J Med Dent Sci 2006; 53: 135−140

Original Article

Preliminary study of the short tandem repeat region of human growth hormone gene in Japanese

Keita Sato[1,2,6], Seiko Katsumura[3], Yoh Tamaki[4,5,6], Fusao Nishikawara[5,6], Yoshiaki Nomura[5,6], Akihisa Tsurumoto[5] and Nobuhiro Hanada[6]

1) Department of Forensic Medicine and Dentistry, Tsurumi University School of Dental Medicine, Kanagawa, Japan
2) Oral Implantology and Regenerative Dental Medicine, Graduate School, Tokyo Medical and Dental University
3) The Second Department of Anatomy, Tsurumi University School of Dental Medicine, Kanagawa, Japan
4) Division of Oral Health, Department of Health Science, Kanagawa Dental College, Kanagawa, Japan
5) Department of Preventive Dentistry and Public Health, Tsurumi University School of Dental Medicine, Kanagawa, Japan
6) Department of Oral Health, National Institute of Public Health, Saitama, Japan

The human growth hormone genes (*hGH*) are located on chromosome 17q22-24. A tandem repeat linked to *hGH* is highly polymorphic and the basic structure of the repeat is reported to be $(AAAG)_3ACAA(AG)_3[(AAAG)_x(AG)_y(AAAG)_z]AAGG(AAAG)_3$ in Caucasians, but details of allelic structure have not been clarified in Japanese. The objective of this study was to provide the initial data of the *hGH* alleles and the structure of the tandem repeat in Japanese. In this study, the sequences of *hGH* alleles were analyzed in 56 healthy, unrelated Japanese. A total of 57 alleles were found and the degree of heterozygosity was 96.4%, however, 2 samples could not be sequenced. Our results provide the basic data of the *hGH* alleles in Japanese and point out the future improvement for forensic analysis of this locus.

Key words: human growth hormone gene; STR-polymorphism; forensic genetics; micro-variant; Japanese

Corresponding Author: Yoh Tamaki, DDS, PhD
2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, Japan
Tel: +81-45-581-8379 Fax: +81-45-573-9599
E-mail: pxz11337@nifty.com
Received February 3; Accepted March 17, 2006

Introduction

The human growth hormone gene (*hGH*) is located on chromosome 17q 22-24 and presents a short tandem repeat (STR) polymorphism based on an $(AAAG)_3ACAA(AG)_3[(AAAG)x\ (AG)y\ (AAAG)z]AAGG(AAAG)_3$ sequence[1-4]. Eighteen alleles have been reported in Caucasians, according to typing by electrophoresis of PCR products[4]. However, in complex mutational systems, some alleles defined by the length of DNA fragments (length alleles) may be further sub-typed by sequencing into base sequence alleles or micro-variants, thus providing a higher degree of polymorphism[5-7].

This study examined the length alleles of the *hGH* system and their base sequences using DNA samples from healthy, unrelated Japanese. The aim of this study was to clarify the structure of the *hGH* alleles and to obtain the initial data of the *hGH* system in Japanese population. This report provides the first basic data of the *hGH* alleles in Japanese and point out the future improvement for forensic analysis of this locus.

Materials and Methods

DNA samples

The study population consisted of randomly selected healthy Japanese living in the Tokyo metropolitan area. Genomic DNAs were extracted from fresh peripheral blood of 56 healthy, unrelated Japanese blood donors by a phenol/chloroform method. All subjects gave written informed consent to participate in the study. This study was approved by the ethical committee of Tsurumi University School of Dental Medicine (No. 236)

First PCR amplification and electrophoresis

The reaction mixture contained 1×buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin), 50 μM each of dNTP, 10 pM each of primers A (5'-TCCAGCCTCGGAGACAGAAT-3') and B (5'-AGTCCTTTCTCCAGAGCAGGT-3'), 1 U rTaq polymerase (Takara, Shiga, Japan), and 80 ng of DNA as the template, in a final volume of 50 μl. The PCR reaction involved an initial incubation at 94°C for 3 min, followed by 94°C 1 min, 58°C 45 sec, and 72°C 1 min, repeated for 30 cycles[8]. The primer sequences were those reported by Polymeropoulos *et al.*[4]. The PCR products were electrophoresed on 10% polyacrylamide gel (acrylamide:bis-acrylamide; 29:1, gel size; 20 cm×4 cm×0.3 mm) at 1500 V for 2 hrs. Bands were developed by silver staining, and *hGH* fragment lengths were assigned by comparison with a pBR322/MspI digested DNA marker.

Analysis of heterozygous hGH micro-variants by PCR-SSCP

Single bands detected by electrophoresis after the first PCR amplification were submitted to single-strand conformation polymorphism (PCR-SSCP)[9-10] analysis in an attempt to detect heterozygous micro-variants or sequence alleles. The conditions of gel electrophoresis used in PCR-SSCP were 10% polyacrylamide gel (acrylamide: bis-acrylamide; 99:1 containing 10% glycerin, gel size; 20 cm×4 cm×0.3 mm) in 0.5× TBE buffer (50 mM Tris, 42 mM boric acid, 0.5 mM-EDTA・2Na) at 450 V for 20 hrs at 4°C.

Second PCR and sequencing.

All bands detected by electrophoresis after the first PCR and PCR-SSCP were cut out from the gel. Each band was washed with TE buffer (10 mM Tris-HCl, 1 mM EDTA) and incubated in TE buffer at 55°C overnight to elute DNA fragments into the buffer. The eluate was extracted with phenol/chloroform to obtain the DNA fragments. The extracted DNA was amplified by *hGH*-specific primers as used in the first PCR. The second PCR products were purified by filtration with Ultra Free C3 (Millipore, Billerica, USA.). A portion of the products was submitted to both forward and reverse cycle-sequence reactions, using an Auto Cycle TM Sequencing Kit (Amersham Biosciences, Piscataway, USA) and the same primers as those used in the PCR. The sequence reaction products were analyzed by an A.L.F. DNA Sequencer (Amersham Biosciences, Piscataway, USA), and the hGH fragment length was finally confirmed from the sequence data.

Results

First amplification and electrophoresis

All samples were amplified clearly. Among 56 healthy, unrelated Japanese, 48 samples were detected as heterozygotes (multiple bands) and 8 samples as single bands (Figure 1).

Analysis of heterozygous hGH micro-variants by PCR-SSCP

The 8 single bands (from 8 samples) obtained from fragment length typing were subjected to PCR-SSCP analysis. Six samples showed double bands on electrophoresis reflecting a single-strand conformational difference between alleles with the same fragment length but heterozygous sequences (Figure 2).

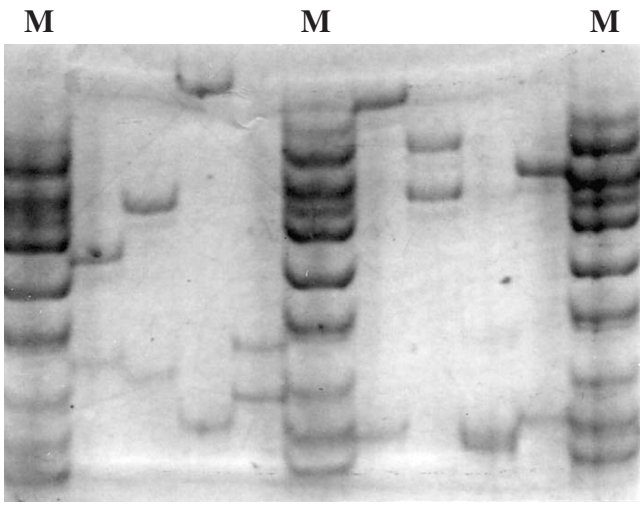

Fig. 1. Result of electrophoresis after the first PCR
M: Original ladder markers(285bp, 282bp, 280bp, 278bp, 276bp, 270bp, 265bp, 256bp, 248bp, 240bp)

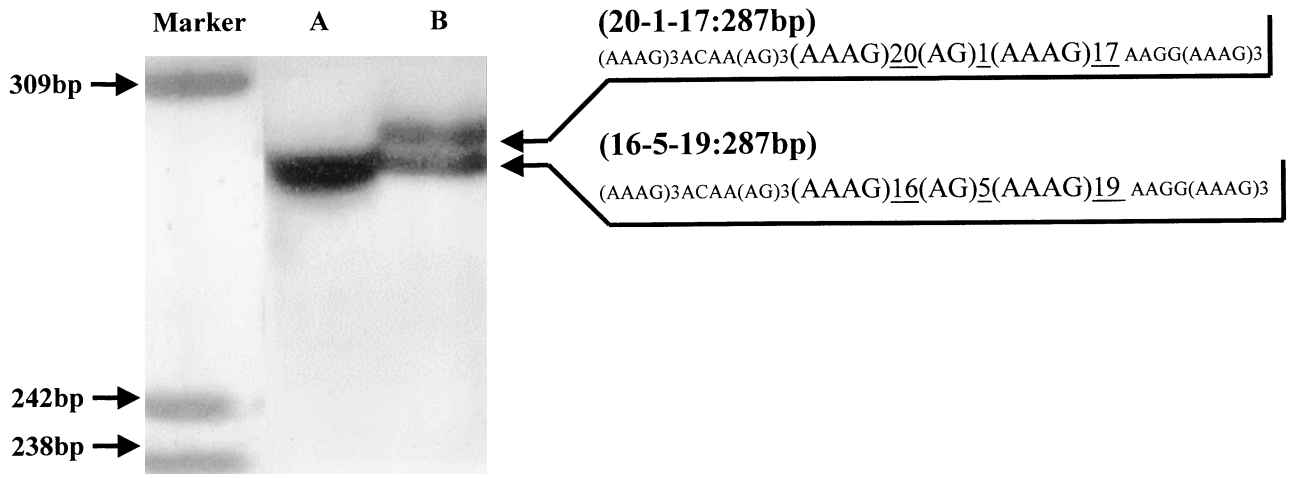

Fig. 2. Separation of heterozygous sequence allele by SSCP A: Not separable sample (homozygous sequence allele?) B: Separable sample(heterozygous sequence allele)
*Sequence and fragment size were found by sequence analysis Marker: pBR322/MspI digest.

Table 1-1. Data of 25 length alleles and 57 sequence alleles found in Japanese subjects, showing different numbers of repeats in each unit

Allele designation	Number of alleles	Fragment length (Length allele)	Sequence structure of repeat region ···(AAAG)x(AG)y (AAAG)z···			Frequency
6-12-13	1	237bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)12(AAAG)13	AAGG(AAAG)3	0.0089
6-11-14	3	239bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)11(AAAG)14	AAGG(AAAG)3	0.0268
6-13-13	1	239bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)13(AAAG)13	AAGG(AAAG)3	0.0089
7-11-13	2	239bp	(AAAG)3ACAA(AG)3	(AAAG)7(AG)11(AAAG)13	AAGG(AAAG)3	0.0179
7-12-13	2	241bp	(AAAG)3ACAA(AG)3	(AAAG)7(AG)12(AAAG)13	AAGG(AAAG)3	0.0179
6-17-12	2	243bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)17(AAAG)12	AAGG(AAAG)3	0.0179
6-14-14	3	245bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)14(AAAG)14	AAGG(AAAG)3	0.0268
6-18-12	2	245bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)18(AAAG)12	AAGG(AAAG)3	0.0179
6-16-13	2	245bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)16(AAAG)13	AAGG(AAAG)3	0.0179
6-17-13	2	247bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)17(AAAG)13	AAGG(AAAG)3	0.0179
6-14-15	3	249bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)14(AAAG)15	AAGG(AAAG)3	0.0268
6-16-14	2	249bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)16(AAAG)14	AAGG(AAAG)3	0.0179
6-18-13	3	249bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)18(AAAG)13	AAGG(AAAG)3	0.0268
6-13-16	3	251bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)13(AAAG)16	AAGG(AAAG)3	0.0268
6-15-15	2	251bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)15(AAAG)15	AAGG(AAAG)3	0.0179
6-15-16	1	255bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)15(AAAG)16	AAGG(AAAG)3	0.0089
7-17-14	1	255bp	(AAAG)3ACAA(AG)3	(AAAG)7(AG)17(AAAG)14	AAGG(AAAG)3	0.0089
6-16-16	1	257bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)16(AAAG)16	AAGG(AAAG)3	0.0089

Second amplification and sequencing

A total of 112 bands were obtained from the gels of the first PCR and PCR-SSCP. Among them, 108 bands were successfully sequenced for allele genotyping. The two samples that showed no band shift by PCR-SSCP could not be sequenced.

From the 56 healthy, unrelated Japanese, 25 length alleles were identified. Fragment lengths of the alleles were distributed in the range of 237 to 289 bp. Sequencing of these length alleles identified 57 sequence alleles (Table 1-1, -2 and -3), showing that some length alleles contain micro-variants with different sequences although they are categorized to be the same allele by fragment length. All the sequenced alleles contained a repetitive sequence based on $(AAAG)_3ACAA(AG)_3$ [(AAAG)x (AG)y (AAAG)z]AAGG $(AAAG)_3$ (Table 1). Therefore, the *hGH* alleles could be designated by the fragment length (base pairs) and by the number of repeats in the polymorphic unit [x-y-z for the repeat region $(AAAG)_x$ $(AG)_y$ $(AAAG)_z$].

Discussion

In this study, a total of 57 *hGH* sequence alleles were identified from 56 healthy, unrelated Japanese. Furthermore, we demonstrated that the heterozygosity of *hGH* was as high as 96.4%. The high degree of heterozygosity implies that this highly polymorphic locus may be subject to frequent rearrangement during meiosis[11-13]. Furthermore, in the process of human evolution, the newly generated variants are maintained by the equilibrium selection in the population through time[14-17].

Short tandem repeats (STRs) are scattered in the introns, intergenic regions and coding regions. The mechanisms for generating different numbers of tandem repeats are generally assumed to be through

Table 1-2. Data of 25 length alleles and 57 sequence alleles found in Japanese subjects, showing different numbers of repeats in each unit

Allele designation	Number of alleles	Fragment length (Length allele)	Sequence structure of repeat region ···(AAAG)x(AG)y (AAAG)z···			Frequency
6-13-18	2	259bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)13(AAAG)18	AAGG(AAAG)3	0.0179
6-15-17	1	259bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)15(AAAG)17	AAGG(AAAG)3	0.0089
6-19-15	1	259bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)19(AAAG)15	AAGG(AAAG)3	0.0089
6-16-17	1	261bp	(AAAG)3ACAA(AG)3	(AAAG)6(AG)16(AAAG)17	AAGG(AAAG)3	0.0089
14-1-17	2	263bp	(AAAG)3ACAA(AG)3	(AAAG)14(AG)1(AAAG)17	AAGG(AAAG)3	0.0179
15-1-16	2	263bp	(AAAG)3ACAA(AG)3	(AAAG)15(AG)1(AAAG)16	AAGG(AAAG)3	0.0179
15-2-16	2	265bp	(AAAG)3ACAA(AG)3	(AAAG)15(AG)2(AAAG)16	AAGG(AAAG)3	0.0179
15-1-17	2	267bp	(AAAG)3ACAA(AG)3	(AAAG)15(AG)1(AAAG)17	AAGG(AAAG)3	0.0179
16-1-16	2	267bp	(AAAG)3ACAA(AG)3	(AAAG)16(AG)1(AAAG)16	AAGG(AAAG)3	0.0179
19-1-13	2	267bp	(AAAG)3ACAA(AG)3	(AAAG)19(AG)1(AAAG)13	AAGG(AAAG)3	0.0179
16-2-16	2	269bp	(AAAG)3ACAA(AG)3	(AAAG)16(AG)2(AAAG)16	AAGG(AAAG)3	0.0179
19-1-14	2	271bp	(AAAG)3ACAA(AG)3	(AAAG)19(AG)1(AAAG)14	AAGG(AAAG)3	0.0179
17-1-17	3	275bp	(AAAG)3ACAA(AG)3	(AAAG)17(AG)1(AAAG)17	AAGG(AAAG)3	0.0268
18-7-13	1	275bp	(AAAG)3ACAA(AG)3	(AAAG)18(AG)7(AAAG)13	AAGG(AAAG)3	0.0089
19-1-15	2	275bp	(AAAG)3ACAA(AG)3	(AAAG)19(AG)1(AAAG)15	AAGG(AAAG)3	0.0179
20-1-14	3	275bp	(AAAG)3ACAA(AG)3	(AAAG)20(AG)1(AAAG)14	AAGG(AAAG)3	0.0268
20-5-12	3	275bp	(AAAG)3ACAA(AG)3	(AAAG)20(AG)5(AAAG)12	AAGG(AAAG)3	0.0268
22-1-12	2	275bp	(AAAG)3ACAA(AG)3	(AAAG)22(AG)1(AAAG)12	AAGG(AAAG)3	0.0179
23-1-11	2	275bp	(AAAG)3ACAA(AG)3	(AAAG)23(AG)1(AAAG)11	AAGG(AAAG)3	0.0179

Table 1-3. Data of 25 length alleles and 57 sequence alleles found in Japanese subjects, showing different numbers of repeats in each unit

Allele designation	Number of alleles	Fragment length (Length allele)	Sequence structure of repeat region ···(AAAG)x(AG)y (AAAG)z···			Frequency
15-6-17	1	277bp	(AAAG)3ACAA(AG)3	(AAAG)15(AG)6(AAAG)17	AAGG(AAAG)3	0.0089
17-9-14	1	279bp	(AAAG)3ACAA(AG)3	(AAAG)17(AG)9(AAAG)14	AAGG(AAAG)3	0.0089
17-7-15	3	279bp	(AAAG)3ACAA(AG)3	(AAAG)17(AG)7(AAAG)15	AAGG(AAAG)3	0.0268
18-5-15	2	279bp	(AAAG)3ACAA(AG)3	(AAAG)18(AG)5(AAAG)15	AAGG(AAAG)3	0.0179
18-3-16	3	279bp	(AAAG)3ACAA(AG)3	(AAAG)18(AG)3(AAAG)16	AAGG(AAAG)3	0.0268
18-1-17	2	279bp	(AAAG)3ACAA(AG)3	(AAAG)18(AG)1(AAAG)17	AAGG(AAAG)3	0.0179
16-4-18	2	281bp	(AAAG)3ACAA(AG)3	(AAAG)16(AG)4(AAAG)18	AAGG(AAAG)3	0.0179
17-4-17	1	281bp	(AAAG)3ACAA(AG)3	(AAAG)17(AG)4(AAAG)17	AAGG(AAAG)3	0.0089
18-5-16	2	283bp	(AAAG)3ACAA(AG)3	(AAAG)18(AG)5(AAAG)16	AAGG(AAAG)3	0.0179
19-1-17	2	283bp	(AAAG)3ACAA(AG)3	(AAAG)19(AG)1(AAAG)17	AAGG(AAAG)3	0.0179
20-1-16	1	283bp	(AAAG)3ACAA(AG)3	(AAAG)20(AG)1(AAAG)16	AAGG(AAAG)3	0.0089
21-1-15	2	283bp	(AAAG)3ACAA(AG)3	(AAAG)21(AG)1(AAAG)15	AAGG(AAAG)3	0.0179
19-4-16	2	285bp	(AAAG)3ACAA(AG)3	(AAAG)19(AG)4(AAAG)16	AAGG(AAAG)3	0.0179
19-2-17	2	285bp	(AAAG)3ACAA(AG)3	(AAAG)19(AG)2(AAAG)17	AAGG(AAAG)3	0.0179
17-5-19	1	287bp	(AAAG)3ACAA(AG)3	(AAAG)26(AG)5(AAAG)19	AAGG(AAAG)3	0.0089
20-1-17	3	287bp	(AAAG)3ACAA(AG)3	(AAAG)20(AG)1(AAAG)17	AAGG(AAAG)3	0.0268
23-1-14	1	287bp	(AAAG)3ACAA(AG)3	(AAAG)23(AG)1(AAAG)14	AAGG(AAAG)3	0.0089
25-1-12	2	287bp	(AAAG)3ACAA(AG)3	(AAAG)25(AG)1(AAAG)12	AAGG(AAAG)3	0.0179
17-10-16	1	289bp	(AAAG)3ACAA(AG)3	(AAAG)17(AG)10(AAAG)16	AAGG(AAAG)3	0.0268
19-8-15	1	289bp	(AAAG)3ACAA(AG)3	(AAAG)19(AG)8(AAAG)15	AAGG(AAAG)3	0.0179

unequal crossing over and replication slippage,[13] but their role in genetic system is largely unknown.

In forensic area, the STRs are especially important for identification of individuals in paternity test and criminal investigation. Because of the smaller fragment size of STR alleles compared to variable number of tandem repeat (VNTR)[20], they are well suited to the amplification of DNA extracted from degraded specimens[18-19]. The *hGH* allele structure that we identified in our sample of unrelated Japanese subjects is basically represented by $(AAAG)_3ACAA(AG)_3$ [(AAAG) x (AG)y (AAAG)z]AAGG$(AAAG)_3$, which is the same as that of Caucasians reported previously[3-4]. The results of this study suggest that the hGH system may well be useful in the practice of forensic medicine in Japan.

We also demonstrated a large number of microvariants in *hGH* alleles. This signifies that alleles of the same size may in fact contain multiple sequence alleles. This accounts for the higher degree of polymorphism in the *hGH* system than in the common simple repeat DNA polymorphic systems[20-21]. On the other hand, this characteristic also implies that electrophoresis of PCR products (depending on fragment length) alone is not adequate for typing, and that sequence analysis is necessary. This poses a problem particularly when alleles show a single band of the same chain length. When we performed PCR-SSCP in this study, band shifts were observed in 6 of the 8 single band samples. However, considering the general characteristics of PCR-SSCP, the 2 samples that showed no band shift cannot immediately be concluded to be homozygotes containing alleles of identical length and sequence. Therefore, we analyzed these two cases by direct sequencing. However, homozygosity was not confirmed, because sequence data could not be obtained. One possibility is that these non-

shift samples are new variants occurring in the *hGH* system.

A limitation of the techniques we used in this study is that direct sequence may be inappropriate to analyze some minority variant alleles. To solve this technical issue, we need to analyze the non-shift samples by cloning techniques. A second limitation is that direct sequencing requires several days to complete. To further develop this system for practical application, we need to search for alternative techniques that are rapid, reliable and adaptable for larger number of samples; for example, modification of the procedure of PCR-SSCP used in this study.

Before the *hGH* system can be used effectively in paternity testing and other forensic purposes, allele frequencies should be determined. Since individual hGH alleles have low frequencies in Japanese, utilization of this system would yield a markedly higher paternity probability than by using a simple repeat DNA polymorphism.

Acknowledgments

The authors express sincere thanks to Dr. Boettcher B., professor emeritus of the University of Newcastle (New South Wales, Australia) for language advice and Dr. Hasekura H., professor emeritus of Tokyo Medical and Dental University (Tokyo, Japan) for medical advice.

References

1. Hirt H, Kimelman J, Birnbaum MJ, *et al*. The human growth hormone gene locus: Structure, evolution, and allelic variations. *DNA*. 1987;6:59-70.
2. Vnencak-Jones CL, Phillips JA, Chen E.Y. Molecular basis of human growth hormone gene deletions. *Proc Nat Acad Sci USA*. 1988;85:5615-5619.
3. Chen EY, Liao YC, Smith DH, *et al*. The human growth hormone locus: nucleotide sequence, biology, and evolution. *Genomics*. 1989;4:479-497.
4. Polymeropoulos MH, Rath DS, Xiao H, *et al*. A simple sequence repeat polymorphism at the human growth hormone locus. *Nucleic Acids Research*. 1991;19:689.
5. Brinkman B, Meyer E, Junge A. Complex mutational events at the HumD21S11 locus. *Hum Genet*. 1996;98:60-64.
6. Zhou HG, Sato K, Nishimaki Y, *et al*. The HumD21S11 system of short tandem repeat polymorphism in Japanese and Chinese. *Forensic Sci Int*. 1997;86:109-118.
7. Klintshar M, Glock B, Dauber EM, *et al*. Genetic variation and sequence studies of a highly variable short tandem repeat at the D17S976 locus. *Int J Legal Med*. 1998;112:50-54.
8. Sato K, Nishimaki Y, Fang L, *et al*. Complex STR polymorphism in HGH genes in Japanese. *Acta Crim Japon*. 1997;63:164-168
9. Orita M, Iwahana H, Kanazawa H, *et al*. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Nat Acad Sci. USA*. 1989;86:2766-2770.
10. Orita M, Suzuki Y, Sekiya T, *et al*. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics*. 1989;5:874-879.
11. Stephan W. Tandem-repetitive non-coding DNA: forms and forces. *Mol Biol Evol*. 1989;6(2):198-212.
12 . Stephan W. Quantitative variation and chromosomal location of satellite DNAs. *Genet Res*. 1987;50(1):41-52.
13. Tachida H, Evolution of repeated sequences in non-coding regions of the genome *Jpn J Genet Vol.* 1993; 68 (6):549-565.
14. Tajima F, Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics.* 1989;123(3): 585-595.
15. Kimura M, The natural theory of molecular evolution: a review of recent evidence. *Jpn J Genet*. 1991;66(4):367-386.
16. Takahata N, Relaxed natural selection in human populations during the Pleistocene. *Jpn J Genet.* 1993;68:539-547.
17. Richman A, Evolution of balanced genetic polymorphism. Mol Ecol. 2000;9(12):1953-1963.
18. Sivolap Y, Krivda G, Kozhuhova N, Chebotar S, Benecke M. A Homicide in the Ukraine: DNA-based Identification of a boiled, skeletonized, and varnished human skull, and of bone fragments found in a fireplace. *Am. J. Forensic Med. Pathol*. 2001; 22(4):412-414.
19. Opel KL, Chung DT, Drabek J, Tatarek NE, Jantz LM, McCord BR. The application of miniplex primer sets in the analysis of degraded DNA from human skeletal remains. *Forensic Sci.* 2006 ;51(2):351-356.
20. Budowle B, Smerick JB, Keys KM, Moretti TR. United States population data on the multiplex short tandem repeat loci—HUMTHO1, TPOX, and CSF1PO—and the variable number tandem repeat locus D1S80. *J Forensic Sci.* 1997;42(5):846-849.
21. Puers C, Hammond HA, Jin L, *et al*. Identification of repeat sequence heterozygosity at the polymorphic short tandem repeat locus HUM TH01[AATG] and reassignment of alleles in population analysis by using a locus specific allelic ladder. *Am J Hum Genet*. 1993;53:953-958.