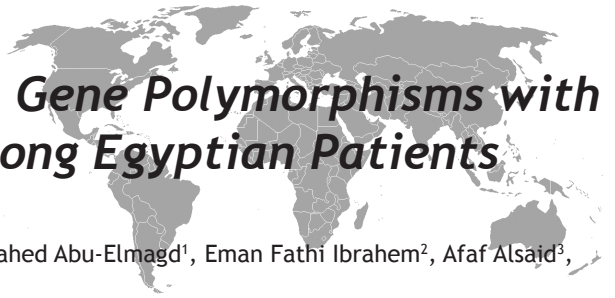

Association of IL-10 and IL-6 Gene Polymorphisms with Type 2 Diabetes Mellitus among Egyptian Patients

Mohamed Abdel-Halem Helaly[1], El-Sayed Zaki Hatata[1], Megahed Abu-Elmagd[1], Eman Fathi Ibrahem[2], Afaf Alsaid[3], Ibrahim Ahmed Abd El-Aal[4], Ahmad Settin[3]

ABSTRACT

Recent findings indicate that certain cytokines are capable of interfering with insulin sensitive glucose uptake and induce insulin resistance. To test for the association of IL-10-^{1082} and IL-6-^{174} genetic polymorphisms with type 2 diabetes mellitus in Egyptian patients. Participants included 69 cases with type 2 DM (31 males and 38 females) with a median age of 57 years in addition to 98 healthy unrelated controls from the Nile Delta region, Egypt. Compared to controls, cases with type 2 diabetes mellitus showed a highly significant increase in the frequency of IL-10-^{1082} GG genotype (p<0.001, OR = 11.24); higher frequency of IL-10-^{1082} G allele (p<0.001, OR = 2.2) with a significantly lower frequency of IL-10-1082 GA genotype (p<0.001, OR = 0.22). On the other hand, the IL-6-^{174} CC genotype frequency was significantly higher in the diabetic group (p<0.001, OR = 5.41) in contrast to the IL-6-^{174} GC genotype that showed significantly lower frequency (p=0.005, OR = 0.31) with a non significant differences in either G or C allelic frequencies between diabetic and control groups (p>0.05). Polymorphisms related to IL-10-^{1082} GG and IL-6-^{174} CC genotypes may be considered as a risk factor for type 2 diabetes mellitus among Egyptian subjects with a potential impact on family counseling and management.

Key words: *IL-10, IL-6, gene polymorphism, type 2 diabetes, Egypt*

Mısır'lı Hastalarda Tip 2 Diyabet ile IL-10 ve IL-6 Gen Poliformizmi Arasındaki İlişki

ÖZET

Son yıllarda bazı sitokinlerin insülin duyarlı glikoz alınımı ile ilişkiye girebilme ile birlikte insülin direnci meydana getirebilme yeteneğine sahip olduğu bildirilmiştir. Mısırlı tip 2 diabetli hastalarda L-10-^{1082} and IL-6-^{174} genetik poliformizm ilişkisini test etmek için ortalama yaşı 57 olan 69 hasta (31 E ve 38 K) ve sağlıklı 98 kişi Nil deltasından çalışmaya alındı. Kontrol grubu ile karşılaştırıldığında tip 2 diabetli hastalar yüksek oranda L-10-^{1082} GG genotipe (p<0.001, OR = 11.24), IL-10-^{1082} G allele (p<0.001, OR = 2.2) ve düşük oranda IL-10-1082 GA genotype (p<0.001, OR = 0.22) sahip olduğu gösterilmiştir. Diğer bir sonuç, IL-6-^{174} CC genotipi sıklıkla diabetli grupta yüksekti (p<0.001, OR = 5.41), IL-6-^{174} GC ise düşüktü. IL-6-^{174} GC genotipinde ise her grupta anlamlı farklılık göstermedi. Diğer bir sonuç ise IL-6-^{174} CC genotip sıklığı diabetikli hastalarda oldukça yüksekti (p<0.001, OR = 5.41), bunun aksine IL-6-^{174} GC genotipi anlamlı derecede düşüktü (p=0.005, OR = 0.31). G or C allelic sıklığı her iki grupta aynı idi (p>0.05). IL-10-^{1082} GG ve IL-6-^{174} CC genotipi ile ilişkili poliformizm Mısır'lı hastalarda bir risk faktörü olabilir.

Anahtar kelimeler: *IL-10, IL-6, gen poliformizmi, tip 2 diabet, Mısır*

[1]*Mansoura University, Diabetes and Endocrinology Unit, Specialized Medical Hospital,* [2]*Bany Swef University, Internal Medicine Department,* [3]*Paediatric Hospital, Genetics Unit,* [4]*Mansoura University, Clinical Pathology Departmen, Mansoura, Egypt*

Received: 07.03.2012, Accepted: 08.05.2013

Correspondence: *Mohamed A. Helaly,*
Internal Medicine departement, Specialized Medical Hospital, B.O.Pox 35516, Al-Gomhoria Street, Mansoura, Egypt.
Tel: 00201062216791
E-mail: *helaly70@yahoo.com*

INTRODUCTION

Diabetes mellitus is a growing disease that was estimated to affect roughly 6% of the world's population (1). Type 2 diabetes is by far the most common type of diabetes which accounts for about 90-95% of those with diabetes (2), and is characterized by variable degrees of insulin deficiency and resistance. It is a common disorder with a prevalence that rises markedly with increasing degree of obesity and sedentary lifestyle (3). Type 2 DM has also been recognized as an immune mediated disease leading to impaired insulin signaling and selective destruction of insulin producing beta cells in which cytokines play an

important role (4). Cytokines are immunomodulatory proteins or glycoproteins which control or modulate the activities of cells within the immune system (5). The production of many cytokines is under genetic control and polymorphisms have been identified within a large number of these genes (6). Pro and anti-inflammatory cytokines play a key role in identifying patients at elevated risk for several diseases (7). Previous studies indicate that certain pro and/or anti-inflammatory cytokines are capable of interfering with insulin sensitive glucose uptake and induce insulin resistance (8).

IL-6 is an interleukin that acts as both a pro-inflammotry and anti-inflammotry cytokine encoded by IL-6 gene. Its location at 7p21 and -174 polymorphism is located in its promoter region. It controls transcriptional activity of IL-6 which has an important role in type 1 DM (9). IL-10 is an anti-inflammatory and immunosuppressive substance produced within the body and plays a role in the regulation of immune responses (10). As its expression is relatively delayed, release of IL-10 provides an efficient autocrine mechanism for regulating proinflammatory cytokine production (11). The aim of the present work is to investigate the association of polymorphisms of IL-6–174 and IL-10–1082 genes to the susceptibility and severity of type 2 DM among cases from the Nile Delta region of Egypt.

MATERIALS AND METHODS

Participants included 69 cases with type 2 diabetes; 31 males and 38 females. Their age ranged between 40–78 years (mean±SD = 57.38 ± 7.67 years, median= 57 years). They were selected from the Department of Endocrinology, Specialized Medical Hospital, Mansoura University, Egypt. All patients had a diagnosis of established type 2 diabetes mellitus on the basis of medical history, clinical examination stressing on items as pulse, mean blood pressure (diastolic BP + 1/3 pulse pressure) (12), body mass index (BMI = weight (kg) / height2 (m) and laboratory tests as blood and urine glucose, insulin, glucagon, c-peptide and haemoglobin A1C (HBA1C). Exclusion criteria included any patient with gross or microscopic proteinuria, with renal affection related or not related to DM, with systemic or blood disease that may affect the kidneys as SLE, leukemia and lymphoma and cases with heavy urinary tract infection. Diagnosis of microalbuminuria was done by testing the first fasting mid-stream urine sample with Micral-2 test strips which is specific semi-quantitative test for detection of microalbuminuria. Cases were compared to a control group of 98 unrelated healthy subjects of matched age and sex from the same locality. They were proved healthy and euglycemic by clinical and laboratory tests. Written consent was taken from every participant in this study. 3 ml venous blood sample on EDTA was taken from all patients and controls for analysis of cytokine gene polymorphisms by PCR. Another sample of 5 ml venous blood was taken fasting for assessment of glucose, insulin, glucagon, C-peptide and HBA1C. Quantitative determination of glucose was done by glucose oxidase method using spin react kit (Madrid, Spain) (13). Quantitative determination of insulin, C-peptide and glucagon was done by Amplified Sensitivity Immunoassay performed on microtitre plates (INS-EASIA Biosource, Belgium). The assay used monoclonal antibodies directed against distinct epitopes of the corresponding hormone. The amount of substrate turnover is determined colorimetrically by measuring the absorbance which is directly proportional to hormone concentration (14). HBA1C (glycosylated Haemoglobin) is measured by quantitative colorimetric method, determined as percent of glycohemoglobin in relation to total hemoglobin (Glycohemoglobin % = R of unknown/R of standard X concentration of glycohemoglobin of standard (Human GmbH, Germany). Homeostasis Model of Assessment of Insulin resistance (HOMA) was calculated as = Fasting glucose (mg/dl) X Fasting insulin /450 (15).

DNA Extraction and amplification

DNA was extracted from anticoagulated whole blood by Axygen kit (Axygen Scientific, USA). The IL-6-^{174} (G/C) SNP genotypes were determined in an allele-specific PCR including the G and C alleles. Primers sequences were (IL-6 F): 5-GAGCTTCTCTTTCGTTCC-3 and either

Table 1. *Frequencies of IL-10-^{1082} (G /A) genotype and allelic polymorphisms among type 2 diabetic patients compared to control subjects*

IL-10	*Cases n (%)*	*Control n (%)*	*Fisher Exact (p)*	*Odds ratio (95% CI)*
Genotypes				
GG	*26 (37.7)*	*5 (5.1)*	*<0.001***	*11.24 (4.04-31.28)*
GA	*41 (59.4)*	*85 (86.7)*	*<0.001***	*0.22 (0.11-0.48)*
AA	*2 (2.9)*	*8 (8.2)*	*0.198*	*0.34 (0.07-1.63)*
Alleles				
G	*93 (67.39)*	*95 (48.47)*	*<0.001***	*2.2 (1.40-3.46)*
A	*45 (32.61)*	*101 (51.53)*	*<0.001***	*0.46 (0.29-0.72)*

*** p highly significant <0.001*

(IL-6 R1): 5-CCTATTGTGTCTTGCC-3 or (IL-6 R2):5-CCCTAGTTGTGTCTTGCG-3 Reactions contained 1 umol/L of each primer, 0.5 U Taq polymerase, 200 umol/L dNTPs mixture, and 1.5 mmol/L MgCl2 in 1X NH4 Cl buffer in addition to test DNA, to a final volume of 25 ul. Aamplification was performed on thermal cycler with 30 cycles each of 94°C for 30 seconds, 54°C for 60 seconds, and 72°C for 60 seconds, followed by a final extension of 72°C for 7 minutes. PCR products were resolved by agarose gel electrophoresis (3%) and visualized ethidium bromide with a positive band at 234 bp. IL-10-^{1082} polymorphism were determined in an IL-10 allele specific PCR including the IL-10 G or A alleles. Primers sequences were: (IL-10 F) 5- AGCAACACTCCTCGTCGCAAC-3 with (IL-10 R1) 5- CCTATCCCTACTTCCCCC-3 or (IL-10 R2) 5-CCTATCCCTACTTCCCCT-3 Reactions contained 20 umol/L of each primer, 0.5 U Taq Polymerase and 200 umol/L dNTPs mixture with 1.5 mmol/L MgCl2 in 1X NH4 Cl buffer in addition to test DNA, to a final volume of 25 ul. Amplification was performed on thermal cycler with 30 cycles each of 94°C seconds, 60°C for 60 seconds, 72°C for 60 seconds, followed by a final extension of 72°C for 7 minutes. PCR products were resolved by agarose electrophoresis (3%) and visualized ethidium bromide with a positive band at 179 bp.

Statistical analysis

Data were processed and analyzed using the Statistical Package of Social Science (SPSS, version 15). Quantitative variables were described by their mean and standard deviation and compared using student t-test. On the other hand, the frequency of studied allelic polymorphisms among cases was compared to that of controls using Fisher's Exact test and odds ratio (OR) with 95% confidence intervals (CI). A minimum level of significance is considered at a p level of < 0.05.

RESULTS

Investigations related to the diagnosis of type 2 DM showed a highly significant increase in the fasting glucagon level compared to controls (222.48±48.30 ng / L vs. 73.77±28.92 ng / L, p<0.001) with a significantly lower C-peptide level (3.06±1.18 ng/ml vs. 6.49±1.60 ng/ml, p<0.001). Regarding studied genetic polymorphisms, cases with type 2 DM showed a significantly higher frequency of IL-10-^{1082} GG genotype in comparison to controls (37.7% vs. 5.1%, P < 0.001) with a significantly lower frequency of the GA genotype (59.4% vs. 86.7%, p<0.001). Cases showed also a significant increase in

Table 2. *Frequencies of IL-6-^{174} (G/C) genotype and allelic polymorphisms among type 2 diabetic patients compared to control subjects*

IL-6	*Cases n (%)*	*Control n (%)*	*Fisher exact (p)*	*Odds ratio (95% CI)*
Genotypes				
GG	*2 (2.9)*	*5 (5.1)*	*0.701*	*0.56 (0.10-2.95)*
GC	*49 (71)*	*87 (88.8)*	*0.005**	*0.31 (0.14-0.70)*
CC	*18 (26.1)*	*6 (6.1)*	*<0.001***	*5.41 (2.02-14.50)*
Alleles				
G	*53 (38.40)*	*97 (49.49)*	*0.057*	*0.64 (0.41-0.99)*
C	*85 (61.6)*	*99 (50.51)*	*0.057*	*1.57 (1.01-2.45)*

**p significant <0.05 ** p highly significant <0.001*

Table 3. *IL-10^{-1082} and IL-6^{-174} genotype frequencies among cases with controlled versus uncontrolled diabetes and cases with variable degrees of insulin resistance*

	IL-10^{-1082}			IL-6^{-174}		
	GG n (%)	GA n (%)	AA n (%)	GG n (%)	GC n (%)	CC n (%)
HbA1C %						
< 7% (n=33)	14 (42.4)	17 (51.5)	2 (6.1)	1 (3)	24 (72.7)	8 (24.2)
> 7% (n=36)	12 (33.3)	24 (66.7)	0 (0)	1 (2.8)	25 (69.4)	10 (27.8)
x2 (P)		3.225 (0.20)			0.11 (0.95)	
HOMA						
< 20 (n=33)	14 (32.6)	28 (65.1)	1 (2.3)	1 (2.3)	33 (76.7)	9 (20.9)
> 20 (n=36)	12 (46.2)	13 (50)	1 (3.8)	1 (3.8)	16 (61.5)	9 (34.6)
x2 (P)		1.547 (0.46)			1.82 (0.40)	

the frequency of IL-10^{-1082} G Allele (67.4% vs. 48.5%, p<0.001) as shown in Table 1.

Cases showed also a significantly higher frequency of IL-6^{-174} CC genotype when compared to controls (26.1% vs. 6.1%, p<0.001) with a significantly lower frequency of the GC genotype (71% vs. 88.8%, p= 0.005). However, no significant difference was found comparing the IL-6^{-174} G or C alleles frequencies between diabetic and control group p value (>0.05) as shown in Table 2. Cases with good control of diabetes (HBA1C<7%) had a higher frequency of IL-10^{-1082} GG with a lower frequency of IL-6^{-174} CC genotypes compared to others with relatively uncontrolled diabetes (HBA1C>7%), (42.4% vs. 33.3% and 24.2% vs. 27.8% respectively) but still statistically insignificant (p>0.05) (Table 3). Also cases with marked insulin resistance (HOMA>20) had statistically non-significant higher frequency of the frequency of IL-10^{-1082} GG and IL-6^{-174} CC genotypes (46.2% vs. 32.6% and 34.6% vs. 20.9% respectively, p>0.05) as shown in Table 3.

DISCUSSION

Type 2 DM has been recognized as an immune mediated disease leading to impaired insulin signaling and selective destruction of insulin producing beta cells in which cytokines play an important role (4). The most prominent feature from the current study is the significant higher frequency of IL-10^{-1082} GG genotype with higher frequency of G allele among Egyptian type 2 DM cases versus control non-diabetic group. This was apparent in patients with poor control and cases with marked insulin resistance. These findings are in agreement with Kolla et al. who found significant increase in IL-10^{-1082} GG genotype in Indian patients with type 2 diabetes mellitus (16). The higher level of IL-10 GG genotype that is known to be a high producer may emphasize the immune modulatory action of IL-10. It was reported by Wogensen et al. that IL-10 should not simply be regarded as an immune inhibitory cytokine, since, it possesses, powerful immunostimulatory properties as well (17). Nonetheless, Settin et al found no association with IL-10^{-1082} polymorphic variants in type 1 DM (18). This stresses the fact that type 1 and type 2 DM may be considered different entities with a probable different pathophysiologic pattern. Similarly, Scarpelli et al. concluded that there was no significant differences between genotype frequencies of the IL-10^{-1082} variants between type 2 diabetics and non diabetic Caucasian Italian subjects (19). On the other hand, Tsiavou et al. concluded that G/A mutation at position –1082 of IL-10 promoter gene region might be participating to the pathogenesis of Latent Autoimmune Diabetes of Adult (LADA) but not type 2 DM in Greece population(20).

Regarding IL-6^{-174} genetic polymorphisms, this study showed a significant increase in IL-6^{-174} CC genotype in diabetic cases particularly among cases with poor control and cases with high insulin resistance. These findings go in hand with the finding of Kubaszek et al. who established that IL-6^{-174} CC genotype is risky for type 2 diabetes mellitus than other genotypes and that IL-6^{-174} GC polymorphism was found to be associated and correlated with insulin resistance in Finnish subjects (21). Also, Herbert et al. stated that IL-6^{-174} GG polymorphism is protective for type 2 DM in American population (22), a finding coincides with our results that IL-6^{-174} GG genotype was higher in control versus diabetic cases in spite of being statistically insignificant. Nevertheless, Stephens et al. in contrast to our finding stated that IL-6^{-174} GG genotype was associated with a small but

significant increase in risk of type 2 DM in British subjects compared to other genotypes. Also they stated that IL-6$_{-174}$ CC and GC genotypes is protective against type 2 DM, that is partially in agreement with our finding that IL-6$_{-174}$ GC polymorphism was associated with decreased risk of type 2 DM (23). Similarly, Qi et al. stated that IL-6$_{-174}$ G/C polymorphisms were not associated with risk of type 2 DM in American population (24). Vozarova et al. also -in contrast to our results- reported that IL-6$_{-174}$ GC polymorphism and G allele was associated with increased risk of type 2 DM in Native Americans and Caucasians (25). Taking into consideration the fact that genetic polymorphisms are population specific, we can come to the conclusion that IL-10$_{-1082}$ GG and IL-6$_{-174}$ CC gene polymorphisms are associated with type 2 DM among Egyptian population. In this respect, we recommend further wide scale studies in multiplex families using segregation and linkage analysis to document these observations.

Conflict of Interest

Authors declare this work to be free from any issues related to or concerned with any sort of conflict of interest.

REFERENCES

1. *Harrison SA. Liver disease in patients with diabetes mellitus. J Clin Gastroenterol 2006; 40(1): 68-76.*
2. *Williams textbook of endocrinology. (12th ed. ed.). Philadelphia: Elsevier/Saunders pp. 1371-435.*
3. *Sullivan PW, Morrato EH, Ghushchyan V, Wyatt HR, Hill JO. Obesity, inactivity, and the prevalence of diabetes and diabetes-related cardiovascular comorbidities in the U.S., 2000-2002. Diabetes Care 2005; 28(7): 1599-603.*
4. *Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 2004; 27: 813-23.*
5. *Abdallah AN, Cucchi-Mouillot P, Biteau N, Cassaigne A, Haras D, Iron A. Analysis of the polymorphism of the tumor necrosis factor (TNF) gene and promotor and of circulating TNF-alpha levels in heart transplant patients suffering or not suffering from severe rejection. Eur J Immunogen 1999; 26: 249-55.*
6. *Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, et al. Cytokine gene polymorphism in human disease: on-line databases. Genes Immunity 1999; 1(1):3-19.*
7. *Ding Y, Kantarci A, Badwey JA, Hasturk H, Malabanan A, Van Dyke TE. Phosphorylation of pleckstrin increases proinflammatory cytokine secretion by mononuclear phagocytes in diabetes mellitus. J Immunol 2007; 179(1): 647-54.*
8. *Shiba T, Higashi N, Nishimura Y. Hyperglycemia due to insulin resistance caused by interferon-gamma. Diabet Med 1998; 15: 435-6.*
9. *Short AD, Catchpole B, Kennedy LJ, et al. Analysis of candidate susceptibility genes in canine diabetes. J Hered 2007; 98(5): 518-25.*
10. *Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001; 19: 683-765.*
11. *Donnelly RP, Freeman SL, Hayes MP. Inhibition of IL-10 expression by IFN-gamma up-regulates transcription of TNF-alpha in human monocytes. J Immunol 1995; 155: 1420-7.*
12. *Ross J A. Dynamics of peripheral circulation B. Electrocardiography and disorders of cardiac rhythm. In West JB (ed.). Best and Taylor's, Physiological basis of medical practice. 11th edition, 1985; PP: 132-136.*
13. *Trinder P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J Clin Pathol 1969;22(2):158-61.*
14. *Frier BM, Ashby JP, Nairn IM and Blairs JD. Plasma insulin, C-peptide and glucagons concentration in patients with insulin independent diabetes treated with chlorpropamide. Diab Metab 1981; 7(1): 45-9.*
15. *Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model of assessment : insulin resistance and beta-cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 1985; 28: 412-9.*
16. *Kolla VK, Madhavi G, Pulla Reddy B, et al. Association of tumor necrosis factor alpha, interferon gamma and IL-10 gene polymorphisms with peripheral neuropathy in south Indian patients with type 2 DM. Arch Med Res 2009; 40(4): 276-84.*
17. *Wogensen L, Lee MS, Sarvetnick N. Production of IL-10 by islet cells accelerates immune-mediated destruction of beta cells in non obese diabetic mice. J Exp Med 1994 1;179(4):1379-84.*
18. *Settin A, Ismail A, El-Magd MA, El-Baz R, Kazamel A. Gene polymorphisms of TNF-alpha-308 (G/A), IL-10(-1082) (G/A), IL-6(-174) (G/C) and IL-1Ra (VNTR) in Egyptian cases with type 1 diabetes mellitus. Autoimmunity 2009; 42(1): 50-5*
19. *Scarpelli D, Cardellini M, Andreozzi F, et al. Varients of the interleukin-10 promotor gene are associated with obesity and insulin resistance but not type 2 diabetes in Caucasian Italian subjects. Diabetes 2006; 55(5): 1529-33.*
20. *Tsiavou A, Hatziagelaki E, Chaidaroglou A, et al. TNF-a, TGF-beta1, IL-10, IL-6 gene polymorphisms in latent autoimmune diabetes of adults (LADA) and type 2 diabetes mellitus. J Clin Immunol 2004; 24(6): 591-9.*
21. *Kubaszek A, Pihlajamaki J, Punnonen K, Karhapää P, Vauhkonen I, Laakso M. The C-174G promoter polymorphism of the IL-6 gene affects energy expenditure and in-*

sulin sensitivity. Diabetes 2003; 52: 558-61.

22. Herbert A, Liu C, Karamohamed S, Schiller J, Liu J, Yang Q, et al. The -174 IL-6 GG genotype is associated with a reduced risk of type 2 diabetes mellitus in a family sample from the National Heart, Lung and Blood Institute's Framingham Heart Study. Diabetologia 2005; 48(8):1492-5.

23. 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.

24. Qj 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(11):1914-20.

25. Vozarova B, Fernandez-Real JM, Knowler WC, Gallart L, Hanson RL, Gruber JD, et al. The interleukin-6 (-174) G/C promotor polymorphism is associated with type 2 diabetes mellitus in Native American and Caucasians. Hum Genet 2003; 112: 409-13.