

Brief Genetics Report

Interleukin-6 Gene Polymorphism and Insulin Sensitivity

José-Manuel Fernández-Real, Montserrat Broch, Joan Vendrell, Cristina Gutiérrez, Roser Casamitjana, Michel Pugeat, Cristóbal Richart, and Wifredo Ricart

Type 2 diabetes and the insulin resistance syndrome have been hypothesized to constitute manifestations of an ongoing acute-phase response. We aimed to study an interleukin-6 (IL-6) gene polymorphism in relation to insulin sensitivity (IL-6 is the main cytokine involved in an acute-phase response). Subjects homozygous for the C allele at position -174 of the IL-6 gene (SfaNI genotype), associated to lower plasma IL-6 levels, showed significantly lower integrated area under the curve of serum glucose concentrations (AUC_{glucose}) after an oral glucose tolerance test, lower blood glycosylated hemoglobin, lower fasting insulin levels, lower total and differential white blood cell count (a putative marker of peripheral IL-6 action), and an increased insulin sensitivity index than carriers of the G allele, despite similar age and body composition. A gene dosage effect was especially remarkable for AUC_{glucose} (6.4 vs. 9.3 vs. 9.7 mmol/l in C/C, C/G, and G/G individuals, respectively). The serum concentration of fully glycosylated cortisol binding globulin (another marker of IL-6 action), suggested by concanavalin A adsorption, was lower in C/C subjects than in G/G individuals (32.6 ± 2.9 vs. 37.6 ± 4.6 mg/l, $P = 0.03$). In summary, a polymorphism of the IL-6 gene influences the relationship among insulin sensitivity, postload glucose levels, and peripheral white blood cell count. *Diabetes* 49:517-520, 2000

It has been hypothesized that type 2 diabetes and the insulin resistance syndrome are partly a manifestation of an ongoing acute-phase response (1,2). This hypothesis is based on the findings of increased blood concentrations of markers of the acute-phase response,

From Unitat de Diabetologia, Endocrinologia i Nutricio (J.M.F.-R., W.R.), University Hospital of Girona "Dr Josep Trueta," Girona, Spain; Unitat d'Endocrinologia (M.B., J.V., C.G., C.R.), Hospital of Tarragona "Joan XXIII," Facultat Medicina, Universitat Rovira i Virgili, Tarragona, Spain; Laboratoire de la Clinique Endocrinologique (M.P.), Hospices Civils de Lyon, Hôpital de l'Antiquaille; INSERM U329 (M.P.), Lyon, France; and the Hormonal Laboratory (R.C.), University Hospital Clinic, Barcelona, Spain.

Address correspondence and reprint requests to J.M. Fernández-Real, MD, PhD, Unitat de Diabetologia, Endocrinologia i Nutricio, University Hospital of Girona "Dr Josep Trueta," Carretera de França s/n, 17007 Girona, Spain. E-mail: endocrino@htrueta.scs.es.

Received for publication 22 June 1999 and accepted in revised form 4 November 1999.

AUC_{glucose} , integrated area under the curve of serum glucose concentrations; CBG, cortisol binding globulin; ConA, concanavalin A; FSIVGT, frequently sampled intravenous glucose tolerance test; IL, interleukin; PCR, polymerase chain reaction; RFLP, restriction fragment-length polymorphism; WBC, white blood cell.

including C-reactive protein, serum amyloid-A, α -1 acid glycoprotein, sialic acid, and cortisol (1-4).

Interleukin (IL)-6 is a pleiotropic cytokine involved in the regulation of the acute-phase reaction, immune responses, and hematopoiesis. Plasma IL-6 levels are elevated in type 2 diabetic patients, particularly in those with features of the insulin resistance syndrome (1,2). Although the concentrations of multiple components of the acute-phase response increase together, not all of them increase uniformly in all patients. These variations indicate that the components of the acute-phase response are individually regulated, and this may be explained in part by differences in the pattern of production of specific cytokines (5). Recently, it has been reported that a polymorphism in the 5' flanking region of the IL-6 gene alters the transcriptional response to stimuli such as endotoxin and IL-1 (6).

In addition to its role in acute-phase response, IL-6 has been recently shown to be released by adipose tissue, and this release is greater in obese subjects, especially in those with a higher waist-to-hip ratio (7). Furthermore, IL-6 increases postprandially, in parallel to glucose and insulin levels, in the interstitial fluid of subcutaneous adipose tissue (8). This increase suggests that IL-6 might modulate adipose glucose metabolism in the fed state.

A G/C polymorphism of the IL-6 gene at position -174 has been found to be associated with different transcription rates. Specifically, subjects with the C/C genotype showed lower plasma IL-6 levels compared with G/C or G/G subjects (6). Given the higher IL-6 levels found in patients with the insulin resistance syndrome and the possible role of IL-6 in postprandial adipose glucose metabolism, we aimed to study the G/C polymorphism of IL-6 in relation to postload glucose levels and insulin sensitivity.

Anthropometric and biochemical characteristics of the subjects at the time of entry into the study are shown in Table 1. The sample of 32 subjects was divided into two groups on the basis of IL-6 genotype. Of the subjects, 21 had a G at position -174 of the IL-6 gene: 13 heterozygous (C/G) and 8 homozygous (G/G). There were 11 subjects homozygous for the presence of C at this position.

Subjects homozygous for the C allele, associated with lower plasma levels of IL-6 in a recent study (6), were similar in age, sex, BMI, fat mass, and waist-to-hip ratio in comparison with carriers of the G allele (Table 1). However, these subjects showed significantly lower fasting insulin levels and an increased insulin sensitivity index than carriers of the G allele (Table 1). The integrated area under the curve of

TABLE 1
Anthropometric and biochemical variables of the study subjects

Variable	C/C	G/C and G/G	P
n	11	21	—
Men/women	5/6	10/11	NS
Age (years)	36.8 ± 8.6	37.5 ± 6.7	NS
BMI (kg/m ²)	27.2 ± 5.7	28.7 ± 5.1	NS
Fat mass (kg)	23.8 ± 12.5	24.9 ± 9.9	NS
Fat-free mass (kg)	58 ± 15.1	56.4 ± 11.7	NS
Waist-to-hip ratio			
Men	0.98 ± 0.03	1.01 ± 0.05	NS
Women	0.89 ± 0.06	0.91 ± 0.06	NS
Systolic blood pressure (mmHg)	118.6 ± 12.6	128.1 ± 11.4	0.064
Diastolic blood pressure (mmHg)	70.5 ± 5.4	77.5 ± 12.7	0.059
Fasting glucose (mmol/l)	4.92 ± 0.43	5.56 ± 0.99	0.051
Fasting insulin (mU/l)	8.2 ± 2.8	12.7 ± 7.7	0.02
AUC glucose during OGTT (mmol/l)	6.4 ± 1.2	9.5 ± 3.3	0.001
AUC insulin during OGTT (mU/l)	69.1 ± 30.6	93.7 ± 71.7	NS
Insulin sensitivity (min · mU ⁻¹ · l ⁻¹)	3.49 ± 1.6	2.27 ± 1.8	0.016
HbA _{1c} (%)	4.62 ± 0.24	5.18 ± 0.66	0.002

Data are means ± SD. AUC, area under the curve; OGTT, oral glucose tolerance test.

serum glucose concentrations (AUC_{glucose}) after an oral glucose tolerance test (Fig. 1) and blood glycosylated hemoglobin were significantly lower in these subjects (Table 1) in the presence of nonsignificantly different integrated insulin levels. A gene dosage effect was especially remarkable for AUC_{glucose} (6.4 vs. 9.38 vs. 9.7 mmol/l in C/C, C/G, and G/G individuals, respectively) despite similar age, BMI, fat mass, and fat-free mass among these groups.

We measured peripheral white blood cell (WBC) count and used it as a marker of peripheral IL-6 action. As shown in Table 2 and Fig. 2, subjects homozygous for the C allele dis-

played significantly lower total and differential WBC count in comparison with carriers of the G allele. Insulin sensitivity significantly correlated with peripheral WBC count (r = -0.48, P = 0.001), neutrophil count (r = -0.50, P = 0.001), and lymphocyte count (r = -0.34, P = 0.02).

Plasma levels of glycosylated cortisol binding globulin (CBG) were not significantly different among groups of subjects. However, C/C homozygous individuals showed significantly lower plasma glycosylated CBG (32.6 ± 2.9 vs. 37.6 ± 4.6 mg/l, P = 0.03) than G/G individuals.

We describe here that a polymorphism of the IL-6 gene at position -174 is associated with insulin resistance, postload glucose levels, and with two markers of putative IL-6 action, peripheral WBC count, and glycosylated CBG (12-14). When comparing constructs of the 5' flanking region of IL-6 in a luciferase reporter vector transiently transfected into He La cells, the -174 C construct showed lower expression than the -174 G construct (6). This different transcription rate was supported by the in vivo observation that IL-6 levels were lower in normal subjects with the C/C genotype (6). From our findings, it could be speculated that those individuals with the G allele would be prone to develop insulin resistance and increased plasma markers of the acute-phase response. The mechanism by which IL-6 would induce insulin resistance is unknown. The M value (a measure of insulin sensitivity), obtained using a euglycemic-hyperinsulinemic glucose clamp, was significantly lower in the patients with cancer that displayed increased circulating IL-6 levels (9). IL-6 stimulates the release of ACTH. In fact, higher cortisol levels, a well-known inducer of insulin resistance, are described in type 2 diabetic patients with the insulin resistance syndrome (1). However, it should be kept in mind that cytokines operate both as a cascade and as a network and can regulate the production of other cytokines and cytokine receptors (5). In this sense, perhaps the increased production of IL-6 is merely reflecting the actions of other cytokines more closely involved in insulin resistance, such as tumor necrosis factor-α.

Smaller numbers of peripheral blood neutrophils in IL-6 knockout mice have been observed (10,11). A higher peripheral WBC count has been associated with insulin resistance since the preliminary observations of Facchini et al. (12). In particular, it was observed that WBC count significantly correlated with insulin-mediated glucose disposal during a euglycemic clamp (12). In a subsequent study, it was demonstrated that neutrophil and lymphocyte count correlated positively with several components of the insulin resistance syndrome and that plasma insulin concentration was specifically asso-

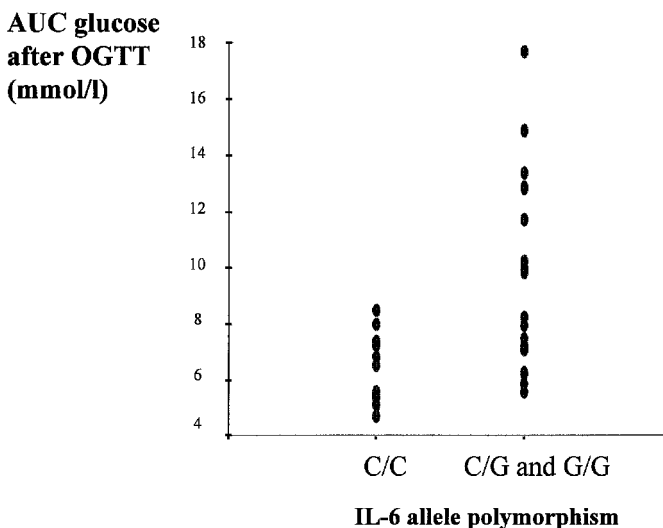


FIG. 1. Area under the curve of glucose during an oral glucose tolerance test (OGTT) according to the IL-6 genotype.

TABLE 2
Indirect parameters of IL-6 action

Variable	C/C	G/C and G/G	P
White blood cell count (×10 ⁹ /ml)	5,490 ± 1,553	7,528 ± 1,854	0.002
Neutrophils (×10 ⁹ /ml)	3,085 ± 881	4,270 ± 1,348	0.008
Lymphocytes (×10 ⁹ /ml)	1,812 ± 529	2,384 ± 451	0.001
Monocytes (×10 ⁹ /ml)	449 ± 199	633 ± 210	0.01
CBG (mg/l)	33.4 ± 2.9	35.7 ± 6.7	NS
Glycosylated CBG (mg/l)	32.6 ± 2.9	34.9 ± 6.5	NS

Data are means ± SD.

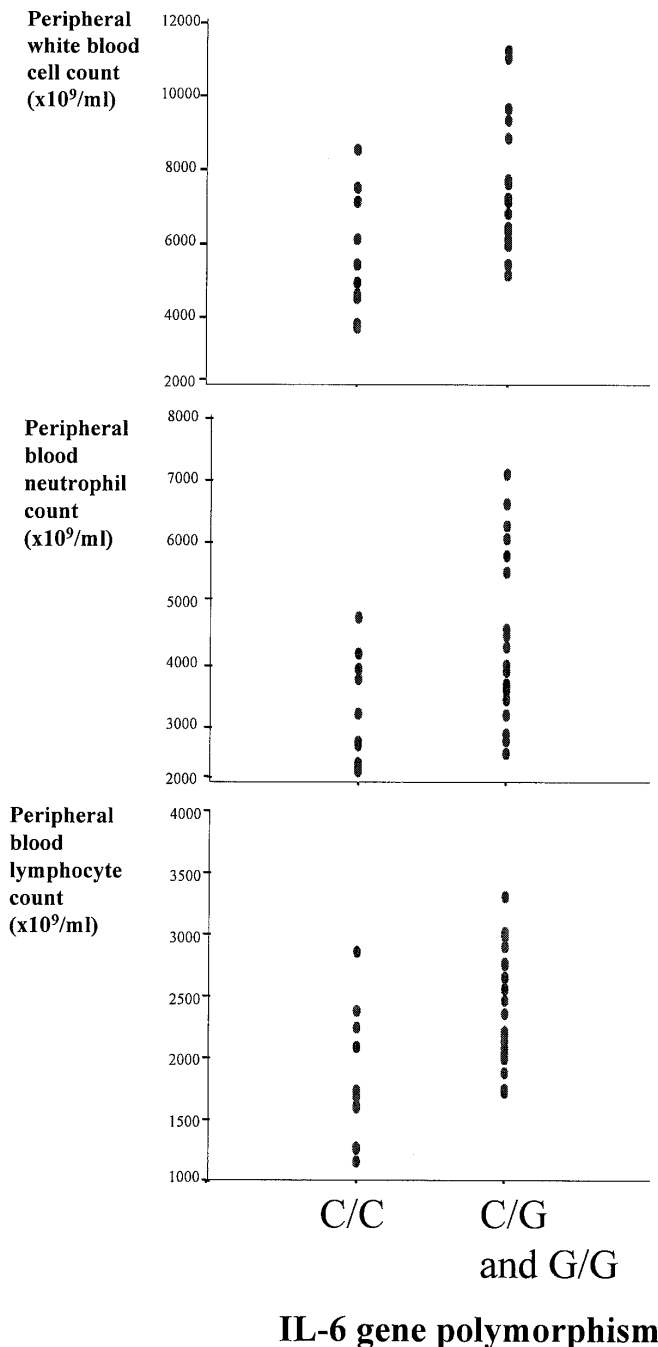


FIG. 2. Peripheral WBC count according to the IL-6 genotype.

ciated with lymphocytes and monocytes (13). These associations were confirmed in our study subjects. Given that IL-6 is involved in hematopoiesis (14), we suggest that these associations might in part be due to different IL-6 levels attributed to the IL-6 polymorphism at position -174. Those subjects with the G allele would be prone, simultaneously, to insulin resistance and higher peripheral WBC counts.

Given that IL-6 induces posttranslational changes in acute-phase protein glycosylation through oligosaccharide branching (15), we studied the glycosylation of CBG, a negative acute-phase protein (16). We did not find significant differences in plasma glycosylated CBG among subjects with dif-

ferent IL-6 genotypes. However, when comparing C/C homozygotes with G/G homozygotes, subjects of the latter group displayed significantly higher plasma concentrations of glycosylated CBG. In addition, the individuals that showed the highest glycosylated CBG levels were those with the G allele—presumably those with the higher IL-6 levels. Thus, the percentage of fully glycosylated CBG, as suggested by concanavalin A (ConA) adsorption, was higher in those G/G subjects who are believed to show a high IL-6 secretion rate. By increasing CBG glycosylation, IL-6 may decrease the half-life (or plasma clearance rate) of CBG with a consequent increase in its serum concentration.

We did not measure plasma IL-6 levels because of their low plasma half-lives and the presence of blocking factors. The circulating cytokine molecules are seldom found in the unbound state. They are almost always bound to binding or carrier proteins, autoantibodies, and soluble receptors. The usual sandwich format immunoassays recover free and some predictably bound cytokines, but miss other cytokines bound by unpredictable binding entities and cytokines not revealed by the kinetics of the assay protocol. We indirectly measured the effects of IL-6 through peripheral WBC count and glycosylated CBG.

Considerable interethnic variation in the frequencies of polymorphisms of IL-6 has been demonstrated (6,17), which is consistent with the different allelic frequency obtained in our study in comparison with other populations (6).

In summary, a polymorphism of the IL-6 gene influences the relationship among insulin sensitivity, postload glucose levels, and peripheral blood cell count. In evolutionary terms, we have hypothesized that genetic predisposition to insulin resistance and inflammation could be beneficial in the response to starvation and injury for our ancestors (18), a matter that merits further research.

RESEARCH DESIGN AND METHODS

Subjects. There were 32 healthy subjects (15 men and 17 women) prospectively studied. All subjects were of Caucasian origin from Catalonia (northern part of Spain). Inclusion criteria were as follows: 1) BMI (weight in kilograms divided by the square of height in meters) <40 kg/m², 2) absence of any systemic disease, and 3) absence of any infections in the previous month. None of the subjects were taking any medication (including glucocorticoids or estrogens) or had any evidence of metabolic disease other than obesity. Liver disease and thyroid dysfunction were specifically excluded by biochemical workup. The protocol was approved by the Hospital Ethics Committee, and informed consent was obtained from each subject.

Procedures. Anthropometric parameters, blood pressure, and post-oral load glucose and insulin levels were measured as previously reported (19).

Insulin sensitivity was analyzed using the frequently sampled intravenous glucose tolerance test (FSIVGT) with minimal model analysis as described elsewhere (19).

The serum glucose level during the FSIVGT was measured in duplicate by the glucose oxidase method with a glucose analyzer 2 (Beckman, Brea, CA). The coefficient of variation was 1.9%. The serum insulin level during the FSIVGT was measured in duplicate by monoclonal immunoradiometric assay (IRMA; Medgenix Diagnostics, Fleunes, Belgium). Intra- and interassay coefficients of variation were similar to those previously reported (19). Blood HbA_{1c} concentrations were analyzed by high-pressure liquid chromatography (Merck Diagnostics, Rahway, NJ), with coefficients of variation <4%. The range for HbA_{1c} in glucose tolerant subjects was 3.8–5.43%.

White blood total and differential cell count. White blood total and differential cell count was determined by routine laboratory tests (Coulter Electronics, Hialeah, FL).

CBG and glycosylated CBG measurements. Plasma CBG concentration was measured by using a radioimmunoassay as previously described (20). To analyze glycoform variation of CBG, serum samples (50 μ l) were subjected to ConA sepharose adsorption (300 μ l in 50% Tris buffer [50 mmol/l Tris HCl, pH 7.4, 0.5 mol/l NaCl, 1 mmol/l CaCl₂, 1 mmol/l MgCl₂, 1 mmol/l MnCl₂]). The con-

centration of CBG was measured in the supernatant of the ConA gel for calculating the percentage of CBG that was adsorbed by ConA and referred to as "glycosylated CBG."

Restriction fragment-length polymorphism IL-6 gene analysis. The SfaNI polymorphism is due to a replacement of G by C at position 174, and primers were designed to amplify the promoter region of the IL-6 gene. The primers used in the polymerase chain reaction (PCR) were as follows: 5' TGACTTCAGCTTTA CTCTTTGT 3' and 5' CTGATTGGAAACCTTATTAAG 3'. The reaction was carried out in a final volume of 50 ml containing 1.5 mmol/l MgCl₂, 0.2 mmol/l of each dNTP (Boehringer Mannheim, Mannheim, Germany), 0.2 mmol/l of each primer, and 2.5 U Taq polymerase (Gibco BRL, Gaithersburg, MD). DNA was amplified during 35 cycles with an initial denaturation of 10 min at 94°C and a final extension of 10 min at 72°C. The cycle program consisted of 1 min denaturation at 94°C, 1 min and 35 s annealing at 55°C, and 1 min extension at 72°C. PCR products were digested with SfaNI restriction enzyme at 37°C overnight and electrophoresed on a 2% agarose gel. SfaNI restriction fragment-length polymorphism (RFLP) was detected by ethidium bromide staining.

DNA was extracted from cellular blood components by the salting-out method. PCR was used to detect the IL-6 SfaNI RFLP. The identified genotypes were named according to the presence or absence of the enzyme restriction sites, so SfaNI G/G, G/C, and C/C are homozygous for the presence of the site (140/58 bp), heterozygotes for the presence and absence of the site (198/140/58 bp), and homozygous for the absence of the site (198 bp), respectively. The frequency of the alleles was C: 0.55 and G: 0.45. The population was in the Hardy-Weinberg equilibrium. X values were 1.30 and P = 0.52.

Statistical analysis. Descriptive results of continuous variables are expressed as mean ± SD. Before statistical analysis, normal distribution and homogeneity of the variables were tested. Parameters that did not fulfill these tests (WBC count, CBG, glycosylated CBG, and insulin sensitivity index) were log-transformed. Comparison of variables between groups of subjects was performed using the Student's t test.

ACKNOWLEDGMENTS

This work was partially supported by Comisión Interministerial de Ciencia y Tecnología Grant SAF 98-0130.

REFERENCES

- Pickup JC, Mattock MB, Chusney GD, Burt D: NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* 40:1286–1292, 1997
- Pickup JC, Crook MA: Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia* 41:1241–1248, 1998
- Jonsson A, Wales JK: Blood glycoprotein levels in diabetes mellitus. *Diabetologia* 12:245–250, 1976
- McMillan DE: Increased levels of acute-phase serum proteins in diabetes. *Metabolism* 38:1042–1046, 1989
- Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340:448–454, 1999
- Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P: The effect of novel polymorphisms in the interleukin 6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 102:1369–1376, 1998
- Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppel SW: Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor- α , in vivo. *J Clin Endocrinol Metab* 82:4196–4200, 1997
- Orban Z, Remaley A, Sampson M, Trajanoski Z, Chrousos GP: The differential effect of food intake and beta-adrenergic stimulation on adipose-derived hormones and cytokines in man. *J Clin Endocrinol Metab* 84:2126–2133, 1999
- Makino T, Noguchi Y, Yoshikawa T, Doi C, Nomura K: Circulating interleukin 6 concentrations and insulin resistance in patients with cancer. *Br J Surg* 85:1658–1662, 1998
- Romani L, Mencacci A, Cenci E, Spaccapelo R, Toniatti C, Puccetti P, Bistoni F, Poli V: Impaired neutrophil response and CD4+ T helper cell 1 development in interleukin-6-deficient mice infected with *Candida albicans*. *J Exp Med* 183:1345–1355, 1996
- Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei X-F, Achong MK: IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. *J Clin Invest* 101:311–320, 1998
- Facchini F, Hollenbeck CB, Chen YN, Chen YD, Reaven HM: Demonstration of a relationship between white blood cell count, insulin resistance, and several risk factors for coronary heart disease in women. *J Intern Med* 232:267–272, 1992
- Targher G, Seidell JC, Tonoli M, Muggeo M, De Sandre G, Cigolini M: The white blood cell count: its relationship to plasma insulin and other cardiovascular risk factors in healthy male individuals. *J Intern Med* 239:435–441, 1996
- Kishimoto T, Akira S, Tani T: Interleukin-6 and its receptor: a paradigm for cytokines. *Science* 258:593–597, 1992
- Van Dijk W, Mackiewicz A: Interleukin-6-type cytokine-induced changes in acute phase protein glycosylation. *Ann N Y Acad Sci* 762:319–330, 1995
- Vranckx R, Savu L, Maya M, Nunez EA: Alpha-fetoprotein and transcortin behave as acute phase reactants in the maternal and fetal compartments of the inflammatory pregnant mouse. *Endocrinology* 120:1782–1789, 1987
- Bowcock AM, Kidd JR, Lathrop GM, Daneshvar L, May LT, Ray A, Sehgal PB, Kidd KK, Cavalli Sforza LL: The human "interferon-beta 2/hepatocyte stimulating factor/interleukin-6" gene: DNA polymorphism studies and localization to chromosome 7p21. *Genomics* 3:8–16, 1988
- Fernández-Real JM, Ricart W: Insulin resistance and inflammation in an evolutionary perspective: the contribution of cytokine genotype/phenotype to thriftiness. *Diabetologia* 42:1367–1374, 1999
- Fernández-Real JM, Broch M, Ricart W, Casamitjana R, Gutierrez C, Vendrell J, Richart C: Plasma levels of the soluble fraction of tumor necrosis factor receptor 2 and insulin resistance. *Diabetes* 47:1757–1762, 1998
- Pugeat M, Bonneton A, Perrot D, Rocle-Nicolas B, Lejeune H, Grenot C, Dechaud H, Brebant C, Motin J, Cuilleron CY: Decreased immunoreactivity and binding activity of corticosteroid-binding globulin in serum in septic shock. *Clin Chem* 35:1675–1679, 1989