Investigation of genetic association between PRODH gene and schizophrenia in Iranian population

Saeid Rahman zadeh[1], Hamid Shahbaz Mohammadi[2], Morteza karimipour[3], Saeed Heidari keshel[4,5] , Eskandar Omidinia [2, *]

[1]Department of biology , Faculty of sciences , Islamic azad university, Science & Research campus , Tehran , IRAN
[2]Department of Biochemistry Pasteur Institute of IRAN.
[3]Molecular Medicine Group, Biotechnology research Centre, Pasteur Institute of IRAN, Tehran, IRAN.
[4]proteomics research center, Shahid Beheshti University of Medical Sciences, Tehran, IRAN.
[5]Tissue engineering department, school of advanced medical technology, Tehran University of medical sciences, Tehran, IRAN.

*Corresponding Author: email address: skandar@pasteur.ac.ir (E. Omidnia)

ABSTRACT

Schizophrenia is a complicated, debilitative mental disorder. Evidence is emerging for the association of polymorphisms in PRODH gene and increased risk of schizophrenia. In the present research, we investigated relationship between of this gene and schizophrenia disease by means of a gene polymorphism using PCR-RFLP technique.150 persons suffering from acute Schizophrenia and 160 healthy persons volunteering for this project were bled. . Based on intended SNP, pair of primers was designed by *Oligo7* program and polymerase chain reaction (PCR) was performed by thermo cycler. Then the resulted reactive mixture was exposed to a special enzyme, which we had intended for our study. Finally, the fragments of enzyme cut were transferred on the gel (4%) and migration pattern of resulted components were compared in healthy and patient subjects , whereby obtaining genotypes of different persons in polymorphic position. We utilized SPSS 16.0 program for statistical investigation of the work and studied SNP 1945T>C and its relation with the disease in statistical population. Our findings showed a meaningful relation between the occurrence of this nucleotide mutation and its frequency in patients (given *P* value=0.00). Results of this work indicate that PRODH gene can be considered to be a significant candidate in our population as a factor influencing the occurrence of Schizophrenia.

Keyword: Schizophrenia, PRODH, Proline, Hyperprolinemia

INTRUDUCTION

Schizophrenia is a powerful, very severe mental disease with prevalence of approximately 1% worldwide[1, 4]. This disease generally occurs in late youth and early maturity, followed by psychological symptoms which result in a hard, painful life for both patient and his/her family. According to report of WHO concerning the losses inflicted by diseases on developed countries, Schizophrenia is ranked 5th among all existing diseases in terms of the years in person's life debilitated by diseases [12]. This is because of high mortality (about 1-14%) among the people suffering this disease who commit suicide[5]. The most important feature of this kind of diseases is change of formation and content of thought and destruction of psychological functions, which in turn results in abnormalities in social life of such persons. The reports of brain imaging show that this disease is the result of disorder in brain structure and function in the left temporal loop. On the other hand, investigation of one-ovum twins indicates that Schizophrenia is genetic based disease. Studies conducted on heredity of Schizophrenia denote that this disease is a very complex genetic disorder that may be affected by several genes or, in other words, has a multigenetic pattern. hereditability of this disease has been estimated to be about 82-84% [15]. According to meta analyses of scan genome and gene linkage analyses[11] , there are highly

susceptible genes on chromosomes 1q, 3p, 5q, 6p, 8p, 11q, 14p, 20q and 22q contributing to Schizophrenia. [6,14]. One of the most important genes contributing to the disease is PRODH Gene which is located on chromosome 22q11. The expression of this gene is brain is high. It codes a protein named Proline Dehydrogenase (Oxidase) which catalyzes the first step of proline breakup. Proline oxidase is localized within the mitochondria where it catalyses the conversion of Prolin to D-1-pyroline-5-carboxylate(P5C). P5C then converts to glutamate or γ-aminobutric acid, two neurotransmitters critically implicated in the pathophysiology of schizophrenia[17] . A defect in this gene may naturally affect this process. It has been reported that some mutations in this gene may cause a decrease in its expression and consequently an increase in proline of blood plasma, resulting in hyperprolinemia typeI . Such people have been seen to be highly susceptible to Schizophrenia. On the other hand, there are some evidences , indicating that some mutations in this gene may cause its hyperactivity and increase of its expression, as the result of which the expression of relevant enzyme increases and naturally resulting in plasma proline decrease and glutamate increase instead. Some evidences have shown that proline has a functional role in brain, including adjustment of Ach performance and acting as a metabolic prerequisite for glutamate in a subgroup of glutamate neurons. Major function of this gene in pathology of this disease is based on the role of –L of proline as regulator of glutamate transmission in brain and expression of Proline oxidize enzyme and Proline/P5C route in suppression of growth affiliated to P53 and apoptosis. Researches on this gene in different populations have shown that some mutations of this gene have made the person susceptible to Schizophrenia, whereas some other ones have not affected the process of the disease[3,7,19] . This gene has 15 exons and is about 23.77 kb. It is located near the end of an area which has been deleted in DIGeorge and Locardia syndrome. In fact, the first suspicion toward the area and identification of this gene as a candidate for this disease took place when the people suffering the DIGeorge and Locardia syndrome were seen to be 20-30% more susceptible to Schizophrenia than others[2,13]. Then a gene screening in the area resulted in identification of a number of genes contributing to the occurrence of this disease, one of the most important of which was Proline dehydrogenase gene[10,11]. As mentioned above, various studies have been conducted on polymorphism of this gene and its relation with Schizophrenia. In this research we investigated the relation of SNP 1945 T>C which is located in exon 2 of this gene. Replacement of nucleotide C for nucleotideT results in replacement of amino acid isolosin for lucin in the position of amino acid 581 of Proline oxidize enzyme. It seems that this replacement causes performance of this enzyme. It was for the first time that such research was carried out in Iran.

MATERIALS AND METHODS

In this project, all cases were of Iranian descent and were recrutited from the throughout of country. A total of 150 unrelated patient with schizophrenia (mean age ±SD **:** 41.7±13.5 years , including 80 female and 70 male,aged 18-60 years) was obtained of psychiatry hospitals and clinics . These patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) ,and their diseases were confirmed by psychiatrists. Then control cases were collected of population volunteers of different regions of country, that this samples were healthy for this illness .For this object, a total of 160 unrelated persons were collected. (mean age ±SD : 40.5±13.2 , including : 82 female and 78 male, aged 20-58 years) They were anonymous healthy volunteers and had not been evaluated by psychiatrists. Patients and controls almost resided in the same area of Iran . All participants provided written informed consent .The study was approved by ethics committee of . Razi university and hospital . Also , this study is the first association scrutiny of PRODH gene and schizophrenia in the Iranian population .

Genotyping

For all participants , Blood samples (5-8 ml)were obtained and collected in EDTA- containing tubes . Then DNA was extracted by using the salting

out procedure . In the continuation , for the PRODH genotyping , on the basis of respective SNP and one pairs primers that we were designed, previously, we were accomplished PCR reactions for amplifying related exon of this gene , and genotypes were determined by restriction fragment length polymorphism and digestion with proper restriction enzymes .

In this study , for analysis of this gene and its relation with schizophrenia ,We genotyped one SNP marker , including 1945 T>C (rs372055) that were located in exon number of 2 on PRODH gene.this SNP marker was selected based on previous investigations which demonstrated possible association between this SNP and schizophrenia, in other populations . Genotyping of this genetic marker in our sample populations were determined with the RFLP method After PCR reaction of the regions including polymorphism with desigened primers (list in table 1) . This primers was desigened by Oligo7 and gene runner soft ware. After amplifying , PCR amplicons were digested with *P*vuII , and for observe of migration pattern of resulted components, was transferred on the agars gel (4%). For genotyping of SNP marker of C1945 T in our population , we was performed PCR reaction with PI& PII primers (table 1). in the continuation , PCR amplicons were digested with *PvuII* restriction enzyme for 3 hours at 37ºc and electrophoresed on ethidium bromide-stained agars gels (4%) to separate according to size .

Table 1. properties of primers

T1945C Amplicon lengh: 499 bp RE : PvuII	PI)Forward primer: 5'–3	CTCCCTGGTGCGATGGGGTAC Tm: 71 GC : 66.7% 21 mer attachment site : 22877
	PII)Reverse primer: 5'-3'	GGGCCCACACATTCGAGGAG Tm: 71.2 GC: 65% 20 mer attachment site : 23375

Statistical analysis

Hardy-weinberg equilibrium(HWE) test based chi-squared test for this SNP marker in this study , were determined with the Haploview software program (http://www. Broad. Mit. Edu/mpg/haploview/). Statistical analysis were performed using SPSS16.0 software (Statistical Package for the Social Sciences) . statistical significance was accepted at $p < 0.05$.

RESULTS

Demographic characteristics

In this work , we recruited 150 unrelated patient with schizophrenia, including 80 female and 70 male , that their ages ranged from 18-60 years old and mean age 41.7 $\pm$ 13.5 and 160 unrelated individual with out schizophrenia , including 82 female and 78 male that their ages ranged from 20-58 years old with mean age 40.5$\pm$ 13.2 . Totally , there were 162 female and 148 male participants . Years of education in patients was less than control subjects . average age of expression of illness in females was 26.5 $\pm$ 10.2 and in males was 22.8$\pm$7.6 and 32% of patiens were married but this value in healthy individuals was 79.5% .

Risk of outbreak schizophrenia in relation to PRODH gene and respective SNP marker

In this work , we were studied one SNP marker on PRODH gene in relation to schizophrenia , including T1945C. In PRODH 1945T>C polymorphism that located on exon 2 , in normal condition this codon is codes for lue 581 amino acid in Prolin dehydrogenase whereas , previous studies was demonstrated that substitution T>C in this position is caused to Ile is located in this position . Genotype of this codon determined using of *PvuII* restriction enzyme . In individuals with wild type hemozygote genotype (TT) we were observed a cut pattern with 2 band including 200 and 300 bp , approximately, on agarose gels and for individuals with hetrozygote genotype (TC) , we were observed 3 band on agarose gels , including , 100,200 and 300 bp , approximately.also , in recessive hemozygotes with CC genotype were observed a 200 bp band that

was bold (two 200 bp band overlapping) and a 100 bp band that was colourless. (Fig.1) . On the based of this data , there were 7 individual with CC genotype in patient group but there weren't this genotype in control group ,also there were 26 TC genotype in patient group while there were 3 TC genotype in control group . results of statistical analysis for this SNP marker are shown in table 2.

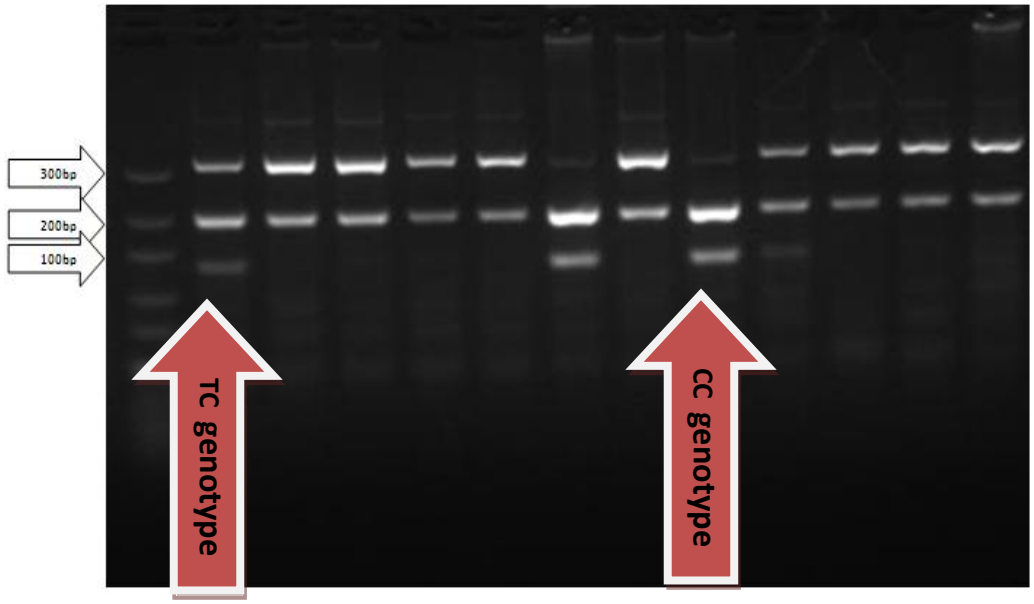

Fig .1. Digestion with *PvuII* restriction enzyme : In this figure were observed that CC genotype displayed two band ,including one bold band, that received of overlapping of 2 bands 200bp , and one 100 bp band that was colourless.and also in TC genotype were observed 3 band including 100,200,300 bp , approximately. In wild type TT genotype were observed 2 band including 200,300 bp .

Table 2. PvuII * Patients/Controls

Crosstab

			Patients/Controls		
			Control	Patients	Total
PvuII	CC	Count	0	8	8
		% within Pv	.0%	100.0%	100.0%
	TC	Count	3	29	32
		% within Pv	9.4%	90.6%	100.0%
	TT	Count	157	113	270
		% within Pv	58.1%	41.9%	100.0%
Total		Count	160	150	310
		% within Pv	51.6%	48.4%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Squa	36.010[a]	2	.000
Likelihood Ratio	42.419	2	.000
N of Valid Cases	310		

a. 2 cells (33.3%) have expected count less tha minimum expected count is 3.87.

On the base of stistical analysis for this genotype in our samples , we are observed a meaningful differences between patient and control subject for this ferequency of mutation allele . Ferequency of this mutation allele in patient group is very more than control group , that this suggested T1945C polymorphism in this population can be in relation to schizophrenia . In other words , PRODH marker T1945C show a significant association with schizophrenia (*P*= 0.00).

Investigation of effect of different independent elements on this illness

In this work , beside of study of genetic marker in relation to schizophrenia , we were analyzed effect of independent elements on this disorder in compare of control group and patient group, including season of birth , gender , age and smoking.

Table 3. Statistical comparision of seasons of birth between control and patient group . in this table 3,4,5,6 numbers refered to spring, summer, autumn and winter,respectively

Crosstab

			Patients/Controls		
			Control	Patients	Total
MONTH OF BIRTH	3.00	Count	29	34	63
		% within MONTH OF BIRTH	46.0%	54.0%	100.0%
	4.00	Count	47	29	76
		% within MONTH OF BIRTH	61.8%	38.2%	100.0%
	5.00	Count	49	29	78
		% within MONTH OF BIRTH	62.8%	37.2%	100.0%
	6.00	Count	35	58	93
		% within MONTH OF BIRTH	37.6%	62.4%	100.0%
Total		Count	160	150	310
		% within MONTH OF BIRTH	51.6%	48.4%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Squ	15.170[a]	3	.002
Likelihood Ratio	15.312	3	.002
Linear-by-Linear Association	1.970	1	.160
N of Valid Cases	310		

a. 0 cells (.0%) have expected count less than minimum expected count is 30.48.

Season of birth

In north hemisphere , most of schizophrenia patients is born in winter and initial of spring ,wheras, in south hemisphere this illness is expressed in these monthes lesser than north hemisphere. Probably , this differences is resulted of effect of environment on mother or growth of germ . Base of this analysis , we find that most of patients in our statistical populations was born in winter season and base of this data there was meaningful different between control and patient group . [15]

The relationship between smoking and its effect on schizophrenia

In some of studies that accomplished by several individuals, demonstrated that smoking can be in relation to express of symptoms of schizophrenia[9]. This subject were also analyzed in our statistical population . Study of effect of smoking, as a environmental potential, on this illness and compare of between control and patient groups demonstrated that number of smoking individuals in patient group was very more than control group and there was meaningful relation with illness (table 4) .

Study of interplay effect of smoking and gender with respective polymorphism

Results of this survey, demonstrated that there was not meaningful relationship between smoking and sex and polymorphism in compare of control and patient group.

Tabel 4. statistical comparison of patient /control smoking

Patients/Controls * smoke Crosstabulation

			smoke		Total
			NO	OK	
Patients/Contro	Control	Count	128	32	160
		% within Patients/Contro	80.0%	20.0%	100.0%
	Patients	Count	61	89	150
		% within Patients/Contro	40.7%	59.3%	100.0%
Total		Count	189	121	310
		% within Patients/Contro	61.0%	39.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Squa	50.332[b]	1	.000		
Continuity Correc[a]	48.693	1	.000		
Likelihood Ratio	51.898	1	.000		
Fisher's Exact Te				.000	.000
N of Valid Cases	310				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expe 55.

Table 5. Patients/Controls = Patients (PvuII * smoke)

Crosstab[a]

			smoke		
			NO	OK	Total
PvuII	CC	Count	3	5	8
		% within Pv	37.5%	62.5%	100.0%
	TC	Count	14	15	29
		% within Pv	48.3%	51.7%	100.0%
	TT	Count	44	69	113
		% within Pv	38.9%	61.1%	100.0%
Total		Count	61	89	150
		% within Pv	40.7%	59.3%	100.0%

a. Patients/Controls = Patients

Chi-Square Tests[b]

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Squa	.869[a]	2	.648
Likelihood Ratio	.859	2	.651
N of Valid Cases	150		

a. 2 cells (33.3%) have expected count less tha minimum expected count is 3.25.

b. Patients/Controls = Patients

Table 6 . Statistical comparision betweensex and polymorphism (*PvuII* ***** sex)

Crosstab[a]

			sex		
			1.00	2.00	Total
Pvull	CC	Count	5	3	8
		% within Pv	62.5%	37.5%	100.0%
	TC	Count	19	10	29
		% within Pv	65.5%	34.5%	100.0%
	TT	Count	46	67	113
		% within Pv	40.7%	59.3%	100.0%
Total		Count	70	80	150
		% within Pv	46.7%	53.3%	100.0%

a. Patients/Controls = Patients

Chi-Square Tests[b]

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Squa	6.558[a]	2	.038
Likelihood Ratio	6.603	2	.037
N of Valid Cases	150		

a. 2 cells (33.3%) have expected count less tha minimum expected count is 3.73.

b. Patients/Controls = Patients

DISCUSSION

Proline dehydrogenase gene is located on chromosome 22q11.2 in an area highly susceptible to mutations and microdeletions which cause mental diseases, especially Schizophrenia[10,19]. In some populations, this gene had been reported as one of the most important genes susceptible to the disease and numerous polymorphisms of this gene has been investigated. The results of these investigations in some populations indicated their relation with the disease and in other populations indicated no relation between them[2,16,18,19]. Our hypothesis was that proline dehydrogenase gene as one of the most important genes contributing to the occurrence of Schizophrenia, which has been proved to play role in susceptibility to Schizophrenia in some populations, may contribute to the occurrence of this disease in Iran population too. In fact, we are stating that proline dehydrogenase gene is one of the genes that make a person susceptible to Schizophrenia.

In this hypothesis we used a uni-nucleotide polymorphism reported in other populations. Of course, in some populations these SNPs have no relation with the disease. This SNP is 1945T/C rs372055). Also, such factors as gender, smoking and birth season were taken into consideration.

As mentioned before, allele T1945C is located in exon 2 of this gene. This codon naturally codes amino acid lucin in situation 581 in this enzyme. This polymorphism has been investigated in other populations too. For example, some research was carried out in 2006 to investigate this codon in European population. To this end they used 488 persons in Bulgarian population in the form of three-member families consisting of parents and patient child. The results showed that this mutated allele has no meaningful frequency in the occurrence of this disease, or in 2006 a research group in China studied the relation between this polymorphism and Schizophrenia in eastern China population. They used 166 parents together with their patient children and made genotypic investigation of that area of their genes. here no

meaningful relation in allele 1945 was seen too. The results of our work, however, indicated that this codon may contribute in the occurrence of this disease in our statistical society. The results were as follows: there were 8 CC defeated homozygote cases. We didn't saw this genotype in our control group. There were also 28 TC heterozygote cases in patient population and 3 heterozygote cases in control group. The number of homozygote healthy persons in patient population was 113 and in control population was 157. According to Chi-Square test, this T>C uni-nucleotide polymorphism may, given *P*=000, be statistically effective in the occurrence of disease in the population under study.

Again another polymorphism in this gene was investigated in another population. the results showed that codon A1766G is located in exon 13 of this gene. In 2009, this codon together with codons 1945 and 1852 was investigated in 217 persons in Greece[16]. They reported that haploid CGA for these three codons, compared to control group, has a meaningful relation in making a person susceptible to Schizophrenia. In a Chinese population, this codon was reported to have no relation.

Codon C1482T in exon 11 was also reported to be of high risk for the occurrence of different mutations in this gene. The conclusion we can draw from these results is that proline dehydrogenase gene can be a considerable candidate in the occurrence of Schizophrenia in the population under study. Of course, as previously mentioned, this disease may occur as a result of environmental factors. Also, it has been reported as a disease with complex hereditary pattern and different genes have been taken into consideration. For example, in the area that proline dehydrogenase genes is located, there are important genes such as COMT and ZDHHC8 which have been reported to play some roles in the disease [2]. We can not therefore state that the only gene that causes the disease is proline dehydrogenase, but the results show that this gene plays a significant role in the occurrence of this disease.

The factors of age, gender, birth season and smoking were also investigated. Statistical results show that there is a meaningful relation between age and this disease. Average age of patients are 13.5+41.7 and that of healthy people is 13.2+40.5. The average age in which the disease occurs is 7.6+22.8 in males and 10.2+26.5 in females. The difference was statistically meaningful (p=0.009). As to gender, it was not seen to have a meaningful relation with related polymorphisms. As regards birth season, there is a meaningful correspondence to previous hypotheses stating that in southern hemisphere the disease is more likely to occur in those born in winter, as 58% of our patient population had been born in winter. Smoking had a meaningful relation with the disease in general, but had no relation with analyzed polymorphisms.

Overally, this gene can be elected as an important molecular target for the treatment of these patients.

ACKNOWLEDGEMENT

The authors wish to gratefully acknowledgement the Biochemistry Department of Pasteur of Iran. we would like to thanks Razi hospital for its assistant in the preparation of blood sample .The authors wish to thank the patients for providing the samples for this study .

REFERENCES

1.Ad GeurtsvanKessel, HanG.Brunner, Roel A.Ophoff, Recurrent CNVs Disrupt Three Candidate Genes In Schizophrenia Patients . (2008) The American Journal of Human Genetics 83, 504–510.

2. BeateGlaser , Valentina Moskvina , George Kirov , Kieran C. Murphy , HywelWilliams , Nigel Williams , Michael J. Owen , Michael C. O'Donovan. Analysis of ProDH, COMT and ZDHHC8 risk variants does Not support individual or inter active effects on schizophrenia susceptibility. Schizophrenia Research87(2006)21–27

3. Colm M.P.O'Tuathaigh , Daniela Babovic, Gillian O'Meara, Jeremiah J. Clifford, DavidT.Croke,JohnL.Waddington. Susceptibility genes for schizophrenia :Characterisation of

mutant Mouse models at the level of phenotypic behavior . Neuroscience and Biobehavioral Reviews31(2007)60–78.

4. Goldner, E.M.,Hsu,L.,Waraich,P.and Somers ,J.M.(2002)Prevalence and incidence studies of schizophrenic disorders :asystematic review of theliterature.CanJ.Psychiatry,47:833–843.

5. Hafner,H.andHeiden,Wad.(2003)Course and outcome of schizophrenia .In:HirschS.R.and Weinberger D.R.(Eds.), Schizophrenia .Blackwe llPublishing,Malden,MA,pp.101–141.

6. Harrison,P.J .and Owen,M.J.(2003) Genes for schizophrenia Recent findings and their pathophysiological implications .Lancet, 361:417–419

7. H.Ishiguro , Y. Horiuchi , M. Koga , T. Inada , N. Iwata ,N.Ozaki , H. Ujike , T. Muratake , T. Someya , T. Arinami. RGS4 is not a susceptibility gene for schizophrenia in Japanese:A ssociation study in alarge case-control population. Schizophrenia Research 89(2007)161–164

8. Joseph A.Gogos and David J.Gerber, Schizophrenia susceptibility genes: Emergence of positional candidates and future directions. TRENDS in Pharmacological Sciences Vol.27No.4

9. J. H. Williams, N. A. Wellman, L. M. Allan, E. Taylor, J. Tonin, J. Feldon and J. N. P. Rawlins, 2002 . Tobacco smoking correlates with schizotypal and borderline personality traits . Personality and individual differences . vol 20

10. Liu,H .,Abecasis,G.R .,Heath,S.C.,Knowles ,A.,Demars,S.,Chen,Y.J.,Roos,J.L.,Rapoport,J.L., Gogos,J.A.,andKarayiorgou, M.(2002b).Genetic variation in the22q11 locus and susceptibility To schizophrenia. Proc.Natl.Acad.Sci.USA 99, 16859–16864.

11. Liu,H .,Heath,S.C., Sobin,C., Roos,J.L. , Galke,B.L., Blundell,M.L.,Lenane,M.,Robertson, B.,Wijsman,E.M.,Rapoport,J.L.,Gogos,J.A.,andKa rayiorgou,M.(2002a).Genetic Variation at the 22q11PRODH2/DGCR6 locus presents an unusual pattern and increasesSusceptibility to schizophrenia. Proc.Natl.Acad.Sci.USA 99, 3717–3722.

12. Murray ,C.J.L., Lopez, A.D.,Murray ,C.J.L.,Lopez,A.D., Murray ,C.J.L. and Lopez, A.D.S. (1996) The Global Burden Of Disease .Harvard University Press, Boston.

13. Nigel M. Williams ,Michael C.O'Donovan ,andMichaelJ.Owen CHROMOSOME 22 DELETION SYNDROME AND SCHIZOPHRENIA. NEURO BIOLOGY, VOL.73

14. O'Donovan ,M.C.,Williams ,N.M.andOwen ,M.J.(2003) Recent advances in the genetics of schizophrenia .Hum. Psychiatry,8:217–224.

15. Owen ,M.J.,O'Donovan ,M.C.andGottesman ,I.I.(2002)Schizophrenia .In :McGu ? nP .,OwenM.J. andGottesmanI.I.(Eds.), Psychiatric Genetics and Genomics .Oxford University Press ,NewYork,pp.247–266.

16.PanosRoussos, Stella G. Giakoumaki, and Panos Bitsios , 2009 A Risk PRODH Haplotype Affects Sensorimotor Gating ,Memory ,Schizotypy, and Anxietyin Healthy Male Subjects. BIOLPSYCHIATRY, 355-362

17. Pearlson GD (2000): Neurobiology of schizophrenia. *Ann Neurol* 48:556–566.

18. Tsuyuka Ohtsuki,Syunsuke Tanaka, Hiroki Ishiguro, Emiko Noguchi ,, Tadao Arinami , 2004. Failure to find association between PRODH deletion and schizophrenia . Schizophrenia Research 67 .111–113

19. Xiaohong Ma , Jinhua Sun , Jing Yao , Qiang Wang , Xun Hu , Wei Deng ,XueliSun, Xiehe Liu , Robin M. Murray , David A. Collier , Tao Li , A quantitative association study between schizotypal Traits and COMT ,PRODH and BDNF genes in a healthy Chinese population . Psychiatry Research 153(2007)7–15.