

Williams 증후군 환아와 그 가족에서의 Fluorescent in Situ Hybridization을 이용한 Elastin 유전자 결손의 검출 및 임상 양상에 대한 평가

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Detection of Microdeletion of Elastin Gene in Patients with Williams Syndrome and Their Family by Fluorescent in Situ Hybridization and Evaluation of Clinical Manifestations

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ABSTRACT

Background : Williams syndrome is characterized by supravalvular aortic stenosis, mental retardation and peculiar facial appearance. Its genetic etiology is considered to be hemizygotic deletion in Chromosome 7q11.23 which includes the *elastin* gene. We examined the deletion in Korean Williams syndrome patients and their parents. **Materials and Method :** Sixteen patients were selected through careful clinical examination including echocardiography and cardiac angiography. Hemizygotic deletion of *elastin* gene was determined in patients and 21 parents with fluorescent *in situ* hybridization (FISH) technique using the bacterial artificial chromosome clone 244H3 probe or commercial WSCR probe. **Results :** FISH showed hemizygotic deletion of chromosome 7 in all sixteen patients but none of their parents showed deletion. **Conclusion :** Hemizygotic deletion of elastin gene can be determined by FISH with new probe 244H3 in clinically suspected Williams syndrome patients. (Korean Circulation J 2000;30(4):507-516)

KEY WORDS : Williams syndrome · Elastin · Hemizygotic deletion · Fluorescent in situ hybridization.

서론

가

80%

Williams

가

64%

: , 110 - 799

28

.¹⁾ Williams

: (02) 760 - 3570 · : (02) 743 - 3455

가

Williams

elastin

1990 Fa -
zio²⁾ *elastin* 가 7q11.23
가 Williams
가
가 *elastin*
가 Ewart³⁾ Cur -
ran⁴⁾ Morris⁵⁾
가 *elastin*
(reciprocal translocation)가
Ewart⁶⁾ pulsed fi -
eld gel electrophoresis, polymerase chain reaction
Southern blot analysis
가 *elastin*
(hemizygotic deletion) *elastin*
fluorescent *in situ* hybridization
(FISH), Southern blot analysis

Williams 74 100%
7 - 10) Williams
가
Williams
FISH
elastin
가

대상 및 방법

대상 환자의 선정

1986 1998 Williams
가 가
16 2
25 9 , 7

Williams

1) 가 (doli -
cocephaly), (epicanthal fold),
(periorbital fullness),
(asymmetric bitemporal hypoplasia),
(malar flatness), (full nasal tip),
(full cheek), (long philtrum),
(full lips), (wide mouth),
(dental malocclusion) 11가
7가 , 4
6가 (equi -
vocal)
2) 가 가 ,
가
가
psychom -
etry 가
3)
가
4)
5)

Phenotype scoring

Lowery⁷⁾ phenotype scoring
system Williams . Lo -
wery phenotype scoring system
3
2 , 2 ,
1 , mental retardation/develop -
mental delay 1 , 1
2 가 10 4 10
Williams

핵형 분석(karyotyping)

Williams

가
heparin
5 6 phytohemagglutinin 20%
FBS가 가 RPMI 1640 5 ml flask
37 68 72
37 0.75M kcl 5 ml 10
(MeOH : acetic acid = 3 : 1) 6 ml
pellet 2
3
7 trypsin
Giemsa G - banding
Epstein-Barr virus를 이용한 림프구의 형질전환
, Epstein - Barr virus(EBV)
11)
B95 - 8 / 2 × 10⁶/ml
0.45 µl membrane filter
2 4
Histopaque - 1077(Sigma)
hemacytometer
2 × 10⁶ 6 ml
membrane filter 가
1 ml 20% FBS가 RPMI
1640 1 ml 24
20% FBS, 2 µg/ml cyclosporin A가
RPMI 1640
1 20% FBS 1 µg/ml
cyclosporin A가 RPMI 1640
가
가
4 cycl -
osporin A가
15%, 10%

Fluorescent *in situ* hybridization
DNA 244H3 WSCR(Williams Syn -
drome Critical Region ; Oncor) 244H3
human genomic DNA bacterial artificial ch -
romosome(BAC) clone library elastin
sequence 가 clone elastin
3'
100 kb 가 244H3
BAC clone DNA Nick translation
biotin - 14 - dATP
200 ng salmon sperm DNA 15 µg, human
Cot - 1 DNA 5 µg
16 µl hybridization mixture[50%(v/v) deionized
formamide/2 × SSC/10% dextran sulfate]
75 10 37 15
preannealing
(metaphase chromosome)
37 RNase(100 µ
g/ml in 2 × SSC) 1 2 × SSC
3 washing (70%, 85%, 100%
5) 70% deionized forma -
mide/2 × SSC 2
hybridization mixture
37 12 14 Hybridiza -
tion 50% formamide/2 × SSC
42 5 washing 60 0.1
× SSC washing 5 µg/ml
FITC - conjugated avidin DCS/0.1% BSA(bovine
serum albumin)/4 × SSC 37 30
0.1% Tween - 20/4 × SSC 42
3 3 washing FISH signal
10 µg/ml biotinylated anti - avidin D/0.1%
BSA/4 × SSC 37
30 0.1% Tween - 20/4 × SSC
42 3 washing FITC - conjugated
avidin DCS washing
mounting buffer[1.5 µg/ml propi -
dium iodide, 1.5 µg/ml DAPI(4',6 - diamidino - 2 -
phenyl indole dihydrochloride), 1.5 µg/ml actino -
mycin - D, 1 mg/ml p - phenylenediamine]
Olympus BX 50

Fuji negative(ASA 200) mounting buffer
(OLYMPUS, AH3 - RFCA)

WSCR 7 Fuji negative(ASA 200)
7q11.23 elastin 7q11.23 244H3
7q36 D7S427 DAPI banding 7
(control probe)가 FISH signal
10 μ l WSCR
37 humidified chamber 12~16 hybridization (D7S427) si -
dization Hybridization slide signal
42 50% formamide/2 \times SSC 5 3 , 60 가 12가 244H3
0.1 \times SSC 5 3 elastin gene 4가
digoxigenin hybridization WSCR
detection digoxigenin detection kit(On -
cor) FITC가 sheep
anti - digoxigenin antibody 37 15 임상 양상에 대한 평가
0.1 Tween - 20/4 \times SSC 5 3 Williams 16
FISH signal rabbit anti -
sheep IgG antibody FITC가 anti - rabbit Table
IgG antibody 37 15 1
0.1% Tween - 20/4 \times SSC 5 3 14

Table 1. Profiles and echocardiographic findings of williams syndrome patients

Case	Age (yr)	Sex	Echocardiographic findings	
			α -Ao Dop. (m/sec)	Other findings
Case 1	6	M	4.0	Peripheral PS
Case 2	18	F	ND	
Case 3	12	M	2.0	
Case 4	8	M	3.0	Peripheral PS
Case 5	14	F	2.5	
Case 6	11	M	5.2	
Case 7	9	F	1.8	SVPS
Case 8	11	F	3.0	Peripheral PS
Case 9	7	F	Normal	Coarctation of Ao
Case 10	11	M	4.0	
Case 11	25	F	4.0	
Case 12	6	M	2.5	
Case 13	3	F	3.0	Valvular PS
Case 14	2	M	4.8	
Case 15	3	M	4.5	
Case 16	7	M	2.0	Severe MI

Abbreviations) α -Ao Dop : Doppler velocity at ascending aorta
Ao : aorta, MI : mitral insufficiency, ND : not determined
PS : pulmonary stenosis
SVPS : supralvalvular pulmonary stenosis

Table 2. Phenotyping scores of the williams syndrome patients

Case (score)	Peculiar face (3)	MR (1)	SVAS (2)	CHD (1)	I. hernia (1)	Hypercalcemia (2)	Total (10)
Case 1	+	+	+	Peri. PS	+	-	8
Case 2	+	+	+	-	-	NA	6
Case 3	+	+	+	-	+	-	7
Case 4	Equivocal	+	+	Peri. PS	+	-	7
Case 5	+	+	+	-	+	NA	7
Case 6	+	+	+	-	+	NA	7
Case 7	+	+	-	SVPS	-	NA	5
Case 8	+	+	+	Peri. PS	-	NA	7
Case 9	+	+	-	COA	+	-	6
Case 10	+	+	+	-	+	NA	7
Case 11	Equivocal	+	+	-	-	-	5
Case 12	+	+	+	-	+	NA	7
Case 13	+	+	+	Valv. PS	+	NA	8
Case 14	+	+	+	-	-	-	6
Case 15	+	+	+	-	-	-	6
Case 16	+	+	+	Severe MI	+	-	8

Abbreviations) + : present, - : not present, NA : not available, CHD : other congenital heart disease, COA : coarctation of aorta, I : inguinal, MI : mitral insufficiency, MR : mental retardation, Peri. PS : peripheral PS, SVAS : supravalvular AS, SVPS : supravalvular PS, Valv. PS : valvular PS

2
Williams 가 4 (Case 1, 6, 11, 15)

가 13 (Case 9)

가 1 (Case 16)

Williams 10

가 8

visuo - 가 1

motor coordination 가 가

shape distortion integration difficulty가 8

organic brain damage

Williams Low -

16 14 ery 가

1 (Case 7) Williams (Table 2).

1 (Case 9)

3 (Case 1, 4, 8) Epstein-Barr virus를 이용한 림프구의 형질전환

EBV

1 (Case 13),

1 (Case 16) FISH

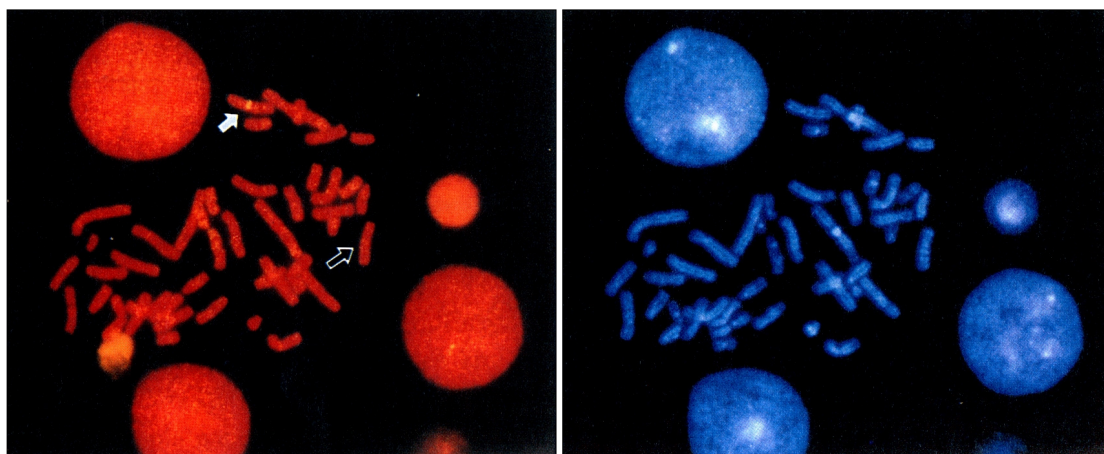


Fig. 1. FISH shows a positive result in Case 4. Left picture shows FISH signal in only one Chromosome 7 (filled arrow) but the other Chromosome 7 does not show FISH signal (empty arrow). Chromosome 7 was determined by DAPI-banded chromosomal patterns in right picture.

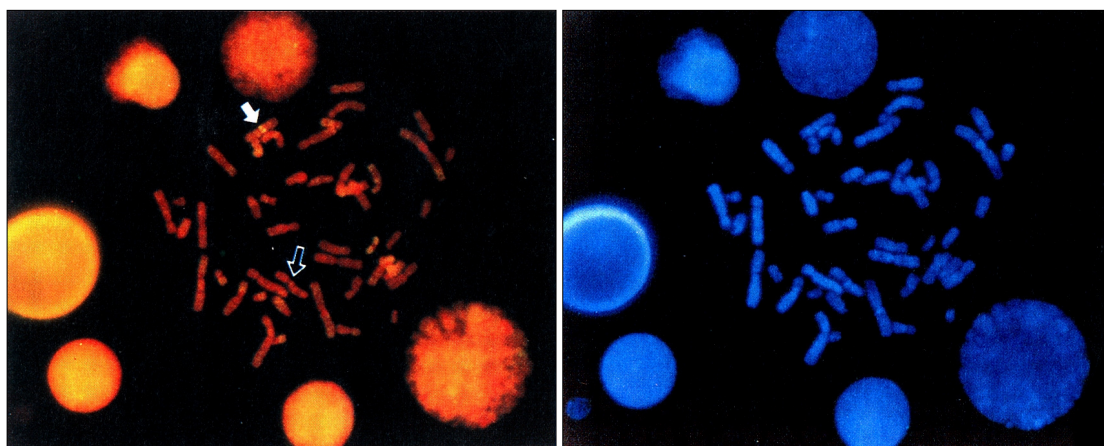


Fig. 2. FISH shows a positive result in Case 6. Left picture shows FISH signal in only one Chromosome 7 (filled arrow) but the other Chromosome 7 does not show FISH signal (empty arrow). Chromosome 7 was determined by DAPI-banded chromosomal patterns in right picture.

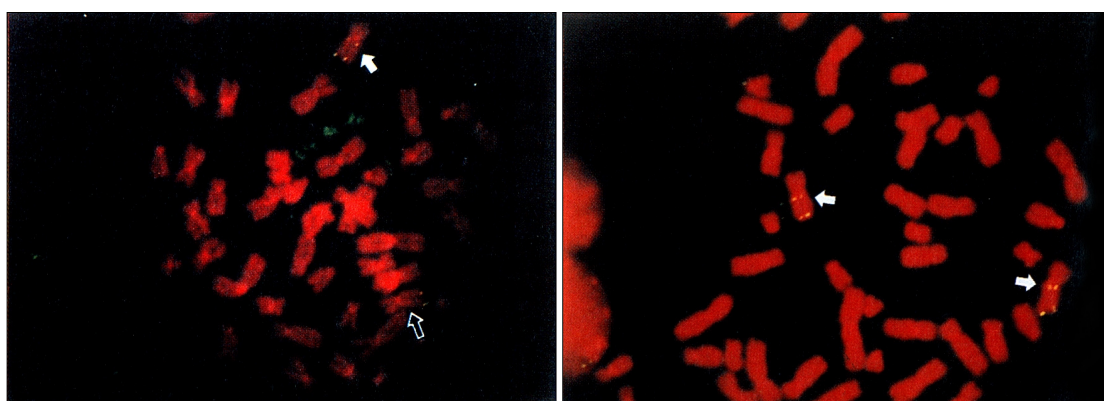


Fig. 3. FISH shows a positive result in Case 13 (left picture) and negative in her father (right picture). Left picture shows FISH signal near centromere in only one Chromosome 7 (filled arrow) and not in the other (empty arrow) but right picture shows FISH signal near centromere in both Chromosome 7 (filled arrows).

Table 3. Results of FISH in williams syndrome patients and their parents

Case	Probe	Patient	Father	Mother
Case 1	244H3	Del	Non-Del	Non-Del
Case 2	244H3	Del	Non-Del	Non-Del
Case 3	244H3	Del	Non-Del	Non-Del
Case 4	244H3	Del	Non-Del	Non-Del
Case 5	244H3	Del	Non-Del	Non-Del
Case 6	244H3	Del	Non-Del	Non-Del
Case 7	244H3	Del	Non-Del	Non-Del
Case 8	244H3	Del	Non-Del	Non-Del
Case 9	244H3	Del	NA	Non-Del
Case 10	244H3	Del	Non-Del	Non-Del
Case 11	244H3	Del	NA	Non-Del
Case 12	244H3	Del	NA	Non-Del
Case 13	WSCR	Del	NA	NA
Case 14	WSCR	Del	NA	NA
Case 15	WSCR	Del	NA	NA
Case 16	WSCR	Del	NA	NA

Abbreviations) Del : deletion, Non-Del : no deletion, NA : not available

핵형 분석(karyotyping)과 Fluorescent *in situ* hybridization

16 Williams

244H3 WSCR

FISH

Williams

FISH 7

(Table 3).

FISH 7

(Table 3).

Figs. 1 and 2 244H3

FISH DAPI banding

7 FISH signal

Fig. 3 WSCR FISH

Fig. 3 7

signal

(centromere) 가 sig -

가 hemizygotic deletion

FISH 7

가 signal

Figs. 1 and 2 FISH FISH

signal fluorescence가

BAC

clone 244H3

Alu repeat sequence가

fluorescence

고 안

Williams ,

가

가

(autosomal do -

minant)

Williams *elastin*(*ELN*)

가

Martin ¹²⁾ 9 ; 17

Jefferson ¹³⁾

Colley

4 ¹⁴⁾ 13 ; 18

13

가

Williams

1991 Fazio ²⁾

elastin 가

가 7q11.23

Williams

Ewart ³⁾

가 *elastin*

가 Curran ⁴⁾ Mo -

rris ⁵⁾ 가 *elastin* 3'

Ewart ⁶⁾ pulsed field gel electrophoresis,

polymerase chain reaction Southern blot analysis

가

elastin 3' 100 kb가

DNA exon 28 36 *elastin* recombination ¹⁷⁾
 Dutly ¹⁸⁾ 15 Williams
 3 가 7
 (homologous chromosome) unequal cro-
 ssingover(interchromosomal rearrangement)가
 cent *in situ* hybridization Southern blot
 analysis 가 Williams 4
 5 7q11.23 intrachromosomal rearrangement가
elastin
 FISH Williams
 Williams *elastin* sequence
elastin bacterial artificial chromo-
 WSCR(Williams syndrome some clone 244H3 WSCR FISH
 critical region, Oncor) 가 cosmid 7q11.23 Williams
 Borg ¹⁶⁾ Wi -
 Williams 5 Williams FISH
 5 3 7q11.23
elastin Lowery ⁷⁾
 114 Williams 96% WSCR *elastin*
 39 3 *elastin* *elastin*
 Mari ⁹⁾
 60 54 *elastin* WSCR FISH
 Williams 100% deletion
 가 Williams Wi -
 Williams , 가
 Nickerson ¹⁰⁾ FISH dinu - WSCR
 cleotide repeat sequence polymorphic marker
 44 91% *elastin*
 39%가
 , 61%가 244H3 Williams
 FISH FISH
 Williams 74 100%
elastin Williams
 Williams
Elastin genomic DNA가 45 kb
 가 36 exon 가 2Mb
 intron 가 *Alu* repeat sequence가 kb
 3' . *Alu* repeat sequence ge - Williams
 nomic DNA 4 kb 가
elastin gene 가 4
Alu repeat sequence
 “contiguous gene syndrome”
⁸⁾¹⁵⁾

가 가 . Peoples ²⁰⁾
 18 Williams replication
 factor C subunit 2(*RFC2*) 가 DNA
RFC2
 DNA elongation multimeric complex

(cognitive function) LIM -
 kinase 1 (*LIMK1*) . Frangiskakis ²¹⁾
 Williams 가
 83.6 kb DNA
 DNA sequence analysis
ELN *LIMK1* 가 가
LIMK1

Williams
 visuospatial constructive cog -
 nition 가 ²²⁾
 Williams

가

요 약

연구배경 :
 Williams ,
 가 *elastin*
 7q11.23
 (hemizygotic deletion)
 Williams
 fluorescent *in situ* hybridi -
 zation(FISH)
 방 법 :
 Williams 16 21
 FISH *elastin*
 가 . FISH
elastin genomic DNA sequence
 bacterial artificial chromosome clone 244H3
 WSCR(Oncor)
 결 과 :
 Williams 16 7

결 론 :

Williams
 244H3 FISH

중심 단어 : Williams · Elastin ·
 · Fluorescent *in situ* hybridization.

감사문

1998 (MSD)

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