Interleukin-6 (IL-6) is a pleiotropic cytokine involved in the pathophysiology of various human diseases such as type 2 diabetes and obesity. IL-6 signals via a heterodimeric receptor complex consisting of a soluble IL-6 α-subunit (IL-6 receptor [IL6R]) and a signal transducing subunit (gp130). The IL6R gene maps to an important candidate locus for type 2 diabetes on chromosome 1q21. An Asp358Ala polymorphism of the IL6R has been reported to associate with obesity in Pima Indians. We investigated the Asp358Ala polymorphism in relation to type 2 diabetes, obesity, and other pre-diabetic quantitative traits among Danish whites. By applying a recessive genetic model in a case-control study of 1,349 type 2 diabetic patients and 4,596 glucose-tolerant control subjects, we found a significant difference in genotype distribution ($P = 0.008$) and in allele frequency (Ala-allele 38.3% [95% CI 36.5–40.1] in diabetic subjects vs. 41.2% [40.2–42.2] in control subjects; $P = 0.007$). The odds ratio for the Asp/Asp carriers versus Ala/Ala carriers was 1.38 (1.09–1.71). Among 4,251 middle-aged glucose-tolerant subjects, the Asp358Ala polymorphism was not associated with estimates of obesity, post–oral glucose tolerance test serum insulin release, or the homeostasis model assessment of insulin resistance index. In conclusion, the Asp358Ala polymorphism of the IL6R associates with type 2 diabetes in Danish whites. *Diabetes* 53: 3342–3345, 2004

Interleukin-6 (IL-6) is a pleiotropic cytokine secreted by various cell types, including leukocytes and endothelial cells, muscle tissue, and adipocytes (1,2). IL-6 is a mediator of some of the inflammatory response involved in the pathophysiology of metabolic disorders such as type 2 diabetes and obesity (3,4).

IL-6 exerts its signal transduction effect by binding to the IL-6 receptor (IL6R), and this complex subsequently binds to the gp130 subunit (also named IL6ST) (5,6). The gp130 plays an important role in stabilization of the ligand complex (7). The expression of IL6R is predominantly confined to hepatocytes and subpopulations of leukocytes (8). However, a biological active soluble form of the IL6R also exists (sIL6R). The IL6/sIL6R complex potentiates the effect of IL-6 and is capable of activating cells not expressing membrane-bound IL6R (8). Interestingly, a significantly higher plasma level of IL-6 and IL6/sIL6R complex has been shown among type 2 diabetic patients (9); in addition, sIL6R correlates with BMI among end-stage renal disease patients (10).

IL6-IL6R double-transgenic mice overexpressing IL-6 and sIL6R are not diabetic but remarkably smaller and have reduced body fat compared with single-transgenic or nontransgenic mice. IL-6 and IL6R single-transgenic mice do not differ from their wild-type littermates according to weight gain, food and water intake, or behavior (11).

The IL6R gene is located on chromosome 1q21 (12) in a region where several family studies in different ethnic populations have reported linkage to type 2 diabetes. The IL6R is located within a 1-logarithm of odds interval of the linkage peak in at least four of these studies (13–17).

Several single nucleotide polymorphisms (SNPs) of the IL6R have been identified among Pima Indian, Korean, African-American, and white subjects (18–20). One of these SNPs, a nonsynonymous variant in exon 9, Asp358Ala, has in a preliminary communication (18) been reported to associate with type 2 diabetes in whites and with indexes of obesity but not with diabetes in Pima Indians (20).

The objective of the present investigation was in a large-scale setting to replicate whether the IL6R Asp358Ala polymorphism, which is localized to a functional domain of the protein (21), associates with type 2 diabetes, obesity, or other quantitative traits of metabolism among Danish whites.
Genotyping. DNA was isolated from leukocytes using standard procedures. The genotyping method used for detection of the IL6R Asp358Ala polymorphism (rs8192284) was a chip-based matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (DNA MassARRAY) analysis of PCR-generated primer extension products as previously described (24). The genotyping success rate was 94%, and among 80 replicate samples there were no mismatches. The genotypes observed in both the Inter99 cohort and in the type 2 diabetic group were in Hardy-Weinberg equilibrium.

Statistical analysis. Fisher’s exact test was applied to test for significance of differences in allele frequencies, and logistic regression analyses (with sex and age as covariates) were tested with a general linear model including sex and genotype as fixed factors and age and BMI as covariates. For genotype quantitative trait studies, normal distribution of the residuals was verified and logarithmically transformed if appropriate. A two-sided P value < 0.05 was considered significant in case-control studies. Because the examination for the pre-diabetic quantitative traits had an explorative character, all significant levels were considered after Bonferroni correction for multiple testing (25). All analyses were done using SPSS for Windows, version 12.0.

RESULTS

In the case-control study we found significant differences in allele frequency and genotype distribution of the Asp358Ala polymorphism of the IL6R between 1,349 type 2 diabetic patients and 4,596 glucose-tolerant subjects applying a codominant as well as a recessive and dominant model of inheritance (Table 2). The Asp358-allele was more frequent among type 2 diabetic than control subjects (Table 2). The genotype and allele frequencies in the various examined subgroups are given in online appendix Table 1 (available from http://diabetes.diabetesjournals.org). For Asp/Asp carriers versus Ala/Ala carriers, the OR was 1.38 (95% CI 1.09–1.71) (Table 2). When the analysis was restricted to 1,365 glucose-tolerant subjects (794 men and 571 women) who were age and sex matched to the type 2 diabetic patients, a significant association of the Asp358 allele with type 2 diabetes was also found. Genotype distribution and allele frequencies of this subset of glucose-tolerant subjects were as follows: Asp/Asp 34.8%, Ala/Ala 47.8%, and Ala/Ala 17.4% (Ala-allele 41.3% [39.4–43.1]; P = 0.02).

The impact of the Asp358Ala variant on quantitative traits of metabolism was evaluated in a study involving the group of 4,251 glucose-tolerant subjects from the Inter99 cohort. After correction for multiple testing, the variant did not associate with levels of plasma glucose and serum.
insulin in the fasting state, during an OGTT, or with indexes of insulin resistance or insulin secretion. Similarly, the variant was not associated with alterations in serum lipids or indexes of body weight and body composition (Table 3).

### DISCUSSION

Our finding that the Asp-allele of the Asp358Ala variant of the IL6R associates with type 2 diabetes among white Danes suggests that this polymorphism may contribute to the genetic susceptibility of common diabetes. The magnitude of this association, i.e., the OR, is, as reviewed by McCarthy (17), comparable with the assessed significance of other reported genetic variants conferring an increased risk for type 2 diabetes.

Whether our observation reflects a true diabetogenic impact of the Asp358Ala polymorphism of IL6R gene per se cannot be determined from the present study, and attempts for replication are obviously needed. In a preliminary communication of a study among U.S. whites, a similar association of the Asp358Ala variant of the IL6R to type 2 diabetes was reported (18), whereas the same variant was associated with obesity but not with diabetes among Pima Indians (20). Other IL6R functional variant(s) in high linkage disequilibrium with the Asp358Ala variant might explain the present association. However, previous molecular studies indicate that individuals carrying the Ala-allele at codon-358 may have reduced circulating levels of the soluble form of IL6R. This receptor, sIL6R, is released from the cell surface after proteolytic cleavage, called shedding, in the Gln357/Asp358 juncture (21,26). Thus, the Gln357/Asp358 juncture is obviously an important region for the IL6R cleavage, and interestingly, it has been reported that substitution with glycine at codon 358 reduces shedding to 26% (21). Furthermore, an Ala357/Ala358 double mutation attenuated shedding by 66% (21). These findings may indicate that carriers of the Ala358 allele have impaired proteolytic cleavage and thereby lower sIL6R production (21), which in turn might cause reduced IL-6 signal transduction. Hence, the diminished risk of type 2 diabetes linked with the presence of the Ala358 allele might be mediated by reduced IL-6 signal transduction or by reduced signaling of other cytokines that also mediate their biological impact via the IL6R (8).

To elucidate the potential pathophysiological mechanisms underlying the association of the Asp358Ala variant and type 2 diabetes, we investigated the effect of the polymorphism on metabolic phenotypes in groups of NGT subjects in a codominant model, recessive model ($P_{recessive}$), and dominant model ($P_{dominant}$).

### TABLE 2

Genotype distribution and allele frequencies of the Asp358Ala polymorphism of the IL6R in 1,349 type 2 diabetic patients and in 4,596 glucose-tolerant subjects.

<table>
<thead>
<tr>
<th></th>
<th>Type 2 diabetes</th>
<th>NGT</th>
<th>$P$</th>
<th>$P_{recessive}$</th>
<th>$P_{dominant}$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (men/women)</td>
<td>1,349 (819/530)</td>
<td>4,596 (2,136/2,460)</td>
<td>0.019</td>
<td>0.008</td>
<td>0.068</td>
<td>1.38 (1.09–1.71)</td>
</tr>
<tr>
<td>Asp/Asp</td>
<td>509 (37.7%)</td>
<td>1612 (35.1%)</td>
<td>1.10</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Asp/Ala</td>
<td>647 (48.0%)</td>
<td>2180 (47.4%)</td>
<td>0.48</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ala/Ala</td>
<td>193 (14.3%)</td>
<td>804 (17.5%)</td>
<td>0.08</td>
<td>0.84</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ala allele</td>
<td>38.3 (36.5–40.1)</td>
<td>41.2 (40.2–42.2)</td>
<td>0.007</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are number of subjects with each genotype (% of each group) and % (95% CI) unless otherwise indicated. $P$ comparing case versus control subjects in a codominant model, recessive model ($P_{recessive}$), and dominant model ($P_{dominant}$).

### TABLE 3

Clinical and biochemical data of 4,251 middle-aged glucose tolerant white subjects from the Inter99 cohort when classified in accordance to their genotype of the Asp358Ala polymorphism of IL6R.

<table>
<thead>
<tr>
<th></th>
<th>Asp/Asp</th>
<th>Asp/Ala</th>
<th>Ala/Ala</th>
<th>$P^*$</th>
<th>$P_{recessive}$</th>
<th>$P_{dominant}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (men/women)</td>
<td>1,495 (682/813)</td>
<td>2,013 (942/1,071)</td>
<td>743 (348/395)</td>
<td>45 ± 8</td>
<td>45 ± 8</td>
<td>45 ± 8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.6 ± 4.1</td>
<td>25.5 ± 4.0</td>
<td>25.4 ± 4.0</td>
<td>0.84 ± 0.09</td>
<td>0.84 ± 0.08</td>
<td>0.84 ± 0.08</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.15 ± 13,358</td>
<td>20.87 ± 13,288</td>
<td>20.78 ± 12,811</td>
<td>31 ± 21</td>
<td>31 ± 19</td>
<td>31 ± 18</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.16 ± 0.70</td>
<td>1.18 ± 0.80</td>
<td>1.21 ± 1.53</td>
<td>31 ± 21</td>
<td>31 ± 19</td>
<td>31 ± 18</td>
</tr>
</tbody>
</table>

Data are means ± SD. *Comparing Asp/Asp, Asp/Ala, and Ala/Ala; †comparing Asp/Asp and Asp/Ala with Ala/Ala; and ‡comparing Asp/Asp with Asp/Ala and Ala/Ala, all after adjustment for age, BMI, and sex. AUC, area under the curve; HOMA-IR, homeostasis model assessment of insulin resistance index; NS, not significant.
IFG, and IGT subjects. However, after Bonferroni correction we failed to show any significant associations.

In contrast to a recent study of 786 Pima Indians that showed an association of the codon 358 IL6R variant to obesity but not to diabetes (20), we failed to show any impact of the IL6R variant on estimates of obesity in any of our study groups. The reason for this inconsistency is unclear but might be explained by differences in study populations. In this respect, the marked difference in allele frequency of the Asp558Ala polymorphism in the two populations is also emphasized. Furthermore, complex interactions between ethnic-specific polymorphisms in the promoter regions of IL6R, IL-6, or in the complex of IL6-IL6R signaling proteins might contribute to this discrepancy.

Based on simulation studies, it was estimated that the present study has a power of 90% to detect a relative risk of 1.2 for an association with type 2 diabetes or obesity. Therefore, if the variant possesses an effect on obesity risk in the Danish population, the effect size is relatively subtle. It is well known that spurious associations can even be found in well-designed case-control studies. However, we believe that the risk that our findings of an association with type 2 diabetes are due to population stratification is relatively modest, as all studied subjects are Danish whites and all have been collected from the same region of the western part of Copenhagen County. In addition, >20 other SNPs have been analyzed in the same study samples, and no associations with type 2 diabetes were found. Nevertheless, attempts to validate the present finding of an association to diabetes are essential.

In conclusion, the common Asp558Ala polymorphism of the IL6R gene associates with an increased risk of type 2 diabetes, with an OR for the Asp-allele carriers of 1.30 in Danish whites. This finding needs replication in other study populations, especially due to the lack in the present study of an association with metabolic phenotypes in middle-aged glucose-tolerant subjects.

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