Psychiatria Danubina, 2020; Vol. 32, No. 1, pp 130-132

Letter to Editor

HETEROZYGOSITY AT 9 CODIS STR LOCI AND RISK OF SCHIZOPHRENIA

Zhilin Luan[1,2,*], Yunxin Zhai[3,*], Di Dai[4], Chunqing Liu[1], Qiaoling Chen[5], Weijian Yu[6] & Hui Liu[1]

[1]*Faculty of Medical Laboratory, Dalian Medical University, Dalian, P.R. China*

[2]*Advanced Institute for Medical Sciences, Dalian Medical University, Dalian, P.R. China*

[3]*Department of Neurology, Shenyang Brain Institute, Shenyang First People's Hospital, Shenyang, P.R. China*

[4]*Entry-exit Inspection & Quarantine of Liaoning Province, Dalian, P.R. China*

[5]*Dalian Seventh Hospital, Dalian, P.R. China*

[6]*Dalian Blood Center, Dalian, P.R. China*

**These authors contributed equally*

* * * * *

Schizophrenia is a complex mental disease affecting approximately 1% of the population worldwide (Tandon et al. 2008). It has a strong genetic component, which has been consistently indicated by genetic epidemiological studies (International Schizophrenia et al. 2009). The heritability of schizophrenia has been estimated between 66% and 85%. The inheritance pattern of schizophrenia suggests a non-Mendelian mode of transmission and makes simple major gene dependency impossible. Thus, the risk for schizophrenia probably results from mutual effect of various genetic loci with small contribution. Identifying potential susceptibility genes for schizophrenia is conductive to the pathogenesis and pathophysiology of schizophrenia.

Microsatellites or short tandem repeats (STR) consist of tandemly repeated DNA units ranging from two to six nucleotides, scattered through the genome with high heterozygosity and evolutionary information (Edwards et al. 1992). They are both powerful tools for human genetics study with highly polymorphic variable number of tandem repeats. In this study, we chose 9 CODIS STR loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820) from Combined DNA Index System (CODIS), the United States national DNA database created and maintained by the Federal Bureau of Investigation. These 9 loci have been reported with reliable assay for genotype by using commercial multiple kits, which facilitates unambiguous allele designation and consistency (Perez-Lezaun et al. 1997). To verify the association between these CODIS microsatellite alleles and schizophrenia in the Chinese Han population, an case-control study was performed in a sample-set including 150 diagnosed schizophrenia patients with 300 chromosome sets (78 males and 72 females, 28.9±8.9 years old) and 100 controls (50 males and 50 females, 37±8.2 years old). The patients were recruited from the Dalian Seventh Hospital (psychiatric hospital) in Dalian, China. The controls were healthy blood donors selected by a simple non-structured interview to exclude individuals with mental health problem or neurological diseases. Approval for the current study was obtained from the Ethical Committee of Dalian Medical University. The patients and their relatives signed informed consent before volunteering in this study.

DNA was extracted from 3 ml of peripheral blood by the Chelex100 procedure (Walsh et al. 2013). The PCR analysis was performed by using the AmpFISTR Profiler plus PCR Amplification Kit (Perkin Elmer, Foster City, CA, USA) under conditions recommended by the manufacturer in a reaction volume of 50 µl, using a 9600 Perkin Elmer thermal cycler. Amplification products (1.5 µl) were added to 10 µl formamide and 1 µl of an internal size standard (Genescan-500 ROX, Applied Biosystems). Genescan Analysis 2.1 software (Applied Biosystems) was applied to determine fragment sizes. Allele identification was achieved by comparison of the amplified fragments with the allelic ladders included in the reagent set and alleles were labeled according to the international nomenclature using the Genotyper Software package (Perkin Elmer). Two types of STRs are normally identifiable in one subject from one STR locus (heterozygote). Two alleles were considered identical (homozygote) if only one type of STR was found in a locus. Allele frequencies were calculated. Observed heterozygosity values, heterozygote / (heterozygote + homozygote), were compared between patients and control subjects using the Pearson Chi-Square Test with the SPSS statistical software package.

The 9 CODIS STR loci were genotyped, and the number of alleles observed, data of the repeated motifs, and chromosomal location for these 9 STR loci were obtained. For each locus, Hardy-Weinberg equilibrium was tested by comparing the observed genotype numbers with those expected under the hypothesis of panmixia (Hardy-Weinberg equation). No deviation from the Hardy-Weinberg equilibrium were observed in control group. The heterozygosity of these 9 STR loci was investigated, and the data showed that heterozygosity of D21S11 was lower in schizophrenia group than in control group ($p<0.05$). No other statistical difference was observed in other loci between the two groups (Table 1).

Table 1. Number of alleles observed and basic data for 9 CODIS STR loci

Locus	Chromosomal Location	GenBank Accession	Number of Alleles		H-W Equation (*p* value) Control	Heterozygosity (%)		*p* Value
			Reported	Observed		Schizophrenia	Control	
D3S1358	3p	NW_105591	10	8	>0.50	74.0	68.0	0.319
vWA	12p13	AH005287	9	9	>0.75	80.0	79.0	0.874
FGA	4p28	AY749636	26	16	>0.9	81.3	90.0	0.073
D8S1179	8q24.1-24.2	NT_008046	11	9	>0.97	76.0	82.0	0.276
D21S11	21p11.1	M84567	20	14	>0.97	76.7	94.0	<0.001
D18S51	18q21.3	X91254	21	17	>0.75	79.3	82.0	0.630
D5S818	5q22	NT_034772	10	9	>0.75	79.3	79.0	0.949
D13S317	13q	NT_086804	9	8	>0.5	76.0	84.0	0.153
D7S820	7q11.21-22	NT_079595	9	6	>0.75	76.0	79.0	0.646

The difference in terms of age at onset of schizophrenia between the patients carrying homozygosity or heterozygosity of these 9 STR loci was also analyzed. Significant differences of the patients in mean age at onset for D21S11 was observed. Significant increase showed in mean age at onset with D21S11 heterozygosity ($p<0.001$).

Genetic markers, such as SNPs (single nucleotide polymorphisms) and microsatellites (or short tandem repeats, STRs), are high-resolution molecular tools in gene mapping related to diseases and useful in genetic studies due to their quantity and stable inheritance over generations. We focused on 9 CODIS STR polymorphism loci, and the esults of these 9 loci were achieved by AmpFISTR Profiler Plus kit, and all loci were highly polymorphic as reported in literature (Wang et al. 2003). According to the statistical tests, no deviation from Hardy-Weinberg equilibrium was detected in control group. These implied dependable date obtained in our study. For complex polygenic disorders, homozygosity was often associated with disease, while the heterozygosity not (Wakitani et al. 1998). Thus, the heterozygosity probably decreases in correlative loci and strong signals may be obtained. The heterozygosity value of D21S11 was markedly lower in schizophrenia group reaching statistical significance ($p<0.05$) than in control group. D21S11 also showed significant difference in terms of age at onset of schizophrenia between patients with heterozygosity and homozygosity. The lower allele frequency and higher age at onset for D21S11 in schizophrenia group cross-validated the importance of the genetic region harbored D21S11 in Chromosome 21p11.1, indicating that schizophrenia susceptibility genes may be observed in this gene region. STR DNA sequences have been previously applied for hereditary linkage balance analysis in studies for locating schizophrenia genes, and some candidate genes have been successfully been screened out (Kaufmann et al. 1998, Hattori et al. 2001). However hereditary research on schizophrenia with specific CODIS STR on heterozygosity is not available yet. The investigations on possible contribution and potential role of these microsatellites in psychiatry may represent an example of different approaches required to validate genetic targets in the "post-genomic era". The efforts to increase integration between genetics, epidemiology and clinical trials through virtual data among laboratories may lead to genetically informative designs not only identify susceptibility genes but also contribute to analyze genetic features of complex hereditary diseases for instance as hybrid vigor. Nevertheless, more extensive studies are required further for resolving the schizophrenia genetics, prevention, prognosis and therapy.

Acknowledgements:

We are grateful to all the subjects participating in the current study, and extend our gratitude to the psychiatrists involved in diagnosing the patients. We would like to thank Nan Xiao and Guang Yang for assisting in completing the experiments.

Source of Funding:
This work was funded by National Natural Science Foundation of China (39670273 and 81601174).

Conflict of interest: None to declare.

References

1. *Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R: Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 1992; 12:241-253*
2. *Hattori E, Ebihara M, Yamada K, Ohba H, Shibuya H, Yoshikawa T: Identification of a compound short tandem repeat stretch in the 5'-upstream region of the cholecystokinin gene, and its association with panic disorder but not with schizophrenia. Mol Psychiatry 2001; 6:465-470*
3. *International Schizophrenia C, Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC et al.: Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 2009; 460:748-752*
4. *Kaufmann CA, Suarez B, Malaspina D, Pepple J, Svrakic D, Markel PD et al.: NIMH Genetics Initiative Millenium Schizophrenia Consortium: linkage analysis of African-American pedigrees. Am J Med Genet 1998; 81:282-289*

5. *Perez-Lezaun A, Calafell F, Mateu E, Comas D, Bosch E, Bertranpetit J: Allele frequencies for 20 micro-satellites in a worldwide population survey. Hum Hered 1997; 47:189-196*
6. *Tandon R, Keshavan MS, Nasrallah HA: Schizophrenia, "just the facts" what we know in 2008. 2. Epidemiology and etiology. Schizophr Res 2008; 102:1-18*
7. *Wakitani S, Imoto K, Murata N, Toda Y, Ogawa R, Ochi T: The homozygote of HLA-DRB1*0901, not its heterozygote, is associated with rheumatoid arthritis in Japanese. Scand J Rheumatol 1998; 27:381-382*
8. *Walsh PS, Metzger DA, Higuchi R: Chelex 100 as a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material. Biotechniques 2013; 54:134-139*
9. *Wang W, Jia H, Wang Q, Cai Z, Wei L, Wang D et al.: STR polymorphisms of "forensic loci" in the northern Han Chinese population. J Hum Genet 2003; 48:337-341*

Correspondence:
Professor Hui Liu, MD
Faculty of Medical Laboratory, Dalian Medical University
Dalian 116 044, China
E-mail: liuhui60@sina.com