Integrin α2β (ITGA2) Gene Polymorphism and Effects on Various Microvascular Complication among Subjects with Type 2 Diabetes

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Abstract:

We carried out a case-control study in a patient with type 2 diabetes mellitus (T2DM) to evaluate the association of ITGA2 gene polymorphisms in microvascular complications. The BglII gene polymorphism of the a2b1 Integrin, a platelet collagen receptor, has been suggested as a genetic risk factor for diabetic retinopathy. The association study included 125 patients genotyped by PCR-RFLP using sequence-specific primers for ITGA2 C807T variant and BglII restriction enzyme. The 125 participants were consists of 25 participants in each group with diabetic neuropathy, diabetic retinopathy, diabetic nephropathy, newly diagnosed type 2 diabetes, and participants without diabetes.

The T/T genotype of the ITGA2 gene was associated with the diabetic retinopathy group (OR= 2.43, 95% CI 2.56 - 4.11, p= 0.001). Thus, the BglII genotype is related to the prevalence of retinopathy independent of the other variables, but it is not independently associated with the prevalence of nephropathy and neuropathy. The ITGA2 gene polymorphisms were a risk marker for diabetic retinopathy among the various microvascular complications in type 2 diabetes. These ITGA2 gene polymorphisms would further help create an evidence base to support the markers for preventing the development of Diabetic Retinopathy among type 2 diabetes.

Keywords: BglII gene polymorphism of the a2b1 Integrin, Microvascular complication, Diabetic retinopathy, Diabetic Nephropathy, Diabetic Neuropathy, Type 2 diabetes Association study Genetic risk factor

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia due to insulin resistance or inadequate insulin secretion (Berg et al., 2002). Long-term complications of diabetes due to hyperglycemia might lead to retinopathy(DR), lead to vision loss; nephropathy(DNH), which is associated with renal failure; and neuropathy(DN), with risk of neural damage (Alghadyan, 2011). DR is one of the significant complications of DM in which the retina becomes severely damaged progressively, leading to vision loss and hence blindness (Saleem et al., 2015). Approximately one-half of people with diabetes have some form of peripheral neuropathy (PN), either polydiabetic or monodiabetic neuropathy (Dyck PJ et al., 1993). Diabetic nephropathy (DNH) is a severe and progressive complication of type 1 DM and type 2 DM. The integrin α2 subunit is a single-chain transmembrane polypeptide that pairs exclusively with the β1 subunit. In humans, a single copy of the α2 gene (ITGA2) is present in the haploid genome located on the short arm of chromosome 5 (5p11-12) (acquelin B et al., 2001). Associations between the BglII polymorphism and the prevalence of DR had been reported in Japanese (Matsubara Y et al., 2000) and Caucasian (Petrovic MG et al., 2003) diabetic patients, and BglII (+) allele in these two ethnic groups is a risk factor for Diabetic retinopathy. This study has aimed to

determine whether the BglII gene polymorphism of the a2b1 integrin gene is a risk factor among microvascular complications with type 2 diabetes.

Patients and methods

This study was a hospital-based cross-sectional study where a total of 125 (M: F 81:44) subjects were consecutively enrolled between October 2018- June 2019 in a tertiary care center for diabetes in Chennai, South India. The subjects were divided into five groups: Group-I Diabetes retinopathy (DR; n=25), Group-II –Diabetes Neuropathy (DN; n=25), Group-III - Diabetes nephropathy (DNH; n=25), Group-IV - Normal Glucose Tolerance (NGT; n= =25) Newly Diagnosed Diabetes Mellitus (NDM; n=25). Patients with type 1 diabetes, liver cancer, interferon therapy, end-stage renal disease, gestational diabetes, and those unwilling to sign the consent form were excluded from the study. Anthropometric measurements, including height and weight, were obtained using standardized techniques. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Fasting plasma glucose (FPG) (glucose oxidase–peroxidase method), serum cholesterol (cholesterol oxidase–peroxidase–amidopyrine method), serum triglycerides (glycerol phosphate oxidase–peroxidase–amidopyrine method), high-density lipoprotein cholesterol (HDL-C) (direct method-polyethylene glycol-pretreated enzymes), and creatinine (Jaffe's method) were measured using a fully automated biochemistry. Glycated hemoglobin A1c (HbA1c) was estimated by the Immuno turbidimetry method (Roche Diagnosis). All the biochemical investigations were done in a NABL accredited laboratory.

Isolation of DNA from the peripheral blood cells was performed using the Qiagen Kit method. The isolated DNA was subjected to PCR amplification by using a Bio-Rad Thermal cycler. The Coding sequences of this BglII polymorphism of ITGA2 gene was amplified from the genomic DNA, using Sequences specific forward GTCATTTCAGGCCATCGTG and reverse GGTGTGCAAGTTAATAAAGTGACG primers obtained from the xcleris lab, India PCR conditions of 34 cycles in this study were as follows: the first two cycles were 94°C for 1 minute, 69°C for 1 minute, and 72°C for 1 minute; the second 2 cycles were 94°C for 1 minute, 67°C for 1 minute, and 72°C for 1 minute; the remaining 30 cycles were 94°C for 1 minute, 65°C for 1 minute, and 72°C for 1 minute. Amplified DNA was digested with BglII restriction enzyme (BglII) at 37°C for overnight (2uL 10× buffer, 1.0uL BglII, 7uL distilled water, and 10uL PCR product). The expected product sizes after digestion were: normal homozygote CC 580 bp, 104 bp; mutant homozygote TT, 340 bp, 240 bp; and heterozygote CT, 580, 340, and 240 bp, respectively.

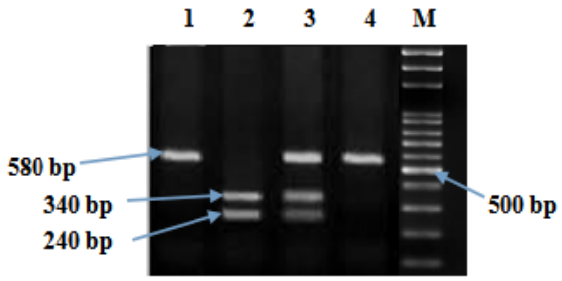

Fig 1- RFLP of ITGA2 gene

Statistical analysis

Data entry was done in Microsoft Excel. IBM SPSS (v 11.5; IBM Corp, Armonk, NY) was the statistical software used and Epi Calc 2000 (Brixton Health, UK). Comparison of the demographic data between the five study groups was made using the x2-test and student's t-test. The polymorphism was tested for deviation from the Hardy- Weinberg equilibrium by comparing the observed and expected genotype frequencies using the x2 - test. For SNP analysis, the frequencies of the genotype and alleles of the itga2 BglII

polymorphism were compared between the five groups using the x2 - test, and the odds ratios (ORs), Relative Risk (RR), and 95% confidence intervals (CIs) were calculated using unconditional logistic regression analysis. A p-value <0.05 was considered to be statistically significant

Result

Biochemical analysis

Among the groups showed significant in fasting Postprandial and HbA1c while comparing with NGT with include BMI in Neuropathy group and eGFR, Urea, HDL and LDL in Nephropathy group also showed significant. While comparing with NDM VLDL, Non-HDL BMI Showed significance in all groups in addition to urea, creatinine, eGFR, HDL, LDL in the Nephropathy group, and Neuropathy Clinical parameters like eGFR, HDL, and LDL showed significantly.

Genotype analysis

Table -1 Genomic and allele frequency between Diabetic Retinopathy and Control

Genotype frequency	Retinopathy (n=25)	Neuropathy (n=25)	Nephropathy (n=25)	NGT (n=24)	NDM (n=24)
CC	2 (8%)	18 (72%)	13 (52%)	20 (80%)	15 (60%)
CT	9 (36%)	4 (16%)	8 (32%)	4 (16%)	5 (20%)
TT	14 (56%)	3 (12%)	4 (16%)	0 (0%)	4 (16%)
Allele Frequency					
C	11 (22%)	40 (80%)	34 (68%)	44 (91%)	35 (72%)

The ITGA2 *BglII* polymorphism is located in intron 7, and the result is the conservative amino acid substitution from Cytosine to Thymine. Genotypic analysis Table -1 showed significant increase in presence of T allele in Diabetic Retinopathy (TT=14 (56%), when compared to the other group like Diabetic Neuropathy (TT=3 (12%), Diabetic Nephropathy (TT= 4 (16%), NGT (TT= 0 (0%) and NDM (TT= 4 (16%). Allele-wise analysis showed a higher frequency of 'T' allele in the Diabetic Retinopathy = 37 (74%) compared to that of other like Diabetic Neuropathy=10 (20%), Diabetic Nephropathy =16 (32%), NGT=4 (9%), NDM=4 (9%).

Comparing Diabetic Retinopathy with NGT

Table-2 Genomic and allele frequency between Diabetic Retinopathy and Control

Diabetic Retinopathy Vs. Control (NGT)					
Genotype	NGT	Retinopathy	OR	95% CI	P-Value
CC	20	2	57.5	9.5-34.8	0.0065
CT	4	9	0.35	0.09-1.37	0.132
TT	0	14	0.01	0.009-0.29	0.0001
Allele Frequency					
T	11	40	37.00	5.86-12.96	0.001

In Table -2, the BglII polymorphism is the statistical analysis for the genotype and allele frequencies for ITGA2 (C807T C<T) in Diabetic retinopathy and control individuals. Out of 24 control individuals, 80% were CC homozygous, 0% were TT homozygous, and the remaining 16% were heterozygous CT. The genotype distribution in the Diabetic retinopathy population was as follows: Out of 25 Diabetic retinopathy, 8% were homozygous for the ancestral (non-risk) CC allele, 56% were homozygous for TT (risk) allele, while 36% subjects were heterozygous CT. The allele frequency for Diabetic Retinopathy cases was 11 (22%) for C and 37 (74%) for T, whereas in healthy controls, it was 44 (91%) and 4 (9%), respectively. Logistic regression analysis of the genotype data under the dominant model (DM) and recessive model (RM) revealed

association of the CC, CT and TT genotypes (Odds Ratio (OR) =57.5, 95% confidence interval (CI) =9.5-347.8, p <0.0001), (OR=0.35, 95% CI=0.09-1.37, p =0.132) and (OR=0.01, 95% CI=0.009-0.29, p =0.005) respectively with Diabetic Retinopathy. The risk allele frequencies of the T allele were compared between DR cases and controls, and significant differences (OR=37.00, 95% CI=10.868-125.96, p=0.001) were observed. Thus, the BglII genotype is associated with the prevalence of retinopathy independent of the other variables, but it is not independently associated with the prevalence of nephropathy and neuropathy.

Conclusion

Several studies have investigated the increased risk of T2DM associated with ITGA2, that the BglII gene polymorphism of the a2b1 integrin gene can be considered a risk factor for diabetic retinopathy (Petrovic et al., 2003). In conclusion, we can confirm the association of the ITGA2 gene polymorphism with Diabetic retinopathy in the population of India. We studied the association of BglII polymorphism of the ITGA2 gene with Microvascular complications for the first time. Our findings suggest that this polymorphism is highly associated with Diabetic retinopathy compare with other microvascular complications like Diabetic Neuropathy and Diabetic Nephropathy in the Indian population. However, the predictive value of ITGA2 C807T polymorphism has to be validated by well-designed further studies with more significant study participants.

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