

조직별 및 나이에 따른 미토콘드리아 DNA 결손 (Δ mtDNA⁴⁹⁷⁷)의 축적

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Accumulation of mtDNA Deletion (Δ mtDNA⁴⁹⁷⁷) showing Tissue-Specific and Age-Related Variation

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Objectives: Controversial arguments exist on both the case for and against on the accumulation of mitochondrial DNA (mtDNA) deletion in association to tissue and age. The debate continues as to whether this mutation is a major contributor to the phenotypic expression of aging and common degenerative diseases or simply a clinical insignificant epiphenomenon. The objective of this study was to determine whether the accumulation of mtDNA deletion is correlated with age-related and tissue-specific variation.

Materials and Methods: One hundred and fifty-seven tissues from blood, ovary, uterine muscle, and abdominal muscle were obtained from patients ranging in age from 31~60 years. After reviewing the clinical reports, patients with mitochondrial disorder were excluded from this study. The tissues were obtained at gynecological surgeries with the consent of the patient. Total DNA isolated from blood, ovary, uterine muscle, and abdominal muscle was amplified by two rounds of PCR using two pairs of primers corresponding to positions 8225-8247 (sense), 13551-13574 (antisense) for the area around deleted mtDNA and 8421-8440 (sense), 13520-13501 (antisense) for nested PCR product. A statistical analysis was performed by χ^2 -test.

Results: About 0% of blood, 94.8% of ovary, 71.4% of uterine muscle, and 86.1% abdominal muscle harbored mtDNA deletion. When we examined the proportion of deleted mtDNA according to age deletion rate was 90% of ovary, 63.6% of uterine muscle, 77.7% of abdominal muscle in thirties and 100% of all tissue in fifties.

Conclusion: The findings of this study suggest that the mtDNA deletion is varied in tissue-specific

pattern and increases with aging.

Key Words: Mitochondrial DNA, Common deletion, PCR

DNA 16.5-kb 가 가
 DNA , , DNA
 가 가 .
 가 2.
 2~10 copy DNA (mtDNA) 1) DNA
 가 .¹ DNA
 homoplasmy .² , , , 55
 DNA , 가 10 mM Tris-HCl, 1 mM EDTA (pH 8.0), 0.1 mg/ml
 . DNA proteinase K (Invitrogen, CA, USA), 0.5% SDS
 heteroplasmy 가 4 . DNA phenol : chloro-
 form : isoamylalcohol (25 : 24 : 1)
 chloroform : iso-
 amylalcohol (24 : 1) . DNA
 가 0.3 M NaCl 2 가 -80
 1 , 4 12,000 rpm
 , 70% alcohol .
 DNA 가 DNA 200~400 µl 10 mM Tris-HCl, 1 mM EDTA
 (pH 8.0) -20 .
 2) (PCR)
 PCR Perkin Elmer GeneAmp 2400 Thermal
 cycler , PCR 2 U Taq polymerase
 (Promega, WI, USA), 1×PCR buffer, 2.5 mmol/L MgCl₂,
 1 mM dNTPs, 0.2 µmol/L primer, 100 ng DNA
 50 µl . , MT-1 (5'-AT-
 TCCCCTAAAAATCTTTGAAATG-3') MT-3 (5'-AG-
 AGTAATAGATAGGGATCAGGGG-3') primer
 first PCR , MQ-1 (5'-AGAGTAATAG-
 ATAGGGATCAGGGG-3') MQ-3 (5'-CCTAGGATT-
 GTGGGGGC-3') primer second PCR
 . PCR 94 5 , 94 30 ,
 1. 60 30 , 72 30 30 cycles
 72 10 . PCR
 157 가 2% agarose gel EtBr , UV

3) 100% (Table 2).

t-test Chi-square (χ^2 -test)
5%

DNA 가
90%

가

0%, ROS (Reactive
Oxygen Species) ROS 가
94.8%, 71.4%,
86.1% 가 가
(Table 1). DNA ROS DNA
가

(p=0.0067)

가 (Table 1). 가 가
30, 40, 50 ³⁻⁶ 가 가
, 30 Δ mtDNA⁴⁹⁷⁷ (common deletion) Common
deletion
가
50 DNA
가

Table 1. mtDNA deletion rate of various tissues

| Tissue | mtDNA deletion (%) |
|------------------|--------------------|
| Ovary | 37* / 39† (94.8) |
| Abdominal muscle | 31 / 36 (86.1) |
| Uterine muscle | 32 / 35 (71.4)‡ |
| Lymphocyte | 0 / 47 (0) |

*: number of mtDNA deletion, †: total number of sample,
‡: inter-tissue variation of ovary and uterine muscle,
p=0.0067 (p<0.05)

Table 2. mtDNA deletion rate according to age

| Age | Ovary | Uterine muscle | Abdominal muscle |
|-------|-----------------|------------------|------------------|
| 30~39 | 10* / 11† (90%) | 7* / 11† (63.6%) | 7* / 9† (77.7%) |
| 40~49 | 20 / 21 (95%) | 14 / 20 (70%) | 17 / 20 (77.7%) |
| 50~59 | 7 / 7 (100%) | 4 / 4 (100%) | 7 / 7 (100%) |

*: number of mtDNA deletions, †: total number of samples

DNA direct repeat DR1 (Direct repeat 1, nt 13447-13459) DNA 가
 DR2 (Direct repeat 2, nt 8470-8482)가 DNA 가
 heavy strand DR1 light strand DR2
 가 heavy strand
 DNA
 가
 DNA DR1
 가¹³ DNA
 가
 ROS
 가¹⁴
 DNA
 DNA
 가
 가

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