The Allele Frequency Variation of Autosomal 19 Short Tandem Repeat (STR) Loci in Drug Abuser: A Pilot Study

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Research article

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Abstract

Backgrounds: Substance use disorders (SUDs) refers to drug abuse of 10 separate classes impacting seriously mental health.

Objective: This study aims to explore the genetic polymorphism of the 19 short tandem repeat (STR) loci (D19S433, D5S818, D21S11, D18S51, D6S1043, D3S1358, D13S317, D7S820, D16S539, CSF1PO, Penta D, VWA, D8S1179, TPOX, Penta E, TH01, D12S391, D2S1338, and FGA) and the risk genetic factors of substance use disorders(SUDs).

Methods: The capillary electrophoresis was applied to analyze 19 STR loci in 156 drug abusers. The data of 6,134 healthy people in a previous study were applied to contrast as the control group. The differences in the frequency of the gene were analyzed by Chi-square test.

Results: The gene distributions of 19 STR in drug addicts were consistent with hereditary rules. The frequency of vWA, FGA, Penta E was significantly different between the drug abuser and the control group ($P<0.05$).

Conclusion: The 19 STR loci might provide highly informative polymorphic data for addicted genetic studies. Furthermore, there might be a predisposing gene for the drug abuser.

Introduction

Substance use disorders (SUDs) is described as the wild range of the disorder after taking 10 separate classes of drugs (alcohol, caffeine, cannabis, hallucinogens, inhalants, opioids, sedatives, hypnotics and anxiolytics, stimulants, tobacco) (DSM-5). It potentially led to abuser's mental health disorder, because brain's function in reward, cognitive and stress pathways [1], as well as personality traits[2] were affected by SUD. More importantly, fathers suffering from SUDs had a great impact on the mental health of their offspring[3]. In addition, the heritability was reported as 54% among drug addicts according to polygenic scores(PGSs) [4, 5]. Therefore, the individual identification and gene polymorphism of SUDs has been the key points in recent years' studies.

The premise of genetic susceptibility in persons with SUDs has been addressed in some literatures. Molecular genetics[6], transcription, and gene expression in the brain concerned with SUDs had been put forward by 1998 [7]. The gene transcription and expression variation was related with the amount of heroin use every day [8] and early age and recurrence[9]. It suggested the probability of hereditary susceptibility of substance use. Moreover, some genetic mutations of identification loci had been found to lead to inherited substance use vulnerability according to the studies of substance abuse father or his offspring[10].

As a method of genetic research, Short tandem repeat(STR) has variety and wide distribution, low mutation rate[11], high degree of polymorphism, allele, ability to identify (on each STR loci, several or

dozens of allelic fragments), the characteristics of high sensitivity for mutation. Notably, the 19 STR includes 13 CODIS loci (CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11) and 6 STR loci in Chinese (D19S433, Penta E, D2S1338, Penta D, D6S1043 and D12S391), which were confirmed by the DNA Analysis Science Working Group (SWGDAM) [12].

The 19 STR has the higher excluding probability of paternity (probability of exclusion, PE) and probability of discrimination power (DP) in various populations [10, 13–18], as well as the polymorphism test of 19 STR is economical and convenient. Especially, STR is widely used in genetic mapping, species, forensic identification, kinship analysis (especially the paternity test), even disease gene location and polymorphism and genetic disease diagnosis, susceptibility genes exploration, such as the genetic risk of gastric cancer [19]. the Penta D loci might be associated with the aggressive behaviors of schizophrenia, and its Allele 10 and genotypes 10–12 may confer a risk for schizophrenia [20]. However, there were few reports on the application of STR in SUD yet.

Consequently, we come up a hypothesis that the 19 STR loci would not only has personal identification and paternity identification, but also some significant mutation and autosomal gene STR loci polymorphism in addicts. We used blood samples from drug abusers in a certain drug treatment center in China to detect gene polymorphism of the 19 STR loci, and compare it with the reported gene polymorphism in normal Han population[21]. Some individual identification data could be furtherly understood through the characteristics of gene polymorphism in addicts in the current study, on the other hand, the predisposing gene polymorphism of SUD was explored.

Methodology

Population

Blood samples were collected from 156 unrelated individuals with drug abuse (110 males and 46 females) among the Han population in southwest, China. An informed consent was obtained from each individual for the blood collection.

DNA extraction and PCR amplification

An AGCU-EX20 STR kit (AGCU ScienTech Incorporation) was used to covers 19 STR loci and one gender loci, which is commonly selected as the polymorphism test for human genomic DNA. This kit adopts multi-color fluorescent labeling and multiple compound amplification, which can be directly amplified without lifting, template extraction and purification. Blood stains or saliva stains can be directly amplified using a filter paper or FTA card as the carrier (<http://www.agcu.cn/cpzx/fyjccp/crsxl/350773.shtml>).

Genotyping and quality control

The PCR products were detected by capillary electrophoresis in an ABI 3130xl Genetic Analyzer (Applied Biosystems) using the CC5 ILS 500 size standard and reference allelic ladder provided along with the AGCU-EX20 STR kit. Data analysis and genotyping were automatically performed using the GeneMapper ID v3.2 software (ABI, Forster City, USA). The internal controls (negative control and the 2800 M DNA positive control) were genotyped along with each batch of samples to ensure the reproducibility and accuracy of the results.

Data analysis

The allelic frequencies, power of discrimination, power of exclusion, polymorphism information content and typical paternity index were calculated using the PowerStatsV12 software (<http://www.promega.com/geneticidtools/>). The *P*-values of the exact test of Hardy-Weinberg's equilibrium, expected heterozygosity and observed heterozygosity were calculated using the Arlequin software (<http://cmpg.unibe.ch/software/arlequin3.5>).

Results

The statistical parameters of the 19 STR loci in the drug abuser and Han population were presented in this study. In the drug abuser, the expected heterozygosity (HE) ranged from 0.598 (TH01) to 0.907 (Penta E), and the observed heterozygosity (HO) ranged from 0.571 (TH01) to 0.910 (Penta E), while the power of discrimination (PD) varied between 0.776 (TPOX) and 0.971 (Penta E), and the combined power of discrimination for the 19 STR loci was over 0.999 999 999 999 999 999 999 999 999. The power of exclusion (PE) varied between 0.301 (TH01) and 0.818 (Penta E), and the combined excluding probability of paternity for the 19 loci was over 0.999 999 999 999 999 999 999 999 999. The polymorphism information content (PIC) varied between 0.550 (TPOX) and 0.895 (Penta E). The typical paternity index (TPI) varied between 1.353 (TPOX) and 5.75 (Penta E). Among all the studied loci, no significant deviations from the Hardy-Weinberg expectations were observed after the Bonferroni correction ($P > 0.0026$).

The differences in allelic frequencies between the drug abuser and Han population are presented in Table 1–3. The three loci (VWA, FGA, Penta E) presented a significant difference ($P < 0.05$, bilateral test) between these two populations. However, no significant difference was found in each shared allele in the three loci between the drug abuser and Han population yet.

Table 1

Comparison of allele frequencies at the VWA locus between subjects and controls (frequency, %).

Allele	Subjects(n = 156)	Controls(n = 6134)	χ^2	P-value	Odds ratio	95%CI	
						low	high
14	39(25)	1627(26.52)	.182	.670	.923	.640	1.333
15	4(2.564)	185(3.023)	.107	.744	.846	.310	2.308
16	30(19.231)	1060(17.286)	.404	.525	1.140	.761	1.706
17	41(25.962)	1438(23.436)	.682	.409	1.164	.811	1.671
18	24(15.705)	1171(19.084)	1.358	.244	.771	.497	1.196
19	15(9.615)	535(8.73)	.152	.696	1.113	.649	1.910
20	3(1.923)	101(1.65)	.072	.789	1.171	.367	3.734
χ^2	1121.018						
P-value	< 0.001						
Note: Figures in brackets indicate frequencies. Allele frequencies < 1% in both subjects and controls were removed. The level of statistical significance for these pairwise tests was set at 0.05/7 = 0.007							

Table 2

Comparison of allele frequencies at the FGA locus between subjects and controls (frequency, %).

Allele	Subjects(n = 156)	Controls(n = 6134)	χ^2	P-value	Odds ratio	95%CI	
						low	high
18	4(2.244)	177(2.88)	.056	.813	.886	.325	2.417
19	10(6.09)	335(5.464)	.264	.607	1.186	.619	2.271
20	6(3.846)	304(4.95)	.400	.527	.767	.336	1.749
21	17(10.897)	739(12.041)	.190	.663	.893	.536	1.486
22	32(20.513)	1128(18.384)	.456	.499	1.145	.772	1.698
23	35(22.436)	1344(21.907)	.025	.876	1.031	.704	1.509
24	22(14.423)	1082(17.644)	1.315	.251	.767	.486	1.209
25	18(11.538)	638(10.397)	.211	.646	1.124	.683	1.849
26	9(5.449)	287(4.681)	.403	.525	1.247	.630	2.470
27	4(2.244)	74(1.201)	2.290	.130	2.155	.778	5.970
χ^2	65.746						
P-value	< 0.001						
Note: Figures in brackets indicate frequencies. Allele frequencies < 1% in both subjects and controls were removed. The level of statistical significance for these pairwise tests was set at 0.05/10 = 0.005							

Table 3

Comparison of allele frequencies at the Penta E locus between subjects and controls (frequency, %).

Allele	Subjects(n = 156)	Controls(n = 6134)	χ^2	P-value	Odds ratio	95%CI	
						low	high
5	6(3.526)	325(5.302)	.644	.422	.715	.314	1.629
9	1(0.321)	71(1.165)	.359	.549	.551	.076	3.991
10	4(2.244)	245(4.001)	.818	.366	.633	.232	1.721
11	32(20.513)	961(15.67)	2.687	.101	1.389	.936	2.061
12	18(11.538)	689(11.236)	.014	.905	1.031	.627	1.695
13	9(5.449)	347(5.654)	.004	.952	1.021	.516	2.019
14	13(8.333)	500(8.147)	.007	.935	1.024	.576	1.821
15	11(6.731)	573(9.348)	.947	.330	.736	.396	1.367
16	13(8.013)	487(7.939)	.032	.857	1.054	.593	1.874
17	11(7.051)	460(7.496)	.044	.834	.936	.503	1.740
18	12(7.372)	419(6.837)	.177	.674	1.137	.626	2.065
19	10(6.09)	331(5.392)	.305	.581	1.201	.627	2.301
20	6(3.526)	310(5.049)	.465	.495	.751	.330	1.713
21	6(3.526)	165(2.691)	.769	.381	1.447	.631	3.320
22	4(2.564)	100(1.635)	.816	.366	1.588	.577	4.370
23	1(0.641)	68(1.102)	.307	.580	.576	.079	4.171
25	2(1.282)	12(0.199)	8.085	.004	6.626	1.470	29.856
χ^2	144.730						
P-value	< 0.001						
Note: Figures in brackets indicate frequencies. Allele frequencies < 1% in both subjects and controls were removed. The level of statistical significance for these pairwise tests was set at $0.05/17 = 0.003$							

Discussion

In the present study, similarly with previous conclusion among different region [11, 22, 23] and ethnic population [24] in literatures, the 19 STR gene seat distribution in the drug abuser conforms to the genetic law, and so it can be applied for personal and paternity identification among drug addicts.

Drug use, preferences and gene expression may link to some genetic variations (Gozen et al., 2016; Montesinos, Gil, & Guerri, 2017), and the mutations were frequent in the paternal reproductive system—specially the addicted father or their offspring [10, 25]. In the 19 STR loci, VWA, FGA, Penta E locus were identified significant associations with drug abuser among Chinese Han ethnicity in our study. Because Penta E has the highest polymorphism information content (PIC), discrimination ability (PD) [26] and the most prone to mutation[27]. As well as, FGA and VWA sites are the most prone to change in size ($p = 0.002$)[28]. Genetic variations may be just happened in abuse liability loci or other important loci, and then, produce some epigenetic variation, individual identification data, and so on, as the susceptible factors of drug abuse (Raabe, Mathies, Davies, & Bettinger, 2014). Therefore, the associated gene polymorphism in drug abuse is necessarily worth of further explorations to provide some strategies for early prevention and treatment of SUD.

Previous research suggested that different classification of drug addiction may have a different biological basis. The reduction in excitatory neurotransmitters in the Nucleus Accumbens (NAc) may separately predicted the effects of repeated cocaine exposure[29] and changed the role of central proinflammatory immune signaling[30]. Alcohol consumption/addiction could be modulated by blocking the Toll-like 4 (TLR4) response.

Additionally, internal environment changes may lead to sequence variation and increase the heterozygosity of STR loci and become a possible mechanism of drug abuse [31, 32], namely, increased heterozygosity of some alleles may be a risk factor for susceptibility and recurrence [33], while the homozygous genotype of the gene reduces the risk of addiction [34, 35].

However, the risk heterozygosity and frequency of loci need to be further explored in future through increasing the cases, due to the small sample size in this study, as well as the biological risk of various drug addiction.

Limitation

The occurrence and development of SUDs is a complex process that involves multiple genes and many factors. The STR loci associated with SUDs, the difference in gender and drug types were yet not very clear in the present study because of the sample size of pilot study. However, it is greatly worth of further research after collecting a large amount of data.

Conclusion

The 19 STR loci might provide highly informative polymorphic data for addicts, and there might be predisposing genes of SUDs.

Declarations

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

Ethical approval was granted by the medical faculty of the North Sichuan medical University, China (local number 2018JY0497).

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