

Association of GR GENE Polymorphism with T2DM among North Indian Population

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Abstract

Background & Objective: Diabetes mellitus is a complex metabolic disorder and is escalating at a very fast rate across the globe in 21st century and is a pressing problem for the world and especially for our nation. Genetics has given us a solution to most of the unsolved mysteries and therefore it was very rational to investigate the association of GRL gene polymorphism with T2DM among north Indians.

Methods: A total of 50 blood samples in each group of T2DM cases and of healthy controls were collected randomly from Diabetes clinic in Medicine Department of ELMC&H. Genomic DNA was isolated from whole blood using the standard phenol-chloroform extraction method. The DNA concentration was determined by spectrophotometer and stored at -20°C. Ethical committee's clearance was obtained from Institutional Ethics Committee, prior to the commencement of the study.

Results: Frequencies of GRL GG, GT and TT genotypes in T2DM cases and controls were 2%, 24%, 74% and 0%, 8%, 92% of GRL G and T allele was 4%, 96% in cases and 14%, 86% in the controls respectively. Odd Ratio of GT was 3.63(95% CI=1.08-12.18, X²=4.76, p values=0.029), for GG NA (95% CI=NA, X²=1.01, p values=0.315) and for TT 0.25 (95% CI=0.07-0.82, X²=5.74, p values=0.017).

Interpretation & Conclusion: On the basis of the findings of this study it is concluded that GRL GT and TT genotype is significantly (p=0.029, 0.017) associated with T2DM. The short study period, small sample size and financial restrains were the limitations of the study. There is need to carry out a multicentric study on larger sample size and for a longer duration to find out a genetic solution of the escalating problem of T2DM.

Key Words: Diabetes mellitus, DNA, Gene, Glucocorticoid Receptor Gene Polymorphism, Metabolic Disorder

Introduction:

Diabetes mellitus is a chronic condition affecting an individual's body ability to use energy found in food. The most common form is type 2 diabetes (T2DM), accounting for 95% of diabetes cases in adults. Diabetes mellitus is fast gaining the status of a potential epidemic in India with more than 62 million individuals currently diagnosed with the disease. In 2000, India topped the world with the highest number (31.7 million) of people with diabetes followed by China (20.8 million) and United States (17.7 million) [1]. Various studies by scientists have predicted that by year 2030 diabetes mellitus may afflict up to 87 million Indians [2]. As we all know not only lifestyle changes but also the genetic makeup of an individual is responsible for the occurrence of the disease [3]. Therefore it becomes essential to assess the association of T2DM with gene polymorphism.

The glycoprotein receptor gene (GRL) encodes glucocorticoid receptor, which functions both as a transcription factor that binds to glucocorticoid response elements in the promoters of glucocorticoid responsive genes and as a regulator of other transcription factors [4]. It is located on chromosome 5q31.3,

also named nuclear receptor subfamily 3, group C, member 1 (NR3C1). There are three known polymorphisms in the GRL gene -BclI, ER22/23EK, N363S-[5]. Mutations in this gene are associated with generalized glucocorticoid resistance. The N363S variant of the GRL gene has been shown to be associated with measures of increased glucocorticoid effects such as more body fat, a larger insulin response to dexamethasone and a less lean-body mass has been reported [6]. Since obesity is a predisposing factor for T2DM, therefore the present study will help us to investigate the association of GRL gene polymorphism in T2DM and its role in increasing the susceptibility to T2DM.

Aims and Objectives:

1. To investigate association of GRL gene polymorphism with T2DM among north Indians.
2. To study different allele and genotype frequencies of GRL gene in T2DM.
3. To assess the role of GRL gene in increasing susceptibility to T2DM.

Material and Methods:

Patient's Selection:

A total of 50 blood samples of T2DM cases and 50 samples of healthy controls were collected from Diabetic clinic in Medicine Department of ELMC&H. Data collection was done for each patient on clinical variables including age, alcohol consumption, body mass index, height, weight, cigarette smoking and family history etc. Informed consent was obtained from each subject before the study. Ethical committee's clearance was obtained from Institutional Ethics Committee, prior to the commencement of the study.

Study Design: A case- control study was conducted with average age of the source population 40-60 years with M>F 2:1 Ratio in each group. Sample size of the study was 50 in each group (Diabetes & Control), since it was a pilot study under ICMRF STS for duration of 3 months. The patients of T2DM were selected randomly from Diabetic clinic in Medicine Department of ELMC&H. Diagnosis of T2DM was based on the physical and clinical examination of Patients by the doctors followed by appropriate laboratory and other investigations. All the cases with T2DM were having fasting plasma glucose level of more than 126mg/dL. The Patients with type 1 presentation, diabetic ketoacidosis, acute presentation with heavy ketonuria (>3+) were excluded from the study.

Place and time of the Study: Study was conducted at ELMC & H, Lucknow in the year 2015 for a period of three months (April-June).

DNA extraction:

Five milliliters of peripheral blood was collected from all the subjects in 0.5M EDTA tubes. Genomic DNA was isolated from whole blood using the standard phenol-chloroform extraction method [7]. The DNA concentration was determined by spectrophotometer and stored at -20°C.

Analysis of Polymorphisms:

GRL Polymorphism

PCR-RFLP method was employed for genotyping of the GRL gene polymorphism. Polymorphism in intron 4 involved G to T substitution, 16 nucleotides upstream from exon 5 was detected by using the following primers was 5'-GAA TAA ACT GTG TAG CGC AG-3' (forward primer), 5'-TAG TCC CCA GAA CTA AGA GA-3' (reverse primer) [8]. The final concentration of the PCR mixture was 0.3 pmol of each primer, 0.2 mmol/L of each dNTP, 0.1 U AmpliTaq DNA polymerase (Perkin-Elmer), 56 mmol/L KCl, 11 mmol/L Tris-HCl (pH 8.3), and 2 mmol/L MgCl₂ in each reaction tube. The PCR amplification was carried out under the following conditions: 10 cycles of 1 minute each at 94°C, 65°C, and 72°C, followed by 15 cycles for 1 minute each at 94°C, 60°C, and 72°C, and then 20 cycles for 1 minute each at 94°C, 58°C, and 72°C, finishing with a step at 72°C for 30 minutes. For intron 4 genotype detection, PCR products was incubated from this region at 37°C overnight with 1 U HinfI enzyme (New England BioLabs). Bands were visualized by ethidium bromide staining after electrophoresis on a 3% high-resolution agarose gel.

Observations and Results:

Frequencies of GRL GG, GT and TT genotypes in T2DM cases and controls were 2%, 24%, 74% and 0%, 8%, 92%. Frequency of GRL G and T allele was 4%, 96% in cases and 14%, 86% in the controls respectively. Odd Ratio of GT was 3.63(95% CI=1.08-12.18, $X^2=4.76$, p values=0.029), for GG NA (95% CI=NA, $X^2=1.01$, p values=0.315) and for TT 0.25 (95% CI=0.07-0.82, $X^2=5.74$, p values=0.017). Significant differences were obtained in the frequencies of GRL GT, TT genotype (p=0.029, 0.017) between cases and control as shown in Table I.

Table I: Genotype & allele frequencies of GRL gene in Diabetes cases and healthy controls.

GRL		Control (50)		T2DM (50)		OR	95% CI	X^2	P values	Bonferroni corrected p values	power
		N	Frequency	N	Frequency						
			(%)		(%)						
Genotype	GG	0	0	1	2	NA	NA	1.01	0.315	0.945	0.974
	GT	4	8	12	24	3.63	1.08-12.18	4.76	0.029	0.087	0.945
	TT	46	92	37	74	0.25	0.07-0.82	5.74	0.017	0.049	0.898
Allele	G	4	4	14	14	3.91	1.24-12.32	6.11	0.013	0.027	0.93
	T	96	96	86	86	0.26	0.08-0.81	6.11	0.013	0.027	0.93

Discussion:

Some but not all studies indicate that glucocorticoids may increase risk of diabetes [9, 10]. In recent years, scientists have recognized that some risk factors for cardiovascular disease and type 2 diabetes cluster together in certain people [11]. This clustering of multiple metabolic risk factors is called metabolic

syndrome. These risk factors include elevated insulin levels (insulin resistance), visceral obesity, high levels of triglycerides, and low levels of high-density lipoprotein (HDL)-cholesterol, as well as hypertension.

Mutations in the GRL were first reported in 1991 [12]. To date, a number of mutations within the human GRL gene have been described. Cross sectional studies in humans have suggested a positive association among obesity, hypertension, and insulin resistance with alleles at the GRL gene. [13] In a large family study of type 2 diabetes in Finland and Sweden, metabolic syndrome was seen in women and men, respectively, in 10% and 15% of subjects with normal glucose tolerance, 42% and 64% of those with impaired fasting glucose/impaired glucose tolerance, and 78% and 84% of those with type 2 diabetes [14].

We have found that the frequencies of GRL GG, GT and TT genotypes in T2DM cases and controls were 2%, 24%, 74% and 0%, 8%, 92%. Frequency of GRL G and T allele was 4%, 96% in cases and 14%, 86% in the controls respectively. The primary reason for considering glucocorticoid receptor variants as an etiologic factor in diabetes relates to the influence of cortisol in glucose and insulin metabolism. Cortisol interferes at several levels of insulin action [15]. In addition, cortisol inhibits insulin secretion from pancreatic b-cells [16]. Significant difference were observed in frequency of GT, TT genotype between cases and control (P=0.029, 0.017). Adequate data of GRL polymorphism and diabetes was not available.

Conclusion

Findings of this study conclude that GRL GT and TT genotype is significantly associated with T2DM. The short study period, small sample size and financial restraints were the limitations of the study. There is need to carry out a multicentric study on larger sample size and for a longer duration to find out a genetic solution of the escalating problem of T2DM.

Acknowledgment:

I am sincerely thankful to the ICMR for providing me an opportunity to do research project under MBBS studentship programme and also to my Institute for extending full help in conducting the study. I am by heart indebted to my Guide and respected teacher Dr.Taslim Raza Assistant Professor Department of Biochemistry, Era's Lucknow Medical College & Hospital, Lucknow for his continuous co-operation and help during the study period. I would also like to thank Ms.Shania Abbas in the Department of Biochemistry who helped me immensely in the standardization of Protocol and Interpretation of Results.

References:-

1. Kaveeshwar SA, Cornwall J. (2014); "The current state of diabetes mellitus in India" AMJ 2014; 7(1):45-48.
2. Ramachandra A, Snehalatha C, Kapur A. et al. (2001); "High Prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes survey" Diabetologia 2001; 44:1094-101.
3. Mohan V, Pradeepa R (2009); "Epidemiology of diabetes in different regions of India "Health administrator 2009; Vol: XXII Number 1 & 2.

4. Rhen T, Cidlowski JA (October 2005). "Anti-inflammatory action of glucocorticoids--new mechanisms for old drugs". *N. Engl. J. Med.* 353 (16): 1711–23.
5. Roussel R, Reis AF, Dubois-Laforgue D, Bellanne-Chantelot C, Timsit J, Velho G (2003); "The N363S polymorphism in the glucocorticoid receptor gene is associated with overweight in subjects with type 2 diabetes mellitus" *Clinical Endocrinology*, Volume 59, Issue 2 August 2003 Pages 237–241
6. Van Rossum EF, Lamberts SW (2004); "Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition" *Recent Prog Horm Res.* 2004; 59:333-57.
7. Sambrook J, Fritsch EF, Maniatis T. (1989); "Molecular cloning: a laboratory manual" 2nd edn. New York: Cold Spring Harbor Laboratory Press, p, 9.14-9.19.
8. Koper JW, Stolk RP, de Lange P, Huizengo NATM, Molijn G-J, Pols HAP, Grobbee DE, Karl M, de Jong FH, Brinkmann AO, Lamberts SWJ. (1997); "Lack of association between five polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance" *Hum Genet.* 99: 663–668.
9. Pagano G, Cavallo-Perin P, Cassader M, (1983); "An in vivo and in vitro study of the mechanism of prednisone-induced insulin resistance in healthy subjects". *J Clin Invest.* 72:1814–1820. 21.
10. Wajngot A, Khan A, Giacca A, et al. (1990); "Dexamethasone increases glucose cycling, but not glucose production, in healthy subjects". *Am J Physiol.* 259:E626–E632
11. Reaven GM. Banting lecture (1988); "Role of insulin resistance in human disease". *Diabetes.* 1988; 37:1595–607.
12. Hurley DM, Accili D, Stratakis CA, et al. (1991); "Point mutation causing a single amino acid substitution in the hormone binding domain of the glucocorticoid receptor in familial glucocorticoid resistance". *J Clin Invest.*; 87:680–6.
13. Rivers C, Levy A, Hancock J, Lightman S, Norman M. (1999); "Insertion of an amino acid in the DNA-binding domain of the glucocorticoid receptor as a result of alternative splicing" *J Clin Endocrinol Metab.* 84:4283–6.
14. Isomaa B, Almgren P, Tuomi T, et al. (2001); "Cardiovascular morbidity and mortality associated with the metabolic syndrome". *Diabetes Care.* 24:683–9.
15. Amatruda JM, Livingston JN, Lockwood DH. (1985); "Cellular mechanisms in selected states of insulin resistance: human obesity, glucocorticoid excess and chronic renal failure". *Diabetes Metab Rev.* 1:293–317. 18
16. Lambillotte C, Gilon P, Henquin JC. (1997); "Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets". *J Clin Invest.* 99:414– 423