Association of 757 C/T polymorphismin PRODH gene with Schizophrenia in Iranian population

Fariba Ghasemvand[1], Saeid Rahman zadeh[2], Zivar Salehi[3],Yousef Fakour[4], Saeed Heidari keshel[5,6] ,Eskandar Omidinia[2],*

[1]Department of Biology , Faculty of Pardis International, University of Guilan , Rasht , Iran
[2]Department of Biochemistry Pasteur Institute of IRAN, Tehran, Iran
[3]Department of Biology, Faculty of Sciences, University of Guilan, Rasht , Iran
[4]Undersecretary of Research and Technology , Ministry of Health and Medical Education, Tehran, Iran
[5]Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[6]Department of Tissue Engineering and Cell Therapy, School of Advanced Medical Technologies,Tehran University of Medical Sciences, Tehran, Iran

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

ABSTRACT

Evidence is emerging for the association of polymorphisms in PRODH gene and increased risk of schizophrenia. In this project, peripheral blood sampling was obtained from 175 schizophrenia patients that their diseases were confirmed by psychiatrists. 185 healthy individuals were considered as control group. The related tests were administered on the basis of PCR-RFLP. In the continuation, statistical analysis was made on the basis of obtained genotypes from two groups of healthy and patient groups using SPSS16.0 software. The administration of this project aims at investigating the hypothesis that proline dehydrogenase gene, as one of the most important genes involved in schizophrenia incidence which is proved in various populations [outside Iran], may also be involved in incidence of schizophrenia in Iran. This study has analyzed one single nucleotide polymorphism in the PRODH gene, including 757C/T in the incidence of this disease in the given statistical population.
According to our results, SNP 757 C/T may be effective in incidence of the disease since *P* value was < 0.01. Ultimately, our results suggest that outbreak of mutation in PRODH gene in our population can be one of causes of increasing risk of Schizophrenia in this population.

Keywords: Schizophrenia; PRODH; Proline

INTRUDUCTION

Schizophrenia is a mental and emotional disease; the incidence is approximately one percent (1%) of the world populations[4]. This disease includes a group of psychiatric symptomatic disorders. The origins of them, their characteristics andpersonality disordersare thought to be different[1]. The common aspect of this group includes changes in form and content of thought and degradation in Psychosocial functioning which in turn results in disturbance in social status. The symptoms of schizophrenia usually appear during adolescence and early adulthood. Cause or Causes of schizophrenia is unknown. The disorderhas been reported in structure and function of brain left temporal lobe in recent studies of brain imaging. Studies on identical twins revealed a strong genetic basis in this disorder. Study of schizophrenia inheritance showed that it is a complex genetic disorder[11]. The transferred pattern is multiple genes and proline dehydrogenase gene is a susceptible gene in this disease which is located in 22q11 chromosome. Meanwhile, the expression of this gene in brain is very high and the coded protein is a proline dehydrogenase (oxidase) which catalyzes the first stage of proline decomposition (it converts proline to Δ-1 pyrolin 5-carboxylate). Defect in this gene causes hyperprolinemia type 1 and probability of schizophrenia[1,5,12]. This research is an attempt to investigate polymorphism pattern of proline dehydrogenase genefor the first time, among Iranian patients suffering from schizophrenia. Evidence suggests a role for prolin in brain function, including the regulation of cortical Ach function and as a metabolic precursor of glutamate in a subpopulation of glutamate neurons[6,13]. Several studies have

been reported an association of specific PRODH variants with increased susceptibility to schizophrenia, while numerous PRODH missense mutations have been identified in patients with schizophrenia. Failure to observe an association between PRODH and schizophrenia has also been reported. The function candidacy of ProDH in the etiology of schizophrenia is mainly based on the putative role of l-proline as modulator of glutaminergic transmission in the brain and implication of ProDH and the proline/P5C pathway in P53-induced growth suppression and apoptosis. Also, this gene carries 15 exons that span 23.77 kb , located at 17.3 Mb in 22q11 , near the centromeric end of the region typically deleted in velocardiofacial syndrome/Digorge syndrome (VCFS/DGS)[2,3,7,11]. Different studies have been carried out regarding the relationship between polymorphism of this gene and the risk of schizophrenia.Prolineoxidase is located in the inner membrane of mitochondria, where catalysis the conversion of prolin to D-l-pyroline-5-carboxilate(P5C). Then, P5C converts to glutamate or γ-amino butiric acid, that these are two important neurotransmitters in nervous system. Recent finding demonstrated that mutation in PRODH gene can be cause of hyperprolinemia, means, result in reduction in function of prolineoxidase , increaseproline level in body.researchers believe that increasing in proline level in brain has influences in function of neurotransmitters (glutamate & GABA) and can increasethe risk of psychiatric disorders including schizophrenia.[1,7,8,9,12] Recentstudies suggest a possible role of the PRODH gene variations , located in the 3'region of the gene , in the etiopathogenesis of schizophrenia . Already, several studieswere accomplished on this gene by different SNPs and on different populations and subsequentlywere obtained variant results on populations. for example , significant association has been shown for haplotypes consisting of three single nucleotide polymorphisms (SNPs) : rs372055(1945T>C), rs450046 (1766A>G) , rs385440(1852G>A), the alleles 1945C,1766G,and 1852A have been shown to be overtransmitted in schizophrenia patients.[9,13] Moreover, the PRODH 1945C-1852A haplotype was an important determinant of executive functions in schizophrenia patients

[10]. However, several other studies have failed to replicate these associations. In other study, PRODH,COMT,ZDHHC8 polymorphisms was researched on Bulgaria population that their results do not support the presence of significant risk effects at PRODH 2026 and 1945 , COMPT ValMet and ZDHHC8 rs175174. In Chinese population, PRODH 1945 T>C polymorphism was studied and no evidence forthe association of this allele has been found [8]. In this work, we study one single nucleotide polymorphism (SNP) in PRODH gene in Iranian populations, including ٤757 C/T. Moreover, in this study, we researche the effect of different independent elements on this disorder, including ٤ smoking, gender, season of birth and level of education.

METHODS AND MATERIALS
Samples:
In this project, all subjects were from Iranian descent and were recruited from the throughout of country. A total of 175 unrelated patients with schizophrenia (mean age $\pm$SD **:** 41.7$\pm$13.5 years , including 85 female and 90 male,aged 18-60 years) were 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 subjects were collected fromvolunteers population of different regions of the country, that thesesamples were healthy for this illness .For this object, a total of 185 unrelated individuals were collected. (mean age $\pm$SD : 40.5$\pm$13.2 , including : 100 female and 85 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 signed 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 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 one pair primers which were designed

previously, PCR reactions were accomplishedfor amplifying one exon of this gene.Also genotypes were determined by restriction fragment length polymorphism and were digested with proper restriction enzymes. We genotyped one SNP marker, as 757 C/T whichwas located in exon 1 on PRODH gene. ThisSNP marker was selected, based on previous investigations in which there was a possible association between 757C/T and schizophrenia in other populations. Genotyping of this genetic marker in our sample population were determined with the RFLP method After PCR reaction of the regions including polymorphisms with designed primers (list in table 1). These primers were designed by Oligo7 and gene runner softwares. After

amplifying, PCR amplicons were digested with *NciI*.for observing of migration patterns of resulted components, we transferred them on the agarose gel (6%). For genotyping the SNP marker of 757C/T in our population, we were performed PCR reaction with NciF,R primers (table 1) . PCR amplicons were digested with NciIrestriction enzyme for 4 hours at 37ºc and electrophoresed on ethidium bromide-stained agarose gels (6%) to separate according to size. PCR was carried out at 95 °C predenaturefor 5 min, followed by 30 cycles of 95 °C denature for 1min , 60 °C annealing for 1min and 72 °C extension 1 min , followed by 72 °C final extension for 7 min.

Table 1. List of primers & their properties

C757 T **Ampliconlengh:** **240bp** **RE *NciI***	Forward primer: 5'–3	GCCCTGTATCCCTGCAC Tm: 59.1 GC:64.7 17 mer attachment site: 22709- 22725
	Reverse primer: 5'-3	ACCTCCATCACGGGGCCA Tm: 61.2 GC: 65.2 18 mer attachment site: 22978-22995

Statistical analysis:
Hardy-weinberg equilibrium(HWE) test based on chi-squared test for all SNPs 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 175 unrelated patients with schizophrenia, including 85 female and 90 male , that their ages ranged from 18-60 years old and mean age 41.7 ± 13.5 and 185 unrelated individuals without schizophrenia , including 100 female and 85 male that their ages ranged from 20-58 years old with mean age of 40.5 ± 13.2 . Totally, there were 185 female and 175 male participants.years of education in patients was less than control subjects. Average age of the expression of the illness in females were 26.5 ± 10.2 and in males were 22.8 ± 7.6 and 32% of patients were married but this value in healthy individuals were 79.5%.

Risk of schizophrenia in relation to PRODH gene:Single marker analysis
In this investigation, we were studied 1 SNP marker on PRODH gene in relation to schizophrenia, including 757C/T. In PRODH 757C>T polymorphism that located on exon1 , in normal condition this codon codes forArg185 amino acid in prolin dehydrogenase whereas , previous studies were demonstrated that substitution of C>T in this position cause to replaceTrpinstead of Arg. Genotype of this codon determined with the use of NciI restriction enzyme. in individuals with wild type hemozygote genotype (CC), we observed one cut site with 2 bands including 80 and 160 bp , approximately, on agarose gels and in individuals with hetrozygote genotype (CT) , we observed 3 bands on agarose gels , including , 80 ,160 and 240 bp .Also , in recessive hemozygotes with TT genotype we observed a 240 bp band which was bolder(see Fig.1) . On the base of this data, there were 29 individuals with TT genotype in patient group but there were 18 individuals in control group ,also there were 92CT genotypes in patient group while there were 70 TC genotypes in control group . Results of statistical analysis for this SNP marker are shown in table 2.
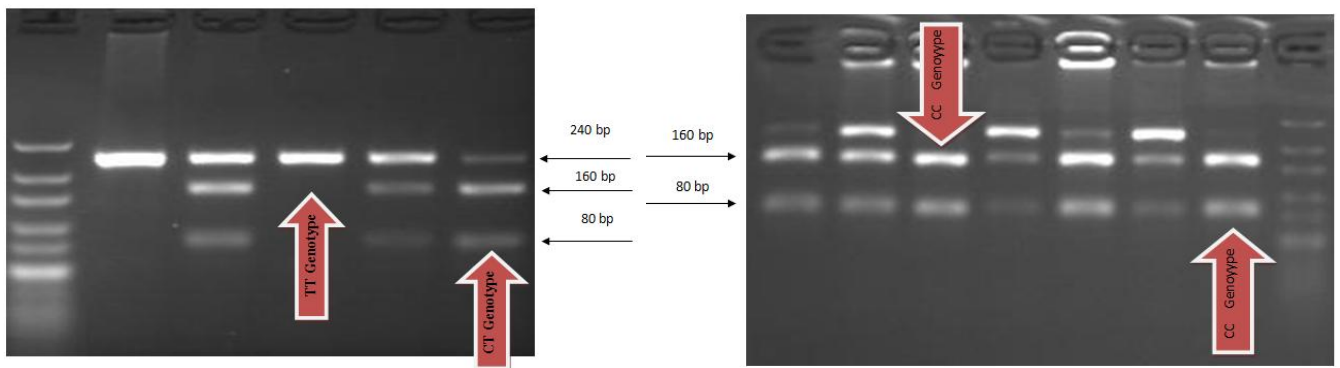
Figure.1. Digestion with *NciI* restriction enzyme : In this figure CC genotype displayed two bands ,including 80 and 160 bp and also CT genotype displayed 3 bands including 80 ,160 ,240 bp. In TT genotype there was 240 bpband.

Table 2:NciI * Patients/Controls

Crosstab

| | | | case.control | | |
			Patients	Controls	Total
NciI	CC	Count	54	95	149
		% within N	36.2%	63.8%	100.0%
	CT	Count	92	71	163
		% within N	56.4%	43.6%	100.0%
	TT	Count	29	19	48
		% within N	60.4%	39.6%	100.0%
Total		Count	175	185	360
		% within N	48.6%	51.4%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Squa	15.805ᵃ	2	.000
Likelihood Ratio	15.963	2	.000
N of Valid Cases	360		

a. 0 cells (.0%) have expected count less than minimum expected count is 23.33.

Table 3: Statistical comparision of seasons of birth between control and patient group

Birth.group * case.control Crosstabulation

			case.control Patients	case.control Controls	Total
Birth. group	Spring+Winter	Count	98	74	172
		% within Birth.group	57.0%	43.0%	100.0%
	Summer+Autumn	Count	64	111	175
		% within Birth.group	36.6%	63.4%	100.0%
Total		Count	162	185	347
		% within Birth.group	46.7%	53.3%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	14.511[b]	1	.000		
Continuity Correction[a]	13.703	1	.000		
Likelihood Ratio	14.613	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	14.469	1	.000		
N of Valid Cases	347				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 80. 30.

On the base of statistical analysis of this genotype in our samples, we observed meaningful differencesin theferequency of mutation allelebetween patient and control sampels . The ferequency of the mutation allelein patient group is higher than control group , this means that the suggested C757T polymorphism in this population can be in relation to schizophrenia . In other word, PRODH marker 757 shows a significant association to schizophrenia (P = 0.00).

Investigation the effect of different independent element on this illness

In this work, beside the study of genetic marker in relation to schizophrenia, we analyzed the effect of independent element on this disorder in compare of control group and patient group, including season of birth

Season of birth

In north hemisphere, most schizophrenia patients were born in winter and early of spring ,wheras, the number of the schizophrenia patients who were born at this time of year in south hemisphere was less thannorth hemisphere. Probably, these differences were resulted from the effect of environment on mother or growth of germ. Based on this data, we find that, most of patients in our statistical populations were born in winter and spring seasons and there was a meaningful difference between control and patient groups.[14]

DISCUSSION

Schizophrenia is a severe psychiatric disorder that has alifetime prevalence of 1% in most of the populationsstudies[1]. Similar to many common complex disorders,schizophrenia is a multifactorial disorder that is characterizedby the contribution of multiple susceptibility geneswhich could act in conjunction to epigenetic processes andenvironmental factors. More than 20 genome-widescans aiming to localize genes for this disorder have beenreported[1].Two recent meta-analyses [2,3] of the combinedresults of several genome-wide studies were performed toattempt to clarify inconsistencies among individualstudies).One meta-analysis study confirmed 8p, 13q and 22q asvalid linkage regions that probably contain one or moresusceptibility genes. The second meta-analysis study(using a different statistical methodology) implicated2p12–q22.1 (under stringent criteria), in addition to loci5q, 3p, 11q, 2q, 1q, 22q, 8p, 6p, 20p and 14q. One interpretation of these findings is that approximately ten regions of the genome are likelyto contain schizophrenia susceptibility genes. Several studies haveestablished that the risk of schizophrenia for a patientwith a 22q11 microdeletion is w25–31 times more thanthe generalpopulation risk of 1% .[13] the rate of 22q11microdeletions in schizophrenia, is w12–

80 times more than the estimated general population rate[10]. This first unequivocal association between a welldefinedgenetic lesion and schizophrenia facilitated finemappingefforts at this locus. LD analysis in familysamples (triads) which tested for preferential transmissionof 72 SNPs and multi-SNP haplotypes from parents toaffected (non-deleted) individuals, identified the overtransmissionof a gene variant located at the 30 end ofthe PRODH gene [5,12]. This finding was recentlyreplicated in two independent family-based samples,including a large collection of 528 families from China[1,3] and 274 families of Ashkenazi Jewish origin [7,8],although one negative family study has also been reported[9]. Moreover, 30-end variants of the gene were alsoidentified as being a risk factor for the development ofpsychotic symptoms during adolescence in children with22q11 microdeletions[10]. Although the implicatedvariants are consistently located at the 30 end of thegene, their functional consequences are still unknown.However, rare variants of the PRODH gene affecting highly conserved amino acids (generated through geneconversion from a nearby pseudogene) that are enriched tovarious degrees in samples of individuals with schizophreniahave been identified [5]. In addition, Prodhdeficientmice show dysregulation of cortical dopamineturnover and transmission that is reminiscent of schizophreniain humans.

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[2,7,9,10,11]. 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 aperson susceptible to Schizophrenia[2,3,5]. In this hypothesis we used a uni-nucleotide polymorphism reported in other populations. Of course, in some

populations these SNPs have no relation to the disease. This SNP is 757 C/T). Also, such factor as birth season were taken into consideration. As mentioned before, allele C757T is located in exon 1 of this gene. This codon naturally codes amino acid Argenine in situation 185 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 andpatient children [3]. 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[5]. 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 757 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 29TT defeated homozygote cases. There were also 92CT heterozygote cases in patient population and 70 heterozygote cases in control group. The number of homozygote healthy persons in patient population was 54 and in control population was 100. According to Chi-Square test, this C>Tuni-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 were investigated in 217 persons in Greece[13]. They reported that haplotype 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. Also,in 2012, rahman zadeh etal reported that 1945T/C polymorphism have relation to this disorder in this population[14].

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 candraw 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 cannot 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 is7.6+22.8 in males and 10.2+26.5 in females. The difference was statistically meaningful (p=0.008). 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 has a meaningful relation with the disease in general, but has no relation with analyzed polymorphisms.Overall, this gene can be elected as an important molecular target for the treatment of these patients.

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