# Usefulness of RPS4Y Gene on Sex Determination in Human Teeth

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Ribosomal Protein S4Y(RPS4Y) gene is the human sex-linked gene on the Y chromosome.

There are a number of reports on the sex determination using RPS4Y gene analysis for prevention and diagnosis in sex-linked disease. Thus RPS4Y gene is a reliable genetic marker for sex determination in forensic medicine. In general, the sex determination of an unidentified body can be achieved based on anatomical characteristics, but sometimes sex determination was considered to be difficult such as pre-adolescent bodies or decomposed, mutilated bodies.

In this case, Sex determination using PCR method in human teeth produces good results. Because human teeth have a great structural durability, the DNA well preserved in the teeth.

So author isolated nuclear DNA from the 20 human teeth (10 males, 10 females), performed to detect RPS4Y gene by PCR method. Samples were divided four group (10 pulp and 10 dentinal tissue in male, 10 pulp and 10 dentinal tissue in female). It was found that detection of RPS4Y gene for sex determination was possible in all the male pulp tissues and 6 out of 10 male dentinal tissues. But there was not detected in female pulp and dentinal tissues.

In the view of this results demonstrates the possibility that detection of RPS4Y gene with other sex chromosome genes from the human teeth is useful to sex determination in forensic medicine.

Key words : Sex determination, Teeth, RPS4Y gene

## Ⅰ. INTRODUCTION

Since the polymerase chain reaction(PCR) method was introduced in 1985, it has been applied for DNA analysis in research and clinical fields. Recently, the development of PCR method led to great simplified the determination of genetic marker. Sex determination by PCR method was used to exclude a sex-linked disorder at first, but currently, it is a useful to sex determination in forensic medicine.[1-5)]

In general, the sex determination of an unidentified body can be achieved based on anatomical characteristics of the external genitalia or whether the gonads are ovaries or testes. By this method, however, sex determination was considered to be difficult in forensic medicine, such as pre-adolescent bodies or decomposed, mutilated bodies. In this case, human teeth are the ideal tissues to sex determination by PCR method. Because the dental hard tissue has a great structural durability, that is the most resistant to heat or decomposition, therefore the DNA well preserved in the teeth.[6-8)]

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Sex determination using PCR method in forensic medicine has been performed by amplification of sex chromosomal genes, such as Y chromosomal repetitive DYZ1 sequence in the long arm of distal Y chromosome long arm, centromeric α-satellite repeats of X and Y chromosomes, the X-Y homologous amelogenin gene and sex determining region Y(SRY) gene.[2,4,7,9,10]

Ribosomal Protein S4Y(RPS4Y) gene is the human sex-linked gene on the Y chromosome(Fig. 1). Insufficient expression of RPS4Y gene may play a role in the development of Turner syndrome, the complex human phenotype associated with monosomy X. The condition is most commonly caused by a 45X chromosome constitution, which has resulted from the loss of an X or Y chromosome before the first mitotic division of the fertilised ovum. Because RPS4Y gene is a reliable genetic marker for sex determination in forensic medicine.[11-13]

Human teeth are good reservoir of DNA, yet there was no study of RPS4Y gene on sex determination in human teeth. So author tried to detect the RPS4Y gene in human teeth.

The purpose of this study was to evaluate the reliability and possibility of sex determination after detection of RPS4Y gene from the extracted human teeth.

## Ⅱ. MATERIAL AND METHOD

### 1. Subject

The pulp tissue(10 males, 10 females) and dentinal tissue(10 males, 10 females) from the human teeth were selected for this study. The ages ranged from 19 to 56 years, each male and female averaged age was 29.3 and 24.6 years.

### 2. DNA extraction

Gingiva, blood component, dental calculus, nicotine, pigments and cementum of the teeth surface were removed using sterilized high speed dental bur. The teeth were rinsed with distilled water, 10% commercial bleach, 100% ethanol and sectioned along the vertical axis and separated with surgical chisel. The pulp tissue was obtained from the teeth, then it was placed in sterilized 1.5 ㎖ eppendorf tube. The remained dentinal tissue was crushed with a hammer until resulting fragment were 0.1 ㎜ or less in diameter. And then it was placed in sterilized 1.5 ㎖ eppendorf tube and dispensed 200 ㎕ distilled water, and the sample was overnight at 4℃.

DNA was extracted from the pulp tissue by using the AccuPrep® Genomic DNA Extraction Kit(Bioneer, Daegeon, Korea). First, added 200 ㎕ Tissue Lysis Buffer(TL) and 20 ㎕ proteinase K in eppendorf tube stored pulp tissue, vortex and incubated at 60℃ for 1 hour, or until the tissue was completely lysis. After the tissue was completely lysis, add 200 ㎕ Binding Buffer(GC) to the sample, immediately mixed, and incubated at 60℃ for 10 minutes. Add 100 ㎕ isopropanol and mix well.

Carefully transferred the sample into the upper reservoir of binding column(fit in a 2 ㎖ tube), close the cap and centrifuge at 8,000 rpm for 1 minute. Open the binding column tube and transferred the column to a new 2 ㎖ tube. Add 500 ㎕ Washing Buffer 1(W1). Close the cap and centrifuge at 8,000 rpm for 1 minute. Open the binding column tube and pour the solution into a 2 ㎖ tube to garbage bottle. Add 500 ㎕ Washing Buffer 2(W2). Close the cap and centrifuge at 8,000 rpm for 1 minute. Centrifuge once more at approximately, 12,000 rpm for 1 minute, to completely remove ethanol. Check if there is no droplet clinging to the bottom of the column. Carefully open the binding column tube and transferred the whole column to a 1.5 ㎖ eppendorf tube. Add 100 ㎕ Elution Buffer to the column, and leave for at least 1 minute at room temperature until it was completely absorbed into the glass fiber. Elute by centrifugation at 8,000 rpm for 1 minute. DNA sample was stored at -20℃.

The other hand, DNA of dentinal tissue was isolated according to a conventional DNA extraction method including an overnight at 55℃ in

nucleolysis buffer(0.5% S.D.S., 10 mM Tris-Cl, 0.1 M EDTA, pH 8.0) and 20 ㎕ proteinase K. Each samples added binding buffer and spin down, then incubated at 60℃ for 10 minutes and added isopropanol 100 ㎕. Phenol : chloroform : isoamylalcohol(25:24:1) 500 ㎕ was added and vortex, 13,000 rpm 20 minutes centrifuge and taken the supernatant(repeat this procedure 2 times for high quality). The supernatant was transferred the binding column and centrifuged at 8,000 rpm for 1 minute.

Then, column was washed with W1 buffer and W2 buffer, each 500 ㎕, and centrifuged at 8,000 rpm for 1 minute. 50 ㎕ elution buffer was added the column and centrifuged at 8,000 rpm for 1 minute after placed at room temperature for 10 minute. Finally, DNA sample was stored at -20℃.

### 3. PCR amplification

Each 20 ㎕ PCR mixture contained AccuPower PCR Premix®[10 mM Tris-HCl (pH 9.0), 40 mM KCl, 1.5 mM $MgCl_2$, 250 ㎛ dNTP, and 1 U Tag DNA Polymerase] (Bioneer, Daegeon, Korea), 5 ㎕ template DNA, 1 ㎕ RPS4Y-F primer(10 pmol), 1 ㎕ RPS4Y-R primer(10 pmol) and 13 ㎕ SDDW.

The primer sequence was RPS4Y-F : 5′-CAGACGGAACTATCTCACAGG -3′ and RPS4Y-R : 5′- GCTGAGAACAGTGCTAAGGG -3′. Each set of amplification reactions contained a negative control. Amplification was performed on a PCR thermocycler(Minicycler™, MJ research Ins. Watertown, MA, U.S.A). All target DNAs were denatured at 95℃ for 10 minutes, then underwent 35 cycles of denaturing at 95℃ for 30 seconds, annealing at 63℃ for 30 seconds and extension at 72℃ for 30 seconds. The final extension was followed at 72℃ for 10 minutes.

### 4. RPS4Y gene detection

1.5% agarose gel containing Ethidium bromide(0.5 ㎍/㎖), 9 ㎕ of the PCR product and 1 ㎕ of 10X DNA loading buffer(20% Ficoll 400, 0.1 M EDTA pH 8.0, 1% S.D.S., 0.25% Bromphenol Blue, 0.25% Xylene cyanol) was mixed and was performed, subsequently, by using a UV Transilluminator, the size of PCR products was analyzed. As the Marker, 100 bp DNA Ladder(Bioneer, Daegeon, Korea) used.

Table 1. Result of detection of RPS4Y gene from the pulp tissue

| Sample No. | Sex | Detection | Sample No. | Sex | Detection |
|---|---|---|---|---|---|
| 1 | M | + | 11 | F | - |
| 2 | M | + | 12 | F | - |
| 3 | M | + | 13 | F | - |
| 4 | M | + | 14 | F | - |
| 5 | M | + | 15 | F | - |
| 6 | M | + | 16 | F | - |
| 7 | M | + | 17 | F | - |
| 8 | M | + | 18 | F | - |
| 9 | M | + | 19 | F | - |
| 10 | M | + | 20 | F | - |

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

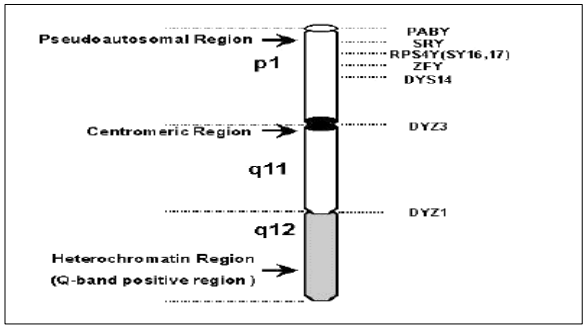
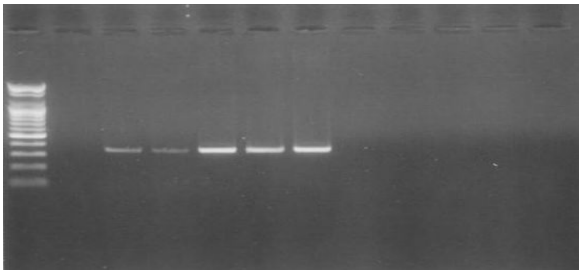



Fig. 1. Diagram of RPS4Y gene on the Y chromosome



1 2 3 4 5 6 7 8 9 10 11 12

Fig. 2. Electrophoresis of PCR products for RPS4Y gene from the pulp tissue.
Lane 1 : Molecular size marker(100 bp DNA ladder)
Lane 2 : Negative control
Lane 3, 4, 5, 6, 7 : Male pulp tissue
Lane 8, 9, 10, 11, 12 : Female pulp tissue

## Ⅲ. RESULT

### 1. Detection of RPS4Y gene from the pulp tissue

Sex determination was performed on the DNA extracted from the 20 pulp tissue(10 males, 10 females). All male sample have distinct band(329 base pair). It was observed 10 cases out of 10 in male, but not observed in female(Table 1, Fig. 2).

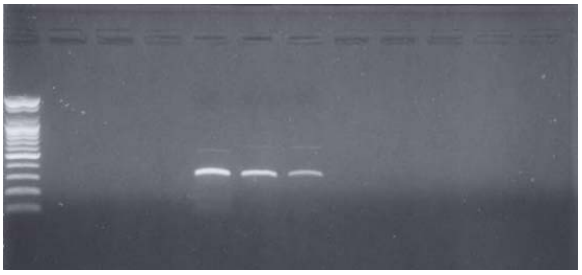
### 2. Detection of RPS4Y gene from the dentinal tissue

Sex determination was performed on the DNA extracted from the 20 dentinal tissue(10 males, 10 females). Male sample has distinct band(329 bp). It was observed 6 cases out of 10 in male, but not observed in female(Table 2, Fig. 3).

Table 2. Result of detection of RPS4Y gene from the dentinal tissue

| Sample No. | Sex | Detection | Sample No. | Sex | Detection |
|---|---|---|---|---|---|
| 1 | M | - | 11 | F | - |
| 2 | M | - | 12 | F | - |
| 3 | M | + | 13 | F | - |
| 4 | M | + | 14 | F | - |
| 5 | M | + | 15 | F | - |
| 6 | M | + | 16 | F | - |
| 7 | M | + | 17 | F | - |
| 8 | M | - | 18 | F | - |
| 9 | M | + | 19 | F | - |
| 10 | M | - | 20 | F | - |

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



1 2 3 4 5 6 7 8 9 10 11 12

Fig. 3. Electrophoresis of PCR products for RPS4Y gene from the dentinal tissue.
Lane 1 : Molecular size marker (100 bp DNA ladder)
Lane 2 : Negative control
Lane 3, 4, 5, 6, 7 : Male dentinal tissue
Lane 8, 9, 10, 11, 12 : Female pulp tissue

## Ⅳ. DISCUSSION

Sex determination is the first step in human identification. The human teeth are the most resistant to external environment after death. For this reason, many studies to determine an individual's sex in human teeth have been carried out. However, sex determination by anatomical morphometric values in human teeth is not distinct except in the canine teeth, and there had been no method in sex determination based on deciduous teeth.[6,14]

Recently, with the development of DNA analysis, sex determination based on the analysis of Y chromosome DNA has become possible. Vergnaud et. al[15] used hybridization of Y-specific repetitive DNA probes to aminotic cells to determine sex in both adults and foetuses. Although this procedure was useful, but it was taken a long time. In 1989, Witt and Erickson[16] reported a method to amplify the specific alphoid centromeric repeat sequence by PCR and the determine sex based on blood stains. The PCR method is an in vitro method for the enzymatic amplification of specific DNA sequences, using two oligonucleotide primers that hybridize to opposite strands of the DNA and flank the region of interest in the target DNA. When PCR method was compared to other sex determination techniques, it provided the highest accuracy rate with the exception of complete skeletal assessment or only deciduous teeth remained.[6,15-17]

Sex determination in human teeth has been performed by sex chromosomal gene amplification of the alphoid satellite family, Y-chromosome specific repeated DNA family and X-Y homologous amelogenin gene. 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. Nevertheless, dually amplified fragments were sometimes closely associated with a cluster of slightly longer or shorter less intense bands, which seemed to confuse the determination. Therefore, when sex determination is performed by DNA analysis, it is need to detect several genes.[2,10,18-22]

The human sex-linked gene RPS4Y encode distinct isoforms of ribosomal protein S4. Insufficient expression of S4 may play a role in the development of Turner syndrome.

Human S4, a component of the 40S subunit, is unique among known mammalian ribosomal proteins. RPS4Y encodes a protein that differs by 19 amino acid substitution. Thus, there are a number of reports on the sex determination using RPS4Y gene analysis for prevention and diagnosis in sex-linked disease.[11,12,23-27]

Author could not find the report to utilize the RPS4Y gene for sex determination in human teeth, and tried to verifying the possibility of sex determination by detection of RPS4Y gene in human teeth. In this study, RPS4Y gene was detected in 10 out of 10 male pulp tissues and 6 out of 10 male dentinal tissues. All the female sample in this study, 10 pulp tissues and 10 dentinal tissues, were not detected. The reason why not detected the RPS4Y gene in some of male dentinal tissue is that the PCR method is very sensitive to some experimental conditions such as DNA quantity and purity, primer sequences and temperatures.[28]

Maybe, RPS4Y gene from the dentinal tissue seems to be sensitive in PCR amplification, and amplification efficacy of RPS4Y gene depend on DNA quantity, storage duration of sample and primer concentration.

Therefore, when failed to detect RPS4Y gene, it could not be interpreted due to either female sample or amplification failure.

In the view of this results demonstrates the possibility that detection of RPS4Y gene from the human teeth is useful for sex determination in forensic medicine. But the high reliability and sensitivity during sex determination of the human teeth, it is necessary to detect RPS4Y gene and internally controlled gene simultaneously.

## V. CONCLUSION

Human teeth could be used as a good samples for identification in forensic medicine.

Especially, sex determination using PCR method in human teeth produces good results. So author isolated nuclear DNA from the 20 human teeth(10 males, 10 females), performed to detect RPS4Y gene by PCR method for sex determination. Samples were divided four group(10 pulp and 10 dentinal tissue in male, 10 pulp and 10 dentinal tissue in female).

It was found that detection of RPS4Y gene for sex determination was possible in all the male pulp tissues and 6 out of 10 male dentinal tissues. But there was not detected in female pulp and dentinal tissues.

This results demonstrated that RPS4Y gene amplification method is useful in human male teeth for sex determination. But, when failed to detect RPS4Y gene, it could not be interpreted due to either female sample or failure of amplification. So if the sex determination is performed by RPS4Y gene, it is recommended to detect RPS4Y gene with other sex chromosome genes.

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국문초록

# 사람치아에서 성별감정시 RPS4Y 유전자의 유용성

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사람 Y염색체상에 존재하는 RPS4Y(Ribosomal Protein S4Y) 유전자는 성별감정시 유용한 유전자로 규명되어 유전질환의 조기 발견이나 예방 및 태아의 성별판정 등에 응용되고 있다. 신원이 불분명한 사체에서 성별감정시, 기존의 성별감정에 이용되고 있는 다른 유전자와 함께 RPS4Y 유전자를 검색함으로써 성별감정의 신뢰도를 높힐 수 있을 것으로 사료된다. 또한 사체의 손상이 심할 때 유전자를 이용한 개인식별은 제한을 받게 된다. 이때 치아는 인체의 기관 중 가장 견고한 구조로 구성되어 있어 외부 환경에 대한 물리적, 화학적 저항성이 높아 법의치과학적 개인식별에 널리 이용되므로, 본 연구에서는 사람 치아에서 중합효소연쇄반응법을 이용한 RPS4Y유전자를 검출하여 법의학적 성별감정에 응용하고자 하였다.

남녀 각각 10개의 치아에서 치수와 상아질을 분리한 후 DNA를 추출하여 중합효소연쇄반응을 시행하였다. RPS4Y 유전자를 검출한 결과, 남자에게서만 특이적으로 유전자가 검출되었으며, 이는 사람 치아에서 RPS4Y 유전자를 이용한 성별감정이 법의학적 개인식별의 성별감정 실무에 있어서 다른 유전자와 함께 유용하게 사용될 수 있을 것으로 사료된다.

주제어 : 성별감정, 치아, RPS4Y