Brief Genetics Report

# Variation in the Interleukin-6 Receptor Gene Associates With Type 2 Diabetes in Danish Whites

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**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 prediabetic 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**

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IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IL-6, interleukin-6; IL6R, IL-6 receptor; OGTT, oral glucose tolerance test; NGT, normal glucose tolerance; sIL6R, soluble IL6R; SNP, single nucleotide polymorphism.

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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.

TABLE 1
The clinical characteristics of the study groups enrolled in the type 2 diabetes case-control study

| | N | n (men/women) | Age (years) | Age at clinical diagnosis (years) | BMI ($kg/m^2$) | $HbA_{1c}$ (%) |
|---|---|---|---|---|---|---|
| Type 2 diabetic patients | 1,349 | | | | | |
| Type 2 diabetic patients from the Inter99 cohort | | 324 (202/122) | 51.1 ± 7.1 | 49.2 ± 8.3 | 30.4 ± 5.6 | 7.0 ± 1.6 |
| Type 2 diabetic patients from Steno Diabetes Center | | 1,025 (617/408) | 58.8 ± 10.7 | 52.7 ± 10.4 | 29.5 ± 5.2 | 8.0 ± 1.6 |
| NGT subjects | 4,596 | | | | | |
| NGT subjects from the Inter99 cohort | | 4,251 (1,972/2,279) | 45.2 ± 7.8 | — | 25.5 ± 4.0 | 5.8 ± 0.4 |
| Elderly NGT subjects | | 345 (164/181) | 62.4 ± 4.9 | — | 26.3 ± 3.6 | 6.1 ± 0.4 |
| NGT subjects matched on age and sex with type 2 diabetic patients | 1,365 | 1,365 (794/571) | 57.0 ± 5.0 | — | 26.1 ± 3.9 | 5.9 ± 0.4 |

Data are means ± SD.

## RESEARCH DESIGN AND METHODS

The Asp358Ala polymorphism was genotyped in the Inter99 cohort of middle-aged subjects, in a population-based sample of elderly glucose-tolerant subjects, and in a group of type 2 diabetic patients recruited from the outpatient clinic at Steno Diabetes Center. Also, a subgroup of glucose-tolerant subjects who was age and sex matched to type 2 diabetic patients was selected from the total group of glucose-tolerant subjects. All subjects who participated in the present protocol were living in the same area of Copenhagen.

The Inter99 cohort is a population-based, randomized, nonpharmacological intervention study for the prevention of cardiovascular disease undertaken at the Research Centre for Prevention and Health in Glostrup, Copenhagen County. Details of this cohort have been reported (22). Glucose tolerance status was evaluated according to the 1999 World Health Organization criteria (23). Oral glucose tolerance and genotype data were available on 5,702 white subjects. Of those, 4,251 subjects had normal glucose tolerance (NGT), 653 had impaired glucose tolerance (IGT), 474 subjects had impaired fasting glucose (IFG), 231 patients had screen-detected type 2 diabetes, and 93 patients had known type 2 diabetes.

The case-control study of type 2 diabetes involved 1,349 case subjects and all glucose-tolerant subjects from the Inter99 cohort, and the group of elderly glucose-tolerant subjects served as control ($n = 4,596$). Clinical characteristics of the study groups included in the present investigation are given in Table 1. Patients with diabetes due to known chronic pancreatitis, hemochromatosis, severe insulin resistance, maturity-onset diabetes of the young, or maternally inherited diabetes and deafness; patients with a family history of first-degree relatives with type 1 diabetes; patients with insulin requirement within the 1st year after diabetes diagnosis; or patients with a fasting serum C-peptide level ≤150 pmol/l at the time of recruitment were excluded in the present study from the category of clinically defined type 2 diabetes.

All study participants were Danish whites by self-report. Informed written and oral consent were obtained before participation. The studies were approved by the Ethical Committee of Copenhagen in accordance with the Helsinki Declaration II.

**Biochemical measurements.** Blood samples for analyses of biochemical variables were drawn in the morning after an overnight fast. Plasma glucose was analyzed by a glucose oxidase method (Granutest; Merck, Darmstedt, Germany) and serum-specific insulin [excluding des(31,32) and intact proinsulin] and serum C-peptide were measured by time-resolved fluoroimmunoassay (AutoDELFIA; Perkin Elmer-Wallac, Turku, Findland). $HbA_{1c}$ was analyzed by principles of ion exchange high-performance liquid chromatography using Bio-Rad variant $HbA_{1c}$ (normal range 4.1–6.4%). The concentration of serum triglycerides was determined by the triglycerides GPO-PAP (glycerol phosphate oxidase para amino phenazone) method, HDL cholesterol by the HDL cholesterol plus method, and the serum total cholesterol by the cholesterol CHOD-PAP (cholesterol oxidase para amino phenazone) method (Roche Diagnostics, Mannheim, Germany).

The insulinogenic index was calculated as fasting serum insulin (in picomoles per liter) subtracted from 30-min post–oral glucose tolerance test (OGTT) serum insulin (in picomoles per liter) and divided by 30-min post-OGTT plasma glucose (in millimoles per liter). Homeostasis model assessment for insulin resistance was calculated as fasting plasma glucose (in millimoles per liter) multiplied by fasting serum insulin (in picomoles per liter) and divided by 22.5.

**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 89 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 applied to evaluate differences in genotype distributions. Odds ratios (ORs) were calculated after adjustment for sex and age. Phenotypic differences between the genotype groups among the NGT subjects 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%, Asp/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

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

| | Type 2 diabetes | NGT | $P$ | $P_{recessive}$ | $P_{dominant}$ | OR (95% CI) |
|---|---|---|---|---|---|---|
| $n$ (men/women) | 1,349 (819/530) | 4,596 (2,136/2,460) | | | | |
| Asp/Asp | 509 (37.7%) | 1612 (35.1%) | | | | |
| Asp/Ala | 647 (48.0%) | 2180 (47.4%) | | | | |
| Ala/Ala | 193 (14.3%) | 804 (17.5%) | 0.019 | 0.008 | 0.068 | |
| Ala allele | 38.3 (36.5–40.1) | 41.2 (40.2–42.2) | 0.007 | | | |
| Asp/Asp vs. Ala/Ala | | | | | | 1.38 (1.09–1.71) |
| Asp/Asp vs. Asp/Ala | | | | | | 1.08 (0.93–1.26) |
| Asp/Ala vs. Ala/Ala | | | | | | 1.25 (1.02–1.53) |

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 ($P_{dominant}$).

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 disequillibrium 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 patophysiological 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

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*

| | Asp/Asp | Asp/Ala | Ala/Ala | $P$* | $P_{recessive}$† | $P_{dominant}$‡ |
|---|---|---|---|---|---|---|
| $n$ (men/women) | 1,495 (682/813) | 2,013 (942/1,071) | 743 (348/395) | | | |
| Age (years) | 45 ± 8 | 45 ± 8 | 45 ± 8 | | | |
| BMI (kg/m$^2$) | 25.6 ± 4.1 | 25.5 ± 4.0 | 25.4 ± 4.0 | NS | NS | NS |
| Waist-to-hip ratio | 0.84 ± 0.09 | 0.84 ± 0.08 | 0.84 ± 0.08 | NS | NS | NS |
| Plasma glucose at 0 min (mmol/l) | 5.3 ± 0.4 | 5.3 ± 0.4 | 5.3 ± 0.4 | NS | NS | NS |
| Plasma glucose at 120 min (mmol/l) | 5.5 ± 1.1 | 5.5 ± 1.1 | 5.4 ± 1.1 | NS | NS | NS |
| Incremental AUC glucose (mmol/l) | 181 ± 104 | 184 ± 99 | 179 ± 98 | NS | NS | NS |
| Serum insulin at 0 min (pmol/l) | 38 ± 24 | 37 ± 23 | 37 ± 23 | NS | NS | NS |
| Serum insulin at 120 min (pmol/l) | 167 ± 121 | 167 ± 133 | 169 ± 139 | NS | NS | NS |
| Incremental AUC insulin (pmol/l) | 21,151 ± 13,358 | 20,878 ± 13,288 | 20,782 ± 12,811 | NS | NS | NS |
| Insulinogenic index insulin | 31 ± 21 | 31 ± 19 | 31 ± 18 | NS | NS | NS |
| HOMA-IR | 9.1 ± 5.7 | 8.8 ± 5.6 | 8.8 ± 5.7 | NS | NS | NS |
| Serum cholesterol (mmol/l) | 5.4 ± 1.0 | 5.4 ± 1.0 | 5.4 ± 1.0 | NS | NS | NS |
| Serum HDL cholesterol (mmol/l) | 1.45 ± 0.40 | 1.47 ± 0.40 | 1.47 ± 0.40 | NS | NS | NS |
| Serum triglycerides (mmol/l) | 1.16 ± 0.70 | 1.18 ± 0.80 | 1.21 ± 1.53 | NS | NS | NS |

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 Bonferonni 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 Asp358Ala 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 Asp358Ala 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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## REFERENCES

1. Mohamed-Ali V, Flower L, Sethi J, Hotamisligil G, Gray R, Humphries SE, York DA, Pinkney J: Beta-adrenergic regulation of IL-6 release from adipose tissue: in vivo and in vitro studies. *J Clin Endocrinol Metab* 86:5864–5869, 2001
2. Steensberg A, Keller C, Starkie RL, Osada T, Febbraio MA, Klarlund-Pedersen B: IL-6 and TNF-alpha: expression in, and release from, contracting human skeletal muscle. *Am J Physiol Endocrinol Metab* 283:E1272–E1278, 2002
3. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP: The pathophysiologic roles of interleukin-6 in human disease. *Ann Intern Med* 128:127–137, 1998
4. Pedersen BK, Steensberg A, Schjerling P: Muscle-derived interleukin-6: possible biological effects. *J Physiol* 536:329–337, 2001
5. Taga T, Hibi M, Hirata Y, Yamasaki K, Yasukawa K, Matsuda T, Hirano T, Kishimoto T: Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell* 58:573–581, 1989
6. Yamasaki K, Taga T, Hirata Y, Yawata H, Kawanishi Y, Seed B, Taniguchi T, Hirano T, Kishimoto T: Cloning and expression of the human interleukin-6 (BSF-2/IFN beta 2) receptor. *Science* 241:825–828, 1988
7. Yawata H, Yasukawa K, Natsuka S, Murakami M, Yamasaki K, Hibi M, Taga T, Kishimoto T: Structure-function analysis of human IL-6 receptor: dissociation of amino acid residues required for IL-6-binding and for IL-6 signal transduction through gp 130. *EMBO* 12:1705–1712, 1993
8. Jones SA, Horiuchi S, Topley N, Yamamoto N, Fuller GM: The soluble interleukin 6 receptor: mechanisms of production and implications in disease. *FASEB J* 15:43–58, 2001
9. Kado S, Nagase T, Nagata N: Circulating levels of interleukin-6, its soluble receptor and interleukin-6/interleukin-6 receptor complexes in patients with type 2 diabetes mellitus. *Acta Diabetol* 36:67–72, 1999
10. Pecoits-Filho R, Barany P, Lindholm B, Heimburger O, Stenvinkel P: Interleukin-6 is an independent predictor of mortality in patients starting dialysis treatment. *Nephrol Dial Transplant* 17:1684–1688, 2002
11. Peters M, Odenthal M, Schirmacher P, Blessing M, Fattori E, Ciliberto G, Meyer-zum B, Rose-John S: Soluble IL-6 receptor leads to a paracrine modulation of the IL-6-induced hepatic acute phase response in double transgenic mice. *J Immunol* 159:1474–1481, 1997
12. Kluck PM, Wiegant J, Jansen RP, Bolk MW, Raap AK, Willemze R, Landegent JE: The human interleukin-6 receptor alpha chain gene is localized on chromosome 1 band q21. *Hum Genet* 90:542–544, 1993
13. Das SK, Hasstedt SJ, Zhang Z, Elbein SC: Linkage and association mapping of a chromosome 1q21–q24 type 2 diabetes susceptibility locus in Northern European Caucasians. *Diabetes* 53:492–499, 2004
14. Hsueh WC, St. Jean PL, Mitchell BD, Pollin TI, Knowler WC, Ehm MG, Bell CJ, Sakul H, Wagner MJ, Burns DK, Shuldiner AR: Genome-wide and fine-mapping linkage studies of type 2 diabetes and glucose traits in the Old Order Amish: evidence for a new diabetes locus on chromosome 14q11 and confirmation of a locus on chromosome 1q21–q24. *Diabetes* 52:550–557, 2003
15. Vionnet N, Hani E, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Lepretre F, Lecoeur C, Gallina P, Zekiri L, Dina C, Froguel P: Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21–q24. *Am J Hum Genet* 67:1470–1480, 2000
16. Xiang K, Wang Y, Zheng T, Jia W, Li J, Chen L, Shen K, Wu S, Lin X, Zhang G, Wang C, Wang S, Lu H, Fang Q, Shi Y, Zhang R, Xu J, Weng Q: Genome-wide search for type 2 diabetes/impaired glucose homeostasis susceptibility genes in the Chinese: significant linkage to chromosome 6q21–q23 and chromosome 1q21–q24. *Diabetes* 53:228–234, 2004
17. McCarthy MI: Progress in defining the molecular basis of type 2 diabetes mellitus through susceptibility-gene identification. *Hum Mol Genet* 13: R33–R41, 2004
18. Wang H, Chu W, Zhang Z, Elbein S: Molecular screening and association studies of IL6R as a functional and positional candidate gene for type 2 diabetes (T2DM) (Abstract). *Diabetes* 52:A256, 2003
19. Kim LH, Lee HS, Kim YJ, Jung JH, Kim JY, Park BL, Shin HD: Identification of novel SNPs in the interleukin 6 receptor gene (IL6R). *Hum Mutat* 21:450–451, 2003
20. Wolford JK, Colligan PB, Gruber JD, Bogardus C: Variants in the interleukin 6 receptor gene are associated with obesity in Pima Indians. *Mol Genet Metab* 80:338–343, 2003
21. Müllberg J, Oberthür W, Lottspeich F, Mehl E, Dittrich E, Graeve L, Heinrich PC, Rose-John S: The soluble human IL-6 receptor: mutational characterization of the proteolytic cleavage site. *J Immunol* 152:4958–4968, 1994
22. Jorgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glumer C, Pisinger C: A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99 (1). *Eut J Cardiovasc Prev Rehabil* 10:377–386, 2003
23. World Health Organization: *Definition, Diagnosis, and Classification of Diabetes Mellitus and Its Comlications: Report of a WHO Consultation. Part 1. Diagnosis and Classification of Diabetes Mellitus.* Geneva, Department of Noncommunicable Disease Survellance, World Health Org., 1999
24. Buetow KH, Edmonson M, MacDonald R, Clifford R, Yip P, Kelley J, Little DP, Strausberg R, Koester H, Cantor CR, Braun A: High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Proc Natl Acad Sci U S A* 98:581–584, 2001
25. Altmanre DG: *Practical Statistics for Medical Research.* New York, Chapman & Hall, 2003
26. Müllberg J, Schooltink H, Stoyan T, Gunther M, Graeve L, Buse G, Mackiewicz A, Heinrich PC, Rose-John S: The soluble interleukin-6 receptor is generated by shedding. *Eur J Immunol* 23:473–480, 1993