

## ORIGINAL RESEARCH

# Genotyping of DRD2 Allele In A Female Indian Population Indicate Significant Association With Obesity and Depression

Priyank Dashora<sup>1</sup>, Devki Kothari<sup>2</sup>, Salil Vaniawala<sup>3</sup>, Arpan Acharya<sup>4</sup>, Harsh Parekh<sup>5</sup>, Pratap N. Mukhopadhyaya<sup>6</sup>

## ABSTRACT

**Introduction:** The aim of this study was to investigate the relation between A1 allele of the DRD2 gene encoding the D2 sub type of the dopamine receptor with obesity and depression in obese, female Indian population.

**Methods:** A resource population of 270 obese female individuals with mean body weight of  $96.3 \pm 9.4$  Kg and body mass index of  $33.1 \pm 2.7$  Kg/m<sup>2</sup> were genotyped for the DRD2 A1 allele using the PCR-RFLP method. Multivariate analysis was performed using a logistic regression model to test the association between phenotype variables and genotypes.

**Results:** Out of 270 obese subjects, 35.55% were heterozygous for the DRD2 A1 allele. No subjects homozygous for this pathogenic allele were encountered. Out of the sub population bearing one copy of the DRD2 A1 allele, 65.62% were suffering from depression.

**Conclusion:** The pathogenic DRD2 allele was found to be significantly associated with conditions of obesity and depression in a section of obese, female Indian population.

**Keywords:** DRD2 allele, Obesity, Depression, PCR-RFLP, Dopamine receptor

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<sup>1-5</sup>Doctoral Research Fellow, <sup>6</sup>PhD Supervisor, Department of Biotechnology, JJT University, Vidyanagari, Churu Jhunjhunu Road, Chudela, District-Jhunjhunu, Rajasthan-333001, India.

<sup>2</sup>Trainee student, <sup>3</sup>Chief Genetic Consultant, <sup>4</sup>Research Scientist, SN Gene Lab, President Plaza, Tower A, Nanpura, Near Jeevan Bharti, Near RTO Circle, Surat, Gujarat, India.

**Corresponding author:** Pratap N. Mukhopadhyaya, PhD Supervisor, Department of Biotechnology, JJT University, Vidyanagari, Churu Jhunjhunu Road, Chudela, District-Jhunjhunu, Rajasthan, India.

## INTRODUCTION

Obesity and depression are currently identified as widely spread problems with significant societal and personal implications.<sup>1,2</sup> Both carry increased risk for cardiovascular diseases and their association is repeatedly implicated in various studies.<sup>3</sup>

Obesity results in poor self image, low self esteem and social isolation that in turn causes depression. Further, such people are often found ostracized, stereotyped and discriminated on various social occasions. Extra body weight results in chronic joint pain apart from other serious diseases such as diabetes and hypertension which again are indirectly associated with depression.

On the other hand, people who are suffering from depression are more susceptible to over eating, making poor choices of food and tend to lead a sedentary life.

The DRD2 gene encodes for the D2 subtype of the dopamine receptor. This receptor functions by inhibiting the adenylyl cyclase activity and is coupled to the G protein. Mutations in this gene has been associated with myoclonus dystonia as

well as schizophrenia.<sup>4</sup>

In recent studies, the DRD2 A1 allele has been documented to be present in around 45.2% of obese subjects, a prevalence that correlates to that found in alcoholics, nicotine and other drug-dependent subjects.<sup>5</sup> Further, this particular allele of the DRD2 gene is also associated with significant carbohydrate craving habits.

In this study, we genotyped an obese female Indian resource population for prevalence of the A1 allele of DRD2 gene and attempted to profile their depression quotient in order to establish a correlation between the DRD2 A1 allele, obesity and depression.

## MATERIALS AND METHODS

A survey was undertaken in Western India for a period of 18 months to identify obese female individuals within the age group of 35-45 years. The inclusion criteria included a body weight and body mass index above 95 kg and 32 Kg/m<sup>2</sup> respectively. Exclusion criteria were defined as diagnosis of diabetes mellitus, PCOS, or descriptors of PCOS, such as hirsutism or oligomenorrhea, infection with the human immunodeficiency virus (HIV) and concomitant antipsychotic medication use. From this survey, a set of 270 obese, female volunteer individuals with mean body weight (Kg) of  $96.3 \pm 9.4$  and body mass index (BMI; Kg/m<sup>2</sup>) of  $33.1 \pm 2.7$  were eventually shortlisted for the study. The research program was conducted at SN Genelab and Research Center, Surat, India and approved by SN Gene laboratory clinical research, institutional bio-safety and bio-ethics committee (Approval number DGL/2013/WZ02).

For genotyping, total DNA was extracted from peripheral blood (n=130) or buccal swab (n=140) and subjected to polymerase chain reaction using gene specific primers. PCR was performed in 25  $\mu$ L reaction mixtures containing 1.5mM MgCl<sub>2</sub>, 200  $\mu$ M dNTP mixture, 5  $\mu$ M of each forward and reverse primers, 1  $\mu$ g of template DNA, 1 unit of Taq polymerase (Invitrogen) and 1X PCR reaction buffer.<sup>14</sup>

Thermal cycling conditions comprised of an initial denaturation at 94°C for 4 minutes followed by 35 cycles each comprising of 30

seconds at 94°C, 30 seconds at 58°C, and 30 seconds at 72°C respectively followed by a final extension step of 5 minutes at 72°C.

The PCR products were digested with 5 U of Taq 1 for 22 hours at 65°C for ascertaining the Taq1A polymorphism. Digestion products were resolved on a 4% agarose gel (5V/cm) containing 0.65- $\mu$ g/ml ethidium bromide.

Three DRD2 Taq1A genotypes were expected to be encountered. These were the predominant homozygote A2/A2 characterized by 2 restriction fragments of 180 and 130 bp, the heterozygous A1/A2 with 3 restriction fragments of 310, 180, and 130 bp and the relative rare homozygote A1/A1 which would be represented by a single uncut fragment of 310 bp size.

For ascertaining the level of depression, a questionnaire named PHQ-9 was used which scores each of the nine DSM-IV (Diagnostic and Statistical Manual) criteria as "0" (not at all) to "3" (nearly every day). This questionnaire has already been validated for use in primary care.<sup>6</sup> Multivariate analysis was performed using a logistic regression model to test the association between phenotype variables and genotypes.

## RESULTS

Blood or buccal swab samples collected from 283 individuals across the study period, who met the inclusion and exclusion criteria predefined for this research program were subjected to isolation of DNA. Total DNA could not be extracted from 7 individuals (4-blood and 3-buccal swab samples) while extracted DNA from 6 individuals (4-buccal swab and 2-blood samples) did not respond to PCR. DNA, successfully extracted from remaining 270 subjects, were subjected to genotyping using the PCR-RFLP technology. Out of these, 96 (35.55%) were found to harbor the DRD2 A1 allele in heterozygous form. No A1/A1 homozygous variants were detected. Out of the sub population with one copy of the DRD2 A1 allele, 63 subjects (65.62%) were found to be suffering from depression. Among them, 39 (61.9%) had level 1, 18 (28.57%), level 2 and 6 (9.52%) level 3 category of depression.

## DISCUSSION

The role of D2 receptor (DRD2) gene locus on the etiology of alcohol addiction has been intensely discussed in recent years. However, the association study reports available in the public domain is still a mix of positive and negative conclusions.<sup>7</sup>

Studies have shown that continued exposure to environmental stress encourage obesity in females through hyperactivation of the hypothalamic-pituitary-adrenocortical (HPA) axis.<sup>8</sup> However, we found that along with such environmental factors, there is a strong association between at least one particular allele of the DRD2 gene with obesity and depression. These studies when seen together help in explaining the reduced penetrance of the DRD2 A1 and pathogenic allele of several other similar genes in dictating obesity and depression in females.

Ponce et al., 2003 investigated the relation of A1 allele of DRD2 gene with predisposition of alcoholism to consolidate the existing data available that are related to phenotypic expression in alcoholism and DRD2 A1 allele.<sup>9</sup> In a study involving 40 patients attempting to investigate the association of A1 allele of DRD2 gene and co-morbid substance use disorder, it was observed that the allele was associated with risk of obesity along with addictive behaviors, earlier known as the Reward Deficiency Syndrome.<sup>10</sup>

In another study, DRD2 A1 allele was demonstrated for its association with early-emerging anxious and depressive symptoms in a group of preschool-aged children. The study indicated gene environment correlation and encouragingly, the positive effect of good parenting on influencing the harmful effects.<sup>11</sup>

In India, considerably study has been undertaken on DRD2 gene variants. Though a focal point of study is the domain of genetics of neuropsychiatry and pharmacology, the potential of this gene and its variants as a promising nuclear DNA marker to study genome diversity has also been explored. Saraswathy and coworkers working on multiple variants of the DRD2 gene in North Indian population indicated increased genetic inflow among the North Indian caste population

in India compared to those in the Southern parts of the country and its different tribal population.<sup>12</sup>

In our study we attempted to focus our intervention towards the association of DRD2 A1 allele with two common problems, namely obesity and depression, that is increasingly getting related with modern living style and stress.

This study is in line with other reports that correlated DRD2 gene polymorphism with obesity and depression. While environmental effect such as social criteria and mental state of obese individuals contribute to mental depression, genetics also appears to be a significant contributor. The relatively high level of DRD2 A1 allele frequency (35.55%) in our study population reflects the related findings from India which were conducted to explore variation of this gene as a tool to investigate degree of genetic diversity.

We did not detect any DRD2 A1 homozygous - person in our resource population. This may be explained by the fact that either the population is yet not saturated with this pathogenic allele such that homozygous individuals are detected even in such semi-enriched population as that of ours where obesity is one of the important inclusion criteria for creating the resource population. Alternatively, the other reason perhaps is that significant out breeding is occurring within the population which has potential to dilute the DRD2 A1 allele given the vastness of our country and changing customs of marriage where caste and creed are playing lesser rules thus indirectly promoting superior gene mixing. However, the darker side of the observation is that even a single copy of the gene is apparently rendering sufficient, phenotypically detectable, pathogenic trait of obesity even though the penetration of this gene appears not to be very high.

The finding of 65.62% of the DRD2 A1 allele-bearing obese individuals in our study population suffering from active depression is an important observation. This indicates the possibility of introducing prediction of this socially harmful stress of depression using genetic analysis although the complex mix of environmental effects and genes that influence depression would continue to challenge such an effort. Nevertheless, the study shows a clear indication of genetic influence of depression and DRD2 A1 allele in obese individuals either by direct or indi-

rect pathways.

It is worthwhile to note that not all genes reported for their association with obesity show similar results in Indian population. For example, a mutation upstream of the insulin-induced gene 2 (INSIG2)(rs7566605) was reported for its association with obesity in as many as four separate cohorts outside India. Interestingly, in contrast to these reports, the same variant with a mutation upstream of the INSIG2 gene was not found to be a determinant of BMI and obesity in Indian population.<sup>13</sup> Because of such phenomenon, it is essential to undertake correlation study of genes such as DRD2, reported for their association with obesity and depression elsewhere, in Indian population also to confirm the extent of phenotype-genotype association.

## CONCLUSION

To our knowledge, this is the first study associating DRD2 genetic variants with obesity and depression in obese, female Indian population. It raises the prospect for genetic prediction of young individuals towards their susceptibility to obesity and depression and underlines the influence of genes even in phenotypes that are believed to be an outcome of living in increasing complex societal settings and modern day stress.

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