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Macf-1 Rs2296172 Variant as Type 2 Diabetes Candidate Gene for Bhargava Population

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ABSTRACT

Introduction: Epidemiological transitions in India in the 21st century have led to non-communicable diseases becoming a major public health problem of growing magnitude. **Aim:** To establish MACF-1 Gene variant rs2296172 as a candidate gene for type 2 diabetes mellitus in Bhargava community. **Methods:** A community based cross sectional study conducted 68 previously diagnosed type 2 diabetic of Bhargava community and 60 non diabetic from general population receiving in the Diabetic Care and Research Center, Department of Medicine, S.P. Medical College attached to P.B.M. Associated Group of Hospitals, Bikaner. A camp was organized at Bhargava dera, KEM road, Bikaner for Bhargava community for sample collection. **Results:** In the population group, the frequency of risk allele (G) was found to be considerably higher in cases (0.235) as compared to that of controls (0.10). The variant was observed to be significantly associated with T2D with OR = 2.76 at 95% CI. **Conclusion:** our study show MACF-1 rs2296172 variant as an ideal type 2 Diabetes Mellites candidate gene for Bhargava population.

Keywords: Bhargava, Candidate Gene, type 2 Diabetes Mellites.

INTRODUCTION:

Epidemiological transitions in India in the 21st century have led to non-communicable diseases becoming a major public health problem of growing magnitude. One of the important diseases in this respect is Diabetes, which is considered a "disease of urbanization".[1] Type 2 Diabetes mellitus is a heterogeneous group of disorder characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased hepatic glucose production. Defects in insulin action and/or secretion give rise to the common phenotype of hyperglycemia in type 2 diabetes mellitus.[2]

The Bhargava community in is considered to be one of the most literate and affluent communities in India. Though the Bhargava community are scattered all over India but majority of them inhabit North India. The Bhargava community are a sub-division of the Brahmin or priest caste, which is at the top of the hierarchically arranged endogamous caste system of social stratification. The level of education is high; 83.8% respondents were educated up to the graduate level.[3]

A study covering 50% of the Bhargava households in the city of Jaipur in previously diagnosed type 2 diabetes suggested 42.3% Bhargava households had one or more known diabetic. The overall prevalence was 16.7%. Pedigree analysis shows that 58% households had at least one primary relative with diabetes. Vertical transmission of diabetes through two or more generations was found in 41.1% diabetics. In 26.8% households both parents were diabetic. The prevalence of diabetes amongst the offspring of these conjugal diabetic parents was 11.7%. A significant contributing factor can be the practice of surname endogamy and marital alliances within closely related gotra (clan) sub- groups. The social custom pertaining to endogamy in marriages can be an important factor in determining the high prevalence of diseases like diabetes.[4]

A cross sectional study in Bhargava community of Bikaner to estimate the prevalence of diabetes identify total load of abnormal glucose tolerance was 46.76% in Bhargava population. In contrast, total load of abnormal glucose tolerance was 21% in general population.[5]

The present study on the MACF-1 gene association with type 2 diabetes mellitus in Bhargava population has been proposed to establish it as a candidate gene. The common belief that the Bhargava community in India for one reason or the other, is more vulnerable to diabetes than others has prompted the present study.

Aim: To establish MACF-1 Gene variant rs2296172 as a candidate gene for type 2 diabetes mellitus in Bhargava community.

METHODS:

A community based cross sectional study conducted 68 previously diagnosed type 2 diabetic of Bhargava community and 60 non diabetic from general population receiving in the Diabetic Care and Research Center, Department of

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Medicine, S.P. Medical College attached to P.B.M. Associated Group of Hospitals, Bikaner. A camp was organized at Bhargava dera, KEM road, Bikaner for Bhargava community for sample collection. The study was approved by ethical committee of SP medical college and PBM hospital Bikaner. Previously diagnosed case of type 2 diabetes mellitus in Bhargava community who fits in ADA 2022 guideline for diabetes were included as cases and patients not suffering from any chronic illness or autoimmune illness like CKD, CLD, CLL, Sjogren syndrome, Rheumatoid arthritis were included as controls. Either subject (case or control) suffering from any acute illness in during or in past 1 months at the time of enrolment were excluded from study.

Study of SNP MACF-1 gene variant rs 2296172 association with type 2 diabetes mellitus:
2ml venous blood sample was taken in EDTA vial and DNA preparation was done on that. Lymphocyte from whole blood was separated by lysing the red blood cells using a hypotonic buffer with minimal lysing effect on lymphocyte. lymphocyte was digested with 100 mg/ml proteinase k in cell lysis buffer for 2-3 hours at 56 degree Celsius and extracted twice with phenol: chloroform : isoamyl alcohol . The DNA was precipitated with two volumes of chilled isopropanolol , and 1/10 volume of 3 M sodium acetate. The precipitated DNA was washed by 70% ethanol and the DNA was eluted by nuclease free water or TE buffer.

Isolated DNA was quantified by spectrophotometry method at 260-280 mm and quantified DNA was then diluted with T10 buffer to 25nm
•Primer used were:(Gtex portal)
MACF-1 gene rs2296172 varying primer
5'AGTATTCCACCTACGGAAACTTCTGTGAGTGCTAA3'
3'TCATAAGGTGCATGCCTTTGAAGATACTCACGATT5'
Primer was diluted with T10 buffer to 10-picomole then annealing temperature of each primer was identified.

PCR amplification was carried out using thermo scientific green master mix in 25ul reaction volumes containing about 100ng of genomic DNA, 10pmol of each primer, 2mM of each Dntp, 0.5U of Taq polymerase with a standard buffer containing 1.5mM MgCl2. The PCR products were analysed by agarose gel electrophoresis. Specific PCR products were purified and subjected to bi-directional sequencing with primers, to identify any alteration of sequence.
STATISTICAL ANALYSIS
Appropriate statistical analysis were applied as and when required using Epi info software from CDC. Unpaired 't' test, chi square test, multiple logistic regression and correlation tests were applied. A p value <0.05 was considered as significant.

RESULT
The mean age of diabetic Bhargava is 60.22±8.51 years, non-diabetic Bhargava is 59.23±6.57 years (p>0.05). In diabetic and non diabetic Bhargava there are 60% males and 40% females(p>0.05).
In the present replication study, we investigated association of variant rs2296172 of MACF1 and T2D in the bhargava population of northwest Rajasthan. The allele frequency distribution has been summarized in Table 1, and it was observed to be fol
lowing Hardy-Weinberg equilibrium (p = 0.218). In the population group, the frequency of risk allele (G) was found to be considerably higher in cases (0.235) as compared to that of controls (0.10). The variant was observed to be significantly
associated with T2D with OR = 2.76 at 95% CI (Table 1) which appeared most appropriate. The PAR percentage observed was 5.82% (2.22–11.33%) at 95% CI.
after correction with age, gender, and WHR (Table 1).

Table 1 Allele frequency distribution

Gene	SNP	Polymorphism	Status	Allelic distribution	
MACF1	rs2296172	A/G		G	A
			Cases	0.23	0.67
			Controls	0.1	0.9
			Odds Ratio at 95% CI	2.76	
			p value	0.001	

DISCUSSION
The allele frequency was observed to be following Hardy-Weinberg equilibrium (p = 0.218). In the population group, the frequency of risk allele (G) was found to be considerably higher in cases (0.235) as compared to that of controls (0.10). The variant was observed to be significantly associated with T2D with OR = 2.76 at 95% CI (Table 1) which appeared

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most appropriate. The PAR percentage observed was 5.82% (2.22–11.33%) at 95% CI. after correction with age, gender, and WHR (Table 1).

It is interesting to note that frequency of the risk allele in patients is equal to the reported (0.23), in the discovery study,[6] but higher than previous Indian studies in Bania population of Punjab (0.17) and Mizo population (0.13).

However, this increased allele frequency could be attributed to gene pool structure and genetic homogeneity that may be existing due to practices of endogamy in Bhargava population. We did post hoc power analyses of the study and observed low power, at the observed allele frequency of the risk allele (approx. 57.5%), in the Bhargava population. To increase the power of study to more than 80% , sample size of 150 cases and 150 controls was estimated. To attain that, we pooled more case and control samples belonging to Indo-European linguistic group from Northwest India, as routinely done in Indian populations based case-control association studies, raising final sample size to 1209 individuals (651 T2D patients and 558 healthy controls). We observed increased significance (p=0.0012) and association of rs2296172 with T2D. However, allelic distribution was not observed to be following the HardyWeinberg equilibrium (p< 0.01) in this extended population set, indicating influence of potential population substructure that might have arisen because of pooling of samples and a limitation of present study.

It was speculate that this variant may have implications in disease etiology through any of the functional roles of MACF1. Variation in MACF1 may affect the F-actin dynamics, affecting insulin secretion. MACF1 has been observed to play key role in determining the position of neurons by regulating dynamics of microtubules through glycolgen synthase kinase (GSK-3) signaling.[7] GSK-3 is a protein kinase which has a major role in the regulation of glycogen synthesis. GSK-3 evidently has shown insulin resistance in skeletal muscles of obese mouse model and in T2D humans.[8] It is speculated that any alteration may have impact on insulin signaling pathways. This is another perspective that needs to be experimentally explored to understand the functional perspectives of the variant.

CONCLUSION

The present study on the Genetic variation, studies MACF-1 gene association with type 2 diabetes mellitus in Bhargava population show MACF-1 rs2296172 variant as an ideal type 2 Diabetes Mellites candidate gene for Bhargava population. Thus, MACF-1 rs2296172 variant gene need to be further evaluated in Indian population, so that it can be attributed as a common biomarker of type 2 diabetes mellites in Indian population. Further, it is emphasized that more genetic variants that are susceptible to type 2 diabetes mellites risk are required to be evaluated in Indian population as type 2 diabetes mellites is a complex multigenic disorder. This study will facilitate us in better understanding of genetic predisposition in Bhargava community of Bikaner population and the genetic heterogeneity in Indian population.

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