

본태성 수전증과 파킨슨병 환자에서 미토콘드리아 DNA 비교 분석

가

김래상 · 유찬종 · 이상구 · 김우경 · 한기수 · 김영보 · 박철완 · 이 언

= Abstract =

The Analysis of Mitochondrial DNA in the Patients with Essential Tremor and Parkinson's Disease

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Essential tremor(ET) is the most common movement disorder however there has been little agreement in the neurologic literature regarding diagnostic criteria for ET. Familial ET is an autosomal dominant disorder presenting as an isolated postural tremor. The main feature of ET is postural tremor of the arms with later involvement of the head, voice, or legs. In previous studies, it was reported that ET susceptibility was inherited in an autosomal dominant inheritance. As with previous results, it would suggest that ET might be associated with defect of mitochondrial or nuclear DNA. Recent studies are focusing molecular genetic detection of movement disorders, such as essential tremor and restless legs syndrome.

Parkinson's disease(PD) is a neurodegenerative disease involving mainly the loss of dopaminergic neurons in substantia nigra by several factors. The cause of dopaminergic cell death is unknown. Recently, it has been suggested that Parkinson's disease many result from mitochondrial dysfunction.

The authors have analysed mitochondrial DNA(mtDNA) from the blood cell of PD and ET patients via long and accurate polymerase chain reaction(LA PCR). Blood samples were collected from 9 PD and 9 ET patients. Total DNA was extracted twice with phenol followed by chloroform : isoamylalcohol. For the analysis of mtDNA, LA PCR was performed by mitochondrial specific primers.

With LA PCR, 1/3 16s rRNA 1/3 ATPase 6/8 and COI 3/4 ND5 regions were observed in different patterns. But, in the COI 1/3 ATPase 6/8 region, the data of PCR were observed in same pattern.

This study supports the data that ET and PD are genetic disorders with deficiency of mitochondrial DNA multicomplexes.

KEY WORDS : Essential tremor · LA PCR · Mitochondrial DNA · Parkinson's disease.

서 론

본태성 수전증(ET)은 가장 흔한 운동장애이지만, 진단 기준에 대해서는 아직까지도 17) . 40 1~6%, 65 2) . 1~22% , 90%가 (hand tremor)

DNA

Hubble⁸⁾ / Louis⁹⁾¹⁰⁾ 가 (family history) DNA 17% 100%

DNA (multiple system atrophy), 가⁵⁾⁷⁾ 1 - methyl - 4 - phenyl - 1, 2, 3, 6 - tetrahydropyridine (MPTP) 가 MPTP glia monoamine oxidase - B(MAO - B) 1 - methyl - 4 - phenylpyridinium(MPP +) MPP +가 DNA NADH CoQ1 reductase(complex) NADH CoQ1 reductase ATP 가¹³⁾ DNA (mitochondrial disorder) complex¹²⁾ complex¹⁴⁾ (multicom - plex) 가²²⁾ PCR (polymerase chain reaction) 가 5kb PCR Long and Accurate PCR(LA PCR) PCR DNA

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가 가

DNA

재료 및 방법

1. DNA 정제

6 9 9 60ml 3,500rpm 15 buffy coat (100 mM Tris · Cl, 50mM EDTA, pH 8.0, 20mg/ml RNase A) . Buffy coat 37 1 incubation 100mg/ml pro - teinase K 65 1 TE (10mM Tris · Cl, 1mM EDTA, pH 8.0) 12,000rpm 10 (24 : 1) 12,000rpm 5 70% DNA TE

2. Polymerase Chain Reaction(PCR)

DNA primer 1 µl(10 pmol), 10X buffer 5 µl, dNTP 4 µl(2.5mM Takara), Taq polymerase(5unit/ µl, Takara) 0.25 µl 50 µl DNA 0.65 µg . PCR Thermal Cycler(Pharmacia, LKB) 94 1 , 94 40 denaturation, 67 40 annealing, 72 2 polymerization 30 cycle 72 15 extension , PCR 10 µl ethidium bromide가 1% agarose gel

. Direct sequencing primer PCR

DNA primer(Table 1)

3. Long and accurate polymerase chain reaction(LA PCR)

DNA primer 1 µl(10pmol),

10X buffer 5 μ l, dNTP 8 μ l(2.5mM Takara), MgCl₂(26 mM, Takara), LA Taq polymerase(5unit/ μ l, Takara) 0.5 μ l, DNA 0.65 μ g, PCR Thermal Cycler(Pharmacia, LKB) 94 1, 98 20 de-naturation, 68 20 annealing, 98 20 denaturation, 68 20 15 polymerization, 16 cycle, 72 15 extension, PCR 10 μ l ethidium bromide가 1% agarose gel

Table 1. Synthesized primers for the PCR and LA PCR

| Primer No. | Primer sequence 5' | 3' |
|------------|--------------------------------|----|
| 1 | 5'-GTCCTACGTGATCTGAGTTCAGAC-3' | |
| 2 | 5'-CACTCTGCATCAACTGAACGC-3' | |
| 3 | 5'-GAATGATCAGTACTGCGGCG-3' | |
| 4 | 5'-CGAGTGCTATAGGCGCTTGCAGG-3' | |

결 과

1. PCR과 LA PCR 분석

() () total DNA . Total DNA DNA specific primer PCR LA PCR COI 3/4 ND5 (Fig. 1) 8kb가 8 (8kb, 2.6kb, 1.4 kb) . 9 8kb 2 (6, 7) 1.5kb DNA COI 3/4 ND5 DNA 6.5kb가 1.5kb

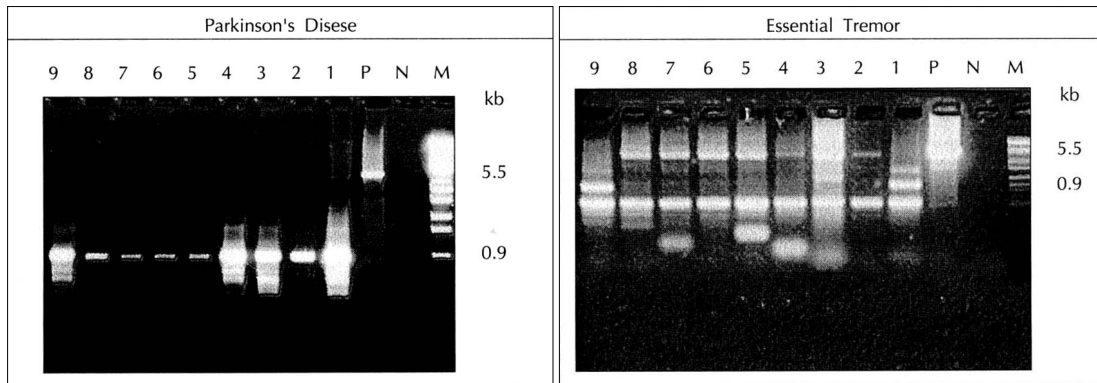


Fig. 1. Selective amplification of a region of mtDNA containing a deletion from the blood cell of Essential tremor and Parkinson's disease patients. : Sequential PCR with 1-3(COI-3/4 ND5) : lane M : -EcoT14 digest marker ; lane N : negative control ; lane P : positive control ; lanes 1-9 : PD and ET patients.

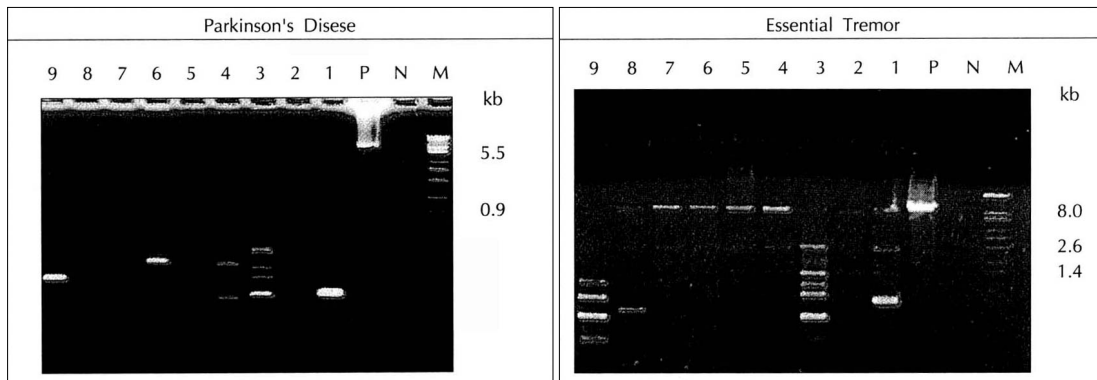


Fig. 2. Selective amplification of a region of mtDNA containing a deletion from the blood cell of Essential tremor and Parkinson's disease patients. : Sequential PCR with 2-4(1/3 16s rRNA-1/3 ATPase 6/8) : lane M : -EcoT14 digest marker ; lane N : negative control ; lane P : positive control ; lanes 1-9 : PD and ET patients.

DNA

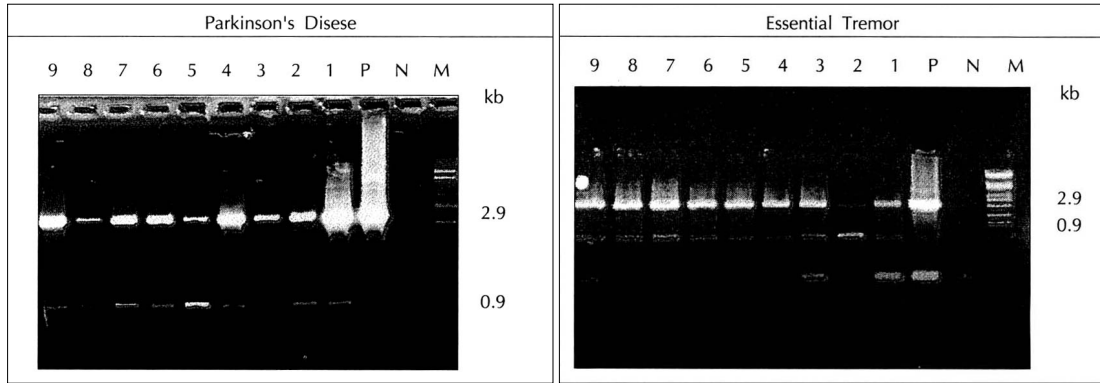


Fig. 3. Selective amplification of a region of mtDNA containing a deletion from the blood cell of Essential tremor and Parkinson's disease patients. : Sequential PCR with 2-3(COI-1/3 ATPase 6/8) : lane M : -EcoT14 digest marker ; lane N : negative control ; lane P : positive control ; lanes 1-9 : PD and ET patients.

8kb
5.4kb 6.6kb가 DNA가 고 찰
1/3 16S rRNA 1/3 ATPase 6/8 PCR 160 , 가
(Fig. 2) , 5.5kb , 가
8 (1) 5.5kb 96%
0.9kb DNA . 8 6 . , 가 가
DNA 2
9 4 (2 : 5.5kb, 1.9kb, 1.5kb,
0.9kb, 9 : 5.5kb, 2.7kb, 1.5kb, 0.9kb)
. 2 (2 , 9) 6 가 (heredofamilial tremor)
. 1 (intention tremor) 3).
5.5kb 3 (1.9kb, 1.5kb, 0.9kb) 가 , 5 , 6
DNA . 1 가 .
8 Dana ,
5.5kb 4 가 , ,
0.9kb DNA . 14)11).
CO 1/3 ATPase 6/8 PCR DNA
(Fig. 3) 2.9kb DNA 2. 11)13)14).
9kb 0.9kb DNA 가 ,
가 (gene
DNA locus) 가
2.9kb가 , 2kb codon(CAG) 5)7).
0.9kb DNA가
DNA (prototypic respiratory chain di-
PCR sease) (mitochondrial ence-
phalomyopathy)

eneous group)

(heterog-

가

가

가

6)

18)

DNA

deletion)

(common
MPTP

가

결 론

DNA

가

DNA

ATP

complex

가

가

15)21).

complex

2

auto - oxidation product melanin

가

neuromelanin

(iron)

23)

가

(ferritin)

가

50 75%

14)

free radical

• : 1999 12 10

• : 2000 9 6

• :

405 - 760

1 1198

가

, Alzheimer's disease

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DNA DNA

free radical

References

, 가

가

19)20)22)

가 가

ATP

6)16)

DNA

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DNA

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