Allele Frequency of the Short Tandem Repeat(STR) Loci FFv Triplex(F13A01, FESFPS, vWA) Gene by Polymerase Chain Reaction in the Korean Population

Chang-Lyuk Yoon, D.D.S., M.S.D., Ph.D., **Geun-Chun Ryu**, D.D.S., M.S.D.

Dept. of Oral Diagnosis and Forensic Odontology, Institute of Forensic Odontology
College of Dentistry, Chosun University

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I. INTRODUCTION

Since restriction fragment length polymorphism(RFLP) was discovered in the mid 1980's, the concept of the deoxyribonucleic acid(DNA) fingerprint was introduced to forensic individual identification of mass disaster and paternity testing.[1,18,19] Individual identification techniques in the 1990's are dominated by the development and rapid establishment of PCR-amplifiable short tandem repeats(STRs) or microsatellites [6,21,32]. Typing polymorphic loci at the DNA level has become a routine in the paternity and identity testing fields.

This has prompted the investigation of various human tissues as potential sources of genetic evidentiary material[16,43].

Originally, highly polymorphic variable number of tandem repeats(VNTR) loci were characterized by restriction fragment length polymorphism(RFLP) analysis. A subgroup of these VNTR loci is the STR loci, which are ideal for use in linkage mapping and DNA fingerprinting. The STR systems are presented according to their chromosomal location, repeat unit, product size, primer sequences and fluorophore tags. The markers - short sequences (2-5bp) that are repeated in a tandem fashion - are abundant, highly polymorphic and uniformly distributed throughout the human genome. Moreover, STR loci, which are generally less than 350 base pairs in length, are amenable to amplication by the polymerase chain reaction(PCR). So this technique is particularly

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useful in the analysis of highly degraded samples or minute stains, where the initial quantity or quality of DNA is unsuitable other DNA profiling methods. STR loci also have an advantage to detect multiple loci at the same time, it can be lowered the possibility that some genotype is accorded with other's one accidentally[6,23,27,31,45,47].

But, STR loci has a demerit that it has low contribution about individual identification when it is used alone in gene analysis, because it has small number of allele, low heterozygosity and high probability which has a same genotypes between individuals. By analysis of many STR loci at the same time, such a problem can be solved. Therefore it is a essential prerequisite to get the gene distribution because the distribution of allele is different according to ethnic group[3,13,32].

The F13AO1 locus[10,17,29,35,37,39,44] one of STR locus, is [AAAG]n polymorphism and present within the 5′ untranslated region of the human coagulation factor XIII A subunit gene on chromosome 6p24-p25 and has 3.2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 alleles. These alleles vary in length from 181 through 231 base pairs. Human c-fes/fps proto-oncogene (FESFPS) [2,8,11,24,33,44] is [AAAT]n polymorphism an located on the chromosome 15q25-qter and has 7, 8, 9, 10, 11, 12, 13, 14 alleles. Length is from 222 through 250 base pairs. Human von Willebrant factor gene(vWA)[4,5,8,9,12,17,20,22,25,26,28,34,41] is [AGAT]n polymorphism and located on the chromosome 12p12-pter and has 13, 14, 15, 16, 17, 18, 19, 20 alleles. Length is from 139 through 167 base pair.

In this study, the distribution of allele and genotype of above Triplex(F13A01, FESFPS, vWA) STR loci in Korean population was obtained to use the basic data for the individual identification and paternity testing

II. MATERIALS AND METHODS

Subjects

1.5~2.0㎖ of blood obtained from 201 unrelated Koreans residing in the Kwang-ju city.

DNA extraction

Genomic DNA was prepared from white blood cell by salt precipitation of protein and treated by 100% ethanol precipitation conventionally. The purified DNA quality and quantity were estimated with UV visible spectrophotometer. Purity of DNA was determined by evaluatly the A260/A280 ratios.

DNA amplification

It was used 1㎛ each primer for F13A01 locus(5′-GAGGTTGCACTCCAGCCTTTGCA A-3′ ; 5′-TTCTGAATCATCCCAGAGCCA-CA3′), FESFPS locus(5′-GGGATTTCCCT-ATGGATTGG-3′, 5′-ATGCCATGCAGATT-AGAAA-3′), vWA locus(5′-CCC TAG TGG ATG ATA AGA ATA ATC-3′, 5′-GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG-3′), 2mM each dNTP(Promega®), 0.01 unit of Taq polymerase(Promega), 500mM KCl, 100mM Tris-HCl, Ph 9.0, 1% Triton X-100 and 15mM $MgCl_2$ in 25㎕ final reaction volume to amplify 20~50ng of genomic DNA. One drop of mineral oil was overlayed the reaction mixtures in thin walled tubes.

The amplification parameters were 96℃ for 2min, 1cycle; 94℃ for 60 sec, 64℃ for 60sec, 7 0℃ for 90sec, 10cycles; 90℃ for 60 sec, 64℃ for 60sec, 70℃ for 90sec, 20cycles, 60℃ for 3min, 1cycle in a GeneAmp PCR System 480 Thermal cycler.(Perkin Elmer Co.)

Electrophoresis of DNA amplification products and stain.

Amplified FFv Triplex allelic profiles were analyzed on 4% denaturing polyacrylamide gel containing 7M urea after mixed 2.5㎖ of 2X loading solution(10mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% Xylene cyanol FF, Promega®). Electrophoresis was conducted for 1 half hour in 1X TBE buffer, FFv Triplex alleles resolved on vertical PAGE gel were visualized after silver staining. The silver staing procedure was as follows;

Statistical evaluation

Evaluation of the Hardy-Weinberg equilibrium was based on comparisons of observed and expected genotypes, using chi-square test. The power of discrimination(PD) and allelic diversity value(h) were calculated using following formula;

$PD = 1-\Sigma Pj^2$ (Pj : genotype frequency)

$h = (1-\Sigma Xi^2)(N/N-1)$

(Xi : allele frequency, N : sample size)

Step	Solution	Time
a. fixing	fix/stop solution	20 min
b. washing	deionized H_2O	2 min ×3
c. staining	staining solution	30 min
d. washing	deionized H_2O	10 sec
e. developing	developer solution(4-10℃)	5 min
f. stoping	fix/stop solution	5 min
g. washing	deionized H_2O	2 min
h. dry		

Ⅲ. RESULTS

After polyacrylamide gel electrophoresis of PCR products, the isolated bands which had individual allelic gene were observed by silver staining(Figure 1.) Allelic genes of F13A01 locus were observed of 3.2 4, 5, 6, 16, but 7, 8, 9, 10, 11, 12, 13, 14, 15 were not detected. The most frequent allele was 6. Allelic diversity value was 0.641(Table 1). The total 12 genotypes were founded in F13A01 locus. Most common genotype was 3.2-6. Power of discrimination value(PD) was 0.814 and heterozygosity was 60.7%(Table 2).

Table 1. Frequency of F13A01 alleles

allele	Number of alleles observed	allelic frequency
3.2	138	0.343
4	46	0.114
5	25	0.062
6	191	0.475
7	0	0.000
8	0	0.000
9	0	0.000
10	0	0.000
11	0	0.000
12	0	0.000
13	0	0.000
14	0	0.000
15	0	0.000
16	2	0.005
all	402	1.000
h	0.641	

h ; allelic diversity value = $(1-\Sigma Xi^2)$ $(N/N-1)$

Xi ; allele frequency, N ; sample size = 201

Table 2. Distribution of F13A01 genotypes

Genotypes	Number observed	Frequency
3.2-3.2	28	0.139
3.2-4	13	0.065
3.2-5	12	0.060
3.2-6	56	0.279
3.2-16	1	0.005
4-4	1	0.005
4-5	3	0.015
4-6	28	0.139
4-16	0	0.000
5-5	1	0.005
5-6	8	0.040
5-16	0	0.000
6-6	49	0.244
6-16	1	0.005
homozygote	79	0.393
heterozygote	122	0.607
total sample	201	1.000
PD	0.814	

PD ; power of discrimination value
$=1-\Sigma Pj^2$, Pj ; genotype frequency

Table 3. Frequency of FESFPS alleles

allele	Number of alleles observed	allelic frequency
7	1	0.002
8	1	0.002
9	2	0.005
10	13	0.032
11	204	0.507
12	106	0.264
13	72	0.179
14	3	0.007
all	402	1.000
h	0.641	

h ; allelic diversity value = $(1-\Sigma Xi^2)$ $(N/N-1)$
Xi ; allele frequency, N ; sample size = 201

Allelic genes of FESFPS locus were observed of 7, 8, 9, 10, 11, 12, 13, 14. The most frequent allele was 11. Allelic diversity value was 0.641(Table 3). The total 15 genotypes were founded in FESFPS locus. Most common genotype was 11-12. Power of discrimination value(PD) was 0.804 and heterozygosity was 66.7%(Table 4).

Allelic genes of vWA locus were observed of 11, 12, 14, 15, 16, 17, 18, 19, 20 and not of 13, 21. The most frequent allele was 17. Allelic diversity value was 0.799(Table 5). The total 23 genotypes were founded in vWA locus. Most common genotype was 14-17. Power of discrimination value(PD) was 0.924 and heterozygosity was 80.1%(Table 6).

Table 4. Distribution of FESFPS genotypes

Genotypes	Number observed	Frequency
7-8	1	0.005
9-11	1	0.005
9-13	1	0.005
10-11	8	0.040
10-12	2	0.010
10-13	2	0.010
10-14	1	0.005
11-11	48	0.239
11-12	58	0.288
11-13	41	0.204
12-12	14	0.070
12-13	17	0.084
12-14	1	0.005
13-13	5	0.025
13-14	1	0.005
homozygote	67	0.333
heterozygote	134	0.667
total sample	201	1.000
PD	0.804	

PD ; power of discrimination value
$=1-\Sigma Pj^2$, Pj ; genotype frequency

Table 5. Frequency of vWA alleles

allele	Number of alleles observed	allelic frequency
11	1	0.002
12	1	0.002
13	0	0
14	88	0.219
15	13	0.032
16	75	0.187
17	112	0.279
18	76	0.189
19	29	0.072
20	7	0.017
21	0	0
all	402	1.000
h	0.799	

h ; allelic diversity value = $(1-\Sigma Xi^2)$ $(N/N-1)$
Xi ; allele frequency, N ; sample size = 201

IV. DISCUSSION

Variable number of tandem repeat(VNTR) loci(so called minisatellite) and restriction fragment length polrmorphism(RFLP) analysis are being used as a good identifying test marker, since it contains many of alleles, high heterozygosity and high power of discrimination[18, 19]. But VNTR loci have a high molecular weight in compared with STR loci, so it is difficult to detect the VNTR loci from the forensic samples occationally , especially in degraded samples. RFLP analysis also fails to detect a large fraction of mutations and polymorphisms and has other limitations such as sensitivity, analysis time or the impossibility of typing highly degraded samples. While on the other hand, it is easy to analyze the STR loci (so called microsatellite) from the degraded one[6, 32]. Power of discrimination of STR loci are lower than

Table 6. Distribution of vWA genotypes

Genotypes	Number observed	Frequency
11-12	1	0.005
14-14	11	0.055
14-15	4	0.020
14-16	14	0.070
14-17	25	0.124
14-18	16	0.080
14-19	7	0.035
15-16	1	0.005
15-17	4	0.020
15-18	4	0.020
16-16	6	0.030
16-17	20	0.100
16-18	20	0.100
16-19	4	0.020
16-20	4	0.020
17-17	15	0.075
17-18	24	0.120
17-19	8	0.040
17-20	1	0.005
18-18	4	0.020
18-19	2	0.005
18-20	2	0.005
19-19	4	0.020
homozygote	40	0.199
heterozygote	161	0.801
total sample	201	1.000
PD	0.924	

PD ; power of discrimination value
$=1-\Sigma Pj^2$, Pj ; genotype frequency

VNTR loci. Therefore, for increasing the power of discrimination, it is recommanded to examine many of the VNTR loci and STR loci at the same time.

Although the sensitivity of amplified fragment length polrmorphism(AmpFLPs) is suitable for

analysing forensic samples, most VNTR loci alleles are too long to be analysed as AmpFLPs; for example, D1S8(MS32)[46] on chromosome 1 consists of a tandem array of 29 bp repeat units, the array varying from 0.3 to 20 kb in length. AmpFLPs from degraded DNA samples may fail to obtain target bands[40]. With the advent of PCR, STR marker polymorphisms replaced VNTRs as the markers of choice. It is estimated that the human genome contains approximately 500,000 STRs(6,000 to 10,000 trimeric and tetrameric repeats). These abundant repeats may be detected using the PCR and polyacrylamide gel electrophoresis. Loci, such as TH01[8, 9, 38], CD4[7], CSF1PO[42], D3S1358[42], F13B[30], HUMAC-TBP2[34, 48], HPRT[14], HUMTPOX[29], CRYG1[15], CYP19[36], LPL[49], have been typed by amplification of DNA by PCR.

Generally forensic samples are mostly corrupted and only a small amount of modified DNA can amplified, the analysis of STR loci by PCR method is indispensable and VNTR loci are not suitable. High ploymorphic STR loci contain 2-5 repetitive base pairs and easy to interpret the type of allele. [6] STR loci have individually different in allele frequency of genotype, heterozygosity and genetic distance, so it have been using for individual identification.

In individual identification and parentage testing, the selection of locus with high heterozygosity is essential. Because forensic samples are mostly corrupted and only a small amount of modified DNA can amplified, the analysis of STR locus by PCR method is very efficient for individual identification. High ploymorphic STR locus is a good marker and contains 2-5 repetitive base pairs and easy to interpret the type of allele. STR loci have individually different in allele frequency of genotype, heterozygosity and genetic distance. so it used for individual identification.

In this study, the allele and genotype of Triplex(F13A01, FESFPS, vWA) STR loci from 201 Korean population was confirmed. A quantitative comparison of allele frequencies for the these genes between this study and Japanese, Chinese population data are showed that no significant differences were observed. This fact showed that genetic distance between two ethnic groups was very close. On the other hand, comparison with American ethnic group and Europian caucacian observed alleles revealed different to Korean[2,4,5,8,17,18,24,25,29,33,39,44].

Authors expected obvious difference of genetic diversity between Oriental and Westerner, and it was verified by this study. Among the American, Hungarian, Chinese and Korean population, some difference was observed Korean heterozygosity of Triplex (F13A01, FESFPS, vWA) STR loci also was different to caucasians' one. PD of Triplex (F13A01, FESFPS, vWA) STR loci were 0.814, 0.804, 0.924. It suggested that several STR loci should be detected simultaneously for accurate individual identification.

Allele frequency is the degree of expression of allele from certain locus, and this is the basic index of measure the genetic diversity of genetic group. Individual identification can be performed as analysis of individual genomic variation by using of allelic frequency. But it must be a prerequisitive condition before identification to reveal whether there is a reliance of allelic frequency of certain locus in genetic group, a similar frequency of Hardy-Weinberg Equilibrium in the next generation. For obtaining this object, genetic analysis of numerous sample size is needed. Results are based on Hardy-Weinberg equilibrium. It was verified by chi-square test to examine the significance about observed value of genotype and expected value of frequency of allele.

STR loci contain many of alleles and high heterozygosity. But VNTR loci have a high molecular size, so called Minisatellite, and are also being used as a good paternity test marke. But, it is difficult to detect the VNTR loci occationally, especially in degraded samples. While on the other hand, is easy to detect the STR locus (so called microsatellite) from the degraded one. Power of discrimination of STR locus is lower than VNTR locus. Therefore, for increasing the power of discrimination, it is recommanded to examine many of the VNTR locus and STR locus at the same time.

These 3 loci were detected as triplex at the same time. Authors tried to detection vWA, F13A01, FES/FPS triplex STR amplification system. It was not difficult to detect from the fresh blood samples. But, it evokes difficult to amplify from some forensic samples, e.g. dried blood, tooth. In order to obtain approximately even signal intensities for all loci within the triplex, specific primer concentrations must be adjusted. The optimal relative primer concentrations will depend upon the method of product detection. It is essential that any new batch of primer, which has been re-synthesized or newly labelled and pruified, is first checked to ensure that it preforms to the required specification within the triplex reaction. The potential requirement to make slight adjustments in relative primer concentrations when employing new batches of primers makes it important to know how variation in primer concentration for one locus will affect the amplification efficiency of the other 2 loci.

Singleplex was well detected compared with multiplex. The main reason for using multiplex is to speed the progress. But, multiplex reactions are a compromise of ideal conditions and loss of efficiency of amplication may result[43]. The use of multiplex and singleplex systems are not mutually exclusive. Singleplex reactions may be required to clarify or enhance a difficult result. When analying poor quality samples the analyst should be familiar with the varied profile morphology that may be expected with forensic samples. With degraded samples or if only limited quantities of DNA of sufficient molecular weight are available the possibility allelic or locus drop-out must be considered and there may be occasions where it is beneficial to use singleplex tests to assist interpretation.

V. CONCLUSIONS

The blood was collected from the 201 unrelated Korean population in Kwang-ju. After extraction of DNA, the Triplex(F13A01, FESFPS, vWA) STR loci was amplified by PCR, and then silver staining was performed after polyacrylamide gel electrophoresis for establishing the Korean population data.

Obtained results were as follows.

(1) 5 allelic genes(3.2, 4, 5, 6, 16) in F13A01 locus were observed . The most frequent allele was 6. Allelic diversity value was 0.641.
(2) The total 12 genotypes were founded in F13A01 locus. Most common genotype was 3.2-6. Power of discrimination value(PD) was 0.814 and heterozygosity was 60.7%.
(3) Allelic genes in FESFPS locus were observed of 7, 8, 9, 10, 11, 12, 13, 14. The most frequent allele was 11. Allelic diversity value was 0.641.
(4) The total 15 genotypes were founded in FESFPS locus. Most common genotype was 11-12. Power of discrimination value(PD) was 0.804 and heterozygosity was 66.7%.
(5) Allelic genes in vWA locus were observed of 11, 12, 14, 15, 16, 17, 18, 19, 20 and not of

13, 21. The most frequent allele was 17. Allelic diversity value was 0.799.

(6) The total 23 genotypes were founded in vWA locus. Most common genotype was 14-17. Power of discrimination value(PD) was 0.924 and heterozygosity was 80.1%.

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국문초록

한국인에서 중합효소 연쇄반응법에 의한 Short Tandem Repeat(STR) 유전좌위 FFv Triplex(F13A01, FESFPS, vWA) 유전자빈도 검색

조선대학교 치과대학 구강진단·구강내과학 교실, 법의치과학 연구소

윤 창 륙 · 유 근 춘

법의학적 개인식별 및 친생자 감정시 여러 개의 single tandem repeats(STR) 유전좌위 검색이 필요하다. 그 이유는 STR 유전좌위는 대립유전자 수가 적고 이형접합도가 낮아 서로 다른 개체간에도 동일한 유전좌위를 가질 확률이 높기 때문에 개인식별에 대한 기여도가 떨어지게 된다. 따라서 여러 개의 다양한 STR 유전좌위들을 동시에 분석함으로써 우연적으로 개체간에 유전자형이 일치할 가능성을 낮추어야 감정의 신뢰성을 높일 수 있으며 이에는 각 STR 유전좌위에 대한 유전좌위의 분포가 인종별, 지역별로 달라 이에 대한 유전자 분포를 구하는 것이 선행조건이다.

이에 본 연구에서는 법의학적 개인식별 및 친자감정시 기초자료로 활용하기 위하여 서로 혈연관계가 없는 201명의 한국인 혈액에서 DNA를 추출하여 STR 유전좌위중 human coagulation factor XIII A subunit gene(F13A01 Locus), human c-fes/fps proto-oncogene(FESFPS Locus), human von Willebrand factor gene (vWA Locus)등 FFv Triplex 유전자를 중합효소반응에 의하여 동시에 증폭하고, 폴리아크릴아마이드겔을 이용한 전기영동 및 질산은 염색을 시행한 후 FFv Triplex유전자의 유전자형 및 대립유전자 빈도 등을 분석하여 다음과 같은 결과를 얻었다.

(1) F13A01유전자는 5개의 대립유전자, 12개의 유전자형을 검출하였으며, 이형접합도는 60. 7%로 나타났고 대립유전자 및 유전자빈도는 3.2, 4, 5, 6, 16 대립유전자에서 각각 0.34 3, 0.114, 0.062, 0.475, 0.005로 나타났으며, 대립유전자 7, 8, 9, 10, 11, 12, 13, 14, 15는 검출되지 않았다.
(2) F13A01 대립유전자다양성(allelic diversity value)은 0.641, 개인식별력(PD)은 0.814를 보였으며 대립유전자다양성 및 이형접합도가 다른 민족과 비교할 때 다소 낮았다.
(3) FESFPS유전자는 8개 대립유전자 모두 나타났으며, 15개의 유전자형을 검출하였으며, 이형접합도는 66.7%로 나타났고 대립유전자 및 유전자빈도는 7, 8, 9, 10, 11, 11, 12, 13, 14 대립유전자에서 각각 0.002, 0.002, 0.005, 0.032, 0.507, 0.264, 0.197, 0.007로 나타났다.
(4) FESFPS 대립유전자다양성(allelic diversity value)은 0.641, 개인식별력(PD)은 0.804를 보였다.
(5) vWA유전자는 9개의 대립유전자, 23개의 유전자형을 검출하였으며, 이형접합도는 80.1% 로 나타났고 대립유전자 및 유전자 빈도는 11, 12, 14, 15, 16, 17, 18, 19, 20 대립유전자 에서 각각 0.002, 0.002, 0.219, 0.032, 0.187, 0.279, 0.189, 0.072, 0.017로 나타났으며, 대립 유전자 13, 21는 검출되지 않았다.
(6) vWA 대립유전자다양성(allelic diversity value)은 0.799, 개인식별력(PD)은 0.924로 매우 높게 나타났다.