JMSCR Vol||05||Issue||10||Page 28969-28976||October 2017

www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 71.58
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i10.84

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

# Role of family history and other metabolic factors in Diabetes in Asian Indian population

Authors
**Shalini Paul[1], Plaban Chaudhuri[1], Riddhi Goswami[1], Mithun Das[2], Indrani Lodh[3]**
[1]Department of Biotechnology, Heritage Institute of Technology, Kolkata, West Bengal
[2]Department of Anthropology, Sree Chaitanya College, Habra, West Bengal
[3]Urvaraa IVF Clinic, Kolkata, West Bengal
Corresponding Author
**Riddhi Goswami**
Department of Biotechnology, Heritage Institute of Technology, Kolkata, West Bengal
Email: *riddhi.goswami@heritageit.edu*

**Abstract**
**Introduction:** *Type 2 diabetes mellitus (T2DM) is a type of metabolic disorder, which is currently imposing a serious threat to the well-being of the world population. Globally, an estimated 422 million adults are living with T2DM, according to the latest 2016 data from WHO, and the number is likely to be doubled by 2030. Variation in the incidence and prevalence of the disease has been associated with the contribution of multigenic predisposition towards it. One such gene is the CRP, where the increased level of CRP has been correlated with the symptoms of T2DM.*
**Objectives**: *To determine the prevalence of T2DM among the Indian obstetric population, To determine the pervasiveness of the gentic polymorphisms in CRP gene in subjects with/without having family history of T2DM (FHD) and to find out any role of the mothers with/without having family history to the newborn babies.*
**Method:** *The investigation has been carried out upon 180 pregnant women and their newborns. Their anthropometric measurements, blood pressure and metabolic profiles were estimated. Alongside, their DNA was isolated for genotyping for CRP followed by statistical analyses.*
**Result**: *A population trend was noticed where individuals with positive family history of diabetes(FHD) had significantly higher prevalence of metabolic risk factors and its confounding factors as compared to their counterparts during pregnancy, which is also affecting their next generation which may lead to childhood obesity.*
**Conclusion**: *Positive FHD and CRP (GG polymorphism) SNPs play an important role in triggering the activation of metabolic risk factors during the pregnancy, and this could be one of the major factors for development of T2DM and metabolic syndrome in the next generations. These findings suggest that family history could be used as a tool for genomic studies among the Asian Indians.*
**Keywords:** *Type 2 diabetes, Family history, Metabolic risk factors, CRP gene*

## Introduction

Type 2 diabetes mellitus is a long term metabolic disorder that consists of an array of dysfunctions characterized by hyperglycemia and resulting from the combination of resistance to insulin action, inadequate insulin secretion, and excessive

or inappropriate glucagon secretion. Common symptoms include increased thirst, frequent urination, and unexplained weight loss. Symptoms may also include increased hunger, feeling tired, and sores that do not heal[1]. Long-term complications from high blood sugar include heart disease, strokes, diabetic retinopathy which can result in blindness, kidney failure, and poor blood flow in the limbs which may lead to amputations.[2]

Globally, an estimated 422 million adults are living with diabetes mellitus, according to the latest 2016 data from the World Health Organization (WHO) [3]. Diabetes prevalence is increasing rapidly; previous 2013 estimates from the International Diabetes Federation put the number at 381 million people having diabetes. The number is projected to almost double by 2030. Type 2 diabetes makes up about 85-90% of all cases [4]. Increases in the overall diabetes prevalence rates largely reflect an increase in risk factors for type 2, notably greater longevity and being overweight or obese.[3]

Until recently, India had more diabetics than any other country in the world, according to the International Diabetes Foundation, although the country has now been surpassed in the top spot by China, Diabetes currently affects more than 62 million Indians, which is more than 7.1% of the adult population. The average age on onset is 42.5 years. Nearly 1 million Indians die due to diabetes every year. [5]

According to the Indian Heart Association, India is projected to be home to 109 million individuals with diabetes by 2035 [6]. The high incidence is attributed to a combination of genetic suscepti-bility plus adoption of a high-calorie, low-activity lifestyle by India's growing middle class. [7]

A considerable variability in the incidence and prevalence of Type II diabetes (T2D) coheres with an important contribution of multigenic predisposition in the development of T2D. Some genes, which probably participate in the pathogenesis of Type II diabetes, also play a role in the regulation of blood pressure, familial hyperlipidemia, familial hypertension and other diseases of the cardiovascular system. [8]

Human C-reactive protein (CRP) is an acute phase reactant whose production is rapidly stimulated in response to infection, tissue injury or inflammation. In a recent study, increased levels of CRP are reported to be associated with metabolic syndrome, obesity, atherosclerosis, unstable angina, insulin resistance and diabetes. Family and twin studies suggest that genetic factors account for 40% of the variance in plasma CRP levels + 1059 G>C (rs1800947) is a single nucleotide polymorphism (SNP) in exon 2 of the *CRP* gene. + 1059 G>C is a silent or synonymous polymorphism at the amino acid level (CTG → CTC, Leu → Leu at codon 184) which has been reported to affect the protein levels of CRP and contribute towards the progression of CAD (coronary artery disease) and T2D. [9]

**Objectives of the Study**

1. To determine the prevalence of Type 2 diabetes (T2DM) among the Indian obstetric population
2. To determine the pervasiveness of the genetic polymorphisms in CRP gene in subjects with or without having family history of T2DM
3. To find out any role of mother in spreading T2DM to newborn babies

**Materials & Methods**

**Study population:** This is a longitudinal follow-up study and present study was being done in continuation with the earlier work. The medical history of the patient's family was recorded. Determination of presence or absence of other risk factors— high cholesterol levels, cigarette smoking, hypertension, diabetes in first-degree relatives (biological parents, siblings, and offspring) was recorded. The present study was conducted on 180 healthy (non-diabetic, normotensive) adult Asian Indian women [including 100 with and 80 without family history

of diabetes (FHD)] living in and around Kolkata, India. This sample size was sufficient to test all the research hypotheses at the 5% level of significance.. During the gestation period they were studied twice, first within 12 weeks and second by 30 weeks. They were then followed up till delivery. During delivery both mothers' venous blood and cord blood were collected to estimate the metabolic variables and genetic polymorphisms of the respective mothers and their new born babies. All the experiments were done according to the "Ethical guidelines for Biomedical Research on Human Participants" published by Indian Council of Medical Research (2006). The Institutional Ethics Committee (IEC) of Heritage Institute of Technology, Kolkata, approved the study.

**Anthropometric measurements:** Demographic profiles including name and age were obtained from participants. Informed consent was obtained from participants prior to the actual commenc-ement of the study. Height was measured to nearest 0.1 cm with a Martin's Anthropometer and weight to the nearest 0.5 kg with a portable weighing machine in light clothing and bare feet.

**Blood pressure measurements:** Left arm systolic (SBP) and diastolic (DBP) blood pressure was taken from each participant with the help of an Omron M1 digital electronic blood pressure/pulse monitor (Omron Corporation, Tokyo, Japan). Two blood pressure measurements were taken and averaged for analysis. A third measurement was taken when the difference between the two measurements was ≥ 5 mmHg, and a subsequent mean was calculated. A five minute relaxation period between measurements was maintained for all participants. The working condition of the instrument was checked periodically using a mercury sphygmomanometer and stethoscope (auscultator procedure). Subjects with SBP/DBP values > 130/85 mm Hg were considered as Hypertensive, according to NCEP and ATPIII guidelines, 2005 [10].

**Estimation of metabolic profiles:** A fasting blood sample (7 ml) was collected from each subject for the determination of fasting blood glucose (FBG), Total cholesterol (TC), Triglycerides (TG), and High density lipoproteins (HDL). All subjects maintained an overnight fast of ≥12 h prior to blood collection. The plasma was separated within 2 h of blood collection using a microcentrifuge at 1000 rpm for about 20 min at room temperature. Estimation of FBG, TC, TG, CRP and HDL were carried out using a Robonik Biochemistry Analyzer (Robonik India, Mumbai, India). Low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) were then calculated by using the standard formula: LDL = TC − (HDL + TG/5) and VLDL = TG/5. All the metabolic profiles were estimated according to NCEP and ATPIII guidelines, 2005 [10].

**Genotyping of CRP**

- The DNA was isolated from whole blood by HiPurA™ Blood Genomic DNA Miniprep Purification Spin Kit. The DNA was amplified by PCR (ABI Biosystems, USA).
- Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) which is based on allele specific amplification of desired fragment using primers corresponding to the alleles has been used for genotyping of +1059 G>C SNP in the CRP gene.
- The sequences of specific primers used are:
  1. Constant forward: 5′-CATTTGTACAAGCTGGGAGT-3′,
  2. Allele C-specific reverse: 5′-ATGGTGTTAATCTCATCTGGTGGG-3′,
  3. Allele G-specific reverse: 5′-ATGGTGTTAATCTCATCTGGTGGC-3′.
- The conditions included initial denaturation (95 °C for 5 min) following a touchdown PCR with denaturation at 94 °C for 45 secs, annealing at 66 °C to 62 °C with 1°C decrease in temperature for the first 4 cycles followed by 29 cycles at 61 °C for 45 secs, extension at 72°C for 45 secs each cycle and final extension at 72 °C for 10 min. [11]

- CRP+1059 G>C genotypes were assessed from the presence/absence of PCR amplicon (237 bp), corresponding to the specific allele (C/G) on 1.5% agarose gel stained with Ethidium bromide.

**Statistical analyses:** Mean and SD for all the metabolic variables, hormones and blood pressure measurements were calculated by conventional statistical methods. Group differences (with and without FHD) for all the variables (18 and 30 months) were tested by One-way ANOVA ($p \pm 0.05$). Differences in CRP values by FHD were estimated by 2X2 Contingency Chi-square analysis ($p \pm 0.05$). Differences in birth outcomes by FHD and CRP values of mothers was estimated by Chi-square analysis ($p \pm 0.05$).

## Observation and Results

### Biochemical analyses of metabolic risk factors

Statistical Analyses of the comparison of the Group differences (with and without FHD) for all the variables (12 and 30 weeks) were tested by One-way ANOVA ($p = < 0.05$). Table 1 shows the anthropometric measurements and biochemical parameters which were considered during the study.

**Table 1.** Baseline characteristics of the study population by family history (n = 180)

| VARIABLES | BY 12 WEEKS | | | BY 30 WEEKS | | |
|---|---|---|---|---|---|---|
| | Both/Either* (n= 100) | None** (n = 80) | ANOVA P | Both/Either (n= 100) | None (n = 80) | ANOVA P |
| AGE (yrs) | 32 (6.7) | 29 (3.4) | 0.262 | 30 (6) | 32 (6) | 0.333 |
| WEIGHT (kg) | 67 (13) | 61 (8) | 0.260 | 76 (10) | 74 (10) | 0.062 |
| BMI (kg/m$^2$) | 25.4 (3.8) | 24.3 (3.7) | 0.405 | 36.05 (4.1) | 32.5 (3.9) | 0.436 |
| WC (cm) | 89 (13) | 80 (9) | **< 0.001** | 127 (14) | 116 (12) | **<0.001** |
| TC (mg/dl) | 210 (16) | 175 (14) | 0.122 | 216 (21) | 210 (20) | 0.069 |
| TG (mg/dl) | 162 (27) | 123 (21) | **<0.001** | 243 (32) | 188 (29) | **<0.001** |
| HDLc (mg/dl) | 44 (9) | 54 (10) | **<0.001** | 40 (8) | 52 (9) | **<0.001** |
| LDLc (mg/dl) | 114 (11) | 108 (12) | **0.001** | 127 (13) | 118 (10) | **0.009** |
| VLDLc (mg/dl) | 34 (5) | 27 (4) | **<0.001** | 57 (10) | 384(7) | **<0.001** |
| FBG (mg/dl) | 110 (10) | 88 (12) | **<0.001** | 118 (12) | 95 (11) | **<0.001** |
| SBP (mmHg) | 126 (11) | 114 (12) | **<0.001** | 148 (14) | 128(16) | **<0.001** |
| DBP (mmHg) | 86 (10) | 78 (9) | **<0.001** | 97 (8) | 84 (6) | **<0.001** |

Read as Mean and ± SD within parentheses
*Both/Either - Participants having positive FHD present in both or either parents;
**None - No FHD.

**Table 2.** Differences in C-reactive protein (CRP) of the babies by mothers' CRP and FHD

| CRP of the Babies | Mothers with +FHD and high CRP (n = 63) | Mothers with -FHD and normal CRP (n = 49) | ANOVA |
|---|---|---|---|
| Mean | 0.47 | 0.26 | P < 0.001 |
| SD | ± 0.088 | ± 0.021 | |

A comparison of the metabolic variables among the subjects with and without FHD revealed that there were significant differences among them with respect to Waist circumference, Triglycerides, HDL-Cholesterol, VLDL-Cholesterol, Fasting Blood Glucose and Blood pressure, both at the gestational period of 12 weeks and 30 weeks. It indicates that family history plays an influencing metabolic factors and triggering the risk factors early in the gestational phase leading to complications in adult life.

Comparison of subjects in the CRP levels between the same groups also revealed a statistically significant difference in subjects with a positive FHD and high CRP levels in comparison to normal cases (Figure 1 & 2).

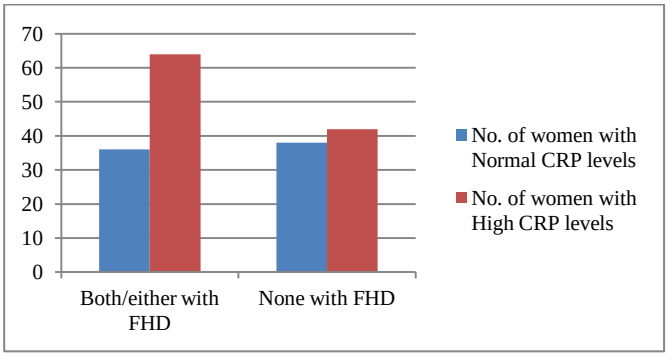


**Fig 1** showing statistically significant difference in C-reactive protein (CRP) levels by FHD (p=0.0109)

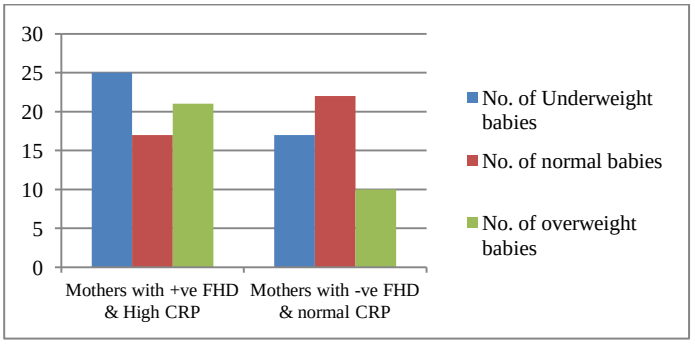


**Fig 2** showing statistically significant differences in birth outcomes (birth weight in kg) by FHD and CRP of the mothers (p=0.0252)

Comparison of birth outcomes in terms of birth weights was done between subjects with and without a positive family history of T2DM and high CRP levels (Figure 1), which revealed a significant difference between the two groups. This shows that family history and perturbations in the gestational period are reflected in the birth outcomes, affecting the next generation (Table 2). It is evident from the results that there is a significant difference in inflammatory status in those newborns whose mothers have a positive FHD compared to their normal counterparts.

**Genotyping of CRP1059 G>C polymorphism-** The genotyping of CRP genes yielded PCR products of size 744 bp. Digestion of the less common 1059C allele produced two smaller fragments, with sizes of 434 and 310 bp. Digestion of the more common 1059G allele produced three fragments, with sizes of 310, 233, and 201 bp.

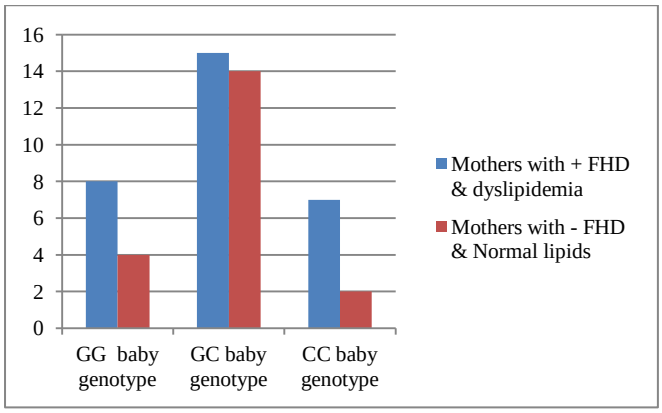


**Fig 3** showing genotyping results of CRP genes of newborn babies, w.r.t their mother's health status

**Discussions**

The Family History of Diabetes (FHD), evidently, has been found to be most useful for predicting T2DM when the disease is premature, that is, it occurs at younger ages than would be expected. This FHD information in combination with other known risk factors could be used to provide more personalized information about our risk for such diseases. Yang *et al.* [12] further suggested that adding FHD could provide significant improvements in detecting undiagnosed diabetes; however, it needs further validation with larger cross-sections of population. In another study [13], it was found that not only the adults but even the young population with a positive FHD showed signs of increased risk for these conditions which indicates the importance of family history approach to screening for children at risk of T2DM and CVD.

In our present study, we noticed a population trend where individuals with positive family history of diabetes had significantly higher prevalence of metabolic risk factors and its confounding factors as compared to their counterparts during pregnancy, which is also affecting their next generation leading to childhood obesity. Therefore, based on this observation, it can be stated that positive FHD and CRP status plays an important role in triggering the activation of metabolic risk factors during the pregnancy period, and this could be one of the major factors for development of T2DM and metabolic syndrome in the next generations. These findings suggest that family history could be used as a tool for genomic studies among the Asian Indians.

CRP is a potential biomarker for prediction of future risk of cardiovascular disease (CVD) both in diabetic and non-diabetic individuals, because even a small rise in plasma CRP levels leads to cardiovascular events. In the present investigation of the association of CRP +1059 G>C polymorphism with the progression to T2D, we have observed that the frequency of G-allele is high in the Asian Indian population (70%). Similarly high frequency for the G-allele has been reported in studies conducted on other world populations (90.0%–96.3%). [14, 15]

Heterozygous carriers of C allele (GC) in the present studied population have shown protection towards the disease manifestation. The GC genotype is highest in controls (9.8%) as compared to cases (2.3%–3.4%) suggesting that some kind of selection pressure is operating on the GC genotype. Considering the thrifty genotype hypothesis, the ancestral version of the alleles proves to be deleterious in the present day

environment while the rarer alleles which may have protective effects against the disease have evolved lately. [16] Fernandez-Real and Ricart have implicated the role of genes encoding for cytokine synthesis as thrifty genes. [17] The hypothesis suggested that higher secretion of cytokines and increased acute phase response were an evolutionary adaptation to phases of acute infections and trauma. In the ancestral period, outbreaks due to infections were higher. Simultaneously due to exposure to famines, metabolic pathways favored insulin resistance to survive in low food situations. It has been proposed that insulin resistance and cytokine responder genotypes were favorable adaptations to low fat, high fiber and high physical activity environment. The genomes of the present day human are still genetically adapted to ancestral conditions which are designed to fight against infection with minimal food intakes and high physical activity. However, environmental transition is more rapid, and evolution being a slow process, our genotypes have not modified according to the present day environments of lower infections, availability of surplus food and low physical activity. In the absence of the favorable conditions and with advancement of age, insulin resistance ensues which further activates inflammatory cascade that eventually results in atherosclerosis. Thus, in the presence of insulin resistance genotypes and western lifestyle, a high cytokine responder genotype would be more prone to develop T2D and atherosclerosis. Although+1059 G>C polymorphism is a silent polymorphism, yet the ancestral allele of the polymorphism (G-allele) has been associated with higher CRP levels in various studies as discussed above. Higher CRP levels are suggestive of inflammatory response which may get accentuated in the background of obesity and insulin resistance condition in T2D. The higher frequency of the G-allele in most of the populations and the protective effect of GC genotype in the present study could be viewed in the background of its role as a thrifty genotype.

**Conclusion**

From our study, we therefore can conclude that the CRP +1059 G>C polymorphism seem to have functional effects on protein production. [18] It has also been proposed that silent SNPs can lead to protein product with the same amino acid sequence, but with different structural and functional properties which might play an important role in defining the protein levels. [19] The reason for discrepancies in association studies across populations may be explained by diverse ethnic background of different populations as different ethnic groups have different susceptibility towards the disease. [20]

Taken together, these observations raise the possibility that these polymorphisms in combination with both family history and environmental factors may account for the phenotype of peripheral insulin resistance and impaired insulin secretion, the two typical features of Type II diabetes.

Also, having observed that family history of diabetes (FHD) is indeed an important and independent risk factor for genomic studies of complex diseases like T2DM, efforts should be made toward translating this knowledge for use in public health programmes designed to detect and prevent diabetes. Once this is achieved, FHD should be incorporated into the screening and prevention programmes for T2DM as rigorously as possible to make these programmes significant and cost-effective.

**Limitations**

However, the present study has several limitations. In the limitations of the present study, firstly, the association of polymorphism with the protein levels needs to be established. Secondly further studies relating the protein levels with studied polymorphism and other functionally relevant polymorphisms which may be in LD with the studied SNP are required on larger sample size to validate the findings of the present study with more statistical power.

**References**


1. "Diagnosis of Diabetes and Prediabetes". National Institute of Diabetes and Digestive and Kidney Diseases. June 2014.-https://www.niddk.nih.gov/health-information/diabetes/overview/tests-diagnosis
2. "Diabetes Fact sheet N°312". World Health Organization. August 2011.-https://web.archive.org/web/20130826174444/http:/www.who.int/mediacentre/factsheets/fs312/en
3. World Health Organization, Global Report on Diabetes. Geneva, 2016. Accessed 30 August 2016.
4. Williams textbook of endocrinology (12th ed.). Philadelphia: Elsevier/Saunders. 1371–1435.
5. "India's Diabetes Epidemic Cuts Down Millions Who Escape Poverty". Gale, Jason. November 7, 2010. https://www.bloomberg.com/news/articles/2010-11-07/india-s-deadly-diabetes-scourge-cuts-down-millions-rising-to-middle-class
6. http://indianheartassociation.org/why-indians-why-south-asians/overview/
7. "Modern Ways Open India's Doors to Diabetes". New York Times. Kleinfield, N. R.. September 13, 2006.
8. Hana Demova,Jana Boleckova,Daniela Rose,Hannes Koeppel,Bohumir Prochazka,Ludmila Brunerova,Ivan Rychlik,Marie Cerna. Gene polymorphisms in patients with Type II diabetes and diabetic nephropathy. Central European Journal of Biology. 2012; 7(2): 210–218.
9. Ramandeep Kaur, Kawaljit Matharoo, Rubina Sharma, A.J.S. Bhanwer. C-reactive protein + 1059 G>C polymorphism in Type II diabetes and coronary artery disease patients. Meta Gene. 2013; 1: 82–92.
10. National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and the treatment of high blood cholesterol in adults (Adult Treatment Program III). Circulation. 2002; 106: 3134-3421.
11. Cao H and Hegele R.A. Human C-reactive protein (CRP) 1059G/C polymorphism. J Hum Genet. 2000; 45: 100-101.
12. Yang Q, Liu T, Valdez R, Moonesinghe R, Khoury MJ. Improvements in ability to detect undiagnosed diabetes by using information on family history among adults in the United States. Am J Epidemiol. 2010; 171:1079–89.
13. Valdez R, Greenlund KJ, Khoury MJ, Yoon PW. Is family history a useful tool for detecting children at risk for diabetes and cardiovascular diseases.A public health perspective? Pediatrics. 2007; 120 ( 2):S78–86.
14. Pasalic D., Marinkovic N., Grskovic B., Ferencak G., Bernat R., Stavljenic-Rukavina A. C-reactive protein gene polymorphisms affect plasma CRP and homocysteine concentrations in subjects with and without angiographically confirmed coronary artery disease. Mol. Biol. Rep. 2009; 36:775–780.
15. Tanja B.G., Winfried M., Wilfried R., Bernhard O.B., Michael M.H. C-reactive protein genotypes associated with circulating C-reactive protein but not with angiographic coronary artery disease: the LURIC study. Eur. Heart J. 2008; 30:170–182.
16. Sharma A.M. The thrifty-genotype hypothesis and its implications for the study of complex genetic disorders in man. J. Mol. Med. 1998; 76:568–571.
17. Fernandez-Real J.M., Ricart W. Insulin resistance and inflammation in an evolutionary perspective: the contribution of cytokine genotype/phenotype to thriftiness. Diabetologia. 1999; 42:1367–1374.

18. Eklund C., Lehtimaki T., Hurme M. Epistatic effect of C-reactive protein (CRP) single nucleotide polymorphism (SNP) + 1059 and interleukin-1B SNP + 3954 on CRP concentration in healthy male blood donors. Int. J. Immuno-genet. 2005; 32:229–232.
19. Komar A.A. Silent SNPs: impact on gene function and phenotype. Pharmacogen-omics. 2007; 8:1075–1080.
20. Radha V., Mohan V. Genetic predisposition to Type II diabetes among Asian Indians. Indian J. Med. Res. 2007; 125:259–274.