial infarction registry was set up. The study subjects of all Augsburg MONICA and KORA surveys and the family studies on myocardial infarction and T2DM are of German nationality and were studied by physical examination, blood testing, and a standardized interview in KORA study centers. All tests were carried out by specially trained personnel. Further details: (10–12). Some individuals were originally recruited for two or more studies, but were assigned to one of the included KORA studies for the purpose of this joint analysis according to a priori defined criteria.
MONICA/KORA Survey S3	The MONICA/KORA Survey S3 originally investigated 4856 individuals. Study participants that are included in the MONICA/KORA Case Cohort Study S123 were eliminated from subjects of the MONICA/KORA Survey S3 for this joint analysis.
KORA MI Family Study	Patients with MI prior to the age of 60 years and their siblings were identified through the acute myocardial infarction registry. The diagnosis of MI was established according to the MONICA diagnostic criteria. Of 1254 patients contacted, 609 agreed to participate in the study (532 men, aged 56.1 ± 0.3 years). Moreover, 540 siblings without MI (251 men, aged 54.6 ± 0.4 years from 325 families) were recruited and examined by the same protocol.
KORA Survey S4 (KORA S4)	The KORA S4 studied a population-representative sample of 4261 subjects, 25–74 years old, during the years 1999–2001. The sample design followed the guidelines of the three previous MONICA Augsburg surveys. In the age-range of 55–74 years, 1653 persons participated in an OGTT. These participants were genotyped for *IL6* -174G > C and included in this joint analysis. Further details: (13).

Table A.II (*Continued*)

Study name (official abbreviation)	Study description[a]
KORA T2DM Family Study (KORA T2DMFAM)	In 2001/2002, 605 nuclear families were enrolled in the KORA T2DM Family Study. Families were ascertained through an index proband with known T2DM, who had at least one full sib or both parents willing to participate in the study. All available members of the index probands' nuclear families, i.e. full sibs and parents, were included. Index probands were all from the city or region of Augsburg. They were recruited from T2DM patients of the Central Hospital of Augsburg, from earlier MONICA and KORA studies, from the acute myocardial infarction registry, or via public relations. All participants were living in Germany, and all were of European origin. Most subjects were extensively phenotyped in the KORA study center, some were examined by their family doctor, who decided whether or not the subject had T2DM and took blood samples for DNA analyses. Data of the sibling generation was included in this joint analysis.
MONICA/KORA Case Cohort Study S123	All participants of at least one of the three MONICA Augsburg surveys were prospectively followed for the MONICA/KORA Case Cohort Study S123. The study was restricted to participants aged 35–74 years at base-line, since the incidence of T2DM is low in younger subjects. A stratified random sample of the source population, containing 1885 subjects, was selected. A total of 555 incident cases of T2DM were observed between participants' study start dates and 31st December 2002. Further details: (14). For the purposes of this joint analysis on quantitative phenotypes, the base-line data of the MONICA/KORA Case Cohort Study S123 (prevalent T2DM and non-diabetic subjects) without the participants who developed their incident T2DM during the follow-up were used to ensure comparability with the other cross-sectional studies (MONICA/KORA-BASE). Analyses of the subjects with incident T2DM are presented separately.
Regensburg MI Family Study	The kindreds of the Regensburg MI Family Study were ascertained through MI index patients, who were identified by screening 93,500 patient charts in seven cardiac in-hospital rehabilitation centers distributed throughout Germany. Index patients had all suffered from MI before 60 years. If at least one sibling had suffered from MI or had severe coronary artery disease or by-pass surgery, the index patient with all available parents and siblings were contacted and invited to participate in the study. All participating individuals filled out a standardized questionnaire that focused on cardiovascular risk factors, medical diagnoses, life-style and medication. Further details: (15). Data of the sibling generation were included in this joint analysis.
Second Northwick Park Heart Study (NPHS II)	For the NPHS II Study, 3012 unrelated healthy Caucasian middle-aged male subjects were recruited from nine general medical practices scattered throughout the UK and prospectively followed from 1989. Sixty-eight subjects with diabetes at base-line were excluded from analysis. Further details: (16).
Tarraco Study	For the Tarraco study, 211 unrelated type 2 diabetic subjects were recruited from the outpatient clinic at Hospital Universitari de Tarragona 'Joan XXIII' during the years 2000–2004. Simultaneously, 118 healthy subjects were recruited from the same hospital. Further details: (2).
University College Diabetes and Cardiovascular Study (UDACS)	The UDACS Study comprises 1011 consecutive subjects recruited from the diabetes clinic at University College London Hospitals NHS Trust (UCLH) between the years 2001 and 2002. Patients completed a questionnaire with details of age, ethnicity, smoking habit, fasting status, duration of diabetes, and other clinical details. Blood was collected for plasma and DNA analysis. Several further parameters, such as BMI, were measured. No subjects requiring renal dialysis were recruited. Further details: (16).

[a]The numbers of participants presented for the original studies do not always match the numbers used in the analyses of this joint analysis. The reason is that some subjects of the original studies were not included in the joint analysis because of study overlap, missing genotype data, or missing phenotype data.

Table A-III. (online appendix). References of included studies and overview on analyzed outcomes.

Study	No. T2DM/Non-diab[a]	Outcome[b] Fasting glucose	2-h glucose	BMI	IL-6 Level	Reference[c]
BOTNIA	731/557	3	3	3	4	n.a.
CAPPP	42/424	2	4	1	2	(4)
DANISH	1212/4399	1	3	1	4	(5)
EDSC	299/0	3	4	3	4	n.a.
EPIC-POTSDAM	0/348	4	4	2	2	(7)
FUSION 1	508/367	3	3	3	4	n.a.
FUSION 2	437/201	3	3	3	4	n.a.
GIRONA	42/123	3	3	3	3	(2)
KORA-MIFAM	95/881	4	4	2	2	(17)
KORA-S4	225/1190	3	3	1	1	(18)
KORA-T2DMFAM	776/513	3	3	3	4	n.a.
MONICA/KORA-BASE	101/1744	4	4	3	3	n.a.
MONICA-S3	151/3551	4	4	2	4	(17)
NPHS II	0/2652	4	4	1	4	(16)
RMIFAM	662/2614	4	4	2	4	(17)
TGN	166/64	4	4	3	4	(2)
UDACS	560/0	4	4	1	3	(16)

[a]Number of type 2 diabetic (T2DM)/non-diabetic (non-diab) subjects included in analyses of the outcome BMI.
[b]Publication of analyses on association between *IL6* -174G>C and outcomes fasting glucose, 2-h glucose, body mass index (BMI), or interleukin-6 (IL-6) levels 1 = as main outcome, 2 = as additional outcome; 3 = completely unpublished before study recruitment for joint analysis. 4 = No or not enough outcome data available for analysis.
[c]References of association studies between *IL6* -174G>C and circulating glucose or IL-6 levels, BMI, or T2DM.
n.a. = not applicable

Table A-IV. (online appendix). Overview of studies and numbers of participants included in the analyses of the quantitative traits fasting glucose, 2-h glucose, body mass index, and circulating interleukin (IL)-6 levels.

	Fasting glucose			2-h glucose			Body mass index			Circulating IL-6 levels		
Study	Non-diab[a]	T2DM[a]	Full[a]	Non-diab	T2DM	Full	Non-diab	T2DM	Full	Non-diab	T2DM	Full
CAPPP	286	(37)[b]	323				424	(42)[b]	466	420	(42)[b]	462
DANISH	4397	1139	5536	4396	366	4762	4399	1212	5611			
EDSC		106						299	299			
EPIC-POTSDAM	65						348		348	346		346
FUSION 1	358	486	844	359	(37)[b]	396	367	508	875			
FUSION 2	194	397	591	192	78	270	201	437	638			
GIRONA	123	(41)[b]	164	89	(12)[b]	101	123	(42)[b]	165	84	(30)[b]	114
KORA-MIFAM							881	95	976	872	94	966
KORA-S4	1186	132	1318	1190	118	1308	1190	225	1415	1183	222	1405
KORA-T2DMFAM	512	152	664	505	56	561	513	776	1289			
MONICA/KORA-BASE							1744	101	1845	1716	101	1817
MONICA-S3	299						3551	151	3702			
NPHS II							2652		2652			
RMIFAM							2614	662	3276			
UDACS								560	560		549	549
Sum main analyses	7420	2412	9440	6731	618	7398	19007	5026	24117	4621	966	5659
Studies with Hardy-Weinberg equilibrium (HWE) violation (included in sensitivity analyses):												
BOTNIA	557	728	1285	539	462	1001	557	731	1288			
TGN		52					64	166	230			
Sum all studies	7977	3192	10725	7270	1080	8399	19628	5923	25635	4621	966	5659

[a]Number of 'Non-diab' = non-diabetic subjects, 'T2DM' = type 2 diabetic subjects, 'Full' = non-diabetic and type 2 diabetic subjects combined (used for full data analysis).
[b]These type 2 diabetic subjects were not included in the T2DM status-specific analysis because there were less than 50 type 2 diabetic subjects in this study with data on the respective outcome.

Table A-V. (online appendix). Characteristics of study participants in the joint analysis.

					Mean ± standard error		*IL6* -174G > C		
Study	Status[a]	T2DM diagnosis[b]	No.[c]	Male (%)	Age (years)	BMI (kg/m^2)	% GC	% CC	*P* HWE[d]
BOTNIA	1	4, 10	731	53	60 ± 10	29 ± 5	53	25	0.18
	2	2, 3, 4, 6	557	48	53 ± 12	26 ± 4	55	24	0.03
CAPPP	1	5, 7, 10	42	81	58 ± 5	30 ± 4	45	26	0.55
	2	4	424	72	57 ± 7	27 ± 4	45	24	0.11
DANISH	1	3, 4, 5, 6, 10	1212	59	57 ± 11	30 ± 5	48	23	0.28
	2	5, 7	4399	47	45 ± 8	26 ± 4	48	23	0.06
EDSC	1	3, 4, 10	299	63	63 ± 14	30 ± 6	45	22	0.13
	2	n.a.							
EPIC-POTSDAM	1	n.a.							
	2	1, 2	348	59	55 ± 7	27 ± 4	55	18	0.07
FUSION 1	1	3, 4, 5, 6, 10	508	55	63 ± 7	30 ± 5	47	30	0.15
	2	2, 3, 4, 7	367	41	66 ± 6	27 ± 4	50	30	1.00
FUSION 2	1	3, 4, 5, 6, 10	437	55	64 ± 9	30 ± 5	49	29	0.89
	2	2, 3, 4, 6	201	38	58 ± 9	27 ± 4	52	28	0.30
GIRONA	1	5, 6, 10	42	76	53 ± 10	31 ± 5	50	10	0.73
	2	4, 6	123	82	47 ± 13	26 ± 4	50	24	1.00
KORA-MIFAM	1	1, 3, 8	95	85	58 ± 6	30 ± 5	50	15	0.66
	2	1, 2, 8	881	67	55 ± 8	28 ± 4	47	17	0.71
KORA-S4	1	2, 3, 5, 6, 10	225	59	65 ± 5	31 ± 5	54	16	0.17
	2	1, 2, 4, 6	1190	51	64 ± 5	28 ± 4	52	17	0.06
KORA-T2DMFAM	1	2, 3, 5, 6, 10	776	58	61 ± 10	31 ± 5	52	16	0.02
	2	1, 2, 4, 6	513	43	58 ± 11	28 ± 5	52	16	0.17
MONICA/KORA-BASE	1	2, 10	101	66	62 ± 7	30 ± 4	49	21	0.85
	2	1	1744	53	52 ± 10	27 ± 4	49	17	0.55
MONICA-S3	1	2, 3, 8, 10	151	58	63 ± 8	30 ± 4	46	23	0.33
	2	1, 2, 8	3551	51	48 ± 14	27 ± 4	50	19	0.26
NPHSII	1	n.a.							
	2	1	2652	100	56 ± 3	26 ± 3	50	18	0.47
RMIFAM	1	1, 9	662	70	61 ± 7	28 ± 4	51	18	0.26
	2	1, 9	2614	68	58 ± 9	27 ± 3	48	19	0.52
TGN	1	1, 4	166	34	60 ± 10	29 ± 5	50	08	0.16
	2	1, 3, 4, 6	64	41	49 ± 14	30 ± 5	61	08	0.04
UDACS	1	4, 10	560	59	66 ± 11	30 ± 6	45	13	0.85
	2	n.a.							

[a]1 = type 2 diabetic subjects, 2 = non-diabetic subjects.

[b]Type of type 2 diabetes mellitus (T2DM)-diagnosis for type 2 diabetic subjects: 1 = interview question; 2 = interview question with diabetes confirmation by doctor; 3 = diabetes medication; 4 = doctor diagnosis; 5 = fasting glucose ≥7.0 mmol/L; 6 = oral glucose tolerance test with 2-hour glucose ≥11.1 mmol/L (WHO-OGTT); 7 = WHO-OGTT in some participants to confirm diagnosis; 8 = random glucose ≥11.1 mmol/L; 9 = glycated hemoglobin (HbA1c) ≥6.2%; 10 = exclusion of subjects with type 1 diabetes.

Type of 'No T2DM'-diagnosis for non-diabetic subjects: 1 = interview question; 2 = no diabetes medication; 3 = doctor diagnosis; 4 = fasting glucose <7.0 mmol/L; 5 = fasting glucose <6.1 mmol/l; 6 = oral glucose tolerance test (OGTT) with 2-hour glucose <11.1 mmol/L; 7 = OGTT with 2-hour glucose <7.8 mmol/L; 8 = random glucose <11.1 mmol/L; 9 = HbA1c <6.2%.

[c]Number of individuals included in analyses for association between *IL6* -174G > C and body mass index (BMI).

[d]*P*-value of exact test for Hardy-Weinberg equilibrium (HWE), using 10,000 Monte Carlo permutations. In the four family-studies FUSION 2, KORA-MIFAM, KORA-T2DMFAM, and RMIFAM, HWE was calculated, including only one randomly drawn type 2 diabetic, or non-diabetic subject, respectively, per family.

n.a. = not applicable.

Table A-VI. (online appendix). Sensitivity analyses, excluding published studies.[a]

Outcome	Egger's test[b]	Excluded studies	Individual *n*	Individual β-estimates (95% CI)	Summary *n*[c]	Summary ß-estimate (95% CI)[c]
Full data analyses: C-allele dominant model						
Fasting glucose (mmol/L)[d]	0.12	DANISH	5536	−0.08 ((−0.20)–(0.03))	3904	−0.09 ((−0.19)–(−0.002))
2-h glucose (mmol/L)[d]	0.23	n.a.	n.a.	n.a.	7398	−0.08 ((−0.20)–(0.05))
BMI[e]	0.06	CAPPP	466	1.03 (0.26–1.81)	13,413	0.01 ((−0.14)–(0.16))
		DANISH	5611	0.04 ((−0.22)–(0.29))		
		KORA-S4	1415	0.13 ((−0.35)–(0.60))		
		NPHS II	2652	0.22 ((−0.06)–(0.50))		
		UDACS	560	0.86 ((−0.05)–(1.77))		
Analysis in type 2 diabetic subjects: CC- versus GG-genotype						
Ln IL-6 (pg/mL)[d]	0.62	KORA-S4	222	0.15 ((−0.20)–(0.51))	744	0.14 ((−0.02)–(0.30))

[a]Published for the respective outcome as a main result at the time of study recruitment.
[b]*P*-value of Egger's regression test for publication bias including all studies of main analysis.
[c]Of unpublished studies.
[d]Adjusted for age, sex, and body mass index BMI (kg/m^2).
[e]Adjusted for age, sex, and type 2 diabetes mellitus (T2DM).
n.a. =not applicable (because all studies were unpublished).

Table A-VII. (online appendix). Full data sensitivity analyses, additionally including studies showing Hardy-Weinberg equilibrium (HWE) violation in non-diabetic subjects (C-allele dominant model).

Outcome	Additionally included studies	Individual *n*	Individual β-estimates (95% CI)	Summary *n*	Summary β-estimate (95% CI)
Fasting glucose (mmol/L)[a]	BOTNIA	1285	0.01 ((−0.38)–(0.39))	10,725	−0.09 ((−0.16)–(−0.02))
2-h glucose (mmol/L)[a]	BOTNIA	1001	0.44 ((−0.37)–(1.25))	8399	−0.06 ((−0.19)–(0.06))
BMI [b]	BOTNIA	1288	−0.02 ((−0.58)–(0.55))	25,635	0.08 ((−0.03)–(0.19))
	TGN	230	−0.27 ((−1.52)–(0.99))		

[a]Adjusted for age, sex, and body mass index BMI (kg/m^2).
[b]Adjusted for age, sex, and type 2 diabetes mellitus (T2DM).

References

1. Kubaszek A, Pihlajamaki J, Komarovski V, Lindi V, Lindstrom J, Eriksson J, et al. Promoter polymorphisms of the TNF-alpha (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. Diabetes. 2003;52:1872–6.
2. Vozarova B, Fernandez-Real JM, Knowler WC, Gallart L, Hanson RL, Gruber JD, et al. The interleukin-6 (−174) G/C promoter polymorphism is associated with type-2 diabetes mellitus in Native Americans and Caucasians. Hum Genet. 2003;112:409–13.
3. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315: 629–34.
4. Wernstedt I, Eriksson AL, Berndtsson A, Hoffstedt J, Skrtic S, Hedner T, et al. A common polymorphism in the interleukin-6 gene promoter is associated with overweight. Int J Obes Relat Metab Disord. 2004;28:1272–9.
5. Hamid YH, Rose CS, Urhammer SA, Glumer C, Nolsoe R, Kristiansen OP, et al. Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. Diabetologia. 2005;48:251–60.
6. Flavell DM, Ireland H, Stephens JW, Hawe E, Acharya J, Mather H, et al. Peroxisome proliferator-activated receptor alpha gene variation influences age of onset and progression of type 2 diabetes. Diabetes. 2005;54:582–6.
7. Mohlig M, Boeing H, Spranger J, Osterhoff M, Kroke A, Fisher E, et al. Body mass index and C-174G interleukin-6 promoter polymorphism interact in predicting type 2 diabetes. J Clin Endocrinol Metab. 2004;89:1885–90.
8. Valle T, Tuomilehto J, Bergman RN, Ghosh S, Hauser ER, Eriksson J, et al. Mapping genes for NIDDM. Design of the Finland-United States Investigation of NIDDM Genetics (FUSION) Study. Diabetes Care. 1998;21:949–58.
9. Silander K, Mohlke KL, Scott LJ, Peck EC, Hollstein P, Skol AD, et al. Genetic variation near the hepatocyte nuclear factor-4 alpha gene predicts susceptibility to type 2 diabetes. Diabetes. 2004;53:1141–9.
10. Wichmann HE, Gieger C, Illig T. KORA-gen—resource for population genetics, controls and a broad spectrum of disease phenotypes. Gesundheitswesen. 2005;67 Suppl 1:S26–30.
11. Holle R, Happich M, Löwel H, Wichmann HE. KORA—a research platform for population based health research. Gesundheitswesen. 2005; 67 Suppl 1:S19–25.
12. Lowel H, Doring A, Schneider A, Heier M, Thorand B, Meisinger C. The MONICA Augsburg surveys—basis for prospective cohort studies. Gesundheitswesen. 2005;67 Suppl 1:S13–18.
13. Rathmann W, Haastert B, Icks A, Löwel H, Meisinger C, Holle R, et al. High prevalence of undiagnosed diabetes mellitus in Southern Germany: Target populations for efficient screening. The KORA survey 2000. Diabetologia. 2003;46:182–9.

14. Thorand B, Baumert J, Kolb H, Meisinger C, Chambless L, Koenig W, et al. Sex Differences in the prediction of type 2 diabetes by inflammatory markers: results from the MONICA/KORA Augsburg case-cohort study, 1984–2002. Diabetes Care. 2007;30:854–60.
15. Broeckel U, Hengstenberg C, Mayer B, Holmer S, Martin LJ, Comuzzie AG, et al. A comprehensive linkage analysis for myocardial infarction and its related risk factors. Nat Genet. 2002;30:210–4.
16. Stephens JW, Hurel SJ, Cooper JA, Acharya J, Miller GJ, Humphries SE. A common functional variant in the interleukin-6 gene is associated with increased body mass index in subjects with type 2 diabetes mellitus. Mol Genet Metab. 2004;82:180–6.
17. Lieb W, Pavlik R, Erdmann J, Mayer B, Holmer SR, Fischer M, et al. No association of interleukin-6 gene polymorphism (−174 G/C) with myocardial infarction or traditional cardiovascular risk factors. Int J Cardiol. 2004;97:205–12.
18. Illig T, Bongardt F, Schopfer A, Muller-Scholze S, Rathmann W, Koenig W, et al. Significant association of the interleukin-6 gene polymorphisms C-174G and A-598G with type 2 diabetes. J Clin Endocrinol Metab. 2004;89:5053–8.