Brief Genetics Report

IL6 Gene Promoter Polymorphisms and Type 2 Diabetes

Joint Analysis of Individual Participants' Data From 21 Studies

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Several lines of evidence indicate a causal role of the cytokine interleukin (IL)-6 in the development of type 2 diabetes in humans. Two common polymorphisms in the promoter of the IL-6 encoding gene *IL6*, −174G>C (rs1800795) and −573G>C (rs1800796), have been investigated for association with type 2 diabetes in numerous studies but with results that have been largely equivocal. To clarify the relationship between the two *IL6* variants and type 2 diabetes, we analyzed individual data on >20,000 participants from 21 published and unpublished studies. Collected data represent eight different countries, making this the largest association analysis for type 2 diabetes reported to date. The GC and CC genotypes of *IL6* −174G>C were associated with a decreased risk of type 2 diabetes (odds ratio 0.91, $P = 0.037$), corresponding to a risk modification of nearly 9%. No evidence for association was found between *IL6* −573G>C and type 2 diabetes. The observed association of the *IL6* −174 C-allele with a reduced risk of type 2 diabetes provides further evidence for the hypothesis that immune mediators are causally related to type 2 diabetes; however, because the association is borderline significant, additional data are still needed to confirm this finding. *Diabetes* 55:2915–2921, 2006

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FHS, Framingham Heart Study; HWE, Hardy-Weinberg equilibrium; IL, interleukin; IPD, individual participants' data.

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Recent studies have investigated the role of variants within genes encoding immune-related markers in mediating increased type 2 diabetes risk. One of the most widely studied immune genes is the interleukin (IL)-6 encoding gene *IL6*, which maps to chromosome 7p21. IL-6 exerts crucial effects not only in inflammation and infection but also within the nervous and endocrine systems (1). A vast number of epidemiological, genetic, rodent, and human in vivo and in vitro studies have investigated the putative role of IL-6 in the pathogenesis underlying type 2 diabetes. The impact of IL-6 on hepatocytes, skeletal muscle cells, β-cells, and the central nervous system has been described, and both protective and pathogenic activity of IL-6 in type 2 diabetes was suggested (2,3). Functional relevance has been ascribed to several *IL6* variants located in the promoter region, including −174G>C (rs1800795) and −573G>C (rs1800796, previously denoted as −572G>C), with in

vitro data demonstrating unequivocally that the *IL6* −174G>C sequence affects promoter strength (4,5). The relation between −174G>C and circulating IL-6 is not completely consistent in the literature. Whereas several studies indicate that −174G>C is associated with plasma levels of IL-6, particularly in inflammatory situations (6,7), no association between −174G>C and IL-6 was found within 718 nondiabetic women of the Nurses' Health Study (8).

Association between −174G>C and type 2 diabetes was first reported in U.S. Pima Indians and Spanish Caucasians (9), the C-allele being statistically significantly associated with a decreased risk of type 2 diabetes. One study subsequently replicated these initial findings (10), although most did not (11–14). The only major study on −573G>C was performed in Danish Caucasians and showed a significantly increased risk of type 2 diabetes by the C-allele, but the −573G>C control genotypes were not in Hardy-Weinberg equilibrium (HWE) (14). Because of the ambiguity in interpreting the role of *IL6* polymorphisms in type 2 diabetes susceptibility based on these disparate reports, we assembled an international *IL6*–type 2 diabetes consortium in order to perform a joint analysis.

The consortium utilized individual participants' data (IPD) and recruited all published and unpublished data on the association of the *IL6* −174G>C or −573G>C polymorphisms and type 2 diabetes. This approach overcomes many of the problems associated with meta-analyses of published estimates such as variability in study design, poor data quality, insufficient or heterogeneous confounder adjustment, and publication bias (15). As of late 2005, investigators from the U.S., Greece, Spain, Germany, U.K., Denmark, Sweden, and Finland participated in the consortium and contributed raw data on >30,000, mostly Caucasian, subjects. As such, this study is one of the largest genetic epidemiologic association studies on IPD ever conducted. The aim of this joint analysis is to provide conclusive evidence whether the two *IL6* variants, −174G>C and −573G>C, are associated with risk of type 2 diabetes.

RESEARCH DESIGN AND METHODS

All available published and unpublished studies fulfilling the following criteria were included in this joint analysis: *1*) association study conducted in humans, *2*) polymorphic genotype data for *IL6* −174G>C or −573G>C, *3*) type 2 diabetic case and nondiabetic control subjects, *4*) published before September 2005 or unpublished, and *5*) availability of IPD. Studies were excluded if the control group consisted only of individuals with pre-diabetes (16) or if ethnic admixture of unrelated study subjects was reported in the original publication (Pima Indian case-control study, 9). Information on search strategy, study recruitment, data collection and cleaning, and genotyping methods is provided in the online appendix (available at http://diabetes.diabetesjournals.org).

Definition of analyzed samples. Included datasets were analyzed as discordant-sib or case-control comparisons. Participants of case-control comparisons were not related to each other or to participants of other included studies. Datasets were edited to ensure that case subjects with type 2 diabetes and control subjects had the same sex and age range. Control subjects consisted of nondiabetic subjects, excluding individuals with pre-diabetes (impaired fasting glucose or impaired glucose tolerance [17]) when glucose values were available (see study-specific details in online appendix Table A2).

Statistical analyses. Statistical analyses were performed using SAS software version 9.1 (Cary, NC). Allele and genotype frequencies were estimated, allowing for the correlation in family data (sibships) by use of an exchangeable structure in a generalized estimating equations approach (SAS Proc Genmod). Linkage disequilibrium was assessed by the squared correlation coefficient r^2, and HWE was tested separately for case and control subjects per study (SAS Proc Allele).

Study-specific odds ratios (ORs) with SEs for association between *IL6* variants and type 2 diabetes were estimated from the IPD by logistic regression for case-control comparisons (SAS Proc Logistic) and by conditional logistic regression for discordant-sib comparisons (SAS Proc Phreg). The correlation due to linkage between disease status and investigated variants among sibs sharing the same marker alleles was accounted for by a jackknife variance estimate (18). All analyses were adjusted for age, sex, and BMI. Effect modification by BMI (quantitative and dichotomized at 28 kg/m^2) and sex was tested.

As the CC genotype of *IL6* −573G>C was rare (<1.5% in all studies), C-allele carriers (CC and GC genotypes) were compared with GG subjects. For −174G>C, ORs comparing either CC or GC with the wild-type GG were calculated, according to which the appropriate genetic model was chosen for the main analysis. Between-study heterogeneity was tested by the χ^2-based Q-statistic, and its impact was quantified by I^2 (19).

For the summary OR, study-specific ORs were combined by using the inverse-variance fixed-effect and the DerSimonian and Laird random-effects models. As the heterogeneity between study-specific ORs was low in all main analyses, the two models provided identical or very similar results. Thus, only the fixed-effect results are reported. The summary ORs of all studies where the control group was in HWE are reported as main results.

Publication bias was investigated by visual inspection of funnel plots and formally tested using Egger's regression method (20). Funnel and forest plots were prepared using Review Manager software version 4.2 (Cochrane Collaboration, Copenhagen, Denmark).

RESULTS

For the *IL6* −174G>C polymorphism, 10 published studies met the inclusion criteria. All of these studies, with the exception of the Framingham Heart Study (FHS) (21), provided IPD and were included in the joint analysis. Additionally, 12 unpublished studies were available for −174G>C and included in our analyses. For −573G>C, only one published study was available. However, data from eight unpublished studies met our inclusion criteria and were additionally used in our analyses. Data from 30,636 (−174G>C) and 21,352 (−573G>C) individuals were initially compiled in the central database; 22,626 and 17,305 subjects met the requirements for the analyzed samples, respectively. Except for one discordant-sib study on admixed Pima Indians, all studies consisted of Caucasian subjects.

Study-specific descriptive statistics. Characteristics of included studies and participants are summarized in Table 1 and online appendix Table A2, respectively. Details on study design and conduct are presented in online appendix Table A3. The estimated r^2 coefficients between the two single nucleotide polymorphisms in control subjects ranged from 0.027 (KORA-T2DMFAM_CC study) to 0.048 (MONICA-S3_CC study). Control genotype frequencies of all studies were in HWE, except for the RMIFAM_DS and TGN_CC studies for *IL6* −174G>C and the Danish_CC study for *IL6* −573G>C (online appendix Table A2).

***IL6* −174G>C polymorphism and risk of type 2 diabetes.** Figure 1*A* shows the ORs and 95% CIs for 18 individual studies for the association between *IL6* −174 C-allele dominant and type 2 diabetes, adjusted for age, sex, and BMI. The pooled OR for 4,746 case and 16,230 control subjects was 0.91 ($P = 0.037$); I^2, the impact of heterogeneity, was 0% (95% CI 0–50). The dominant genetic model appeared most consistent with the data, the pooled model-free OR_{GCvsGG} and OR_{CCvsGG} being 0.92 (0.83–1.01) and 0.90 (0.80–1.01), respectively, and was thus chosen for the main analysis. Visual inspection of the funnel plot of all 18 studies showed that studies with high, as well as low, precision of the OR estimate were distributed symmetrically around the pooled OR (online appendix Figure A1). Thus, no publication bias is suggested, which was further supported by the nonsignificant Egger's regression test ($P = 0.71$).

TABLE 1
Characteristics of included studies

Study name	Contributing studies*	Country	Case/control subjects†	Data on −573G>C‡	Published§	Reference‖
BOTNIA_CC	Botnia Study	Finland	760/539	Yes	3a,b	NA
CAPPP_CC	Captopril Prevention Project	Sweden	45/414	Yes	2a,b	25
DANISH_CC	Danish Study	Denmark	1,094/4,507	Yes	1a,b	14
EDSC_CC	Case: Ealing Diabetes Study of Coagulation; Control: Second Northwick Park Heart Study	U.K.	85/1,326	No	Case: 3a; Control: 1a	Case: NA; Control: 10
EPIC-POTSDAM_CC	European Prospective Investigation into Cancer and Nutrition Potsdam	Germany	175/349	No	1a	12
FUSION_CC	The Finland-United States Investigation of NIDDM Genetics	Finland	506/353	No	3a	NA
FUSION_DS	The Finland-United States Investigation of NIDDM Genetics	Finland	227/132	No	3a	NA
GIRONA_CC	Girona Genetics of Diabetes Study	Spain	43/67	No	1a	9
GREEK_CC	Greek Study	Greece	30/37	No	1a	13
KORA-MIFAM_CC	KORA Myocardial Infarction Family Study	Germany	66/417	No	2a	26
KORA-MIFAM_DS	KORA Myocardial Infarction Family Study	Germany	27/39	No	2a	26
KORA-S4_CC	KORA Survey S4	Germany	230/460	Yes	1a, 3b	11
KORA-T2DMFAM_CC	Case: KORA Type 2 Diabetes Family Study; Control: additionally from KORA Survey S4	Germany	335/421	Yes	3a,b	NA
KORA-T2DMFAM_DS	KORA Type 2 Diabetes Family Study	Germany	344/358	Yes	3a,b	NA
MONICA/KORA-COHORT_CC	MONICA/KORA Case Cohort Study S123	Germany	488/1,585	Yes	3a,b	NA
MONICA-S3_CC	MONICA/KORA Survey S3	Germany	156/3,186	Yes	2a, 3b	26
PIMA_DS	Type 2 Diabetes Susceptibility in Pima Indians Study	U.S.	62/79	No	1a	9
RMIFAM_CC	Regensburg Myocardial Infarction Family Study	Germany	280/983	No	2a	26
RMIFAM_DS	Regensburg Myocardial Infarction Family Study	Germany	412/538	No	2a	26
TGN_CC	Tarraco Study	Spain	156/53	No	1a	9
UDACS_CC	Case: University College Diabetes and Cardiovascular Study; Control: Second Northwick Park Heart Study	U.K.	133/1,326	Yes	1a, 3b	10

IL6 −573G>C was not genotyped in the Ealing Diabetes Study of Coagulation. Thus, for the −573G>C analysis, the complete Second Northwick Park Heart Study (n = 2,652) was used as the control group for UDACS _CC. *For description of contributing studies see online appendix Table A2. †Number of type 2 diabetic case/nondiabetic control subjects included in age- and sex-adjusted analyses for *IL6* −174G>C and/or *IL6* −573G>C. ‡Yes = data on *IL6* −174G>C and −573G>C are available; no = only data on *IL6* −174G>C are available. §Detailed publication of 1a = *IL6* −174G>C, 1b = *IL6* −573G>C and type 2 diabetes; association between 2a = *IL6* −174G>C, 2b = *IL6* −573G>C and type 2 diabetes mentioned in publication with primary outcome other than type 2 diabetes, considered as "unpublished" (mostly, only part of the study participants have been mentioned); unpublished results for 3a = *IL6* −174G>C, 3b = *IL6* −573G>C and type 2 diabetes. ‖References of studies, for which the relationship between the *IL6* −174G>C or the *IL6* −573G>C and type 2 diabetes has been published in detail or mentioned in a publication with primary outcomes other than type 2 diabetes. NA, not applicable.

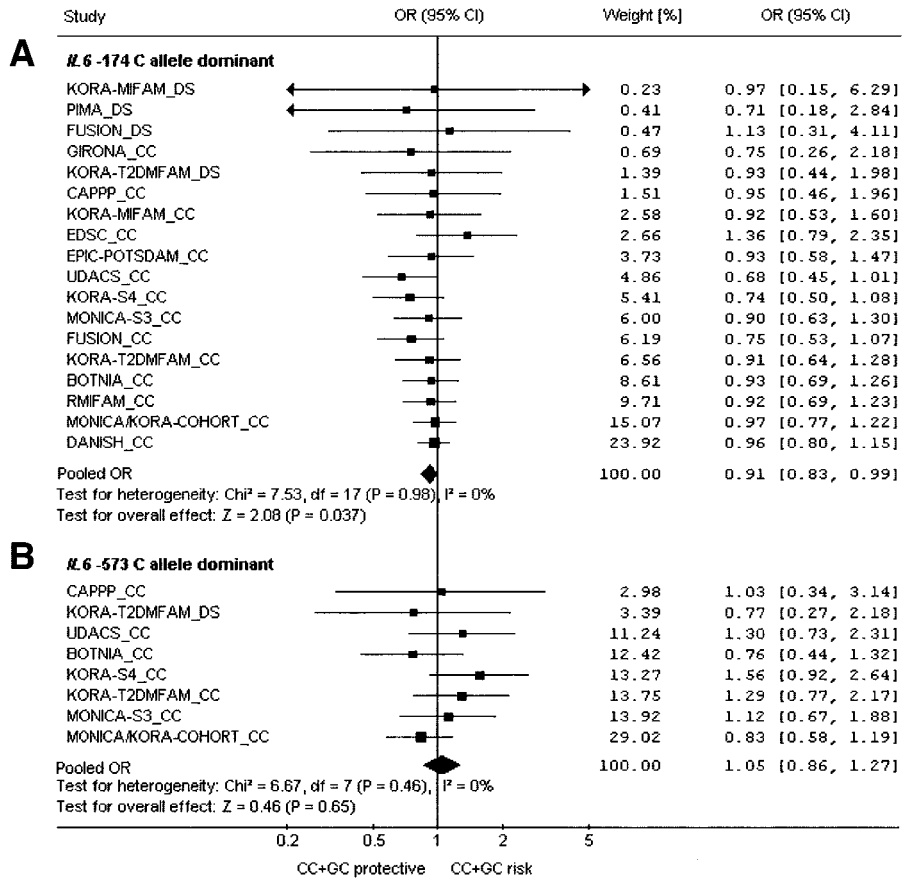

FIG. 1. Forest plot, illustrating the study-specific ORs and 95% CIs for the association between *IL6* −174G>C (*A*) and *IL6* −573G>C (*B*) and type 2 diabetes, dominant model for the C-allele, adjusted for age, sex, and BMI. Additionally, the pooled fixed-effect OR is shown. All studies where the genotypes of control subjects are in HWE, and where the covariates age, sex, and BMI are available, are included. The addenda behind the abbreviated study names denote case-control (CC) and discordant-sib (DS) studies. The studies are sorted according to the weight with which they contribute to the pooled OR estimate. I^2 measures the impact of inconsistency across studies and can range between 0 and 100%.

Two studies were not included in the main analysis due to HWE violation in the control groups and one (GREEK_CC study) because BMI adjustment was not possible. Their study-specific ORs were 1.9 (95% CI 1.2–3.0) for the RMIFAM_DS study, 0.6 (0.3–1.3) for the TGN_CC study (adjusted for age, sex, and BMI), and 2.2

TABLE 2
Pooled ORs of association between *IL6* variants and type 2 diabetes

Analysis type	Studies	Case/control subjects	OR (95% CI)*	*P* for heterogeneity	I^2 (%)
IL6 −174 C-allele dominant					
Main analysis†	18	4,746/16,230	0.91 (0.83–0.99)	0.98	0.0
Influence of studies that are not included in main analysis and of BMI adjustment					
All studies recruited for this joint analysis	21	5,606/17,020	0.94 (0.87–1.02)‡	0.31	11.7
Studies in HWE§	19	5,038/16,429	0.92 (0.85–1.00)‡	0.88	0.0
HWE, BMI available (main analysis but not adjusted for BMI)‖	18	5,008/16,392	0.92 (0.85–1.00)‡	0.93	0.0
IL6 −573 C-allele dominant					
Main analysis†	8	2,392/9,265	1.05 (0.86–1.27)	0.46	0.0
Influence of DANISH_CC study (not included in main analysis), and of BMI adjustment					
All studies recruited for this joint analysis	9	3,509/13,796	1.14 (0.99–1.32)‡	0.07	44.8
Studies in HWE (main analysis but not adjusted for BMI)¶	8	2,458/9,414	1.02 (0.86–1.22)‡	0.19	30.0

I^2 is the measure of heterogeneity and can range between 0 and 100%. *Fixed-effect OR estimate with 95% CI, adjusted for age, sex, and BMI. †All studies with control subjects in HWE, adjusted for age, sex, and BMI. ‡Adjusted for age and sex. §The RMIFAM_DS and the TGN_CC studies are excluded, as the genotypes of the control subjects of these studies are not in HWE for *IL6* −174G>C. ‖GREEK_CC study is excluded, as this study does not have data on BMI for control subjects. ¶DANISH_CC study is excluded, as genotypes of control subjects of this study are not in HWE for *IL6* −573G>C.

(0.7–7.1) for the GREEK_CC study (adjusted for age and sex). Sensitivity analyses, including these studies or showing the impact of BMI adjustment, are presented in Table 2. Further sensitivity analyses are presented in online appendix Table A4; no major difference was found between case-control/discordant-sib studies, between studies which originally were designed/not designed as type 2 diabetes studies, between studies which used/did not use an oral glucose tolerance test to exclude subjects with impaired glucose tolerance from the control subjects, between studies enriched/not enriched for myocardial infarction patients, and between published/unpublished studies, respectively. Analyzing men and women separately also did not appreciably affect the size of the pooled OR. Likewise, there was no major change when excluding each study at a turn, with the pooled ORs ranging between 0.89 (0.81–0.99) and 0.92 (0.84–1.01) (online appendix Figure A2 A). There was no evidence that BMI ($P > 0.4$, no evidence for heterogeneity between studies) or sex ($P = 0.93$, no heterogeneity) significantly modified the relationship between *IL6* −174G>C and type 2 diabetes.

IL6 −573G>C polymorphism and risk of type 2 diabetes. Figure 1*B* shows the ORs and 95% CIs for eight individual studies for the association between *IL6* −573 C-allele dominant and type 2 diabetes, adjusted for age, sex, and BMI. The pooled OR for 2,392 case and 9,265 control subjects was 1.05 ($P = 0.65$); I^2 was estimated as 0% (95% CI 0–68). The DANISH_CC study (OR 1.7 [95% CI 1.3–2.2]) was not included in this main analysis because control genotypes for −573G>C were not in HWE. Sensitivity analyses, presented in Table 2, show that heterogeneity between studies was substantially reduced by eliminating the DANISH_CC study and by adjusting for BMI (reduction from $I^2 = 44.8\%$ [$P = 0.07$] to $I^2 = 0.0\%$ [$P = 0.46$]). Further sensitivity analyses for subgroups of studies and stratification for sex show no remarkable change in the pooled result (online appendix Table A5). Removing each study at a turn yielded pooled ORs ranging between 0.98 and 1.15 with 95% CIs that always included unity, indicating that the pooled OR was not unduly influenced by any single study (online appendix Figure A2 B). There was no effect modification of BMI ($P > 0.6$) or sex ($P = 0.28$) on the relationship between −573G>C and type 2 diabetes.

DISCUSSION

The results presented here, based on IPD from 5,601 type 2 diabetic case and 17,019 control subjects and representing 21 association studies, provide evidence that the *IL6* −174G>C polymorphism is associated with type 2 diabetes and that individuals carrying the C-allele have a 9% lower odds of suffering from type 2 diabetes compared with individuals with the GG genotype ($P = 0.037$). We did not find a statistically significant relationship between *IL6* −573G>C and type 2 diabetes. It is plausible that the shown association of −174G>C with type 2 diabetes reflects a true modulating effect of −174G>C or another variant in linkage disequilibrium with −174G>C. The closest known gene (*TOMM7*) is situated about 100 kb from *IL6* and is located within a different linkage disequilibrium block (http://www.hapmap.org).

Putative impact of unincluded studies. Except for the FHS with data on *IL6* −174G>C (21), all studies investigating the relationship between −174G>C or −573G>C and type 2 diabetes published before September 2005 and fulfilling the inclusion criteria were incorporated in this joint analysis. With only 64 type 2 diabetic cases, the FHS corresponds to a weight of ~2% in this joint analysis. Thus, inclusion of the FHS would have had no major impact on the pooled OR.

Since the deadline for inclusion of newly published studies has elapsed until today (June 2006), only two large studies (>500 participants) fulfilling the inclusion criteria for our joint analysis have been published (8). Their study-specific ORs for association between *IL6* −174 C-allele dominant and type 2 diabetes, adjusted for age and BMI, were 0.95 (95% CI 0.82–1.10) for the Nurses' Health Study (1,315 female case and 2,265 female control subjects) and 0.95 (0.77–1.17) for the Health Professional Follow-up Study (885 male case and 894 male control subjects) (Dr. Lu Qi, personal communication). The pooled OR for the joint analysis, including these studies, was 0.92 (0.86–0.99) and had a slightly lower *P* value of 0.030 than our main analysis.

Analysis strategy. As recommended by Thakkinstian et al. (22), studies with HWE violation in the control group were excluded from the main analyses. This reduced heterogeneity between study-specific ORs for both *IL6* variants. Strikingly, two of the three studies with HWE violation showed ORs that were not compatible with the results of this joint analysis, as their 95% CIs and the 95% CIs of the pooled ORs did not overlap. Koushik et al. (23) investigated the reasons for heterogeneity in the published ORs on the association between the p53 codon 72 polymorphism and cervical neoplasia; the most important factor that contributed to heterogeneity was whether the genotype frequencies of the control groups were in HWE. Several reasons may account for HWE violation, including genotyping error, ethnic admixture in the control group, or chance. The decision to adjust the main analyses not only for age and sex but also for BMI arose from the fact that heterogeneity was remarkably reduced for *IL6* −573G>C.

Strengths and limitations of this joint analysis. This study represents the first joint analysis of IPD designed to address the role of *IL6* variants in type 2 diabetes susceptibility. Using a consortium-based strategy, this analysis was strengthened by the high compliance of investigators to contribute their published and unpublished data. To our knowledge, the present work is the largest IPD study that has been conducted to date to address the role of candidate gene variants in type 2 diabetes susceptibility.

Joint analyses based on IPD have several advantages compared with meta-analyses that are based on published estimates or summary data (15). Here, standardized methods were applied, incoming data were checked and cleaned, genotypes were tested for HWE violation, putative confounders for type 2 diabetes were uniformly adjusted for, stratified and interaction analyses were performed, and a consistent genetic model was applied. The observed low heterogeneity among studies may have resulted from these standardized procedures.

The greatest limitation of any meta-analysis is the risk of publication bias. To avoid this bias, we have strived to include all existing data involving the *IL6* −174G>C and −573G>C variants and type 2 diabetes susceptibility and managed to include predominantly unpublished data. Nevertheless, we conducted analyses to assess the effect of publication bias on our results for −174G>C. Utilizing the funnel plot and Egger's regression test, there was no evidence for publication bias, suggesting that our study sample is comprised of a representative dataset.

Although this study including >20,000 subjects is among the largest genetic association studies performed to date on IPD, the observed inverse association of the *IL6* −174 C-allele with type 2 diabetes, showing a *P* value of 0.037, is borderline significant. Bonferroni correction for the two analyzed single nucleotide polymorphisms would turn the result to statistical nonsignificance. However, the ORs of the recently published Nurses' Health Study and Health Professional Follow-up Study point in the same direction as our joint analysis, thus adding strength to the reported association of −174G>C with type 2 diabetes. The weak OR of 0.91 is plausible, as type 2 diabetes is a complex disease whose etiology is dependent upon multiple genetic and environmental factors and consistent with estimates obtained in other genes that affect susceptibility to type 2 diabetes (24).

In conclusion, this joint analysis is the largest association study on the genetics of type 2 diabetes published to date. We have assessed the role of two widely studied polymorphisms in the *IL6* gene, using IPD from published and unpublished studies, and did not find evidence for an association between *IL6* −573G>C and type 2 diabetes. In contrast, we determined that the GC and CC genotypes of *IL6* −174G>C show an OR of 0.91 for association with type 2 diabetes, which corresponds to a risk reduction of nearly 9%. However, because the association between the *IL6* −174G>C polymorphism and type 2 diabetes is borderline significant, a secondary analysis including additional data is critical. Thus, the present work represents a crucial first step toward elucidating the extent to which the *IL6* −174G>C plays a role in type 2 diabetes susceptibility and provides additional evidence supporting a direct relationship between chronic subclinical inflammation and type 2 diabetes etiology.

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