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Original Article

Pharmaco-genetic analysis of *CYP1A1* and *RGS4* variants and its impact on response to olanzapine and risperidone in Indian schizophrenic cohort

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Abstract: Objectives: Genetic variations contribute significantly to inter-individual responses to drugs and side effects. Pharmacogenomics has the potential to be utilized as a tool in disorders like schizophrenia with a high degree of genetic inheritance, although data on pharmacogenomics of schizophrenia are limited. Olanzapine and risperidone are the frequently used anti-psychotic drugs used in clinics. Studies have observed the variability in the response of both drugs in schizophrenic individuals. Considering the pharmacogenomics importance of both drugs, we aim to examine the cytochrome P 4501A1 (CYP1A1) and regulator of G-protein signaling 4 (RGS4) variants and their metabolizing status in 94 schizophrenic individuals of Indian descent. Methods: The present study is retrospective observational study. The metabolizing status of schizophrenic individuals was examined using Axiom Precision Medicine Diversity Array (PMDA) and the data were analyzed with the help of SNP Axiom Analysis Suite v5.1 (Affymetrix). The pharmacogenomics annotation was performed using PharmGKB. Results: Genotype and allele frequencies were observed. The results reveal the high frequency of poor metabolizers of olanzapine and risperidone in the studied cohort. In lieu of the high distribution of poor metabolizers, we compare observed allele frequencies with global populations' data to understand the variability of the genetic pool attained by Indian schizophrenic individuals. Conclusions: Interestingly, the Indian schizophrenic cohort forms a different cluster compared to global populations, suggesting that pharmacogenomics testing might play an important role in clinical decision making for schizophrenia drug management.

Keywords: Pharmacogenomics, schizophrenia, *CYP1A1, RGS4,* olanzapine, risperidone

Introduction

Schizophrenia is a neurodevelopmental disorder with higher rates of heritability and is usually chronic. It is diagnosed only on the basis of psychiatric symptoms such as impairments in perception, changes in behavior, delusions, hallucinations, and difficulty in cognitive functioning. There are no diagnostic tests and biomarkers available for the disorder [1, 2]. The heritability of this disorder is 70-80% which puts forward genetics as a significant role in its etiology [3, 4]. With the evolution of technology, genetic research has made advances in understanding the genetic architecture and pharmacogenomics of schizophrenia.

For personal well-being, inter and intra relationships, mental health plays an important role. Some disorders interfere with and reduce health, cause pain and discomfort, and sometimes may be fatal [5]. Major mental disorders such as schizophrenia and depression affect more than 30 million individuals globally [6]. The drugs that are used to treat these individuals only work on a limited number of people. Patients diversely respond to drugs. Pharmacogenomics biomarkers could be a potential tool for the treatment of such traits where there is variability in inter-individual response to drug metabolism and with high explained genetic heritability [7].

While treating schizophrenic patients, symptoms such as hallucinations, delusions, and psychotic symptoms are managed to lower the odds of relapse and enhance their functional ability. Olanzapine and risperidone are the

approved antipsychotic drugs that are used to treat psychotic disorders like schizophrenia, bipolar, *etc*. [8-10]. These medications may relieve the patients but have adverse drug reactions [11]. To overcome this variability of drug response, pharmacogenomics plays a pivotal role in identifying new genomic biomarkers (variations) susceptible to the drug response or drug dosages. *CYP1A1* has been validated as one of the significant gene that was observed to be associated with olanzapine concentration in plasma by a genome-wide association (GWAS) [12]. Whereas, *RGS4* variation was observed to be associated with schizophrenia in three populations [13]. *RGS4* variants have shown functional evidence to have a role in antipsychotic treatment efficacy in schizophrenia. Both olanzapine and risperidone are used widely in India to treat negative symptoms and overall clinical severity in schizophrenia [14-16]. Their response to these drugs varies in different studies conducted in Indian population groups [15, 17], indicating a dire need to perform pharmacogenomics evaluation to decipher this variability puzzle in Indians.

Keeping this in mind, we perform genotyping of the two variants of *CYP1A1* (rs2472297) [12] and *RGS4* (rs2842030) [18] in Indian schizophrenic cases (ISC) that have previously shown an association with the olanzapine and risperidone, respectively. These two variations were also annotated in PharmGKB with their genotypes and their effect on the metabolizing status of these two drugs. Such pharmacogenetics study results can aid clinicians in the selection of the drug, optimize the dosage according to the individual need, and may reduce the side effects [19].

Methodology

Subject criteria and selection

The present study was retrospective study conducted among 94 Indian schizophrenic cases recruited from the Department of Psychiatry, Government Hospital Kanpur, India. All recruited cases were diagnosed by psychiatrist using International Classification of Diseases, tenth Edition (ICD-10). All the subjects are descent of North India. The study has been approved by Military Hospital Kanpur (wide letter No. 15965/58th/9/2020/DGAFMS/DG-3B dated March 2019) (Supplementary File 1).

DNA isolation and genotyping

3 ml blood samples were collected from the individuals and stored at 4°C. Genomic DNA extraction was done using QIAGEN DNA MINI Kit according to the recommendations of the manufacturer. Qualitative and Quantitative DNA test was done with the help of agarose gel electrophoresis (Supplementary Figure 1) and Qubit 4 fluorometer. 150 ng of total genomic DNA was hybridized on the Axiom Precision Medicine Diversity Array (PMDA) using Axiom 2.0 Plus assay kit as per the manufacturer instruction. The PMDA was hybridized, washed, and scanned on the Thermo Fisher GeneTitan Multichannel Microarray system, and raw data in the form of '.cel' format were utilized for further analysis. After genotyping accessions, genotype calling and QC metrics were performed with SNP Axiom Analysis Suite v5.1 (Affymetrix) using diploid threshold configurations and default DishQC settings (DQC ≥ 0.82 and call rate > 0.97). Genotype clustering plots were obtained to observe the call threshold for the variant rs2472297 (AX-39946805) of CYP1A1 gene and rs2842030 (AX-88821682) of RGS4 gene (Supplementary Figures 3 and 4). The genotype of all samples was exported in plink format using Axiom Analysis Suite v5.1 (Supplementary Figure 2).

Statistical analysis

Continuous data of socio-demographic and anthropometric were presented as average and count of individuals with frequency in percentage and standard deviation in age, height, and weight. The genotype frequencies were evaluated using Plink v.1.9 [20] for both variations. Global allele frequencies were obtained from gnomAD (https://gnomad.broadinstitute.org/) using whole genome data. The super population groups selected for phylogenetic analysis were Africans/African Americans (AFR), Indian Schizophrenic Cases (ISC), Latino/Admixed Americans (AMR), Ashkenazi Jews (ASJ), East Asians (EAS) and South Asians (SAS). F_{ST} was calculated using Arlequin Version 3.5.2.2 with 10,000 permutations [21], and the model was selected for calculation of ANOVA from our previous study [22]. Metabolizing status of individual subjects with respect to drugs was performed using clinical annotations using PharmGKB (https://www.pharmgkb.org) and was assessed on 01 February 2023.

Table 1. Allele and genotype frequency distribution observed in the studies of schizophrenia cohort

Gene and drug (dbSNP ID) (N = 94)		Poor metabolizer*	Good metabolizer*
CYP1A1 (rs2472297) Olanzapine	Alleles	C = 96.8	T = 3.2
	Genotypes	CC = 93.6	TC+TT = 6.4
RGS4 (rs2842030) Risperidone	Alleles	T = 33.0	G = 67.0
	Genotypes	TT+GT = 55.3	GG = 44.7

*Classification for the poor and good metabolizers based on the annotation provided by PhamGKB.

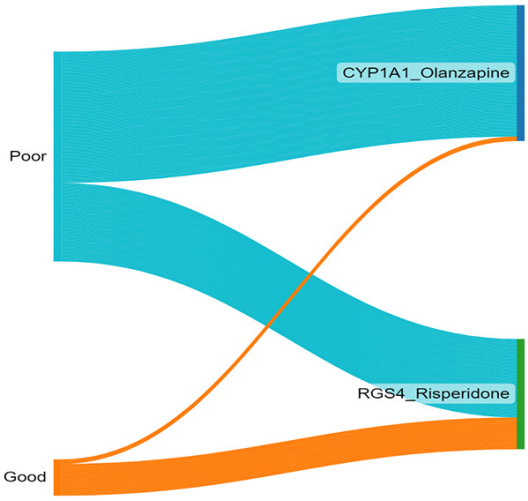

Figure 1. Sankey diagram showing the metabolizing status observed for Olanzapine and Risperidone on the basis of CYP1A1 and RGS4, respectively in the studied schizophrenic cohort. The first column indicts the proportion of metabolization observed, and the second column indicates genes evaluated with their respective drugs.

Results

The study was conducted in 94 unrelated schizophrenic cases belonging to North India, and samples were stratified on the basis of age-matched gender consisted of 42 males and 52 females. The distribution of individuals on the basis of socio-demographics, anthropometrics, and clinical observations is provided in Supplementary Table 1. The observed allele and genotype frequencies of both the variants of *CYP1A1* (rs2472297) and *RGS4* (rs2842030) are summarized in **Table 1**.

The predicted prevalence of allele C (poor olanzapine metabolizer) of variant rs2472297 CYP1A1 was 96.8%, with a genotype frequency of 93.6%. Similarly, for the variant rs2842030 of *RGS4*, the frequency of allele T (poor risperidone metabolizer) was 33.0%, while the risk genotype combination TT+GT was 55.3%. The genotype and allele frequencies observed for the *CYP1A1* and *RGS4* variants suggest that the studied schizophrenia cohort have a greater percentage of patients who are poor metabolizers of olanzapine and risperidone (**Figure 1**).

Moreover, the high frequency of poor metabolizers variations in the studied cohort indicates that the studied population should be compared with global populations' allele frequencies of both variants, to understand the variability of the genetic pool attained by Indian schizophrenic individuals.

Using genomAD, the allele frequencies from the ISC cohort were compared to the worldwide allele frequencies of studied variants (**Tables 2** and **3**). For *CYP1A1* variant, the effective allele frequency of ISC cohort is 96.8% which is in concordance with South Asians (SAS) population *i.e.,* 96.42%. The only population group that has higher allele frequency than ISC and SAS was East Asians (EAS) with effective allele prevalence of > 99%.

Furthermore, the observed *RGS4* variant's effective allele frequency in ISC cohort was 55.3% which is higher than SAS. The global population groups that have higher frequencies than ISC are Ashkenazi Jews (ASJ) and Admixed Americans (AMR) (**Table 3**). Based on comparative results, the higher genetic heterogeneity was observed among different population. To estimate the extent of genetic variability attained by the studied cohort F_{ST} was calculated, and the average number of pairwise differences within and between populations using Slatkin's linearized F_{ST} model [22] was further used for generating the multi-dimensional scaling (MDS) plot (**Figure 2**). The results obtained from the MDS showed that the ISC forms a different cluster than other global populations when evaluated for both the variants of *CYP1A1* and *RGS4* (**Figure 2**).

Table 2. Allele frequency distribution of *CYP1A1* variant rs2472297 associated with pharmacogenomics of schizophrenia in global population groups

Population	Allele frequency of C (in percentage)	Allele frequency of T (in percentage)
AFR	95.81	4.19
ISC	96.8	3.2
AMR	90.45	9.55
ASJ	92.08	7.92
EAS	99.92	0.08
SAS	96.42	3.58

AFR: Africans/African Americans; ISC: Indian Schizophrenic Cases; AMR: Latino/Admixed Americans; ASJ: Ashkenazi Jews; EAS: East Asians; SAS: South Asians.

Table 3. Allele frequency distribution of *RGS4* variant rs2842030 associated with pharmacogenomics of schizophrenia in global population groups

Population	Allele frequency of G (in percentage)	Allele frequency of T (in percentage)
AFR	56.55	43.45
ISC	44.70	55.30
AMR	43.84	56.16
ASJ	41.67	58.33
EAS	48.16	51.80
SAS	62.38	37.62

AFR: Africans/African Americans; ISC: Indian Schizophrenic Cases; AMR: Latino/Admixed Americans; ASJ: Ashkenazi Jews; EAS: East Asians; SAS: South Asians.

Discussion

Olanzapine is the first-line drug used for the treatment of schizophrenia and shows improvement in cognitive functions and less extrapyramidal effects [23]. However, in some cases, it affects the metabolic and cardiovascular system [24]. It is an analog of clozapine that is most effective in managing treatment-resistant symptoms but is ranked last due to safety concerns [25]. It is mainly absorbed by hepatic cells with the help of *CYP1A2, CYP2D6*, and *CYP3A4*. Patients show variations in olanzapine concentration and response due to age, gender, ethnicity, and lifestyle, which should be considered while calculating the dose [26].

Likewise, Risperidone is a second generation of antipsychotics and has lower extrapyramidal effects as compared to the first generation [27]. Genetic polymorphism affects the therapeutic response as well as the side effects [28]. *RGS4* gene is a risk gene for schizophrenia [29]. In the patients, its expression level is reduced in the frontal cortex. It regulates the activity of dopamine, acetylcholine, and serotonin receptors. *RGS4* is a target of antipsychotic medications, and decreased amounts of this protein are seen in patients [18].

In presently studied dataset, the variants were selected based on PharmGKB annotations for *CYP1A1* (https://www.pharmgkb.org/clinicalAnnotation/1444704067) and *RGS4* (https://www.pharmgkb.org/clinicalAnnotation/655384979). The observed genotype and allele frequencies for the *CYP1A1* and *RGS4* variations suggest that ISC might have a higher number of people who were poor metabolizers of olanzapine and risperidone.

The frequency of the C allele of rs2472297 is 96.8%. Since, practically every member of the cohort carries at least one copy of the C allele, which is linked to poor olanzapine metabolism. Assumed that impaired metabolism can lead to higher medication concentrations and a higher risk of side effects, this suggests that olanzapine may be less successful in treating schizophrenia in this population.

Similarly, rs2842030's T allele frequency was 33.0%, which is comparatively higher as compared to some other groups. This shows that a higher percentage of the cohort's members may not metabolize risperidone well. Moreover, the frequency of the TT+GT genotype is relatively high, which is 55.3%. Risperidone's reduced metabolism has been linked to this genotype, which can lead to higher drug concentrations and a high adverse effect.

When compared among them, the allele and genotype frequencies, it was observed that on the basis of pharmacogenomics, the studied cohort has less poor metabolizers of risperidone than olanzapine. This indicates that risperidone may be a better treatment option for this specific population of patients if the cohort has a lower frequency of poor metabolizers of the drug than olanzapine. Overall, the reported allele and genotype rates indicate that caution should be used when giving risperidone or olanzapine to this population since some people may be more susceptible to side effects because of poor drug metabolism.

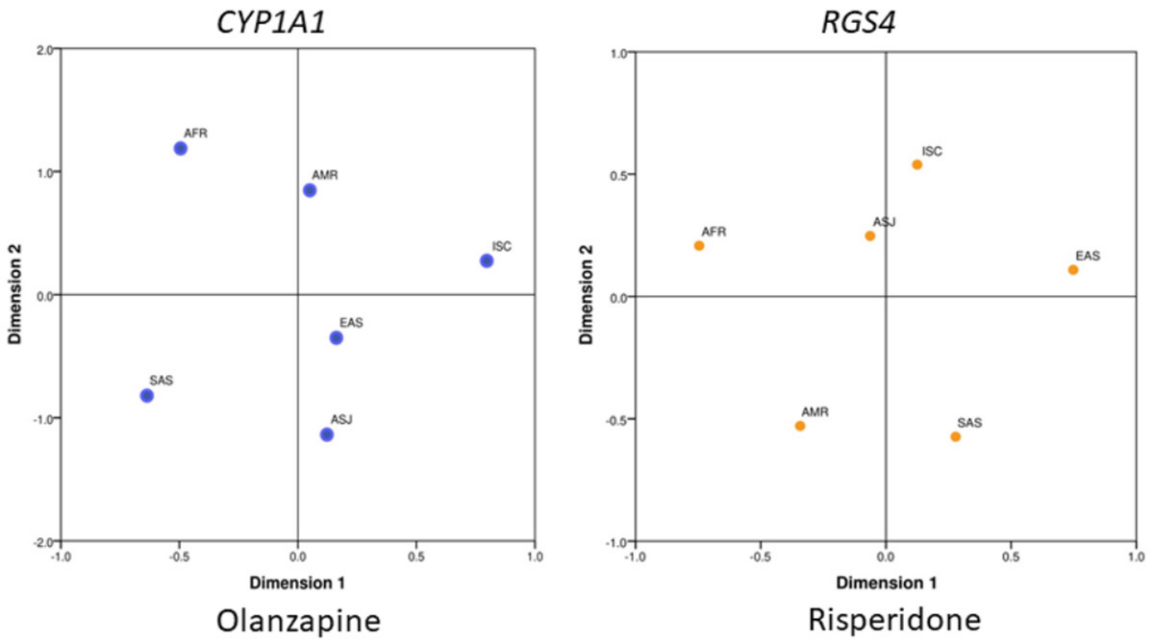

Figure 2. MDS analysis performed among global population and Indian schizophrenic cases. Plot was generated from the F_{ST} values obtained from different population groups retrieved from gnomAD (https://gnomad.broadinstitute.org/) and 94 schizophrenic cases from India. Plot was generated using 05 different super population sets and 94 samples from the present dataset as a whole set for both Olanzapine and Risperidone. AFR: Africans/African Americans; ISC: Indian Schizophrenic Cases; AMR: Latino/Admixed Americans; ASJ: Ashkenazi Jews; EAS: East Asians; SAS: South Asians.

Considering the variability observed in the allele frequencies of different super populations when compared with a studied cohort, it becomes pertinent to understand the genetic variability the ISC possesses and has been suggested by many studies conducted in Indian population groups [22, 30]. When comparing the genetic diversity of our cohort with that of other populations, F_{ST} was utilized to spot any plausible genetic variations that can account for the higher frequency of poor metabolizers in the population. Interestingly, the ISC forms a different cluster for both variants indicating that the schizophrenia cohort of India is genetically distinct from the other five populations around the world in terms of pharmacogenomics variations, according to the observed results. These findings may have a number of effects on how schizophrenia is identified, treated, and managed in India. It implies that pharmacogenomics testing might be crucial in this demographic to guarantee that patients get the best medications possible.

Additionally, it underlines the necessity of additional studies to comprehend the genetic causes of schizophrenia in India and create more specialized treatments for this group. This work may have a few limitations that need to be addressed, such as the necessity to assess more pharmacogenomics variations in the Indian schizophrenic cohort with higher sample counts. Moreover, genome-wide studies focused on the psychiatry pharmacogenomics of Indian sub-population groups may provide insight into the more effective management of psychic traits like schizophrenia.

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Disclosure of conflict of interest

None.

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Appendix 'C'to O/o DGAFMS/DG-3B
letter No. 15965/58th/9/2020/DGAFMS/
DG-3B dated Mar 2019

7 AIR FORCE HOSPITAL KANPUR
CERTIFICATE FROM INSTITUTIONAL ETHICS COMMITTEE

Name of Principal Worker: SQN LDR RAJAT GARG MD(PSY) ,
VIBHA SINGH, M.Phil (Clinical Psychology)

TOPIC: IDENTIFICATION OF POTENTIAL MARKERS ASSCOCIATED WITH SCHIZOPHRENIA AND BIPOLAR DISORDER VIA GENOME – WIDE SCREENING IN NORTH ASIAN INDIANS

1. Research design
   a) Scientifically sound enough to address the objective stated: Y/N
   b) Relevant to contribute to further knowledge: Y/N
   c) Research of Armed Forces significance Y/N
2. Participant selection
   a) Inclusion/exclusion criteria addressed appropriately: Y/N
   b) Special groups/Vulnerable persons adequately protected: Y/N
3. Risk and benefits
   a) Physical/ Social/Psychological risk/discomfort minimized/addressed Y/N
   b) Overall risk/benefit ratio: Acceptable/Un acceptable
4. Privacy and confidentiality
   a) Privacy of the research participants maintained: Y/N
   b) Confidentiality of the research participants maintained Y/N
5. Informed consent form
   a) Informed consent form component/process addressed adequately: Y/N
6. Conflict of Interest: Y/N

DECISION; APPROVED/NOT APPROVED

DECISION; UNANIMOUS/DISSENT

IF DISSENT; REASONS FOR DISSENT

COMMENTS IF ANY:

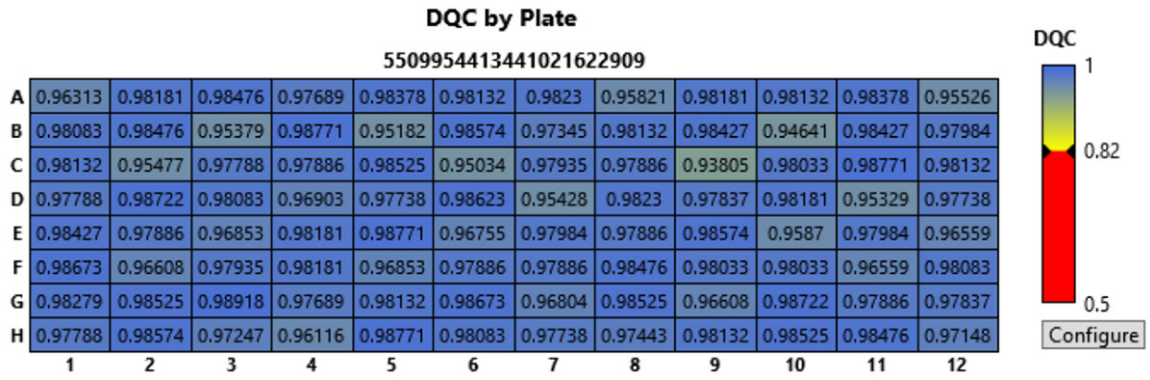
SIGNATURES OF ETHICAL COMMITTEE MEMBERS

(R Somashekhara)	(Nithya Nair)	(Sunita D Patil)	(Navin Bhati)	(Sabreen B)
Gp Capt	Wg Cdr	Gp Capt	Sqn Ldr	Sqn Ldr
SMO, AFS Chakeri	Cl Spl (Opthal)	MD (Microbiology)	MD(Medicine)	MD(Community Medicine
Chairperson	Member Secy	Basic Medical Scientist	Clinician	Scientific Member

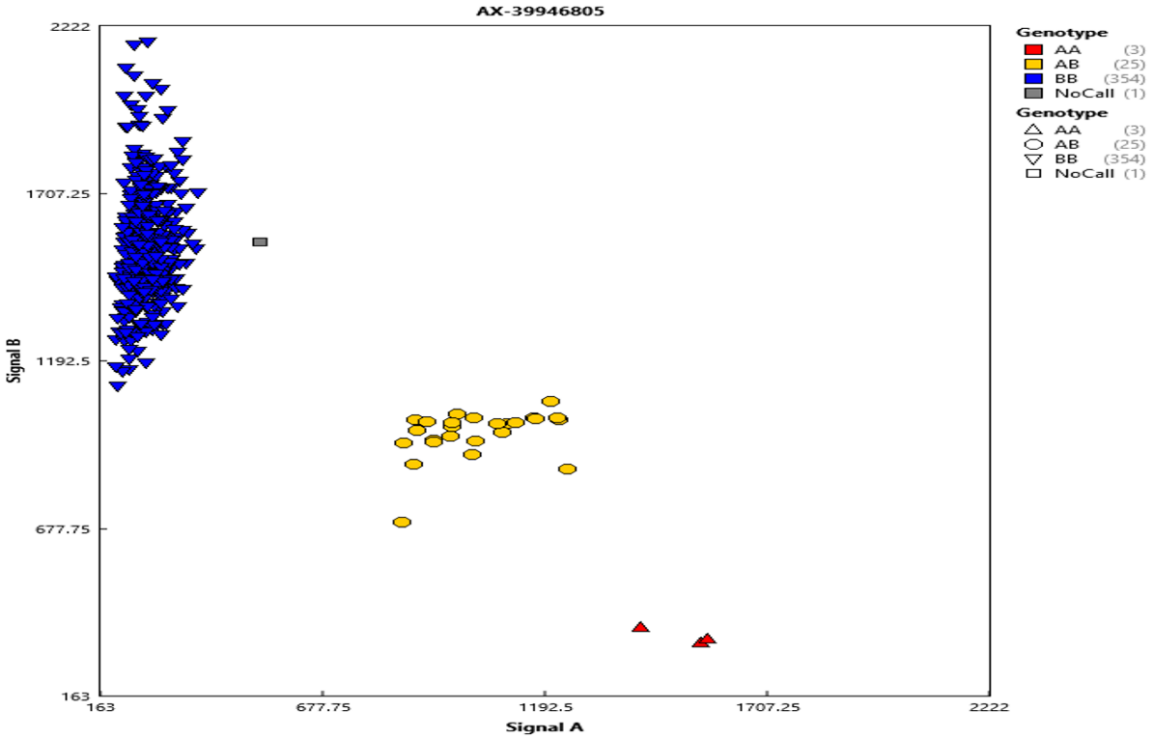
(Nishi Sengar)
LLB
Legal Expert

Supplementary File 1. Certificate from Institutional Ethics Committee.

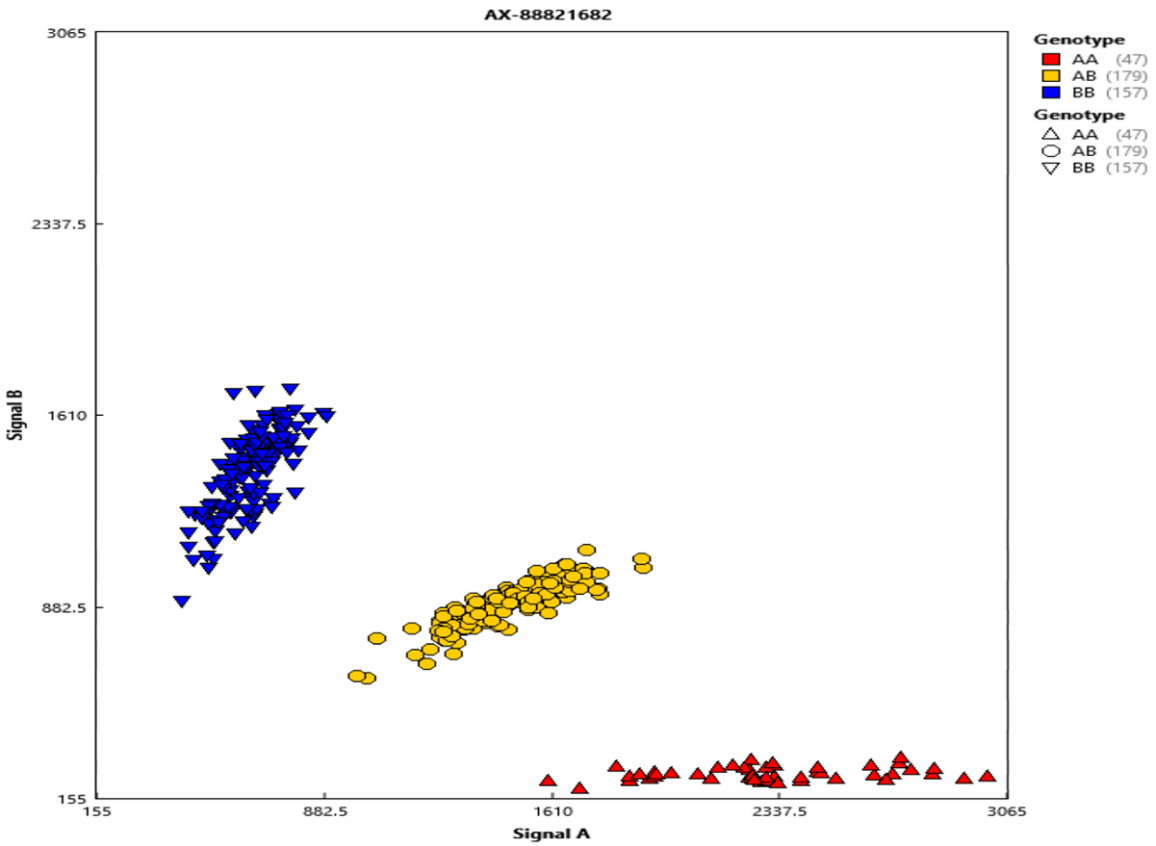
Supplementary Figure 1. 94 samples' extracted DNA from 1% Agarose gels are shown, along with a ladder (L), and two positive controls.



Supplementary Figure 2. Representative genotyping quality observed in sample positioning map derived from SNP Axiom Analysis Suite v5.1 (Affymetrix). All samples have DQC score ≥ 0.82 and call rate > 0.97. Samples that are not able to match the DQC score have been removed from the downstream analysis.



Supplementary Figure 3. Cluster plot obtained for the variant rs2472297 (AX-39946805) of *CYP1A1* gene. The cluster plot showing the clear clustering of the three clusters of genotypes indicating good clustering resolution and precision in genotyping methodology.



Supplementary Figure 4. Cluster plot obtained for the variant rs2842030 (AX-88821682) of *RGS4* gene. The cluster plot showing the clear clustering of the three clusters of genotypes indicating good clustering resolution and precision in genotyping methodology.

Supplementary Table 1. Socio demographic, anthropometric and clinical observations in studied schizophrenia cohort

Parameters		Average/Number of individuals	Frequency (in percentage)
	Age	46.55	
Gender	Male	42	44.68
	Female	52	55.32
Total		94	
Education	Graduation & Above	35	37.23
	Illiterate	11	11.70
	Intermediate	16	17.02
	Primary Marticulation	32	34.04
Total		94	
Marital Status	Married	70	74.47
	Unmarried	17	18.09
	Divorced	1	1.06
	Widow/Widower	4	4.26
	Separated	2	2.13
Total		94	
Religion	Hindu	91	96.81
	Muslim	3	3.19
Total		94	

Domicile	Urban	47	50.00
	Semi-urban	17	18.09
	Rural	30	31.91
Total		94	
	Height	163.3	
	Weight	69.3	
Total		94	
Type of Family	Broken	0	0.00
	Extended	11	11.70
	Joint	12	12.77
	Not living with family	2	2.13
	Nuclear	69	73.40
Total		94	
Occupation	Employed	23	24.47
	Ex-serviceman	5	5.32
	Unemployed	66	70.21
Total		94	
Suicidality	Present	28	29.79
	Absent	66	70.21
Total		94	
Range of Schizophrenia (Scale used - BPRS)	Mild	14	27.45
	Mildly	7	13.73
	Mildly ill	1	1.96
	Minimal	4	7.84
	Moderate	12	23.53
	Moderately ill	7	13.73
	Severe	5	9.80
	Severely ill	1	1.96
Total		51	
Duration of illness (Average in years) (N = 91)		10.52	
Age of onset (in years) (N = 92)		36.07	
Nature of onset of illness	Abrupt	2	2.13
	Acute	15	15.96
	Insidious	77	81.91
Total		94	
Course of illness	Continuous	80	86.96
	Episodic	9	9.78
	Static	3	3.26
Total		92	
Progress (as per the consultation)	Deteriorating	6	6.45
	Improving	67	72.04
	Static	20	21.51
Total		93	
Precipitating factor	Present	36	39.56
	Absent	55	60.44
Total		91	
Family history of significant psychiatric/medical illness	Present	51	60.71
	Absent	33	39.29
Total		84	