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Original Research Article

Risk of Depression in Subjects with Type 2 Diabetes is Modulated by a Genetic Variant within DRD4 Gene: North Indian Diabetes-Depression Link Exploration Study (NIDDLES)

Gurpreet Kaur[1], Harjot Dhillon[1], Ritu Sharma[1], Kanchan Mehta[1], Shallu Khullar[1], Sarabjit Mastana[2], Monica Singh[1], Puneetpal Singh[1]

[1]Department of Human Genetics, Punjabi University, Patiala.
[2]Human Genomics Lab., School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough LE11 3TU, United Kingdom.

Corresponding Author: Puneetpal Singh

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ABSTRACT

The role and relevance of DRD4 gene SNPs for the risk of depression in type 2 diabetes remains to be clarified. To investigate its association, present cross sectional study was conducted on 399 type 2 diabetics who were diagnosed for depression using primary health care questionnaire-9 (PHQ-9) $\geq$ 10 criteria. 191 subjects were depressed whereas, 208 subjects were found to be clinically non-depressed. Minor allele frequencies of two DRD4 SNPs rs1800955 and rs747302 were 0.45, 0.42 and 0.42, 0.34 in depressed and non depressed subjects respectively. C allele of rs747302 showed risk of depression (OR 1.41 95% CI 1.05- 1.87, P= 0.024) in comparison to G allele. It has been observed that carriers of CC genotype had approximately double the risk of depression (OR 1.96 95% CI 1.08- 3.56, P= 0.03) than GG carriers and this risk manifests in recessive mode.

Keywords: Type 2 Diabetes, Depression, DRD4 gene polymorphism, Punjab.

INTRODUCTION

Conserving human health has become a challenging task in India where, largest number (40.9 million) of diabetic subjects of the world lives, and if remains unmanaged this number will increase further (70.4 million) by 2030. [1] Of various risk factors associated with type 2 diabetes mellitus (T2DM), depression has been identified as a significant contributor that may influence its pathology leading to micro and macro vascular complications. [2,3] Diabetes and depression frequently co-occur suggesting their mutual relationships and bidirectional interactions. [4,5] Two meta analyses [6,7] have revealed 24 percent higher risk of developing depression in diabetic subjects and 37 percent increased risk of diabetes in depressed subjects, which further increases with the prolonged use of antidepressants. [8]

Several genes and genetic variants have been exposed in relation to either the risk of depression [9,10] or diabetes, [11,12] but reports on the contribution of genes for the risk of depression in type 2 diabetes are limited. Depression in diabetes can worsen its outcomes as these subjects show poor self-management, non-adherence to medication non-compliance of preventive strategies, avoidance of meeting physicians and skipping regular exercise. [13,14] Prolonged depression presents worst outcomes and contributes significantly to cardiovascular disorders analogous to the continuum of severe symptoms of diabetes

to heart diseases. [3]

Examining the role and relevance of those genetic determinants that influence depression in type2 diabetes is a useful tool for making better and effective strategies to curb the menace of diabetes and its imperative clinical outcomes. There is no systematic investigation on understanding the extent of participation of neurotransmitter gene, Dopamine receptor D4 (DRD4) in conferring risk of depression in type 2 diabetic population of Punjab, India, which has been examined in this paper.

MATERIALS AND METHODS

Study Participants

The present cross-sectional study was conducted during October, 2012 to June, 2016 that comprised of 399 T2DM subjects who attended endocrinological departments of Government Medical College and Hospital (GMCH) Patiala, Cardio Diabetic Clinic, Moga and Deep Hospital, Patiala. All these hospitals are tertiary health care provider and cater to the referral cases. 668 subjects were screened initially and amongst them 550 qualified for inclusion as per the diagnostic criteria of American Diabetes Association. [15]

Inclusion criteria were: subjects belonging to Punjab, consenting and having T2DM. ***Exclusion criteria were:*** subjects not from Punjab or not falling within the age range 35 to 65 years. Unwilling and non-consenting subjects were excluded. Those subjects were also excluded who had any neurological, psychiatric, endocrinological and cardiovascular disease or suffered from cerebrovascular accidents. Finally 399 T2DM subjects were included in the present study.

These T2DM patients were examined for depression using Patient Health Questionnaire-9 (PHQ-9). This test is self-administered version of Primary Care Evaluation of Mental Disorders (PRIME-MD) and has a good concordance with other tests used to diagnose depression. [16,17] It has been confirmed that PHQ-9 score ≥10 has 88 percent sensitivity and specificity for identifying depression in diabetes. [16] According to PHQ-9 ≥10 criteria, 191 subjects were diagnosed with depression, whereas 208 subjects were clinically non depressed. All the subjects gave their written consent before participation. The study protocol was approved by the Institutional Ethical Committee.

Risk Variables

Information regarding age, smoking and alcohol intake was recorded by interviewing the subjects. Height and weight were measured and body mass index (BMI) was calculated according to the equation given by Quetelet (BMI = weight in kilograms/height in meters squared). Systolic and diastolic blood pressure was noted down as a mean of two tests conducted after an interval of 3 minutes in sitting position after 15 minutes of rest.

SNP genotyping

Genomic DNA was isolated from the whole blood by salting out method. Subjects were genotyped to determine SNPs within DRD4gene; rs1800955 (-521 C/T) and rs747302 (-616 C/G) using PCR-restriction fragment length polymorphism (PCR-RFLP). Polymerase chain reaction was conducted in 25 µl of reaction mixture containing 0.1-0.3 µg genomic DNAwith10 pmol of each primer, 12.5 µl of *Taq*polymerase 2Xmastermix, and 5.5 µl of nuclease free water. For SNPs rs1800955 and rs747302, 380bp fragment was amplified using the primers (forward : 5'-TCAACTGTGCAACGGGTG3', Reverse : 5'GAGAAACCGAGAAGGATGGAT - 3') with PCR cycle conditions of 94^{O}C for 4 minutes followed by 35 cycles of 94^{O}C for 40 seconds, 58^{O}C for 40 seconds, 72^{O}C for 40 seconds with final extension of 72^{O}C for 10 minutes. The digestion of the PCR product was performed with 5 units of *Ava*IIenzy me for -616 C/G and *Fsp*Ienzy me for -521C/T SNPs and the products were separated on 3 percent agarose gel. For *Ava*II digest, allele 1(C), 380 bp fragment was uncut, for allele 2 (G), the 380 bp fragments cuts into 2 fragments of 54 and

326 bps. For *Fsp*I digest, allele 1 (C), 380bp was uncut and allele 2 (T) was cut into 2 fragments of 228 and 152 bps.

Statistical analysis

Data is presented as Mean ± standard deviation or percentages. The difference between the groups was examined using Chi-square test for categorical variables, and student's t test for continuous variables. The internal consistency reliability for PHQ-9 was checked using Cronbach's alpha. Allele frequencies and their departure from Hardy-Weinberg Equilibrium were estimated by gene counting and $\chi 2$ test, respectively. The association of genotypes between diabetic subjects with and without depression was calculated with odds ratio. P<0.05 was considered significant except for multiple comparisons where threshold of significance was adjusted to P<0.01. For testing type 1 and type II errors, all depression scores were verified by another experienced psychologist blinded to disease/normal status, which showed that enrollment procedure in the present study has higher sensitivity and specificity (85%).

RESULTS AND DISCUSSION

Baseline characteristics and their differences

Baseline characteristics of the diabetic subjects with and without depression are presented in Table 1. No dissimilarity of ages between two groups suggest that all the subjects were well matched for age (P>0.05). Almost 42 percent (89/213) men and 55 percent (102/186) women were found to be depressed. The results between both genders were found to be statistically significant (P=0.009). Alcohol drinkers were found to be more depressed than non depressed subjects. BMI values were found to be higher in depressed group than non depressed subjects (P<0.001). SBP values were higher in depressed group (P<0.001) however, no statistical significant difference was evident for DBP. Minor allele frequencies for both the SNPs rs1800955 and rs747302 differed significantly (P< 0.001) between both the groups.

Table 1: Baseline characteristics of the study participants

Characteristics	Diabetes with depression (N=191)	Diabetes without depression (N=208)	P values
Age (years)	52.9±7.91	51.9±8.23	0.22
Men, n (%)	89 (46.60)	124 (59.61)	0.009
Women, n (%)	102 (53.40)	84 (40.39)	0.009
Current Smokers, n (%)	118(61.79)	132(63.47)	0.73
Current alcohol users, n (%)	88(46.07)	75(36.05)	0.04
Body mass index(kg. m^{-2})	27.93±4.87	24.99±4.04	<0.001
Diastolic blood pressure (mmHg)	88.16±14.19	89.32±11.74	0.37
Systolic blood pressure (mmHg)	130.55±16.74	118.40±15.9	<0.001
rs1800955 (MAF±SE)†	0.45±0.036	0.42±0.034	<0.001
rs747302 (MAF±SE)†	0.42±0.036	0.34±0.033	<0.001

Values are either mean ± SD or numbers and percentages except † where values are minor allele frequency ± standard error. P values were generated by Chi square analysis for categorical variables and *t* test for continuous variables.

Allele frequencies and Genetic model analysis

The minor allele frequencies of DRD4 SNPs rs1800955 and 747302 were 0.45, 0.42 and 0.42, 0.34 in depressed and non depressed diabetic subjects respectively (Table 2). All the genotype frequencies were in Hardy Weinberg Equilibrium (P>0.05). None of the genotype or allele of rs1800955 were observed to influence the risk of depression. CC genotype of rs 747302 was observed to be higher in depressed subjects and was found to confer 2.24 times (OR 2.24 95% CI 1.18-4.26, P= 0.02) enhanced risk of depression than GG genotype. Dominance of this allele for the risk of depression was evident in recessive model (0R = 1.96, 95% CI 1.08-3.56, P= 0.03). It was observed that those diabetic subjects who carry C allele have 1.41times (OR 1.41 95% CI 1.05-1.87, P= 0.024)

higher risk of developing depression than those subjects who carry G allele.

Table 2: Genetic effects of each DRD4 SNP for the risk of depression in type 2 diabetic population of Punjab

DRD4 Gene SNPs and alleles	Diabetes with depression (n=191)	Diabetes without depression (n=208)	Odds ratio	95%CI	P value
rs1800955					
TT	63	73	Referent	-	-
CT (additive model)	82	94	1.01	0.65-1.58	0.95
CC (additive model)	46	41	1.30	0.76-2.23	0.41
TT vs. CT + CC (dominant model)			1.10	0.73-1.66	0.74
TT + CT vs. CC (recessive model)			1.29	0.80-2.08	0.35
T	208 (54.46)	240 (57.70)	0.88	0.66-1.16	0.39
C	174 (45.54)	176 (42.30)	1.14	0.86-1.51	0.39
rs747302					
GG	64	87	Referent	-	-
GC (additive model)	94	101	1.27	0.82-1.94	0.33
CC (additive model)	33	20	2.24	1.18-4.26	0.020
GG vs. GC + CC (dominant model)			1.43	0.95-2.14	0.11
GG + GC vs. CC (recessive model)			1.96	1.08-3.56	0.03
G	222 (58.11)	275 (61.10)	0.71	0.53-0.95	0.024
C	160 (41.89)	141 (33.90)	1.41	1.05-1.87	0.024

The present study is the first from this region, which investigated the contribution of two promoter SNPs (rs1800955, rs747302) of DRD4 gene for the risk of depression in T2DM subjects of Punjab. It has been revealed that C allele of rs7474302 (-616 C/G) is a potential marker that increases the risk of depression by 1.41 times. The carriers of CC genotype had two fold higher risk of depression than their GG counterparts and this risk manifests in its recessive mode. There is not even a single report available so far with which these results can be compared. The SNP rs1800955 was observed to be non-determinant for the risk of depression in the present population. This inference corroborates with the findings of Lai et al. [18] which revealed no association of -521 C/T (rs180055) polymorphism with depression in Chinese population.

CONCLUSION

In conclusion the present study exposed that there is dominance of C allele of rs747302 (-616 C/G) SNP of DRD4 gene for the risk of depression in the population of Punjab which will be clarified by future studies comprising these SNPs and larger dataset.

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Conflict of interest

All the authors confirm that there is no conflict of interest in the submission or publication of this paper.

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