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Sex Determination Used Sex Determining Region Y Gene on the Y-chromosome of Human Teeth

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사람 치아 Y염색체상의 sex determining region Y(SRY)유전자를 이용한 성별감정

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최근 중합효소연쇄반응을 이용한 분자생물학적 유전자분석기술의 발달로 성염색체상의 유전좌위 증폭을 통한 성별감정이 활발히 이루어지고 있다. 그중 사람 Y염색체상에 존재하는 남성 고환의 형성을 유도하는 sex-determining region Y(SRY) gene이 규명되어 유전질환의 조기 발견이나 예방 및 태아의 성별판정 등에 응용되고 있다. 그러나, 치아는 외부 환경에 대한 저항성이 가장 높은 장기로 성별감정 등 법의치과학적 개인식별에 널리 이용되고 있음에도 불구하고, SRY 유전자를 이용하여 치아에서의 성별감정에 대한 연구는 시도된 바 없다. 따라서, 본 연구에서는 사람 치아에서 중합효소연쇄반응법을 이용한 SRY 유전자를 검출하여 성별판정에 응용하고자 하였다.

남녀 각각 20개 치아의 치수와 상아질에서 DNA를 추출하여 중합효소연쇄반응을 시행하고 SRY 유전자를 검색한 결과, 남성에서는 치수 13개중 8개, 상아질 7개중 4개에서 SRY 유전자가 검출되었고, 여성에서는 검출되지 않았다.

이러한 결과는 중합효소연쇄반응법을 이용하여 사람 치아에서 SRY 유전자를 검색할 때, 남성판별에 유용하고 치아를 이용한 성별감정시 기존의 성별감정에 이용되고 있는 다른 유전자와 함께 SRY 유전자를 검색함으로써 성별감정의 신뢰도를 높힐 수 있을 것으로 사료된다.

Introduction

DNA analysis has been applied to forensic practices such as individual identification, parentage testing and sex determination. Since restriction fragment length polymorphisms(RFLP) analysis was introduced in the mid 1980's,[18] it was used for forensic scientific filed. Although RFLP-based DNA analysis offers a high degree of discrimination, it shows demerits such as need of high molecular weight of DNA, complicated procedures and the difficulty of DNA typing of degraded samples.[4],[28] These problems were solved by advent of polymerase chain reaction(PCR) method. In recent years, the development of PCR method greatly simplified the determination of genetic marker and offers a potential alternative to overcome limit

of the RFLP analysis.[1,2,3,25,26,29,33,38,40] It also makes possible to analyze an infinitesimal quantity of DNA.

In the spot, DNA is often remained in trace amounts and contaminated. In this case, it is impossible to detect the DNA from the forensic sample. If the human teeth can be used, these are useful subject for DNA study. The dental hard tissue encloses physically the pulp and has an great structural durability, therefore the DNA well preserved in the teeth.[7,13,16,35,41]

Sex determination is the first step in the identification of event of a mass disaster such as an air crash or severe charred fire victims. At this time, teeth can provide an important information for sex determination. DNA from teeth is an excellent source for sex determination. It was performed by PCR amplification of Y chromosomal repetitive DYZ1,[25,28] centromeric α-satellite repeats of X and Y chromosomes,[11,27] DYZ1/DXS424[30] and the X and Y homologous amelogenin gene.[1,3,16,30,32,41]

At first, sex determination by PCR method is used to exclude the likelihood of a sex-linked disorder. Especially several genes including sex determining region Y(SRY) gene are DNA marker for sex identification in a range of mammals. SRY gene as testis determining factor(TDF) on the mammalian Y chromosome was isolated by genetic analysis of sex-reversed individuals.[9,16,23,34,38] Studies using deletion mapping and chromosome walking find out that SRY gene is responsible for male initiation testis determination and differentiation. This gene is expressed in the testis during the critical period of sex determination and is able to direct male development in an XX transgenic male and mouse.[11,22,24,34,38]

SRY gene is a reliable genetic marker for sex determination. Teeth are good reservior of DNA, but there was no study of SRY gene in the human teeth. So author tried to detect the SRY gene from the teeth. The purpose of this study is to evaluate the reliability and possibility of sex determination after detection of SRY gene from the human extracted teeth. In this study, the author performed to amplified fragment length polymorphisms (AmpFLPs) by PCR method from the pulp and dentin and detected of SRY gene for sex determination.

Materials and Methods

1. Subjects

The pulp tissues(13 males, 12 females) and dentinal tissues(7 males, 8 females) from the human teeth were selected for this study.

2. DNA extraction

Gingiva, blood component, dental calculus, nicotine, pigments and cementum of the teeth surface were removed using high speed dental bur. The teeth were rinsed with distilled water and sectioned along the vertical axis and seperated with surgical chisel. The pulp tissue was placed in 1.5ml eppendorf tube.

The remained dentinal tissues were crushed with a hammer until resulting fragments were 0.1mm or less in diameter. This dentinal tissue was placed in 1.5ml eppendorf tube and was dispersed 100-200μl distilled water. DNA was isolated from pulp and dentinal tissues according to a conventional DNA extraction method including an overnight at 55℃ in nucleolysis buffer(0.5% S.D.S., 10mM Tris. HCl, 0.1M EDTA, pH 8.0) and proteinase K. Each sample was spun at 10,000 rpm in a centrifuge for 10 minutes to further purify the DNA and discarded the supernatant. DNA precipitated by added 5M NaCl and 100% ethanol. After washed in 70% ethanol and dried, followed overnight at 37℃ for 24 hours, and dissolved in 100μl of distilled water.

3. SRY gene detection

The PCR mixture contained follows ; 50μl reac-

tion containing 7-8μl template DNA, buffer(100mM Tris-HCl(pH 9.0), 500mM KCl, 1.0% Triton) (POSCO), 1.5mM $MgCl_2$, 500ng SRY specific primers(5′-GATCAGCAAGCAGCTGGGAT ACCAGTG-3′ and 5′-CTGTAGCGGTCCCGT-TGCTGCGGTG-3′) according to Koopman et al,[23] 200μM dNTPs and 1.5 unit of Taq DNA polymerase. The PCR was performed under condition of 95℃ for 4 min, 1 cycle; 94℃ for 60 sec, 65℃ for 60 sec, 72℃ for 2min, 30 cycles; 72℃ for 10 min, 1 cycle in PCR thermocycler(Minicycler™, MJ research Ins, Watertown, MA, U.S.A).

The PCR products(8μl) were loaded onto 12% natual polyacrylamide gel, 1mm thickness using vertical electrophoresis unit(Mighty small Ⅱ, Hoffer Scientific Ins, Sanfransico, California, U.S.A). Electrophoresis was carried out at 100V, 3 hours, TBE buffer(90mM Tris-Borate, 2mM EDTA). The gel was subsequently stained with Ethidium Bromide(0.5μg/ml).

Table 1. Results of the detection of SRY gene from pulp and dentinal tissues of the human teeth

Sample No.	Sex	Detection	Sample No.	Sex	Detection
1	F	-	21	F	-
2	F	-	22	F	-
3	M	-	23	M	-
4	M	+	24	M	-
5	M	+	25	M	+
6	M	+	26	F	-
7	M	+	27	F	-
8	M	+	28	F	-
9	M	+	29	F	-
10	M	-	30	M	-
11	F	-	31	M	-
12	F	-	32	F	-
13	F	-	33	F	-
14	F	-	34	F	-
15	F	-	35	M	-
16	M	-	36	M	+
17	M	-	37	M	+
18	M	+	38	M	+
19	M	+	39	F	-
20	F	-	40	F	-

+ : A distinct band was observed
- : No specific band was observed

Results

1. Detection of SRY gene.

Male samples have distinct bands. It was observed 12 cases out of 20 in male(8 out of 13 and 4 and 7, in pulp and dentin, respectively), but not observed in female(Table 1).

Discussion

The testis-determining factor gene(TDF) lies on the Yp chromosome and is responsible for initiating male sex determination, termed sex determining region Y(SRY) gene. SRY gene is located in the sex-determining region of the human and mouse Y chromosome.[5,10,23,24,34,37,38] The position of TDF on the human Y chromosome was defined by analysing the genomes of XX males and XY females which generated by abnormal genetic exchange between the X and Y chromosomes in male meiosis.[4] Page et al[10] studied the sequences present in an XX male and absent in an XY female, previously localized TDF to within 140kilobases(kb), subsequently named Y-borne zinc finger protein(ZFY). But the role of ZFY in male sex determination has questioned, since X-borne zinc finger protein(ZFX)-a homologue of ZFY-was found on the eutherian X chromosome and in metatherian mammals, ZFY-related sequences were found not on the Y or X chromosome but on the autosomes.[22,29,35] Palmer et al[29] described four sex-reversed XX individuals that had inherited Y-derived sequences not including ZFY and TDF is located in 60kb proximal to the pseudoautosomal boundary. Sinclair et al[34] also reported that SRY gene shares homology with the mating-type gene from the fission yeast schizosaccaromyces pombe and with a conserved DNA-binding motif present in non-histone proteins related to high-mobility group 1(HMG1) and high mobility group 2(HMG2) and is roughly located in 35kb of the pseudoauto-

somal boundary. Thus, the sex-determining region Y(SRY) gene was isolated from the sex-determining region of the human Y chromosome by analysis of 35kb of DNA adjacent to the pseudoautosomal boundary of this chromsome.[10,34]

The SRY gene encodes a protein that has been identified as the TDF in mammals. Using cosmid-mediated gene transfer technique, Su et al[37] have determined the organization of the transcriptional unit, promoter and structure of the human SRY gene. Its findings demonstrates that the human SRY gene is an intronless gene, encodes a protein of 204 amino acids and contains a small GC-rich promoter at its 5' sequence.[8,14,15,17,27,37]

This SRY gene is the major gene in mammalian male sex determination and point mutations in the corresponding region of the SRY gene cause sex reversal in humans.[5,11,24] Molecular analysis of XY sex-reversed patients had identified several specific mutations within the segment of the SRY gene coding for the HMG motif.[17] These mutations within the SRY gene have been postulated to be the causes for gonadal dysgenesis in these patients.[10] Thus, there are a number of reports on the sex determination using SRY gene analysis for prevention and diagnosis in sex-linked disease. Recently, SRY gene is highly specific and give accurate results in sex determination and its use provides a new reliable method for routine preimplantation and general prenatal sex determination in man and contributes to diagnosis and prevention of sex-linked disorder.[6,19,20,48,49]

Author could not find the report to utilize the SRY gene for sex determination from human teeth. Maybe, this is the first study to try sex determination using SRY gene from the teeth. Sex determination is the first step in individual identification. Nowadays, with the progress of gene analysis, sex determination methods using DNA analysis techniques have been developing and applying to forensic samples including teeth.[1,2,3,7,13,30,34,41,52,53,36,40] It is not rare to find the human teeth as the only evidence available in the spot. Because the enamel is the hardest substance of the human body and teeth is a rich source for genomic and mitochondrial DNA.[7,33] Sex determination from the teeth has been performed by sex chromosomal gene amplificaiton of the alphoid satellite family(ASF), Y-chromosome specific repeated DNA family(DYZ1) and X-Y homologous amelogenin gene. Because of a higher repeat organization, the ASF amplification provided a very useful for sex determination, but has a high risk of misinterpretation.[14] The DYZ1 amplication provided the sensitivity of the assay, reducing the need for template DNA, but risk of false negative demonstrated.[25,26] Especially, X-Y homologous amelogenin gene amplification offers the advantage of an internal positive control because both X-and Y-specific sequences can be amplified at the same time and generates different length products from the X and Y chromosomes.[11,31,30,32] So X-Y homologous amelogenin gene locus is more reliable for sex determination in compared with above mentioned gene.[11] Morever, dual PCR using two sets of primers for amplification of X-Y homologous amelogenin gene, minimum template DNA is required 0.005ng.[11] However, dually amplified fragments were sometimes closely associated with a cluster of slightly longer or shorter less intense bands, which seemed to confuse the determination.[11,29] Therefore, when sex determination is performed by DNA analysis, it is need to detect several genes.

Author verified the possibility of sex determination by means of SRY gene detection from the human teeth. In this study, SRY gene was detected in 8 out of 13 male pulp tissues and 4 out of 7 male dentin tissues. All of the 20 female samples are not detected. Thus, in order to obtain successful result of undetected samples, author tried to change amplification condition as decreased annealing temperature, increased SRY primer concentration and number of thermal cycling, but resulted in nonspecific PCR product or false-negative. Generally, PCR method is very sensitive to some experimental conditions such as DNA quantity and purity,

primer sequences, temperatures and Mg^{2+} concentration.[1,33] SRY gene was not detected in all male teeth. Maybe, SRY gene from the teeth seems to be sensitive in PCR amplification, and amplification efficacy of SRY gene depend on DNA quantity, storage duration of sample and primer concentration. Also, SRY gene amplication method for sex determination lacks an internal control. Therefore, when failed to detect SRY gene, it could not be interpreted due to either female sample or amplification failure.

As compare with other results of blood sample and embryos, all results conformed the correct sex of origin(100%), only 12 out of 20 of teeth(60%) could determined the sex.[6] This suggests that extraction of DNA from the teeth is difficult and the condition of DNA of the teeth is important.

Anyhow, this results demonstrates the possibility that detection of SRY gene from the teeth is useful for sex determination. But for high reliability and sensitivity during sex determination of teeth, it is necessary to detect SRY gene and internally controlled gene(X-specific or autosomal control gene) simultaneously.

Conclusions

Author isolated nuclear DNA from the 40 human teeth, performed AMP-FLPs by PCR method and electrophoresed to detect SRY gene for sex determination. Samples were devided two group(13 pulp and 7 dentin in male, 12 pulp and 8 dentin in female).

It was found that detection of SRY gene for sex determination was possible in only male pulp and dentin, 8 out of 13 and 4 out of 7, respectively. But there was not observed in female teeth.

This results demonstrated that SRY gene amplification method is useful in human male teeth for sex determination. But, when failed to detect SRY gene, it could not be interpreted due to either female sample or failure of amplification. So if the sex determination is performed by SRY gene, it is recommended to detect SRY gene with other sex chromosome genes.

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