Study on distribution of Pro12Ala single nucleotide polymorphism of *PPARγ2* gene in randomly sampled diabetic population from Guwahati city

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ABSTRACT

Diabetes is one of the most fatal and vastly heritable metabolic disorders across the globe primarily due to the associated long-term effects including uncontrolled blood sugar levels, blindness, renal and cardiac problems. One of the diabetes related genes peroxisome proliferated activated receptor gamma 2 (*PPARγ2*) plays significant role in insulin metabolism and is expressed predominantly in adipose tissues. Single nucleotide polymorphism (SNPs) like Pro12Ala, is known to be associated with *PPARγ2* gene. In this study, we have made an effort to investigate the prevalence of Pro12Ala allele in the diabetic population of Guwahati, Assam. A total of 50 human subjects with control subjects were included in the study. The data obtained revealed that 49 out of 50 samples carried the Pro/Pro allele, while 1 individual carried the Pro/Ala allele. Results obtained in the study indicate the existence of insignificant link between the targeted SNP Pro12Ala and type 2 diabetes in the sampled population, whereas in contrast we found a strong association of the SNP Pro12Pro with diabetes. The present study shows that there is a need to carry out an extensive study with a large population, which would give us the better picture of occurrence of this SNP in Northeast Indian population.

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Introduction

In the present scenario diabetes is the most prevalent and highly heritable disorder globally. The World Health Organization (WHO) came to a decision of establishing World Congress in India, primarily because one diabetic person out of four globally, is Indian. According to the WHO projections, the 30 million to 33 million diabetic patients in India will shoot up to 40 million by 2010 and 74 million by 2025.[1] WHO has declared a forewarning in 2012 that India will be the diabetes capital of the world by 2025.[2] In both, type 1 and type 2 diabetes, blood sugar levels, blood pressure and blood fats must be well monitored to prevent possible development of blindness, renal failure, and cardiac complications.[3,4] Several genes have been found to be related to type 2 diabetes like *TCF7L2*, *PPARγ2*, *KCNJ11*, *CAPN10*, *TNFα*, *RRAD*, *IGFBP5*, *INSIG1* and *NGFI-B*, out of which *PPARγ2* has the most significant role to play.[5,6]

Peroxisome proliferated activated receptor gamma 2 (*PPARγ2*) is a transcription factor that belongs to the same family of nuclear receptors like steroid and thyroid hormone receptors,[7] and is expressed predominantly in adipose tissues. Its action is triggered by certain fatty acids, prostanoids, and anti-diabetic agents like thiazolidinediones.[8] After activation, it heterodimerizes with the retinoid X receptor and binds to specific

PPAR-responsive elements of DNA to facilitate transcription of several target genes.[9] The isoform *PPARγ1* is ex-pressed in most of the tissues, but the *PPARγ2* is specific for adipose tissue, where it plays a critical role in regulating adipogenesis and insulin functioning.[10] The *PPARγ2* gene is located on chromosome 3, and the specific isoforms are produced due to the result of alternative mRNA splicing. Quite a few genetic variants in the *PPARγ2* gene have been identified which include Pro115Gln, Val290Met, Pro467Leu and the highly prevalent Pro12Ala polymorphism in *PPARγ2*.[11,12] The Pro12Ala polymorphism is the result of a CCA-to-GCA missense mutation in codon 12 of exon B of the *PPARγ2*.[13] Single nucleotide polymorphism (SNPs) related to *PPARγ2* gene have been reported on certain populations from Europe, North America and in Asia,[11,14,15] and SNP like Pro12Ala have been found to be well associated with *PPARγ2* gene.

Genome-wide association studies (GWAS) have immensely enhanced our understanding of the genetic basis of T2D. Most of the GWAS studies related to genes implicated in T2D have been reported from European, Chinese and African populations.[16-19] Such GWAS studies,[20,21] are found to be very limited in case of Indian population, though it is among the countries having the highest diabetic population,[16] the situation is more negligible for Northeast Indian population where many ethnic tribes and communities exist. In India, the occurrence of type 1 is considerably more uncommon, and only about 1/3 of type 2 diabetics are overweight. Diabetes is also beginning to appear much earlier in the lifespan of Indians,[22] meaning that chronic long-term complications are becoming more common. But in the Northeast zone of India the prevalence of such SNPs in relation to type 1 or 2 diabetes has not been reported so far. If such data are generated then it will be statistically easier to determine its distribution along with its function and characteristics in Northeast Indian population and thus, will be helpful in drug administration. The present study is a preliminary investigation to lay the foundation for GWAS studies for *PPARγ2* SNPs implicated to be indicative in type 2 diabetics in Northeastern population.

Materials and Methods

For the study, 2 mL of blood were collected from 50 subjects and from one control subject with assistance from Shristi Diagnostic center, Jonali, Guwahati (26.1833° N, 91.7333° E), Assam, India under the supervision of a pathologist. Blood samples from diabetic patients regularly monitoring blood glucose level in the diagnostic center were chosen for the study. The datasheet for each sample prepared included age, sex, weight, height and fasting glucose. For each patient the basal metabolic index (BMI) was calculated using the formula: BMI=weight (kg)/height (m) (Table 1).

DNA from blood was extracted through phenol-chloroform extraction,[23] and quantified using Nanodrop Quantifier (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The DNA was used as template for polymerase chain reaction (PCR) using primers designed specifically for identifying the Pro12Ala mutation. Three primers were designed for the amplification purpose of our targeted gene sequence through Primer3. The primer sequences used in the study are shown in Table 2.

Amplifications were performed in a total reaction of 30 µL containing: 2.5 µL dNTPs (8 mM), 0.3 µL Taq DNA polymerase (5 U/µL), 3.0 µL 10 × buffer with 15 mM $MgCl_2$, 2.0 µL Primer (10 mM), 2.0 µL Template DNA (50 ng/µL) and up to 30 µL H_2O. PCR amplification was programmed to complete 30 cycles after an initial denaturation cycle for 2 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 30 s, an annealing step at 44°C for 45 s and an elongation step at 72°C for 90 s. The primer extension segment was done for 7 min at 72°C in the final cycle. PCR-product of 15 µL was resolved in 1.5% agarose gel electrophoresis with 1× TAE running buffer. Of the three primers used for the study, primers TD1F and TD1R produced a reproducible amplicon. Hence, the PCR was carried out with all the samples collected with the primers TD1F and TD1R and the product was sent for capillary sequencing to Macrogen Korea (Seoul, Korea).

Results

The PCR conditions for the three sets of primers used for amplicons production are shown in Table 3 and out of these three sets the TD1F and TDIR were found to be more efficient in amplification of the DNA.

Sequence analysis

To see the distribution of this SNP in our samples we performed DNA sequencing. The amplicons upon sequencing produced sequences of readable length of 751 bp. Figure 1 shows the representative chromatogram of the control sample and the mutated case sample.

Statistical analysis

We begin our analysis with the hypothesis that there is no relationship between type 2 diabetes and obesity in the sampled population. For the sampled data, along with allelic and genotypic frequency statistical analysis was also performed to find the relation between diabetes and obesity through Chi-square test. The Chi-square test for the samples is shown in Table 4. Association analysis of the SNP with type 2 diabetes was done by Chi-square test after adjustment for BMI.

Table 1. Clinical characteristics of the sample set.

Sample no.	Gender	Age in years	Glucose amount (FBS) mg/L	BMI	Sample no.	Gender	Age in years	Glucose amount (FBS) mg/L	BMI
1.	Male	58	178	30.4	27.	Female	70	104	31.4
2.	Male	34	185	32.4	28.	Female	48	115	33.0
3.	Male	56	189	28.3	29.	Female	41	94	32.9
4.	Female	66	170	28.5	30.	Male	53	143	31.9
5.	Female	64	177	26.5	31.	Female	30	107	26.8
6.	Female	69	148	35.1	32.	Male	44	116	31.9
7.	Female	62	107	37.3	33.	Female	61	143	34.5
8.	Female	68	142	28.0	34.	Male	68	84	25.0
9.	Male	52	94	31.7	35.	Female	40	404	29.0
10.	Male	49	99	30.5	36.	Male	56	104	26.5
11.	Male	30	106	31.4	37.	Female	55	153	28.7
12.	Female	52	93	26.9	38.	Female	55	153	35.2
13.	Female	66	152	27.6	39.	Female	53	151	31.0
14.	Male	61	194	24.4	40.	Female	70	117	29.3
15.	Male	69	161	24.8	41.	Female	55	184	35.6
16.	Male	47	178	31.5	42.	Female	61	151	29.3
17.	Male	66	242	30.7	43.	Female	40	176	30.9
18.	Male	62	132	31.4	44.	Female	60	105	29.3
19.	Female	61	108	35.1	45.	Female	50	184	30.9
20.	Female	40	142	27.4	46.	Male	53	116	29.9
21.	Male	58	247	29.6	47.	Female	60	158	35.1
22.	Female	50	294	30.4	48.	Female	43	166	29.7
23.	Female	55	158	34.5	49.	Female	48	148	30.2
24.	Female	68	113	24.5	50.	Male	35	96	31.0
25.	Female	74	107	30.9	51.	Male	32	285	28.1
26.	Male	50	110	29.6					

FBS, fasting blood sugar; BMI, basal metabolic index.

Table 2. Primers used for initial screening.

Primer pair	Genome type	Name
5′ACTGAACATGGGTCACCG3′ 3′GGAATAAAAGTGCTGCAAGAGGG5′	Human	TD1F TD1R
5′GGATATTGAACAGTCTCTGC3′ 3′CCTTCAAGTCTAAAAAAGCCC5′	Human	TD5F TD5R
5′ATTCAACCAGGAATAGACACC3′ 3′AAAAGTGACCCTTCAAGTCT5′	Human	TD8F TD8R

Table 3. Polymerase chain reaction amplification conditions for the three set of primers.

Primer set	Annealing temperature and time	PCR amplicons produced
TD1F and TD1R	61.9°C for 40 s	++++
TD5F and TD5R	56.7°C for 40 s	++
TD8F and TD8R	55.3°C for 40 s	++

PCR, polymerase chain reaction.

As a result, we found that the critical P value (0.05) is less than the observed value (0.1<P<0.5) (Table 5), which means that our hypothesis regarding the non-relativeness of the obesity with type 2 diabetes is false.

Discussion and Conclusions

Several recent studies have demonstrated that *PPARγ2* gene is significantly related to type 2 and type 1 diabetes in humans.[24,25] The importance of this gene, which is present in human chromosome number 3, is due to the presence of SNP Pro12Ala. Population study in Europe, Africa and some parts of Asia revealed that these SNP's may be responsible for obesity leading to type 2 diabetes.[26-28] But all the results were not affirmative.[29] In the previous studies, T2D subjects were found to possess a significantly higher frequency of the Pro12 allele risk than non-diabetic controls, thus providing a supportive role for *PPARγ2* in the genetic risk for type 2 diabetes in French Caucasians.[30] So, we hypothesized that diabetic population in Guwahati city is not related to obesity. The total study subjects were 50 along with a control. But out of 50 only one sample was found homozygously mutated (Pro12Pro), though 30 of them were highly obese (data not shown). By Chi-square test the P value was found to be 0.05. By sampling out 50 diabetic samples along with the control and calculating their BMI, we found that our P (0.16) value is much greater than the statistically standardized critical P value (0.05). Thus, our hypothesis became null and concluded that most of the diabetic people are obese. That means the SNP Pro12Ala which is associated with obesity has got modest but significant role in susceptibility to type 1 and type 2 diabetes. But after finding the genotypic (0.98 and 0.02) (Table 6) and allelic frequency (0.98 and 0.02) of the control and the samples respectively, it is seen that the SNP Pro12Ala does not have a significant im-

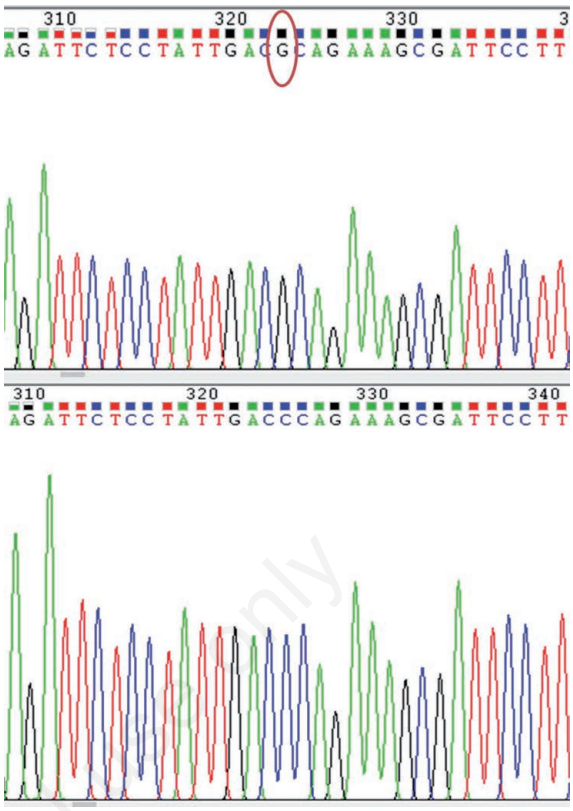

Figure 1. Chromatogram depicting the single nucleotide polymorphism in mutated and control sample.

Table 4. Chi-square test for the samples.

	Obese diabetic	Non-obese diabetic	Total
Observed	30	20	50
Expected	25	25	50

Chi-square value=1.9602; P=0.1<P<0.5.

Table 5. Single nucleotide polymorphism Pro12Ala having significant link with type 2 diabetes.

Gene	SNP	Frequency of case subjects	Risk allele control subject	Risk/non-risk allele	P value (P=0.05)
PPARγ2	rs1801282 Pro12Ala	0.98	0	C/G	0.1<P<0.5

SNP, single nucleotide polymorphism.

Table 6. *PPARγ2* genotype frequencies in the study group.

Types	Pro12Pro (wild-type) C/C	Pro12Ala (heterozygote) C/G	Ala12Ala (homozygote) G/G
Case samples	0.98	0.00	0.02
Control sample	1	-	-

pact on type 2 diabetes and hence it follows Hardy-Weinberg's equilibrium theory. Out of 50 samples only 1 male individual was found to be mutated (CCA/GCA, BMI=31.4), so it was considered insignificant (data not shown). Thus, from the sequencing result we can say that although in some population this SNP Pro12Ala might have shown some significant association with obesity and type 2 diabetes, but within Guwahati city, it does not have any such impact. It is also possible that there might be some errors because of small sample number and random sampling.[31] So, for a better and clear understanding we need to have a large number controls as well as case samples. Thus, like in the European countries,[11,31,32] we were also unable to find any significant link of this SNP Pro12Ala with type 2 diabetes in Guwahati city. Our results show that the Pro12Ala polymorphism is not associated with obesity in the diabetic population of Guwahati city. The results show that 49 individuals out of 50 samples carried the Pro/Pro allele that is (C to C mutation at ID rs1801282), while 1 individual carried the Pro/Ala allele (C to G mutation at ID rs1801282). Till date, no published data are available on the distribution of the Pro/Ala SNP in the diabetic population of Assam. This study is a pilot scale attempt with a small population to understand the prevalence of the SNP in the urban diabetic population. The study was carried out with a secondary aim to elucidate if any link exists between the type 2 diabetics (BMI being an indicator) and the SNP. The results obtained indicated that there is a very insignificant link between the targeted SNP Pro12Ala and type 2 diabetes, but in contrast we found a strong association of the SNP Pro12Pro with diabetes. Hence, though the Hardy-Weinberg's equilibrium theory is not violated, owing to small dataset affirmative conclusion cannot be drawn from the study. However, the results obtained in the study provide the preliminary overview and emphasize the need to have an extensive analysis with a large dataset containing diabetics as well as non-diabetic population. Such a study would provide in depth analysis and will help to elucidate the frequency of existence of this SNP in Northeast India. As Northeast India is hub of many ethnic communities and tribes, understanding this polymorphism may help us in enhanced diagnosis, prevention, and therapeutic approaches toward a more systematic management of type 2 diabetes in different communities.

References

1. Ban HJ, Heo JY, Oh KS, Park KJ. Type 2 diabetes-associated combination of SNPs using support vector machine. BMC Genet 2010;26:1-11.
2. Magon N. Gestational diabetes mellitus: get, set, go from diabetes capital of the world to diabetes care capital of the world. Indian J Endocrinol Metab 2011;15: 161-9.
3. Jesmin J, Rashid MS, Jamil H, et al. Gene regulatory network reveals oxidative stress as the underlying molecular mechanism of type 2 diabetes and hypertension. BMC Med Genomics 2010;3:45.
4. Hovnik T, Dolzan V, Bratina NU, et al. Genetic polymorphisms in genes encoding antioxidant enzymes are associated with diabetic retinopathy in type 1 diabetes. Diabetes Care 2009;32:2258-62.
5. Willer CJ, Bonnycastle LL, Conneely KN, et al. Screening of 134 single nucleotide polymorphisms (SNPs) previously associated with type 2 diabetes replicates association with 12 SNPs in nine genes. Diabetes 2007; 56:256-64.
6. Wu X, Wang J, Cui X, et al. The effect of insulin on expression of genes and biochemical pathways in human skeletal muscle. Endocrine 2007;3:5-17.
7. Stumvoll M, Häring H. The peroxisome proliferator-activated receptor-2 Pro12Ala polymorphism. Diabetes 2002;51:2341-7.
8. Oliver Jr WR, Shenk JL, Snaith MR, et al. A selective peroxisome proliferator-activated receptor δ agonist promotes reverse cholesterol transport. Proc Natl Acad Sci 2001;98:5306-11.
9. Kershaw EE, Schupp M, Guan HP, et al. PPARγ regulates adipose triglyceride lipase in adipocytes in vitro and in vivo. Am J Physiol Endocrinol Metab 2007; 293:E1736-45.
10. Goto T, Lee JY, Teraminami A, et al. Activation of peroxisome proliferator-activated receptor-α stimulates both differentiation and fatty acid oxidation in adipocytes. J Lipid Res 2011;52:873-4.
11. Ludovico O, Pellegrini F, Di Paola R, et al. Heterogeneous effect of peroxisome proliferator-activated receptor γ2Ala12 variant on type 2 diabetes risk. Obesity 2007;15:1076-81.
12. Eftychi C, Howson JM, Barratt BJ, et al. Analysis of the type 2 diabetes-associated single nucleotide polymorphisms in the genes IRS, KCNJ11 and PPARγ2 in type 1 diabetes. Diabetes 2004;53:870-3.
13. Namvaran F, Moghaddama PR, Azarpira N. Genotyping of peroxisome proliferator-activated receptor gamma (PPAR-γ) polymorphism (Pro12Ala) in Iranian population. J Res Med Sci 2011;16:291-6.
14. Sanghera DK, Demirci FY, Been L, et al. PPARγ and ADIPOQ gene polymorphisms increase type 2 diabetes mellitus risk in Asian Indian Sikhs: Pro12Ala still remains as the strongest predictor. Metabolism 2010;59: 492-501.
15. Kooner JS, Saleheen D, Sim X, et al. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. Nature Genet 2011;43:921-89.
16. Tabassum R, Chauhan G, Dwivedi OP, et al. Genome-wide association study for type 2 diabetes in Indians identifies a new susceptibility locus at 2q21. Diabetes 2013;62:977-86.
17. McCarthy MI, Zeggini E. Genome-wide association studies in type 2 diabetes. Curr Diabetes Rep 2009;9: 164-71.
18. Sanghera DK, Blackett PR. Type 2 diabetes genetics: beyond GWAS. J Diabetes Metab 2012;3:6948.

19. Billings LK, Florez JC. The genetics of type 2 diabetes: what have we learned from GWAS? Ann NY Acad Sci 2010;1212:59-77.
20. Saxena R, Saleheen D, Been LF, et al. Genome wide association - study identifies a novel locus contributing to type 2 diabetes susceptibility in Sikhs of Punjabi origin from India. Diabetes 2013;62:1746-55.
21. Gupta V, Vinay DG, Sovio U, et al. Association study of 25 type 2 diabetes related loci with measures of obesity in Indian Sib Pairs. PLoS One 2013;8:e53944.
22. Radha V, Mohan V. Genetic predisposition to type 2 diabetes among Asian Indians. Indian J Med Res 2007;125:259-74.
23. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-9.
24. Tsukahara T, Haniu H, Matsuda Y. Effect of alkyl glycerophosphate on the activation of peroxisome proliferator-activated receptor gamma and glucose uptake in C2C12 cells. Biochem Biophys Res Commun 2013;433:281-5.
25. Muller Y Li, Bogardus C, Beamer BA, et al. A functional variant in the peroxisome proliferator-activated receptor 2 promoter is associated with predictors of obesity and type 2 diabetes in Pima Indians. Diabetes 2003;52:1864-71.
26. Mattevi VS, Zembrzuski VM, Hutz MH. Effects of a PPARγ gene variant on obesity characteristics in Brazil. Braz J Med Biol Res 2007;40:927-32.
27. Salonen JT, Uimari P, Aalto JM, et al. Type 2 diabetes whole-genome association study in four populations: the DiaGen Consortium. Am J Hum Genet 2007;81:338-45.
28. Unoki H, Takahashi A, Kawaguchi T, et al. SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet 2008;40:1098-102.
29. Kao WH, Coresh J, Shuldiner AR, et al. Atherosclerosis risk in communities study, Pro12Ala of the peroxisome proliferator-activated receptor-2 gene is associated with lower serum insulin levels in nonobese African Americans. Diabetes 2003;52:1568-72.
30. Clement K, Hercberg S, Passinge B, et al. The Pro115Gln and Pro12Ala PPAR gamma gene mutations in obesity and type 2 diabetes. Int J Obes Relat Metab Disord 2000;24:391-3.
31. Ghoussaini M, Meyre D, Lobbens S, et al. Implication of the Pro12Ala polymorphism of the PPAR-gamma 2 gene in type 2 diabetes and obesity in the French population. BMC Med Genet 2005;6:1-8.
32. Ereqat S, Nasereddin A, Azmi K, et al. Impact of the Pro12Ala polymorphism of the PPAR-gamma 2 gene on metabolic and clinical characteristics in the Palestinian type 2 diabetic patients. PPAR Res 2009;87412:1-5.