

# The Relationship between Gene Polymorphism of miRNAs Regulating FGA and Schizophrenia

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## Abstract

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**AIM:** To investigate the relationship between the polymorphism of related gene loci of miRNAs regulated fibrinopeptide A and schizophrenia. Lay the foundation for the aetiology of schizophrenia.

**METHODS:** Adapt to the phase match of sex and age case-control study, a total of 513 Chinese Han patients with schizophrenia were selected as the case group, 513 normal healthy persons as a control group. Obtaining SNPs information of the FGA gene by querying the dbSNP database, and reference HapMap database included SNPs site frequency information for screening. The frequency distributions of SNPs were genotyped by iMLDR® SNP detection technology. Two SNPs (pre-hsa-miR-605rs2043556 T>C, pre-hsa-miR-499a/pre-hsa-miR-499b rs4909237 T < C) were analyzed to demonstrate their association with susceptibility to schizophrenia.

**RESULTS:** There were no significant differences between patients and controls in genotype and allele distribution of SNPs (rs2043556 and rs4909237) in the precursor region of hsa-miR-605 and pre-hsa-miR-499a/pre-hsa-miR-499b. Their gene-gene interaction, which suggests that the polymorphisms of miRNA genes might not contribute to schizophrenia susceptibility in the Han Chinese population.

**CONCLUSION:** No significant difference existed between schizophrenic patients and controls in SNP (rs2043556 and rs4909237) in the precursor region of hsa-miR-605 and pre-hsa-miR-499a/pre-hsa-miR-499b. There may not regulate FGA gene expression. Thus, hsa-miR-605 and pre-hsa-miR-499a/pre-hsa-miR-499b may not influence the risks of schizophrenia.

## Introduction

Schizophrenia is a complex mental illness that often comes from big hurt emotional [1]. The behaviour of the main performance by Psychological illusion, altered cognition, delusions, behavioural disorders and emotionally sensitive [2]. Seriously affect people's health and quality of life. Epidemiologic studies show that schizophrenia patients are up to 1%. However, the pathogenesis of schizophrenia is unclear. Which usually causes diagnosis errors.

Medical diagnosis is more and more development. However, the diagnosis of schizophrenia still can only rely on the patient's behaviour to determine because of the pathogenesis and aetiology of schizophrenia remains unclear. No

laboratory diagnostics is available. For the diagnosis of schizophrenia, we need an accurate professional diagnosis or a diagnostic tool. So it is important that the marker should play a significant role in the diagnosis of schizophrenia [3].

Fibrinopeptide A (FPA) is a product of proteolysis by thrombin of fibrinogen [4]. Now some previous studies using the mass spectrum analysis to prove that fibrinopeptide A (FPA) could be a potential biomarker for schizophrenia diagnosis [5]. MicroRNAs (miRNAs) are single-stranded RNA molecules, consisting of 21-23 nucleotides, which has the function of regulation [6]. The highly conservative endogenous non-coding RNA has extensive biological functions. According to some studies, most miRNAs are expressed in neurons and performance obvious patterns in the central nervous system. This means that miRNAs are the importance of brain development

and function [7]. MicroRNAs such as most species of a miRNA have accurately exact ends, behave like traditional polymeric products of gene activity, although there is a little variation [8]. Regulation of miRNA biogenesis is an important issue but no more extensively studied. However, there has an interesting tendency emerging [9]. An amazing much of miRNA genes are formed under the control of the very targets that they regulate [8].

Now this study based on our previous work, as the schizophrenia research object in the Chinese Han population. By adopting the method of a case-control study, using bioinformatics technology, from the level of the miRNA gene sequence, analysing the relationship between the regulating FGA protein of miRNAs and schizophrenia, provide important clues about the molecular mechanisms of schizophrenia occurs.

## Material and Methods

**Study of Population.** Our study included 513 schizophrenia patients that come from 2006 to 2010 years in the sixth hospital of Changchun and encephalopathy hospital of Jilin province. Among patients includes 273 cases of male and 240 cases of women (mean age =  $32.23 \pm 11.16$  years; 53.2% males) that all Han people from in Northeast China. Each patient was diagnosed by two or more physicians according to the criteria of the international classification of diseases from the tenth version of schizophrenia (ICD-10). At the same time, according to the Chinese classification and diagnostic criteria of mental disorders Third Edition (CCMD-3). Healthy people from the 2008 Changchun subhealth survey. Inclusion criteria were not previously diagnosed with chronic disease and A person without the obvious organic disease. Eliminate gestation or lactation women and schizophrenia. A total of 513 control people, includes 245 males and 268 females (mean age =  $31.54 \pm 9.35$  years; 47.76% males), all blood-supply person sign agreement. Venous blood 5 mL, frozen in the refrigerator at  $-20^{\circ}\text{C}$ , use of genomic DNA extraction.

Prediction of miRNA and selection of polymorphic loci. First of all, It was confirmed that the miRNA regulated of FGA gene by using mirtarbase online software query and by experiment; predicted the miRNA by using microcosm, Target, mirSVR and TargetS online software; Then miRNA was BLAST and BLAST-SNP in NCBI's dbSNP database (Build147); evaluated the effect that SNP influenced the interaction of the miRNA- target gene by using energy value ( $\Delta G$ ) of RNAhybrid online software; Finally, the frequency of these SNP in Chinese Han population was examined in the SNP database. Screening criteria:  $\text{MAF} > 10\%$ .

**DNA Extraction and SNP Genotyping.** Extract of genomic DNA from peripheral blood leukocytes and use micropore nuclein analyser (American Bio Tex) to identify the quality of genomic DNA extracted. Identification standard: the concentration of DNA  $> 10 \text{ ng}/\mu\text{L}$ . For compliance with the standard DNA was multiplex SNP typing by using of imLDR™. (IMLDR technology based on the traditional ligase reaction was improved of possessing proprietary intellectual property rights of multiple SNP typing technologies, compared with the traditional ligase reaction technique, IMLDR improves the accuracy and the success rate of typing. After repeated experiments and preliminary validation of a double-blind sample, the accuracy of the data is more than 98%, after sequencing and the Snapshot. Experimental design and operating instructions:

1. The section of the target SNP site was used multiple PCR reaction in a single system and amplification.

2. Amplified products were used follow-up ligase reaction templet after purification by an exonuclease and ExoI/SAP.

3. In a connection reaction, each site contains two 5' allele-specific probes (at the end of 3, there are two allele-specific bases or sequences, in terms of inserting loss polymorphism) and followed a fluorescent labelled specific probes for 3' terminal sites. The products were identified by ABI3730XL capillary electrophoresis. The original data file was analysed by GeneMapper4.1 software (Applied Biosystems).

We selected two tagSNPs located in the fibrinogen alpha chain gene (FGA) included rs2043556, rs4909237. The information of DNA primers was shown in Table 1.

**Table 1: Primers for polymerase chain reaction**

SNPs	Primer sequence (5'-3')
rs2043556	F : 5'-CCCGCCTCTTTTGCTCATTCT -3', R : 5'-TTGCAGAGCAGTTACGCCACAT -3'
rs4909237	F : 5'- AGACACACCCAGGCACACTTCA -3', R : 5'- GGCCACATTCTCTGCCATGT -3'

**Statistical Analysis.** Data processing used SPSS 13.0 software. Hardy-Weinberg (H-W), genetic equilibrium test, was performed by goodness of Chi-Square fit test; the measurement data is expressed as  $a \pm s$ , the count data is expressed as the number of cases and the percentage (%); comparison of measurement data using independent sample t-test; The allele frequency difference of genotype and compared using  $\chi^2$  test; Gene-gene interaction of miRNA was analysed by Logistic regression; Genetic model analysis was performed using SNPs that online genetic analysis software. The statistical significance level was set at 0.05, and all statistical tests were two-sided.

## Results

1. *Demographic Characteristics:* 1026 schizophrenia parents and control group were included in this study; there were no significant differences were found between patients and healthy controls in sex ( $\chi^2 = 3.057$ ,  $P = 0.080$ ).

2. *Hard-Weinberg equilibrium:* No deviation from HWE in schizophrenia and control groups in the two SNPs ( $P > 0.05$ ). The object of the sample study had a good group representative that from a random mating large sample, no migration and cousin marriage.

3. *The Allele and Genotype Analysis:* Table 2 displays genotype distributions and allele frequencies of SNPs in schizophrenic patients and healthy controls. There were no significant difference ( $P > 0.05$ ) in genotypic and allelic frequency in the two SNPs (rs2043556 and rs 4909237).

**Table 2: Comparison of allele frequencies and genotype distributions between case and control group**

SNP	Group	Genotypic frequency			Allelic frequency					
		C/C	C/T	T/T	$\chi^2$	P	C	T	$\chi^2$	P
rs2043556	Case	41	191	278	0.562	0.755	273	747	< 0.001	0.994
	control	36	199	271						
rs 4909237	case	273	202	38	0.840	0.657	748	278	0.810	0.368
	control	283	194	32						

4. *Genetic model analysis:* According to SNPstats online software, analysis polymorphism of two SNPs with the correlation of schizophrenia in codominant, dominant, recessive overdominant and log-additive, at the same time adjusting age and sex, there were not significant statistical with the correlation of schizophrenia ( $P > 0.05$ ). The optimal genetic model was overdominant ( $OR_{C/T-C/C}$  vs  $C/T = 1.10$ , 95%CI 0.85-1.42,  $P = 0.47$ , AIC = 1411.2) at rs2043556. The optimal genetic model of rs4909237 was dominant ( $OR_{C/T-T/T}$  vs  $C/C = 0.91$ , 95%CI 0.74-1.23,  $P = 0.45$ , AIC = 1419.6).

**Table 3: Comparison of genetic models between case and control group**

locus	Genetic model	genotype	case(%)	control (%)	OR (95%CI)	P	AIC
rs2043556	Codominant	T/T	278 (54.5)	271 (53.6)	1.00	0.69	1413
		C/T	191 (37.5)	199 (39.3)	1.08 (0.84-1.41)		
		C/C	41 (8.1)	36 (7.1)	0.89 (0.55-1.44)		
	Dominant	T/T	278 (54.5)	271 (53.6)	1.00	0.7	1411.6
		C/T-C/C	232 (45.5)	235 (46.4)	1.05 (0.82-1.34)		
	Recessive	T/T-C/T	469 (92)	470 (92.9)	1.00	0.54	1411.4
		C/C	41 (8)	36 (7.1)	0.86 (0.54-1.38)		
	Overdominant	T/T-C/C	319(62.5)	307 (60.7)	1.00	0.47	1411.2
		C/T	191(37.5)	199 (39.3)	1.10 (0.85-1.42)		
	rs4909237	Codominant	T/T	38 (7.4)	32 (6.3)	0.80(0.48-1.31)	0.63
C/T			202 (39.4)	194 (38.1)	0.93 (0.72-1.20)		
C/C			273 (53.2)	283 (55.6)	1		
Dominant		C/C	273 (53.2)	283 (55.6)	1.00	0.45	1419.6
		C/T-T/T	240 (46.8)	226 (44.4)	0.91 (0.71-1.16)		
Recessive		C/C-C/T	475 (92.6)	477 (93.7)	1.00	0.43	1419.6
		T/T	38 (7.4)	32 (6.3)	0.82 (0.50-1.34)		
Overdominant		C/C-T/T	311(60.6)	315 (61.9)	1.00	0.72	1420.1
		C/T	202(39.4)	194 (38.1)	0.95 (0.74-1.23)		

5. *Gene-gene interaction:* According to statistical analysis, there were no significant differences were found between gene-gene interaction.

**Table 4: Gene-gene interactions on schizophrenia risks**

rs2043556	rs4909237	Wals	P	OR	95%CI	
					Lower	Upper
C/T	C/T	0.000	0.999	2.671	0.000	
C/T	C/C	0.556	0.456	0.668	0.231	1.931
C/C	C/T	0.000	0.999	5.058	0.000	
C/C	C/C	0.015	0.903	0.883	0.120	6.497
T/T	C/T	0.000	0.999	4.847	0.000	

## Discussion

Our study first presents the correlation between the polymorphism of miRNAs locus regulated fibrinogen A and schizophrenia in the Han Chinese population. We found in the SNPs (rs2043556 and rs4909237): there were no significant difference ( $P > 0.05$ ) in genotypic and allelic frequency; there were no significant difference ( $P > 0.05$ ) in Genetic mode;

In recent years, our group concluded that FPA (Fibrinogen A) has the potential to be a biomarker about the diagnosis of schizophrenia according to comparing of serum protein profiling for first-episode schizophrenia, this protein could also help to determine schizophrenia pathogenesis [3]. Some study found the Fibrinogen in the blood of schizophrenic patients was significantly higher than that of healthy people [10]. Fibrinogen could be a blood index for the diagnosis of schizophrenia. Our group also studied the association between the fibrinogen alpha chain gene polymorphisms and schizophrenia [11]. Another study also further to demonstrate that relationship between polymorphisms in the fibrinogen alpha chain Gene(FGA) and Schizophrenia [5].

Schizophrenia is the complex multifactorial genetic diseases; the author found miRNA regulate gene expression may play a role in brain development [12]. Some studies displayed miRNA-SNP could impact the function of miRNA; it is a newly discovered polymorphism in the human genome. MicroRNA gene polymorphism as a new way of thinking for searching potential regulation of mental disorders and to provide a new reference for the pathogenic mechanism of related diseases. Thibaut et al found a new potentially functional variant ss178077483 located in the pre-mir-30e strongly associated with schizophrenia (allelic  $P = 0.00017$ ; genotypic  $P = 0.00015$ ) [13]. Hansen et al. confirmed rs17578796 in mir-206 and rs1700 in mir-198 were associated with schizophrenia in a Scandinavian population [14]. Jensen et al studies showed that miR-330-3p(rs41305272) associated with severe depression in some Americans ( $OR = 1.95$ ,  $P = 0.007$ ) [15]. There are strict rules of recognition between miRNAs and target genes, therefore, the SNPs (miRNA-SNPs) located in the miRNA gene region includes the miRNAs gene, and the target site of SNP could decorate miRNAs to regulate and control phenotypic type and disease susceptibility

[16]. At the same time, it has been shown that there is a correlation between SNPs located in the miRNAs target gene locus and different diseases [17], especially in the field of schizophrenia [13].

MiR-SNPs may use new or alter function to contribute to the evolution of miRNAs, also may show the generation of clustered miRNAs for the mechanism, family members of miRNAs during evolution, or miRNA homologs [11]. If we figure out the mechanisms clear, the genetic factors of schizophrenia will soon be resolved.

In conclusion, our study had some limitations. First, the low sample size may lead to no significant difference; Second, we used the imLDR<sup>TM</sup> to genotyping; this technology improves the traditional LDRSNP classification technology. We didn't have all the research samples to genotyping; Last, the sample size large differences in three preliminary studies, very few data can be matched, low accuracy of union analysis, our study did not integrate the data. Although we did not find the relationship between SNPs (rs2043556 and rs4909237) and schizophrenia, we will be further to find more SNPs and study them associated with schizophrenia.

## Reference

- Lysaker, PH, Vohs JL, Ballard R, Fogley R, Salvatore G, Popolo R, Dimaggio G. Metacognition, self-reflection and recovery in schizophrenia. *Future Neurology*. 2017; 8(1):103-115. <https://doi.org/10.2217/fnl.12.78>
- Tandon R, Keshavan MS, Nasrallah HA. Schizophrenia "Just the Facts": what we know in 2008 part 1: overview. *Schizophrenia Research*. 2008; 106(2-3):89. <https://doi.org/10.1016/j.schres.2008.07.020> PMID:18799287
- Zhou N, Wang J, Yu Y, Shi J, Li X, Xu B, Yu Q. Mass spectrum analysis of serum biomarker proteins from patients with schizophrenia. *Biomedical Chromatography*. 2014; 28(5):654-9. <https://doi.org/10.1002/bmc.3084> PMID:24254984
- Eisenberg PR, Sherman LA, Schectman KE, Perez JU, Sobel BE, Jaffe AS. Fibrinopeptide A: a marker of acute coronary thrombosis. *Circulation*. 1985; 71(5):912-8. <https://doi.org/10.1161/01.CIR.71.5.912> PMID:3986981
- Rao W, Zhou N, Zhang H, Liu R, Zhang S, Su Y, Yang G, Ma Y, Shi J, Yu Y, Yu Q. A case-control study of the association between polymorphisms in the fibrinogen alpha chain gene and schizophrenia. *Disease markers*. 2017; 2017. <https://doi.org/10.1155/2017/3104180> PMID:28203040 PMID:PMC5288525
- Wang J, Wang Y, Yang J, Huang Y. microRNAs as novel biomarkers of schizophrenia. *Experimental and therapeutic medicine*. 2014; 8(6):1671-6. <https://doi.org/10.3892/etm.2014.2014> PMID:25371713 PMID:PMC4217773
- Follert P, Cremer H, Béclin C. MicroRNAs in brain development and function: a matter of flexibility and stability. *Frontiers in molecular neuroscience*. 2014; 7:5. <https://doi.org/10.3389/fnmol.2014.00005> PMID:24570654 PMID:PMC3916726
- Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. *Cell*. 2009; 136(4):642-55. <https://doi.org/10.1016/j.cell.2009.01.035> PMID:19239886 PMID:PMC2675692
- Beveridge NJ, Gardiner E, Carroll AP, Tooney PA, Cairns MJ. Schizophrenia is associated with an increase in cortical microRNA biogenesis. *Molecular psychiatry*. 2010; 15(12):1176. <https://doi.org/10.1038/mp.2009.84> PMID:19721432 PMID:PMC2990188
- Gao ZS, Zhang LI, Qin CL. The Relationship between Hemorheological Changes and the Anxiety and Depression Symptoms in Schizophrenia. *Chinese Journal of Hemorheology*. 2004.
- Sun G, Yan J, Noltner K, Feng J, Li H, Sarkis DA, Sommer SS, Rossi JJ. SNPs in human miRNA genes affect biogenesis and function. *Rna*. 2009; 15(9):1640-51. <https://doi.org/10.1261/rna.1560209> PMID:19617315 PMID:PMC2743066
- Thibaut F. Schizophrenia: an example of complex genetic disease. *World Journal of Biological Psychiatry the Official Journal of the World Federation of Societies of Biological Psychiatry*. 2006; 7(4):194-197. <https://doi.org/10.1080/15622970600994313> PMID:17071540
- Xu Y, Li F, Zhang B, Zhang K, Zhang F, Huang X, Sun N, Ren Y, Sui M, Liu P. MicroRNAs and target site screening reveals a pre-microRNA-30e variant associated with schizophrenia. *Schizophrenia research*. 2010; 119(1-3):219-27. <https://doi.org/10.1016/j.schres.2010.02.1070> PMID:20347265
- Hansen T, Olsen L, Lindow M, Jakobsen KD, Ullum H, Jonsson E, Andreassen OA, Djurovic S, Melle I, Agartz I, Hall H. Brain expressed microRNAs implicated in schizophrenia etiology. *PLoS one*. 2007; 2(9):e873. <https://doi.org/10.1371/journal.pone.0000873> PMID:17849003 PMID:PMC1964806
- Jensen KP, Kranzler HR, Stein MB, Gelernter J. The effects of a MAP2K5 microRNA target site SNP on risk for anxiety and depressive disorders. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2014; 165(2):175-83. <https://doi.org/10.1002/ajmg.b.32219> PMID:24436253 PMID:PMC4174417
- Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. *Nature Reviews Cancer*. 2010; 10(6):389. <https://doi.org/10.1038/nrc2867> PMID:20495573 PMID:PMC2950312
- Sethupathy P, Collins FS. MicroRNA target site polymorphisms and human disease. *Trends in genetics*. 2008 Oct 1;24(10):489-97. <https://doi.org/10.1016/j.tig.2008.07.004> PMID:18778868