

Cytogenetics and Selected Biochemical Phenotypes in Indian Schizophrenic Patients

Meyyazhagan Arun^{1*}, Mohan Gomathi³, Iyer Mahalaxmi³, Alagamuthu Karthick Kumar², Balasubramanian Balamuralikrishnan^{2,7}, Ramesh Kumar Mithun Kumar⁴, Muruges Easwaran⁵, Nitya Raman⁶, Manivel Gowtham⁵, Shanmugam Sureshkumar^{2,7}, Kuchi Bhotla Haripriya², Pavani Elipilla⁸, Vellingiri Balachandar^{2,3}, Keshavarao Sasikala² and Ramon Cacabelos^{1*}

¹Chair of Genomic Medicine, Camilo José Cela University, Madrid; EuroEspes Biomedical Research Center, Institute of Medical Science and Genomic Medicine, Corunna, Spain

²Human Genetics Laboratory, Department of Zoology, School of Life Sciences, Bharathiar University-641046, Tamil Nadu, South India

³Department of Human Genetics and Molecular Biology, Bharathiar University-641046, Tamil Nadu, South India

⁴Department of Surgery, KMCH Hospital, Coimbatore, South India

⁵Department of Bioinformatics, Computational Biology Lab, Bharathiar University-641046, Tamil Nadu, South India

⁶Microbial Biotechnology Lab, Department of Biotechnology, Dr. G.R.D Arts and College, Coimbatore, Tamil Nadu, South India

⁷Department of Animal Resource and Science, Dankook University, Cheonan, Choongnam, South Korea

⁸Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur - 522510, Andhra Pradesh, South India

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ABSTRACT

Schizophrenia is a multifaceted and multifactorial mental disorder. The aim of the present study was to analyze the age-related features, biochemical changes, chromosomal alterations and genetic polymorphisms of schizophrenia subjects. In total, 254 subjects were taken, based on age group (Group I: <40 years, group II: ≥40 years). Random, numerical and structural aberrations, such as supernumerary markers (47, XXY, +mar, 47, XY, +ace), ploidy, deletions (6q, 21q), inversions (9p), translocations (t (5, 11) (q25, q31.2)) and duplications (Xp, 7(p15p21)), were observed in group I and group II subjects. 66 of the 127 in the subtype category of schizophrenic individuals (51.97%) showed karyotypic abnormalities. In our study, higher levels of dopamine, thyroid stimulating hormone, cortisol and blood pressure were observed in the schizophrenia subjects when compared to controls. Our results showed that three SNPs of *NRXN1* gene were significantly associated with schizophrenia ($p=0.017$, rs2024513: A > G, $p=0.006$, rs13382584: T > C, $p=0.009$, and rs1558852: G > A, $p=0.031$). Furthermore, the association of SNP rs2024513 with schizophrenia remained significant after carrying out Bonferroni correction. In conclusion, our findings suggest that these regions are of interest for identifying important genes and biomarkers involved in schizophrenia.

Keywords: Schizophrenia, Chromosome aberrations, *NRXN1*, Dopamine, TSH, Cortisol, Blood Pressure.

INTRODUCTION

Schizophrenia is a complex neurocognitive disorder, with genetic etiology estimated at >80%, mainly due to its heritability [1]. Schizophrenia has been categorized into five different types: (i) paranoid, (ii) disorganized, (iii) catatonic, (iv) undifferentiated and (v) residual. The global prevalence of schizophrenia is estimated to be 1-2%. It begins in the late teens to early 20s in males, but with a five-year delay in onset in females. The diagnostic criteria for this disease have been established by the Diagnostic and Statistical Manual of Mental Disorders, 4th edition-Text Revised (DSM IV-TR) [2], mainly stating exhibition of psychotic symptoms, but no details are available on deterioration.

Corresponding author: Ramon Cacabelos and Meyyazhagan Arun, Chair of Genomic Medicine, Camilo José Cela University, Madrid, EuroEspes Biomedical Research Center, Institute of Medical Science and Genomic Medicine, Corunna, Spain, Tel: +34-981-780505; Fax: +34-981-780511; E-mail: arun47biotech@gmail.com; rcacabelos@eurospes.com

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Several biological markers have been identified, such as cortisol [3], metabolic syndrome parameter (BP) and thyroid-stimulating hormone (TSH). Hypothyroidism and hyperthyroidism are accompanied by various neuropsychiatric manifestations, such as depression [4], anxiety [5], and psychosis [6].

The co-occurrence of psychiatric and thyroid diseases may be the result of common biochemical abnormalities [7]. The levels of neurotransmitters regulated in the brain by reuptake and metabolism affect pituitary function, leading to reduced TSH levels and an elevated level of dopamine (DA). Higher basal TSH levels may be associated with a poor treatment response in schizophrenia [8]. Neurexins (*NRXN1*) and its other members are presynaptic neuronal adhesion molecules which play an interactive role with the post-synaptic neuroligins (both glutamatergic and GABAergic synapses), leading to synaptic specification as well as well-organized neurotransmission [9,10]. Several studies indicate large deletions in neurexin 1 gene (*NRXN1*) on chromosome 2p16.3 increasing the risk for schizophrenia. Since most *NRXN1* deletions affect the cell-surface receptors in the central nervous system [11], the synaptic functions of neurexin and their proposed role in cognitive disorders were reviewed [10,12,13].

Cytogenetic analysis has identified chromosomal aberrations, asserting the importance of genetic imprinting [14,15]. Earlier studies suggest that several chromosomes, including 1q, 2p, 3p, 6p, 6q, 7q, 9q, 11q, 13q, 14q, 15q, 22q, and Xp, contain susceptible genes for schizophrenia [14,16]. In addition to these observations, cases showing the partial trisomy of 5q11-13 [17], specific translocations such as t(18,21)(p11.1,p11.1) [18], t(1,7)(p22q22) [19] and t(1,11)(q42,q14.3) [20], inversions such as inv(9)(p11q13) [21] and inv(4)(p15q21.3) [22], trisomies of 5p14.1 [23] and deletions at 22q11.1 [17,24] and 5q21-23.1 [25], and sex aneuploidies [26], have been reported in schizophrenic patients.

In the present study, screening of *NRXN1* gene polymorphisms and chromosomal analysis of peripheral blood lymphocytes were investigated in schizophrenia subjects, with an assessment of the relationship between the abnormalities found in disease subtype, gender, age at disease onset and family history. Furthermore, we also investigated the biochemical relationships resulting in co-occurrence of psychiatric diseases, by measuring the levels of DA, cortisol, TSH and BP between age-related groups with age-matched controls and subtypes of schizophrenic patients in a South Indian population.

MATERIALS AND METHODS

Subject

Patients and Controls

The study groups consisted of 254 samples with 127 schizophrenia subjects and an equal number of \pm 2yrs age-matched controls. Records were reviewed independently by two diagnostically-trained psychiatrists blind to family structure, and diagnoses were made according to DSM IV-TR criteria. Among the 127 schizophrenic cases, the subjects were categorized based on their age: group I (<40yrs) [$n=83$, males $n= 57$ (68.67%), females $n=26$ (31.33%)] and group II (\geq 40yrs) [$n=44$, males $n= 31$ (70.45%), females $n=13$ (29.55%)]. Characteristics of the patients and controls are listed in **Table 1**. Pedigree analyses were carried out to visualize the carrier within families, and to understand the mode of inheritance (paternal and maternal) of the diseases. Patients with any other known neurological disorders were excluded.

Patients were selected from Chaitanya Rehabilitation center, Cochin (Kerala), and from hospitals in Coimbatore and Chennai (Tamil Nadu) and Bangalore (Karnataka) during the 2007- 2013 period. The work was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki [27], and written informed consent was obtained from individuals. Healthy controls of similar ethnicity were selected.

Blood samples

10mL of blood were collected from each participant in 2 sterile tubes containing EDTA and Heparin by venipuncture from the antecubital vein, and a follow-up study was conducted. The tubes were analyzed for chemical and biochemical parameters by transporting at 4°C. All samples were kept at room temperature for 24 h prior to processing.

Chromosomal analysis

Cultures of leucocytes were obtained from peripheral blood samples [28] following standard procedure. 0.5 mL blood was added to 4.5 mL of RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1% streptomycin-penicillin, 0.2 mL reagent grade phytohemagglutinin (Sigma Chemicals), and incubated at 37°C. After 71 h, cultures were treated with 0.1 g/mL colcemid (Gibco Laboratory) to block mitosis. Lymphocytes were harvested after 72 h by centrifugation (800-1000 rpm). A hypotonic solution of KCl (0.075 M) was added and maintained at 37°C for 20 min to swell the cells, followed by Carnoy's fixative (methanol: acetic acid (3:1)) treatment and dried on a hot plate (56°C, 2 min). Three days later, slides were stained using the Trypsin - Giemsa technique. For the CA analysis, 100 metaphases in first cell cycle were evaluated per subject under a microscope (100X) to identify numerical and structural CA.

Mutational analysis

Whole genomic DNA was collected as per kit protocol using a frozen blood DNA extraction kit (Genei, India). Considering the report by Kirov and co-workers [10] that

microdeletions spanning the promoter and the first exon of the *NRXN1* gene might increase the risk of developing schizophrenia, three tagSNPs locating the promoter and the

first exon of this gene were selected using dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP>) [10,13].

Table 1. Demographic Characteristics of Schizophrenia Samples

S.No	Patient Details	Total No of Samples	Percentage %
1	Total no. of samples Schizophrenia subjects (n=127)*	254	100
	Group I	83	65.35%
	Group II	44	34.65%
2	Group I <40 yrs; (n=83) (Mean ± S.D)(29.95±5.36)	57	68.67%
	Males (Mean ± S.D)(30.24±5.69)	26	31.33%
	Females (Mean ± S.D)(29.42±4.63)		
3	Group II = 40 yrs (n=44) (Mean ± S.D)(36.45±9.93)	31	70.45%
	Males (Mean ± S.D)(36.90±10.31)	13	29.55%
	Females (Mean ± S.D)(35.38±9.45)		
4	Types of Schizophrenia (n=127)		
	Paranoid	45	35.43%
	Catatonic	31	24.40%
	Disorganized	23	18.11%
	Residual	19	14.97%
	Undifferentiated	9	7.09%
5	Main carrier (n= 73)		
	Paternal carrier	45	61.65%
	Maternal carrier	28	38.35%
6	Age at Onset (n=127)		
	Below 20 years	11	08.66%
	20-29 years	52	40.95%
	30-39 years	33	25.98%
	40-49 years	06	04.72%
	50-59 years	10	07.88%
	Not Specified	15	11.81%

* - equal number of samples with controls

All of the three tag SNPs were genotyped. The information from ABI Taqman assays and context sequences of three SNPs are given in **Table 8**. The standard SNP genotyping assay protocol was performed for polymerase chain reactions (PCRs), which contained 10ng of DNA, 5µL of 2×TaqMan Universal PCR Master Mix, 0.5µL of 20× SNP Genotyping Assay Mix and 4.5µL of water, forming a total volume of 10µL. The conditions of PCR amplification were as follows: 1 enzyme activation step at 95°C for 10 min, and 40 alternating cycles of denaturation at 92°C for 15s and re-annealing and extension at 60°C for 60s.

Biochemical Analysis

Estimation of DA

DA was estimated by reference methodology - HPLC with electrochemical detection [29]. HPLC was performed, fitted with Interface D-7000 IF, dual pump L-7100 and clinlab digital amperometric detector. Catecholamines kit, HPLC column, mobile phase 10L and plasma endocrine controls (normal and experimental) were also used. The HPLC

column was connected to the pump, mobile phase was set at 1 mL/min and the column outlet was attached to the cell inlet of detector - EC3000. Backpressure was set at 46 bar making the column dust-free and ideal for HPLC separation. The detector was set at: 560mV, Filter: 0.05Hz, Cell Type: Passive, Range: ±10 (nA), Offset: 0.00 (nA), Autozero: Disabled. Base current was +0.13 (nA) only, lower than the permitted 0.3 (nA) making the analysis of DA highly sensitive.

Cortisol analysis

Analysis of plasma cortisol was performed according to Petrowski et al. [30]. 50µL of low-viscosity saliva supernatant were removed using a commercially available immunoassay with chemical luminescence detection as described previously in detail by Dressendörfer et al. [31].

Thyroid-Stimulating Hormone (TSH) analysis

Blood samples were drawn after a 12 h overnight fast. TSH serum level was determined immediately. For the normal range three different criteria were used: (1) currently

accepted 0.4–5.0 IIU/mL range, (2) 0.3–3.0 IIU/mL range, recommended by The National Academy of Clinical Biochemistry (NACB) [33].
proposed by the American Association of Clinical Endocrinology [32], and (3) 0.4–2.5 IIU/mL range,

Table 2. The chromosome abnormality found in 57.83% (48/83) patients with Schizophrenia (Group I)

S. No	Case No	Sex/ Age (Experimentals)	Age at Onset	Diagnosis	Type	Family History	Carrier	Karyotype
Supernumary Markers 3/83 (3.62%)								
1	SCZ 9	26/M	21	DSM IV-TR	CTS	+	MC	47,XXY, + mar
2	SCZ 59	35/M	31	DSM IV-TR	UTS	+	MC	47,XY, + ace
3	SCZ 69	37/M	NS	DSM IV-TR	UTS	-	NA	Quadriradial figure
Ploidy 2/83 (2.40%)								
4	SCZ 82	28/M	20	DSM IV-TR	RTS	+	MC	Aneuploidy
5	SCZ 83	31/M	NS	DSM IV-TR	RTS	-	NA	Aneuploidy
Deletion 34/83 (40.96%)								
6	SCZ 41	28/M	NS	DSM IV-TR	DTS	+	PC	46,XY, (1)(q32.1)
7	SCZ 40	32/F	25	DSM IV-TR	PTS	+	MC	47,XX, + 21
8	SCZ 8	25/M	NS	DSM IV-TR	DTS	-	NA	46, XY , (2)(p24.3)
9	SCZ 2	32/M	26	DSM IV-TR	PTS	+	MC	46, XY, del(1q)(42.2)
10	SCZ 4	38/M	NS	DSM IV-TR	RTS	+	MC	46, XY, del (9)(p21.3)
11	SCZ 5	31/M	30	DSM IV-TR	PTS	+	PC	46, XY, del(13q)(34)
12	SCZ 12	23/M	NS	DSM IV-TR	PTS	-	NA	46, XY, del(6p)(23)
13	SCZ 13	30/F	25	DSM IV-TR	RTS	+	PC	46, XX, del (9)(p31.2)
14	SCZ 15	37/M	31	DSM IV-TR	PTS	+	PC	46, XY, del(6q)(15)
15	SCZ 20	31/M	30	DSM IV-TR	PTS	+	PC	46,XY, del(22)(q11)
16	SCZ 25	35/F	30	DSM IV-TR	PTS	+	PC	46, XX, del(13q)(34)
17	SCZ 29	30/M	19	DSM IV-TR	RTS	-	NA	46, XY, del (9)(p21.3)
18	SCZ 31	28/M	NS	DSM IV-TR	CTS	+	PC	46,XY, del(7)(q32)
19	SCZ 32	36/F	31	DSM IV-TR	DTS	+	MC	46,XX, del(10)(p13)
20	SCZ 33	38/M	32	DSM IV-TR	PTS	+	PC	46,XY, del(7)(q32)
21	SCZ 35	29/M	21	DSM IV-TR	CTS	+	PC	46,XY, del(22)(q11)
22	SCZ 39	38/M	31	DSM IV-TR	DTS	-	NA	46,XY, del(22)(q11)
23	SCZ 43	33/M	24	DSM IV-TR	PTS	+	PC	46,XY, del(7)(q32)
24	SCZ 44	36/F	31	DSM IV-TR	RTS	-	NA	46, XX, del (9)(p31.2)
25	SCZ 45	39/M	32	DSM IV-TR	PTS	-	NA	46, XY, del(13q)(34)
26	SCZ 47	31/M	21	DSM IV-TR	CTS	-	NA	46, XY, del(6p)(23)
27	SCZ 49	24/M	21	DSM IV-TR	PTS	-	NA	46,XY, del(11)(q23)
28	SCZ 54	33/F	31	DSM IV-TR	DTS	-	NA	46,XX, del(15)(q26.1)
29	SCZ 56	37/M	33	DSM IV-TR	PTS	+	MC	46,XY, del(7)(q32)
30	SCZ 58	27/M	NS	DSM IV-TR	CTS	+	MC	46,XY, del(6)(p22.3)
31	SCZ 60	37/F	33	DSM IV-TR	PTS	-	NA	46,XX, del(7)(q32)
32	SCZ 62	22/M	20	DSM IV-TR	CTS	+	PC	46,XY, del(15)(q15)
33	SCZ 66	35/F	30	DSM IV-TR	PTS	-	NA	46,XX, del(7)(q31.1)
34	SCZ 70	31/F	22	DSM IV-TR	PTS	+	PC	46,XX, del(21)(q22)
35	SCZ 72	23/M	18	DSM IV-TR	UTS	-	NA	46, XY del(16)(q)
36	SCZ 74	28/M	21	DSM IV-TR	DTS	-	NA	46,XY, del (6)(q)
37	SCZ 77	32/M	23	DSM IV-TR	CTS	-	NA	46,XY, del(6)(p22.3)
38	SCZ 78	38/M	31	DSM IV-TR	PTS	+	PC	46,XY, del(1)(q21)
39	SCZ 79	32/M	23	DSM IV-TR	DTS	-	NA	46,XY, del(7)(q31.1)
Duplication 3/83 (3.62%)								
40	SCZ 16	35/M	32	DSM IV-TR	DTS	-	NA	46, XY, dup(7)(q31.1)
41	SCZ 22	22/M	17	DSM IV-TR	PTS	+	PC	46,XY,

42	SCZ 76	36/M	31	DSM IV-TR	RTS	+	PC	dup(X)(p22.1p11.2) 46,XY,dup(4q)
Translocation 3/83 (3.62%)								
43	SCZ 21	38/M	32	DSM IV-TR	RTS	+	PC	46,XY, der(3)t(1;3)(p11q25)
44	SCZ 24	33/M	NS	DSM IV-TR	CTS	+	MC	46,XY, t(8p22-8p21)
45	SCZ 63	22/M	21	DSM IV-TR	CTS	-	NA	46,XY, t(5;15)
Inversion 3/83 (3.62%)								
46	SCZ 10	35/F	31	DSM IV-TR	PTS	+	MC	46, XX, inv(9)(p12q13)
47	SCZ 17	38/M	NS	DSM IV-TR	RTS	+	PC	46,XY,inv (9) (p11q13)
48	SCZ 26	32/M	23	DSM IV-TR	RTS	+	MC	46, XY, inv(9)(p12q13)

Statistical Analysis

All statistical analyses were performed using SPSS software (version 17) to assess the group statistics for subjects and controls as mean \pm SD. For statistical data inference, the t test for independent variables and ANOVA was used to compare mean values of the quantitative variables. The comparison and deviation of genotype frequencies from the expected were examined by the chi-square test. Multiple regression analysis was performed to determine the correlation of continuous variables, by using them as dependent variables, with several independent variables. A significance level of 0.05 was adopted, all the levels were compared and analyzed for statistical difference. Analysis of variance was carried out to find the significance.

RESULT

Group comparisons of patient demographics and clinical profiles

The demographic characteristics of the subjects and control group, along with sex ratio of 1:8 males to females, are shown in **Table 1**. The research population consisted of 254 schizophrenic subjects. The patients were divided into the following: paranoid, 45 (35.43%), catatonic, 31 (24.40%), disorganized, 23 (18.11%), residual, 19 (14.97%), undifferentiated, 9 (7.09%). The mean \pm SD age of onset in group I was (24.22 \pm 5.09) and group II was (38.35 \pm 12.18). The mean age of group I control and subjects \pm SD was [(30.25 \pm 5.49), (29.98 \pm 5.36)], for males [(30.56 \pm 5.80), (30.24 \pm 5.69)] and for females [(29.57 \pm 4.78), (29.42 \pm 4.63)] respectively. Similarly, the mean age of the group II

controls and subjects was [(56.38 \pm 10.07), (56.45 \pm 9.98)], for males [(56.90 \pm 10.37), (56.90 \pm 10.31)], and for females [(55.15 \pm 9.61), (55.38 \pm 9.45)] respectively.

In a pedigree analysis of 127 schizophrenic subjects only 73 main carriers were identified, in which 45 (61.65%) were the paternal carrier and 28 (38.35%) were the maternal carrier.

Karyotyping results

The detailed chromosomal abnormality (CA), age, sex, age at onset, group, diagnosis, type, family history, and carrier for individual samples were analyzed and presented in **supp.1. Tables 2 & 3** depict the karyotypic results for group I and group II schizophrenic subjects.

Chromosome abnormalities were found in 57.83 % of group I subjects. The aberrations were: 3/83 [3.62%] supernumerary markers from chromosome 47, XXY,+mar, 47, XY,+ace, quadriradial figure, 2/83 [2.40%] ploidy type of aberrations, 34/83 [40.96%] deletions of 1q32.1, 1q42.2, 9p21.3, 13q, 6p, 9p31.2, 6q, 22q, 13q, 7q, 10p, 11q, 15q, 16p, 2p24.3, 3/83 [3.62%] duplication of 7q, Xp, 4q, 3/83 [3.62%] translocations of two balanced der(3)t(1,3)(p11.2,q25), t(5,11)(q25,q31.2), t(5,15), t(8p22-8p21), 3/83 [3.62%] inversions of 9p. Similarly, chromosome abnormality was found in group II 18/44 (40.90%). The subject aberrations were: 3/44 [6.81%] supernumerary markers from chromosome quadriradial figure, 13(p+) and 13(ps+), 11/44 [25%] deletions of 21q, 6p, 9p, 9q, 16p, 1/44 [2.27%] duplication 7(p15p21), 1/44 [2.27%] translocations of two balanced translocation t(5,15), 2/44 [4.54%] and inversions of 9p.

Table 3. The chromosome abnormality found in 40.90% (18/44) patients with Schizophrenia (Group II)

S. No	Case No	Sex/ Age (Experimentals)	Age at Onset	Group Diagnosis	Type	Family History	Carrier	Karyotype
Supernumary markers 3/44 (6.81%)								
1	SCZ 92	60/F	52	DSM IV-TR	CTS	+	MC	46,XX, (13)(p+)
2	SCZ 120	62/F	52	DSM IV-TR	DTS	+	PC	46,XX, (13)ps +

3	SCZ 106	71/M	55	DSM IV-TR	UTS	+	PC	Quadriradial figure
Deletion 11/44 (25%)								
4	SCZ 87	50/M	42	DSM IV-TR	PTS	+	PC	46,XY, del(21)(q22)
5	SCZ 91	57/M	43	DSM IV-TR	DTS	-	NA	46,XY, del(21)(q22)
6	SCZ 98	67/M	23	DSM IV-TR	CTS	+	PC	46,XY, del(6)(p22.3)
7	SCZ 99	63/M	NS	DSM IV-TR	PTS	+	PC	46,XY, del(21)(q22)
8	SCZ 105	64/F	32	DSM IV-TR	PTS	+	MC	46,XX, del(21)(q22)
9	SCZ 107	74/M	NS	DSM IV-TR	PTS	+	MC	46,XY, del(21)(q22)
10	SCZ 111	67/M	59	DSM IV-TR	UTS	-	NA	46, XY del(16)(q)
11	SCZ 96	71/M	54	DSM IV-TR	CTS	-	NA	46,XY, del(9)(q13)
12	SCZ 117	59/M	44	DSM IV-TR	RTS	+	PC	46, XY, del(9)(p31.2)
13	SCZ 127	69/M	56	DSM IV-TR	PTS	+	PC	46,XY, del(21)(q22)
14	SCZ 124	63/M	55	DSM IV-TR	UTS	+	MC	46, XY del(16)(q)
Duplication 1/44 (2.27%)								
15	SCZ 113	65/F	61	DSM IV-TR	CTS	-	NA	46,XX, dup(7)(p15p21)
Translocation 1/44 (2.27%)								
16	SCZ 126	71/F	NS	DSM IV-TR	DTS	+	PC	46,XX, t(5;15)
Inversion 2/44 (4.54%)								
17	SCZ 94	71/M	NS	DSM IV-TR	PTS	+	MC	46, XY, inv(9)(p12q13)
18	SCZ 97	66/M	46	DSM IV-TR	PTS	+	MC	46, XY, inv(9)(p12q13)

S.No-Serial number; M-male; F-female; NS- not specified; DSM IV-TR – Diagnostic Statistical Manual For Mental Disorders – Text Revised; PTS-Paranoid type schizophrenia; CTS-Catatonic type schizophrenia; DTS-Disorganised type schizophrenia; RTS-Residual type schizophrenia; UTS-Unorganised type schizophrenia; + - Positive family history; - - negative family history; PC-paternal carrier; MC-maternal carrier

66 of the 127 subtype category of schizophrenic individuals (51.97%) displayed karyotypic abnormalities (Table 4). Paranoid type of schizophrenia subjects ($n=25$) showed chromosomal abnormality (37.87%), aberrations were 47,XX, +21, 46, XY, del1q, 6q, 13q, 6p, 22q, 7q, 11q, 7q, 21q, duplication in Xp22 and inversion in 9p. Catatonic type of schizophrenia subjects ($n=13$) showed chromosomal

abnormality (19.70%), where aberrations were +21, 13p+, del 22q,7q,6p,15q,6q,9q, dup 7, translocation (8p22-8p21), t(5,15). In disorganized type ($n=11$), the chromosomal abnormality of 16.66% included deletion, duplication and supernumerary markers. In residual type of schizophrenics ($n=11$) the chromosomal abnormality (37.87%) included aberrations such as ploidy, deletion duplication and

inversion 9 and 11.6. Undifferentiated types of subjects showed chromosomal abnormality of 9.10% with supernumerary markers and deletion aberrations.

Biochemical analysis

Dopamine

The individual plasma DA values obtained from group I and group II schizophrenia and control subjects are presented in supplement 2. The mean \pm SD plasma DA levels in both group I (29.95 ± 2.61) and group II (31.06 ± 2.78) were higher than the control groups (Table 5). In the subtypes, the undifferentiated type (35.57 ± 1.81) and paranoid type of schizophrenia (31.53 ± 4.49) showed higher percentages when compared to other subtypes (Table 6).

Table 4. The chromosome abnormality, characteristic features found in subtype category of schizophrenia subjects

S.No	Particulars	SubTypes				
		Paranoid Schizophrenia	Catatonic Schizophrenia	Disorganized Schizophrenia	Residual Schizophrenia	Undifferentiated schizophrenia
1	Characteristic Features	Delusions, hallucinations, grandiose ideas/behaviour, feeling harassed Suspicious towards others, suicidal behavior.	Extremely withdrawn, catatonic stupor, catatonic excitement, echolalia, echopraxia, Extreme negativism, mutism	Disorganized speech, disorganized thought, disorganized behaviour, disorganized emotions, poor in daily activities	psychomotor slowing, underactivity, blunting of affect, avolition, alolia poor self-care	Delusions, hallucination, disorganized speech, disorganized thought, avolition, alolia
2	Total number of affected subjects (chromosomal abnormalities affected individuals)	45 (25)	31 (13)	23 (11)	19 (11)	9 (6)
3	Karyotype Aberrations	47,XX, + 21 46, XY, del(1)(q21) 46, XY, del(1q)(42.2) 46, XY, del(6q)(15) 46, XX, del(13q)(34) 46, XY, del(6p)(23) 46, XY, del(22)(q11) 46, XY, del(7)(q32) 46, XY, del(11)(q23) 46, XY, del(7)(q31.1) 46, XY, del(21)(q22) 46, XY, dup(X)(p22.1p11.2) 46, XY, inv(9)(p12q13)	47,XXY, + mar 46,XX, (13)(p+) 46,XY, del(22)(q11) 46,XY, del(7)(q32) 46, XY, del(6p)(23) 46, XY, del(6)(p22.3) 46, XY, del(15)(q15) 46, XX, (6)(q) 46,XY, del(9)(q13) 46,XY, t(8p22-8p21) 46,XX, t(5;15) 46,XX, dup(7)(p15p21)	46,XY, del(22)(q11) 46, XY, del(15)(q26.1) 46,XY, del(7)(q31.1) 46,XY, dup(7)(q31.1) 46,XX, (13)ps+	Aneuploidy 46, XX, del(9)(p21.3) 46, XX, del(9)(p31.2) 46,XY,dup(4q) 46,XY, der(3)t(1;3)(p11q25) 46, XY, inv(9)(p11q13) 46, XY, inv(9)(p12q13)	47,XY, + ace Quadriradial figure 46,XY, (1)(q32.1) 46, XX, (2)(p24.3) 46, XY, del(16)(q) 46,XY, del(21)(q22)

Cortisol

Table 5 presents group I and group II schizophrenic subjects compared with controls. Group I (6.67 ± 1.80) showed increased levels of cortisol when compared to group I controls (6.14 ± 0.17) and similar results were also observed in group II. The subtypes of schizophrenia indicated

increased levels of cortisol in undifferentiated (8.73 ± 0.06), paranoid (8.48 ± 0.05) and disorganized types of schizophrenia (8.06 ± 0.06). However, catatonic (4.74 ± 0.04) and residual types of schizophrenia (4.56 ± 0.09) showed lower levels (Table 6).

Thyroid Stimulating Hormone (TSH)

Table 5 illustrates reduced levels of mean \pm SD levels of TSH in group I schizophrenia subjects (1.26 ± 0.86) as compared to their controls (1.28 ± 0.61). Group II schizophrenic subjects (1.62 ± 1.61) showed increased level of TSH when compared with group II controls (1.46 ± 0.66) and group I schizophrenic subjects. In the subtypes of schizophrenia, undifferentiated type of schizophrenia (3.72 ± 1.22) showed two times higher values than the other groups (**Table 6**).

Table 5. Biochemical analysis (neurotransmitters, catecholamine, hormone & metabolic testing) Mean \pm SD in Group I and controls; Group II and controls of Schizophrenia subjects.

S. No	Biochemical Parameters	Group	Mean \pm SD		Min.		Max.	
			Group I	Group II	Group I	Group II	Group I	Group II
1	Neurotransmitters Dopamine (ng/L)	Schizophrenia	29.95 \pm 2.61	31.06 \pm 2.78	26	28	37	38
		Controls	21.61 \pm 5.36	20.59 \pm 4.18	14	14	30	31
2	Cortisol (μ U/mL)	Schizophrenia	6.67 \pm 1.80	7.35 \pm 1.64	4.49	4.49	8.85	8.75
		Controls	6.14 \pm 0.17	6.20 \pm 0.17	5.57	5.57	6.38	6.38
3	Hormones Thyroid Stimulating Hormone (TSH) (μ U/mL)	Schizophrenia	1.26 \pm 0.86	1.62 \pm 1.61	0.2	0.2	3.6	4.8
		Controls	1.28 \pm 0.61	1.46 \pm 0.66	0.4	0.5	2.5	2.5
4	Metabolic Testing Blood Pressure (mmHg)	Schizophrenia	123.40 \pm 6.98/ 82.66 \pm 3.22	124.36 \pm 5.31/ 81.93 \pm 3.73	112/74	111/73	135/78	134.84
		Controls	120.40 \pm 3.60/ 79.03 \pm 4.69	119.70 \pm 4.16/ 79.06 \pm 3.91	112/79	111/74	128/81	125.86

Group I (<40yrs); Group II (\geq 40yrs)

Table 6. Biochemical analysis (neurotransmitters, catecholamine, hormone & metabolic testing) Mean \pm SD in subtypes of Schizophrenia subjects.

S. No	Biochemical Parameters	Sub-Types (Mean \pm SD)				
		Paranoid Schizophrenia	Catatonic Schizophrenia	Disorganized Schizophrenia	Residual Schizophrenia	Undifferentiated schizophrenia
1	Neurotransmitters Dopamine (ng/L)	31.53 \pm 4.49	27.74 \pm 1.84	29.73 \pm 1.35	28.63 \pm 1.53	35.57 \pm 1.81
2	Cortisol (μ U/mL)	8.48 \pm 0.05	4.74 \pm 0.04	8.06 \pm 0.06	4.56 \pm 0.09	8.73 \pm 0.06
3	Hormones Thyroid Stimulating Hormone (TSH) (μ U/mL)	1.74 \pm 0.67	0.71 \pm 0.37	1.35 \pm 0.58	0.46 \pm 0.21	3.72 \pm 1.22
4	Metabolic Testing Blood Pressure (mmHg)	127.93 \pm 3.16/ 80.22 \pm 16.14	123.83 \pm 1.82/ 83.39 \pm 2.28	121.82 \pm 11.05/ 81.43 \pm 2.18	117.77 \pm 2.39/ 80.10 \pm 2.94	120.37 \pm 6.84/ 80.5 \pm 4.07

Group I (<40yrs); Group II (\geq 40yrs)

Statistical analysis

t test analysis

The analysis by t-test among the biochemical parameters such as dopamine, cortisol, thyroid stimulating hormone and blood pressure for the two groups of interest in this study showed significant values among the schizophrenic subjects when compared to the controls. Significant p-value summary was obtained for all parameters except for group I schizophrenic subjects analyzed for thyroid-stimulating hormone. The analysis revealed $p=0.0032$ for dopamine in group I, $p=0.034$ for cortisol in group I and $p=0.023$ for blood pressure observations. Among group II subjects, a statistical significance of $p=0.0045$ for dopamine, $p=0.014$ for cortisol, $p=0.023$ for thyroid stimulating hormone and $p=0.043$ for blood pressure was recorded.

Statistical t-test analysis however confirmed a significance of $p=0.003$, $p=0.002$, $p=0.0043$ and $p=0.02$ for dopamine,

cortisol, thyroid-stimulating hormone and blood pressure respectively among the subtypes of schizophrenia subjects.

Regression Analysis

Statistical significance was determined for the dependent and independent variables, in which the schizophrenic subjects showed significant values in TSH levels when compared to the other groups (**Table 7**). Regression analysis showed TSH of group II with Standardized $\beta = 0.058$ and $p=0.710$, having a significant effect. Cortisol group II showed standardized $\beta = 0.687$ and $p=0.00$, DA group II showed standardized $\beta = 0.579$, TSH group I showed standardized $\beta = -0.081$ and $p=0.467$, cortisol group I showed standardized $\beta = 0.580$ and $p=0.00$ and DA group I showed standardized $\beta = 0.332$ and $p=0.02$, inferring a slight influence on the TSH levels in group II subjects.

Table 7. Regression analysis of Group I and Group II Schizophrenia patients and controls

Variables	β	SE	t	P
TSH –Group I	-0.081	0.883	0.467	0.457
Cortisol – Group I	0.580	1.591	6.408	0.00
Dopamine – Group I	0.332	3.985	3.168	0.02
TSH –Group II	0.058	1.193	0.374	0.690
Cortisol – Group II	0.687	1.455	6.135	0.00
Dopamine – Group II	0.579	4.533	4.549	0.00

β - Beta Coefficient; SE – Standard Error; t – Table value; P – Probability; TSH – Thyroid Stimulating Hormone

Table 8. Information of TaqMan assays and context sequences (*DISCI*) of 3 SNPs examined

ID	SNPs	Position	Location	Context Sequence
1	rs2024513	51005523	intron	GAAGTGTTCCTTCTTAGATACATGA[A/G]GTCTTGGTAACCTTAATGGCTATTT
2	rs13382584	51100798	intron	TCTTTACAAATGTAACCACCACCCA[C/T]ATCATGCCCAATGCTCCATTGTTTT
3	rs1558852	51105641	intron	TTAATACATGATCTGTATTGGGAGA[A/G]TCATCCATTCTCAATTAATTATTCA

Mutation Analysis

Three SNPs in the *NRXN1* gene were genotyped in 127 schizophrenia patients and an equal number of controls. The size of our sample was sufficient to detect a significant difference with a power of more than 90%, assuming an odds ratio (OR) value of AA as 1.7 with a minor allele

frequency of 0.1. The genotype distributions of the three SNPs for patients and controls were in Hardy-Weinberg equilibrium (**Table 9**). Significant differences were found in allele frequencies between patients and controls at three SNPs- rs2024513 (A > G, $p = 0.004$), rs13382584 (T > C, p

= 0.007), and rs1558852 (G > A, p = 0.027). However, the difference in the allele frequencies of rs2024513 alone remained significant after Bonferroni correction. Logistic regression was used to identify whether age influenced the marker-diagnosis effects. However, the age of subjects did

not show any significant confounding effects on marker diagnosis association. Furthermore, no association was found between *NRXNI* polymorphisms and early-onset schizophrenia.

Table 9. Comparison of genotype and allele frequencies of three SNPs at the *DISC1* gene between schizophrenic patients and healthy control subject

Makers	Genotype N (Freq.)			Chi-square (df = 2)	p-value	HWEP	Allele N (Freq.)		Chi-square (df = 1)	p-value	OR (95% CI)
	AA	AG	GG				A	G			
rs2024513 Subjects	AA 91 (0.716)	AG 27 (0.212)	GG 9 (0.070)	7.232	0.021	0.717	A 207 (0.814)	G 47 (0.186)	5.543	0.004	1.17 (1.03-1.42)
Controls	85 (0.669)	37 (0.292)	5 (0.039)				0.638	205 (0.807)			
rs13382584 Subjects	CC 3 (0.023)	CT 33 (0.259)	TT 91 (0.716)	6.984	0.027	0.398	T 37 (0.145)	C 217 (0.855)	6.245	0.007	0.63 (0.61-0.87)
Controls	4 (0.032)	40 (0.314)	81 (0.654)				0.578	41 (0.161)			
rs1558852 Subjects	AA 39 (0.307)	AG 63 (0.496)	GG 25 (0.194)	4.105	0.094	0.687	A 140 (0.551)	G 114 (0.449)	4.361	0.027	0.73 (0.71-0.93)
Controls	45 (0.354)	61 (0.480)	21 (0.165)				0.894	150 (0.591)			

Freq. : frequency; HWEP: the p-value for Hardy-Weinberg equilibrium tests; OR, odds ratio; CI, confidence interval

DISCUSSION

Schizophrenia is a seriously debilitating condition that features high on the list of leading causes of lifetime disability. Pathogenesis of schizophrenia has been an ongoing study due to the involvement of genetic factors. To the best of our knowledge, this is the first study for the evaluation of chromosomal alterations and *NRXNI* gene polymorphisms compared with biochemical parameters in Indian schizophrenic patients.

Resurgence of interest in gender differences in schizophrenia is a recent research effort aimed at understanding heterogeneity in schizophrenia. Specifically, it is well known that schizophrenia is highly variable in its clinical presentation, and it is possible to identify the distinct etiological subtypes which would likely have a major impact on researchers' understanding of schizophrenia [34]. This is

perhaps due to the fact that the most robust gender difference in schizophrenia is its earlier age of onset in men compared with women [35]. The present study shows the age distribution of the schizophrenic population and age at onset in **table 1**, similar to the study of Faraone et al. [36] and Szymanski et al. [37].

Some of the chromosome banding which revealed imbalances in earlier studies are 1q21.1, 2p24.3, 3q29, 6p, 7q, 9p21.3, 10p, 11q, 13q, 15q11.2, 16p11.2, 22q and Xp. There are other studies which have also implicated the chromosomal region 11q21-22 as containing genes that increase the liability for schizophrenia. Thus, some balanced translocations at q14.3, q21, q22.3 and q25 sites of chromosome 11 were found in schizophrenia and other psychiatric disorders [38]. Furthermore, our study suggests that different chromosomal regions meet the arbitrarily defined relevance criteria as promising susceptibility genes

for schizophrenia, such as deletions at 1q, 7q, 11q and 22q [17,24,39-42], and sex chromosomes such as 47, XXY,+mar, 47,XY,+ace. Interestingly, some similar reports were observed by Toyota et al. [21] and two types of clonal chromosomal aberration: inv (9)(p11q13) and X chromosome aneuploidy is also displayed in group I subjects, according to Toyato et al. [21] and Demirhan et al. [39].

Additionally, plasma DA levels in both group I and group II subjects were higher than in the control groups. Based on earlier studies, analysis of plasma DA in patients showed a significant decrease ($p < 0.01$), which is in agreement with the findings of Kelly & Cooper [43]. However, some workers have also reported no changes in DA [44], which is in agreement with the findings of Yoshida et al. [45]. Among the undifferentiated type of schizophrenic, paranoid type subjects showed higher levels when compared to other subtypes of schizophrenia.

Here, the relationship between hypertension in schizophrenic subjects and metabolic syndrome was observed. Schizophrenic group I & II subjects show higher BP values ($\geq 130/85$ mm Hg) than in control groups, with mean values of Systolic Blood Pressure (SBP) (124.36 ± 5.31) as well as Diastolic Blood Pressure (DBP) (81.93 ± 3.73). The paranoid type of schizophrenia also showed a higher level ($127.93 \pm 3.16/80.22 \pm 16.14$), although a statistically significant difference was observed [46,47].

The comparison of TSH levels among the age groups of the subjects showed significant differences. In this study the samples revealed that the mean serum TSH levels were higher in patients with undifferentiated type of schizophrenia and lower in group I subjects and controls. This may also be explained by the fact that our study sample was not population-based and reflects associations in thyroid dysfunctions. There are reports which suggest that low rates of TSH are observed in schizophrenia patients [48], but in our study we observed a high TSH rate (6.2%).

Ritsner and co-workers [49] reported that serum cortisol ratios in schizophrenic patients were higher than healthy controls. They associated elevated serum cortisol in schizophrenic patients with anxiety, anger, and hostility. In the current study, Groups I and II showed increased levels of cortisol when compared to respective control units, whereas in the subtypes of schizophrenia, an increased level of cortisol was observed in undifferentiated, paranoid and disorganized types of schizophrenia, whereas catatonic and residual types of schizophrenia showed lower levels of cortisol. It has been reported in some studies that elevated serum cortisol is directly proportional to disease duration in patients with schizophrenia and depression [49]. The significant differences between schizophrenic patients, their first-degree relatives and controls, in terms of serum cortisol ratios analyzed in the existing study indicate no significant

differences between patients with different types of schizophrenia in terms of cortisol ratios.

NRXN1 and its other members are synaptic neuronal adhesion molecules [9,10]. The deletions, along with any kind of point mutations in the neurexin1 (*NRXN1*) genes, are coupled with an increased risk of schizophrenia [50]. Thus, in our study we investigated the association of *NRXN1* polymorphisms in schizophrenia and healthy control subjects (**Table 9**). Significant differences were found in allele frequencies between patients and controls at three SNPs (rs2024513, rs13382584, rs1558852). However, only the difference in the allele frequencies of rs2024513 remained significant after the stringent Bonferroni multiple-test. Several previous studies have suggested that the *NRXN1* gene might be the susceptibility gene for schizophrenia and other neuro-developmental disorders with rare polymorphisms [13,51]. Thus, schizophrenia has been considered to be genetically complicated, as many susceptibility genes, different genetic markers (SNPs and chromosomal alterations), diversity of genotype and allele distributions may be hypothesized to be allied with this disease.

Thus, the genetic conclusions related with the neurological as well as personality changes allied with the phenotypes will help discover the role of more applicable genes in the pathogenesis of schizophrenia [52].

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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