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Type2 Diabetes susceptible loci PPARγ2 is not associated with Type1 Diabetes at age of ~30 in Bangladeshi healthy people

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Abstract

Type 2 diabetes mellitus (T2DM) has become a global public health problem. In Bangladesh the disease has taken a turn of epidemic. This type of diabetes is known as silent killer disease affecting cardiovascular system especially heart and brain, eyes, kidney, nervous system and lower limbs. Genetic factors, sedentary habits, food habit and aging are the risk factors for the disease. In this study it was observed whether type 2diabetes susceptible loci PPARγ2 gene is associated with type 1diabetes. For this study 32 healthy male volunteers (age: 27±5) were included. Genomic DNA was extracted from the blood by Chelax method. For PPARγ2 Pro12Ala polymorphism analysis, PCR followed by Restriction digestion was done and subsequently products were visualized on 2% agarose gel after electrophoresis. Out of 32 subjects 26 (81.25%) were homozygous for Pro/Pro genotype, 6(11.75%) heterozygous for Pro/Ala heterozygous and none for Ala/Ala homozygous allele. No correlation between the polymorphism and age, BMI, systolic blood pressure, diastolic blood pressure, HDL cholesterol, LDL cholesterol, triglycerides, Random blood sugar, Glycosylated hemoglobin was observed. It can be concluded from the findings that, polymorphism in PPARγ2 gene is not associated with type 1diabetes and at the age group of ~30 it is not also associated with type 2 diabetes may be due to onset of type2 diabetes is above 40 age.

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Introduction

Diabetes is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Both new cases of diabetes and the risk of associated complications are increasing at an alarming rate (King *et al*., 1998). Type 1 and type 2diabetes both results from the metabolic consequences of inadequate insulin effect. Type 1diabetes which is also known as Insulin Dependent Diabetes Mellitus (IDDM) results from autoimmune β-cell destruction leading to insulin deficiency (Atkinson and Maclaren, 1994). On the other hand type 2 diabetes also known as Non Insulin Dependent Diabetes Mellitus (NIDDM) is the end point of progressive insulin secretory defect on a background of insulin resistance (Lovejoy, 2002). Type 2diabetes represents about 85% to 95% of the people with diabetes in developed countries and an even higher percentage in developing countries whereas type 1 diabetes accounts for 5-10 % (Wild et al., 2004). In Bangladesh diabetes mellitus patients numbered 3.2 million in 2000 and it is projected that in 2030 it will soar to 11.1 million. There is a sharp rise in the prevalence of type 2 diabetes mellitus in recent years in both the urban 8.5% (Rahman *et al*., 2007) and the rural 6.8% (Rahim *et al*., 2007)**.** Besides other factors genetic causes may account for predisposition to insulin resistance and diabetes in South Asians (Mather and King, 1985).

Peroxisome proliferator-activated receptor γ(PPAR γ) is a member of the nuclear hormone receptor super family of ligand-activated transcription factors.1 It is an important regulator of adipogenesis; by forming a heterodimer with retinoid X receptor, it triggers the adipocyte differentiation program by binding to specific transcription elements in various metabolic genes.2 PPARγ is encoded by a single gene that gives rise through alternative splicing to four isoforms (γ1, γ2, γ3 and γ4), which are transcribed from four different promoters and differ in their first exons. All of the transcripts yield the same protein, except for the γ2 transcript, which codes for 30 additional amino acids on the N terminus (Ackert-Bicknell and Rosen, 2006). The PPARγ2 isoform is almost exclusively expressed in adipose tissue, while PPARγ1 is widely expressed (Fajas *et al*., 1997). A Pro12Ala substitution has been detected in the PPARγ2 gene (Yen *et al*., 1997) and this amino acid is located in the PPARγ2 domain that enhances ligand-independent activation (Werman *et al*., 1997). This amino acid is highly conserved. Codon 12 in the mouse PPARγ2 gene is proline (Yen *et al*., 1997). The protective impact of the Ala genotype is probably based on less efficient stimulation of target genes and lower accumulation of adipose tissue and improved insulin sensitivity (Deeb *et al*., 1998; Hara *et al*., 2000) It is likely that two alleles of PPARγ interact with environmental factors such as a High Fat diet leading to an increase in the incidence of type 2 diabetes mellitus (T2DM) (Heikkinen *et al*., 2009).

The possible relationship between type 1 and type 2 diabetes is a controversial subject. Both diseases are of multifactorial etiology, in which genetic predisposition plays a critical role and behaves as a complex trait. Despite the difference in the basic pathogenetic processes for each type, an overlap in genetic predisposition has been proposed and is quite plausible (Wilkin, 2001). Two recent studies suggests that the type 2 susceptible loci PPAR γ2, Pro12Ala polymorphism is associated with risk of type 1 diabetes (Eftychi *et al*., 2004; Johansen *et al*., 2006). Therefore, purpose of this study was to observe, whether Pro12Ala polymorphism of PPARγ2 gene is associated with Type 1 diabetes in Bangladeshi population.

Materials and methods

Study population

A local Ethical Review Committee approved the case-control study and each participant gave written informed consent to participate in it. The study population consisted of 32 healthy male volunteers of

Bangladesh. On the basis of medical history, clinical examination and routine laboratory tests, healthy volunteers were registered for the undertaking study.

Anthropometric and biochemical measurements

Anthropometric measurements included Body Mass Index, BMI (kg/m2). Systolic and diastolic blood pressures were measured twice in the right arms of the subjects who had been resting for at least 10 min in a comfortable position. Fasting blood samples for the analysis of plasma glucose (GLU) were obtained in the morning after 12-h fasting and 120 min after breakfast. Plasma GLU concentration was analyzed using the GLU oxidase method by Beckman Glucose Analyzer II Glycosylated hemoglobin (HbA1c) was measured following an overnight fast using Olympus AU2700.

DNA analysis

Fasting blood samples were drawn into 10-ml vacuum tubes containing EDTA. Genomic DNA was isolated from whole blood using Chelax-100 DNA extraction kit from BioRad as it is pure, nulclease and ligase inhibitor-free chelating resin, certified not to interfere with downstream PCR. Extracted DNA was stored at -20°C.For the Pro12Ala SNP genotype, exon B was amplified using primers 5'-ACT CTG GGA GAT TCT CCTATT GGC-3'(forward primer) and 5'-CTG GAA GAC AAA CTA CAA GAG-3'(reverse primer), with the underlined mismatched base introducing an *Hae*III recognition site, allowing for restriction digestion and size polymorphism to distinguish between alleles. Samples were amplified for 35 cycles, each of which consisted of denaturing at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s. Amplified products were digested with *Hae*III and visualized in 2% agarose gel. After digestion of the resulting 155-bp fragment, the Pro12 allele yielded 2 fragments with sizes 132 and 23 bp (Pro/Pro homozygous), Pro12Ala allele gave 3 fragments with sizes 155,132,23bp (Pro/Ala- heterozygous) and the Ala12 allele yielded only a single 155-bp fragment(Ala/Ala-homozygous)

Statistical analysis

Differences with two-tailed alpha probability (P) ≤ 0.05 were considered significant. The allelic frequencies and genotype distribution were estimated using the gene counting method. Differences in both alleles with the biochemical markers in the studied group were estimated by the chi-square ($\chi 2$) test.

Results

Genotype frequency

Amplified PCR product was further analyzed by Restriction fragment length polymorphism (RFLP) for Pro12Ala polymorphism detection. Homozygous for Pro/Pro allele gave two products of 132bp and 23bp after digestion while Heterozygous for Pro/Ala allele showed three products of 155bp, 132bp and 23bp.(Fig.1) Genotype distribution for PPAR γ2 Pro12Ala polymorphism in 32 healthy control subjects was: 26 Pro/Pro homozygous (81.25%), 6 for Pro/Ala heterozygous (18.75%) and none for Ala/Ala homozygous allele. The genotypic distribution is given in Table 1.

Table 1. Genotypic frequency of the PPAR γ2 Pro12Ala allele.

	No. of genotype						
	Pro/Pro		Pro/Ala		Ala/Ala		Total (N)
Healthy control (25-30 age)	n	%	n	%	n	%	32
	26	81.25	6	18.75	0	0	

Correlation between PPAR γ2 genotype with Biochemical markers

Clinical parameters were compared with PPAR γ2 Pro12Ala polymorphism for both Pro homozygous and Pro12Ala heterozygous.(Table 2). But no correlation between the polymorphism and age, BMI, systolic blood pressure, diastolic blood pressure, HDL

cholesterol,LDL cholesterol, triglycerides, Random Blood Sugar, Glycosylated hemoglobin could be detected.

Table 2. Anthropometric and biochemical measurements of Healthy people according to the Pro12Ala polymorphism of the PPARγ2 gene.

Characteristics	Genotype		P value
	Pro/Pro	Pro/Ala	
Age	27.5±5	27±5	NS
Body Mass Index(kg/m²)	23.6±2.5	24.1±2.0	NS
SBP (mmHg)	118.25±8.0	119±7.25	NS
DBP (mmHg)	78.2±5.1	78±4.9	NS
HDLC (mmol/L)	1.40±.36	1.45±.30	NS
LDLC (mmol/L)	3.5±.56	3.48±.62	NS
TG (mmol/L)	1.20±.42	1.22±.38	NS
RBS	6.4±.4	6.5±.32	NS
HbA1C(%)	5.5±.8	5.7±.68	NS

BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; HDLC high-density lipoprotein cholesterol; LDLC – low-density lipoprotein cholesterol; TG – triglycerides. RBS- Random Blood Sugar; HbA1C- Glycosylated hemoglobin Fisher exact two-tailed test ($\chi 2$ - test). NS- Non Significant.

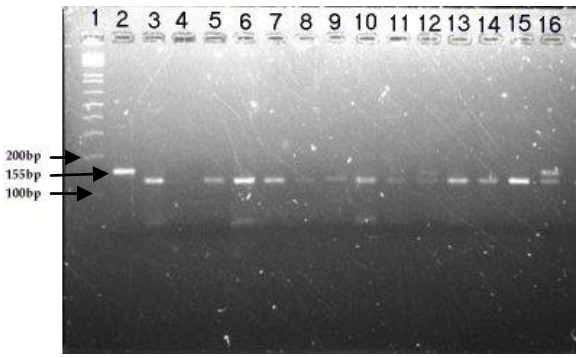

Fig. 1. PCR based RFLP (Restriction Fragment Length Polymorphism) of PPARγ2 gene.PCR followed by restriction digestion with HaeIII was done and visualized in 2% agarose gel. Here Lane 1: Molecular Marker, Lane 2: Undigested PCR product, Lane 4: Negative control. Lane12, 16: Heterozygous Pro/Ala genotype, Lane 3, 5-11, 13-15: Homozygous for Pro/Pro genotype.

Discussion

A cytosine to guanine substitution in the PPARγ2 gene, which is the most common results in an exchange of proline (Pro) to alanine (Ala) in exonB (codon 12) of this gene (Minamikawa *et al*., 1998). Because this mutation is very close to the N-terminal end of the protein, in the ligand independent activation domain, it may cause conformational changes and consequently affect its function. The results showed that frequency of Pro12 allele in healthy person of Bangladeshi population is much higher compare to Pro12Ala allele (81.25% Pro/Pro homozyogus vs 18.75% Pro/Ala heterozygous). The age group of below 30 was chosen to observe the genetic predisposition of type 1 diabetes as affected age group of this type is between 4-30 ages. But from the findings it suggests that there is no correlation between type 1 diabetes and type 2diabetes susceptible loci PPARγ2 gene in Bengali healthy population which is consistent with previous study (Rafiq *et al*., 2008). In this age group Pro12Ala polymorphism of PPARγ2 gene is not also associated with type 2 diabetes mellitus as Pro12 allele is the risk factor for progression of type 2 diabetes and Ala 12 is the protective factor. But here frequency of Pro12 is much higher that Ala12 allele although the subjects are not suffering from disease. These data are consistent with several previous studies (Mancini *et al*., 1999; Ringel *et al*., 1999) although inconsistent with others (Jacob *et al*., 2000; Altshuler *et al*., 2000). Also in this study we did not find any significant difference in the clinical parameters like BMI, Cholesterol, HbA1C with polymorphism although in some studies variation found between polymorphism and BMI (Deeb *et al*., 1998), total cholesterol (Mori *et al*., 1998; Zietz *et al*., 2002), LDL cholesterol (Zietz *et al*., 2002). It can be concluded that this study highlights the importance of differentiation diagnosis of adult-onset type 1 diabetes from type 2 diabetes. Because type 1diabetes does not share common genetic susceptibility with type 2 diabetes, it is important to manage different

treatment for adult type 1 diabetic patients in Bangladeshi people. As only 32 individuals have been included in this study so this small size of population sample can be considered as a weakness of this study. Therefore, large scale genotyping studies are needed for developing a national consensus and database.

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