임상 및 기초연구에서의
유전체분석법 소개 및 사례

의생명과학과

심성한

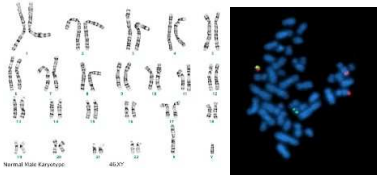
- 강남차병원 유전학 연구실 (~2020)
- 차바이오텍 유전체사업본부 강남센터 (2021~)

차바이오텍 유전체사업본부 강남센터

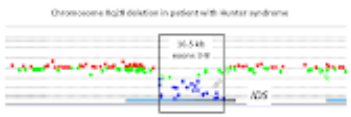
판교종합연구원 624호

Genetic Diagnosis

Clinical Cytogenetics



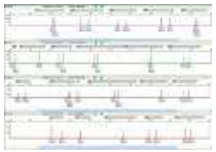
Karyotype analysis FISH



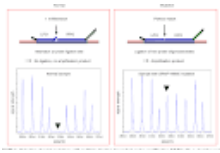
Chromosome microarray

Clinical Molecular Genetics

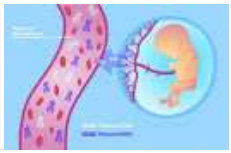
- PCR-RFLP
- Real-time PCR
- Southern blot analysis
- Sanger sequencing
- Quantitative genome analysis



QF-PCR



MLPA



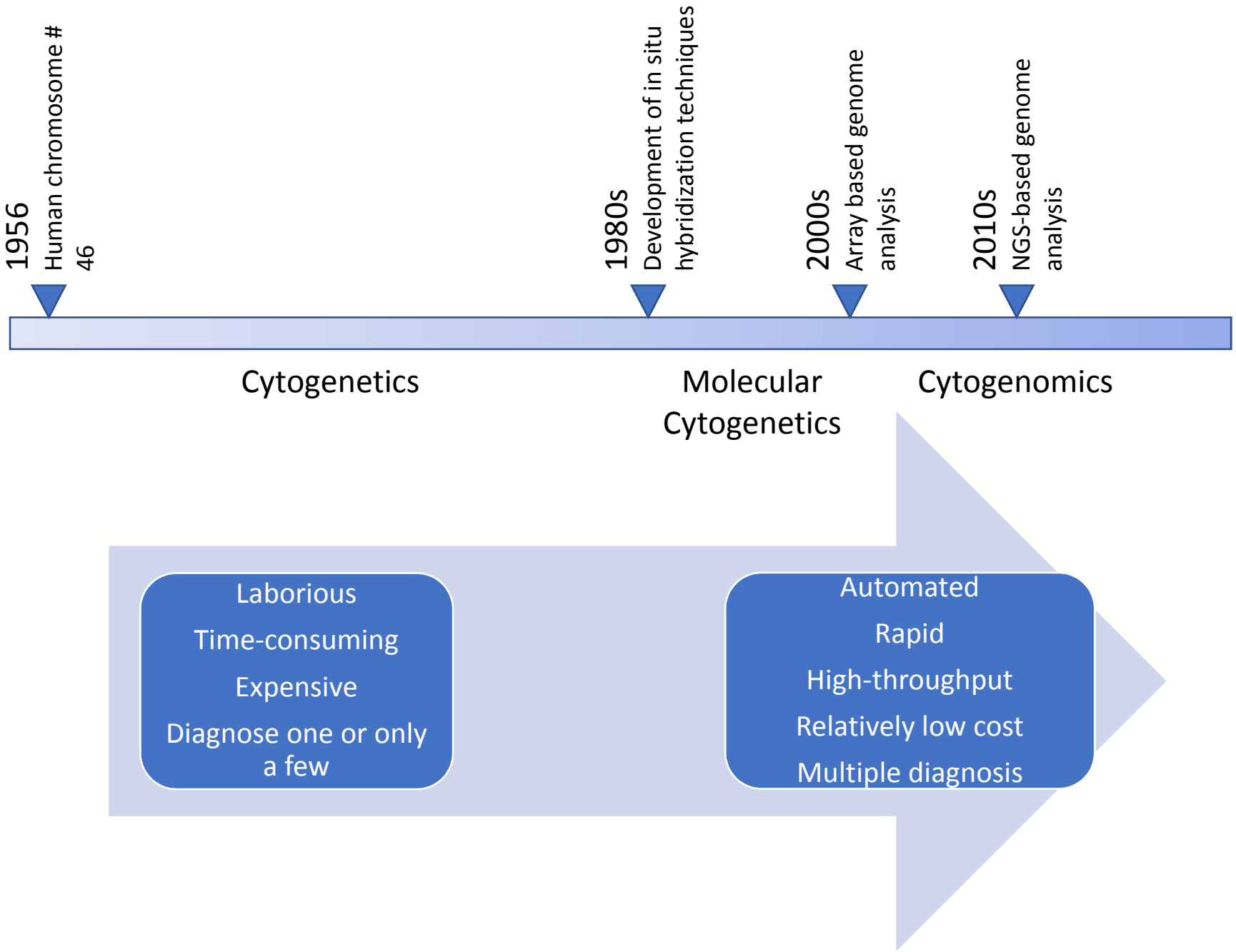
NIPT

Genetic Research

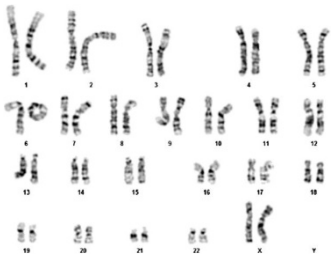
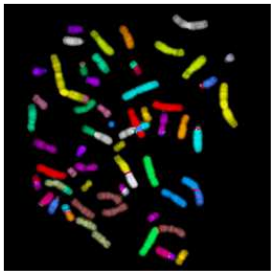
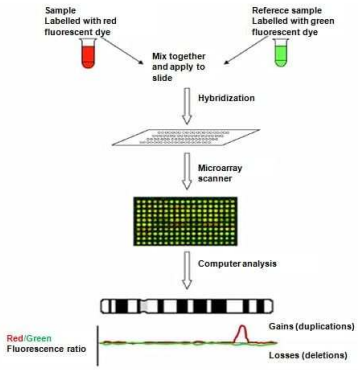
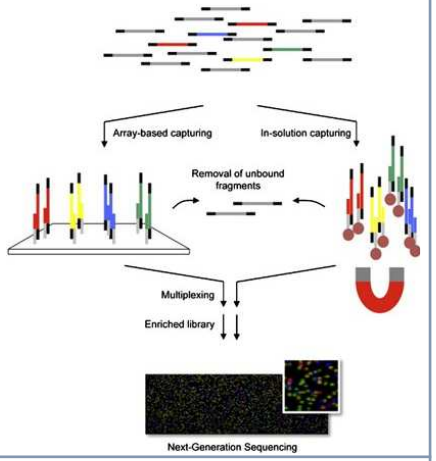
Genome analysis

- Prenatal/preimplantation
- Congenital anomalies

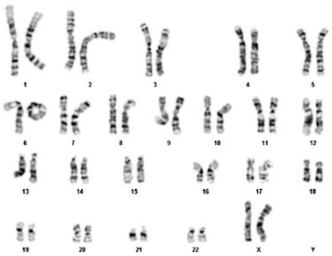
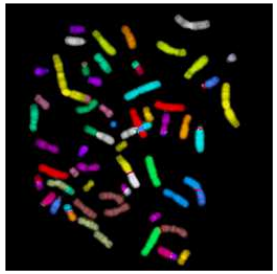
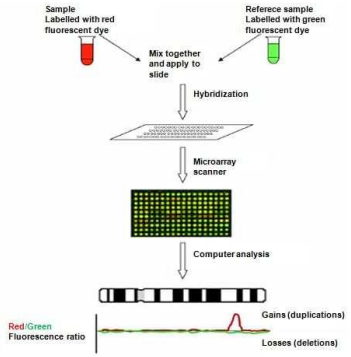
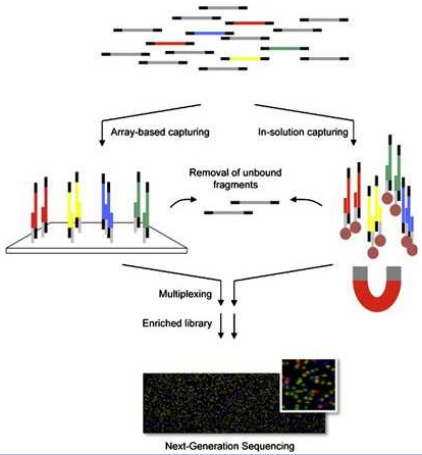
- SNP-array
- Exome sequencing
- Low-depth whole genome sequencing

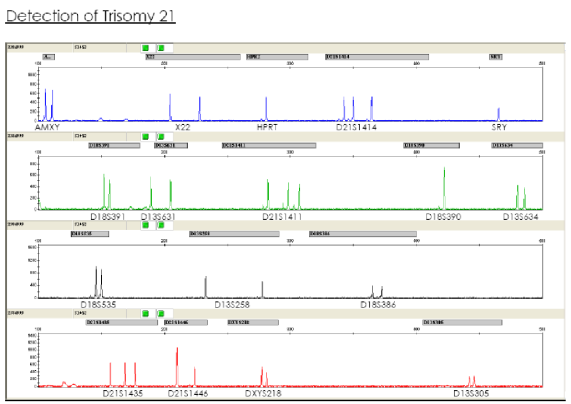
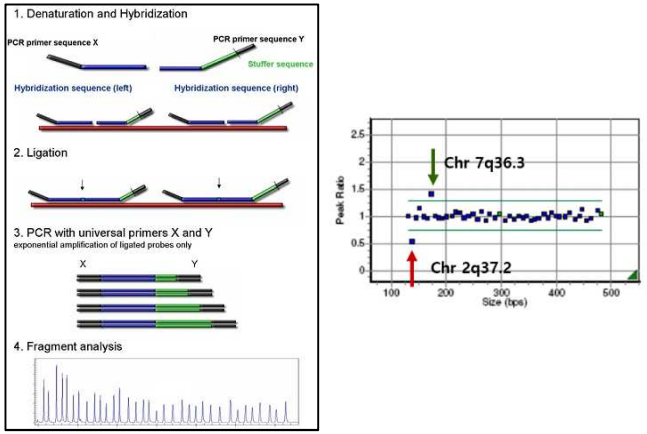

1956
Human chromosome # 46
1980s
Development of in situ hybridization techniques
2000s
Array based genome analysis
2010s
NGS-based genome analysis
Cytogenetics
Molecular Cytogenetics
Cytogenomics
Laborious
Time-consuming
Expensive
Diagnose one or only a few
Automated
Rapid
High-throughput
Relatively low cost
Multiple diagnosis

A comparison of clinically available methods to analyze the genome

	cytogenetics		DNA-based		
	G-banding karyotype	FISH	aCGH	SNP array	Whole exome sequencing
					
Resolution	~10Mb (>5Mb)	Variable (kb ~ Mb)	< 1Mb	Several kb ~1Mb	Single base-pair
Cost	high	high	high	high	high
Sensitivity (true positive rate)	~5%	~5%	>20%	>20%	>20%

A comparison of clinically available methods to analyze the genome

	Cytogenetics		DNA-based		
	G-banding karyotype	FISH	aCGH	SNP array	Whole exome sequencing
					
Unique strength	• Easy and reliable • High sensitivity for mosaic cultures • Detection of balanced chromosomal abnormalities	• High sensitivity for mosaic cultures • **Detection of complex karyotype and markers**	• High resolution • **CNV**	• High resolution • **LOH** • **UPD**	• Ultimate resolution • Point mutations
Unique limitations	• Low resolution	• Inability to identify CNVs	• Low sensitivity for mosaic cultures • Cannot detect polyploidy and balanced abnormalities	• Low sensitivity for mosaic cultures	• Difficulty interpretation (VUS)

	PCR-based	
	QF-PCR (1993~) 	MLPA 
Unique strength	• **Rapid method (within 24 hours) for prenatal aneuploid screening** • Required only small amount of sample – no cell culture • Relative low-cost • High-throughput	• A rapid, high throughput technique for copy number quantification – up to **50 different genomic DNA or RNA sequences** • Many different applications (probe mixes) - tumor profiling, **methylation profiling**
Unique limitations	• Low coverage (only 13, 18, 21, X and Y) • Homozygosity • False result due to MCC, low-rate mosaicism, STR duplication	• Can **not detect balanced** chromosomal abnormalities • A sufficient number of cells is required • Not genome-wide scanning

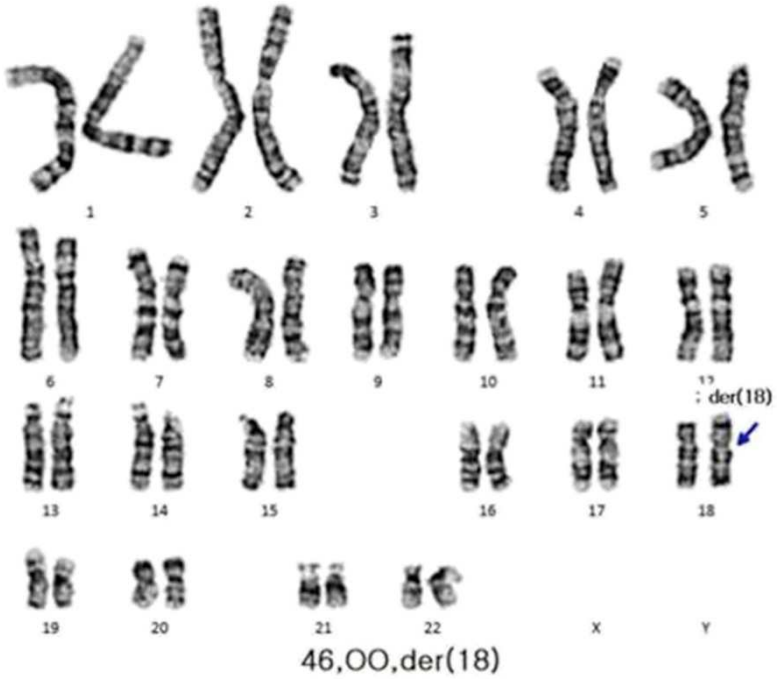
Cases

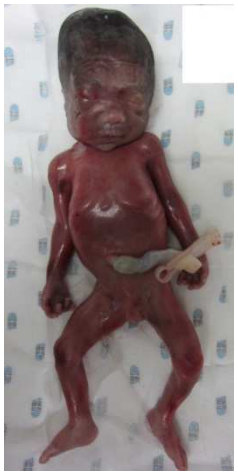
Case Report Taiwanese Journal of Obstetrics & Gynecology 58 (2019) 318e323

Prenatal diagnosis and molecular cytogenetic characterization of partial dup(18q)/del(18p) due to a paternal pericentric inversion 18 in a fetus with multiple anomalies

Min Jin Lee [a], Sang Hee Park [b], Sung Han Shim [b], Myoung-jin Moon [c,**], Dong Hyun Cha [a,*]

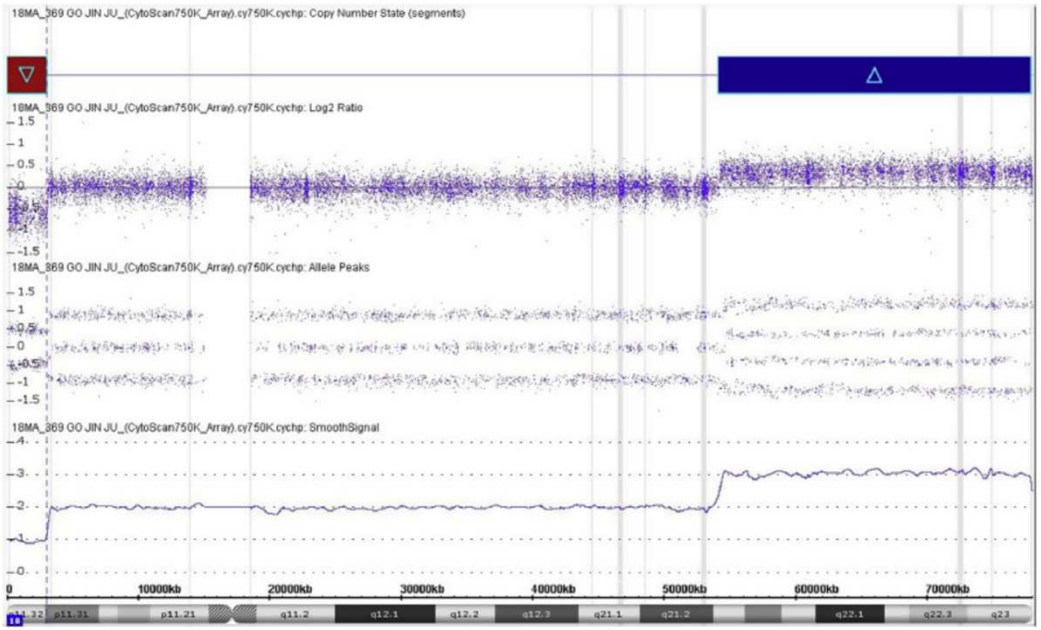
37-year-old F (Gravida 2, para 0, SA 1)
17 weeks and 5 days of gestation
Normal for integrated maternal serum screening
Abnormal ultrasound findings
Amniocentesis and chromosome analysis
→ 46,OO,der(18)



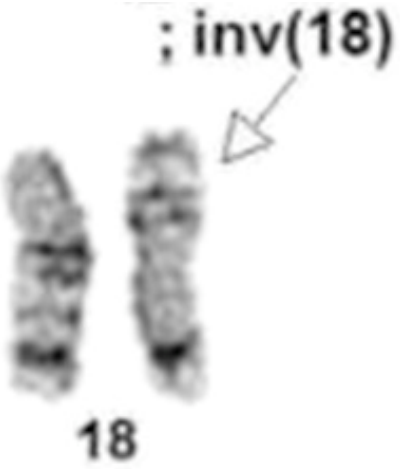


- Hypertelorism
- Clenched hands

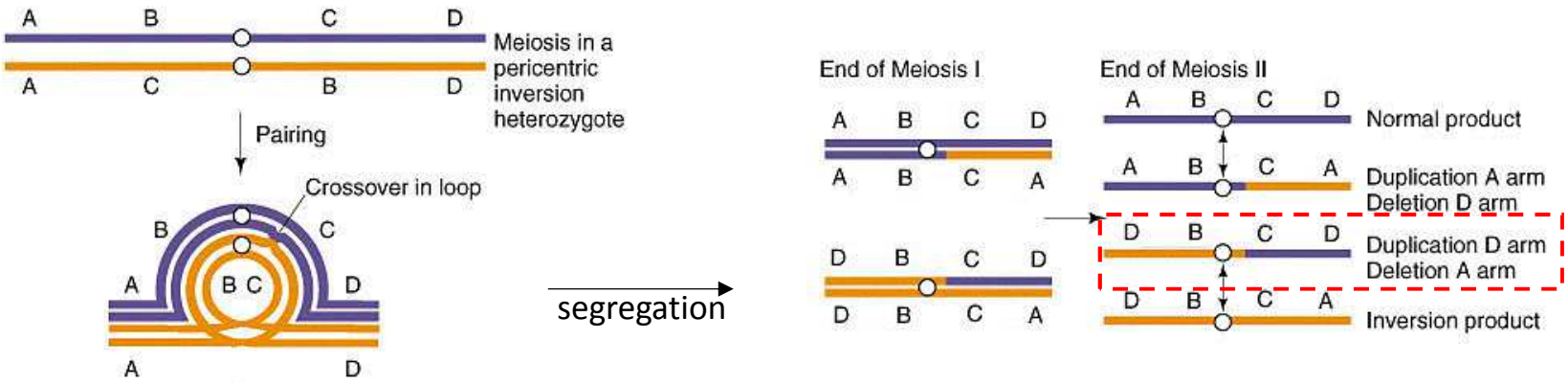
Terminated at 23 weeks and 3 days of gestation



CytoScan 750K SNP array – 3Mb deletion of 18p/23.7Mb duplication of 18q



Father – balanced pericentric inversion carrier; inv(18)(p11.2q21.2)



Contents lists available at ScienceDirect

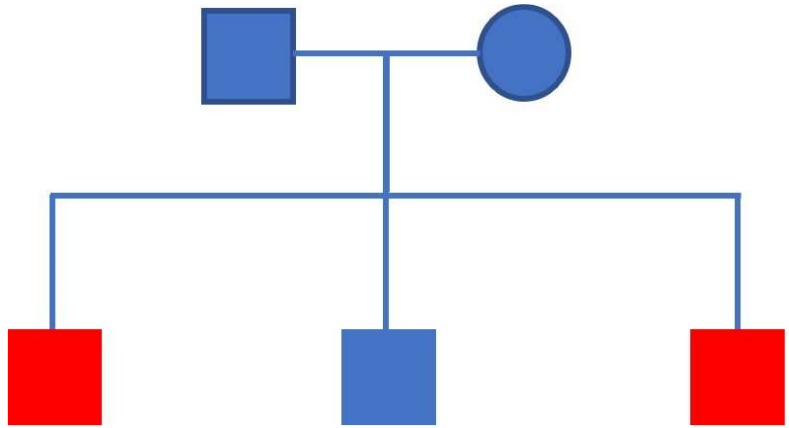
Gene

journal homepage: www.elsevier.com/locate/gene

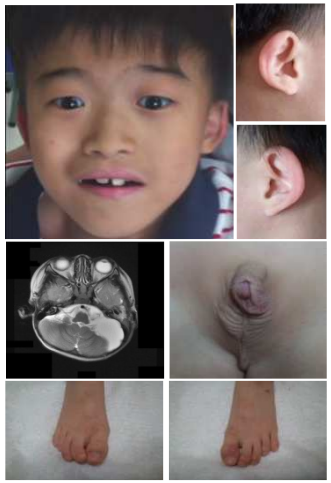
Siblings with opposite chromosome constitutions, dup(2q)/del(7q) and del(2q)/dup(7q)

Sung Han Shim [a,b], Jae Sun Shim [c], Kyunghoon Min [c], Hee Song Lee [c], Ji Eun Park [b], Sang Hee Park [b], Euna Hwang [d], MinYoung Kim [c,*]

[a] Department of Biomedical Science, College of Life Science, CHA University, Republic of Korea
[b] Genetics Laboratory, Fertility Center of CHA Gangnam Medical Center, CHA University, Republic of Korea
[c] Department of Rehabilitation Medicine, CHA Bundang Medical Center, CHA University, Republic of Korea
[d] Department of Plastic and Reconstructive Surgery, CHA Bundang Medical Center, CHA University, Republic of Korea



8 years



40 weeks, 3.0kg, C/sec
Dystocia, apnea, cyanosis

Growth retardation
- Wt <1%
- Ht <1%
- OFC <1%

Moderate MR (IQ 54)

Autism, aggressive behavior

Microcephaly,

epicanthal fold
small palpebral fissures
Hypoplastic alae nasi

Divergent starbismus

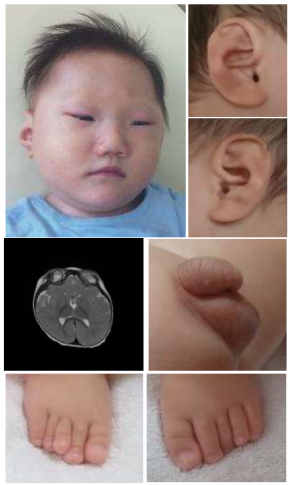
Hypoplastic helix

MRI- Subarachnoid cyst in the left cerebellar hemisphere and peri medullary cistern

Medial deviation of 2nd and 3rd toes in both feet

micropenis

10 months



IUGR,
bilateral hydronephrosis
38 weeks, 2.19kg, C/sec
11 months
Growth retardation
- Wt <1%
- Ht <1%
- OFC <1%

Microcephaly, plagiocephaly

Round face, epicanthal fold
upslanting palpebral fissures,
wide nasal bridge,
broad nasal tip

Retinal detachment with vitreous hemorrhage

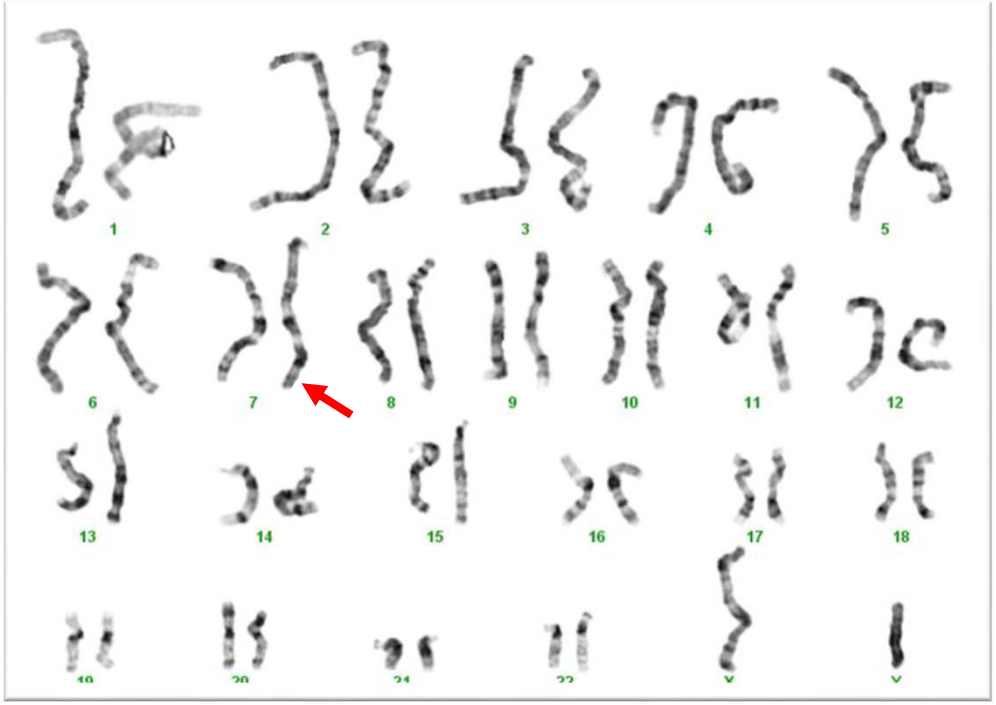
Narrow openings of external acoustic meatus without intertragic notch

MRI- delayed myelination in the brain with abnormal T2 hyperintensities or white matter volume loss

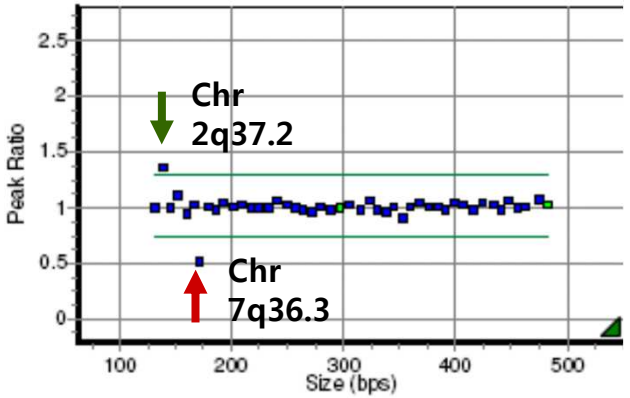
Proximal displacement of right 3rd and left 4th toes

Feeding problems, residual penile septum, hydronephrosis

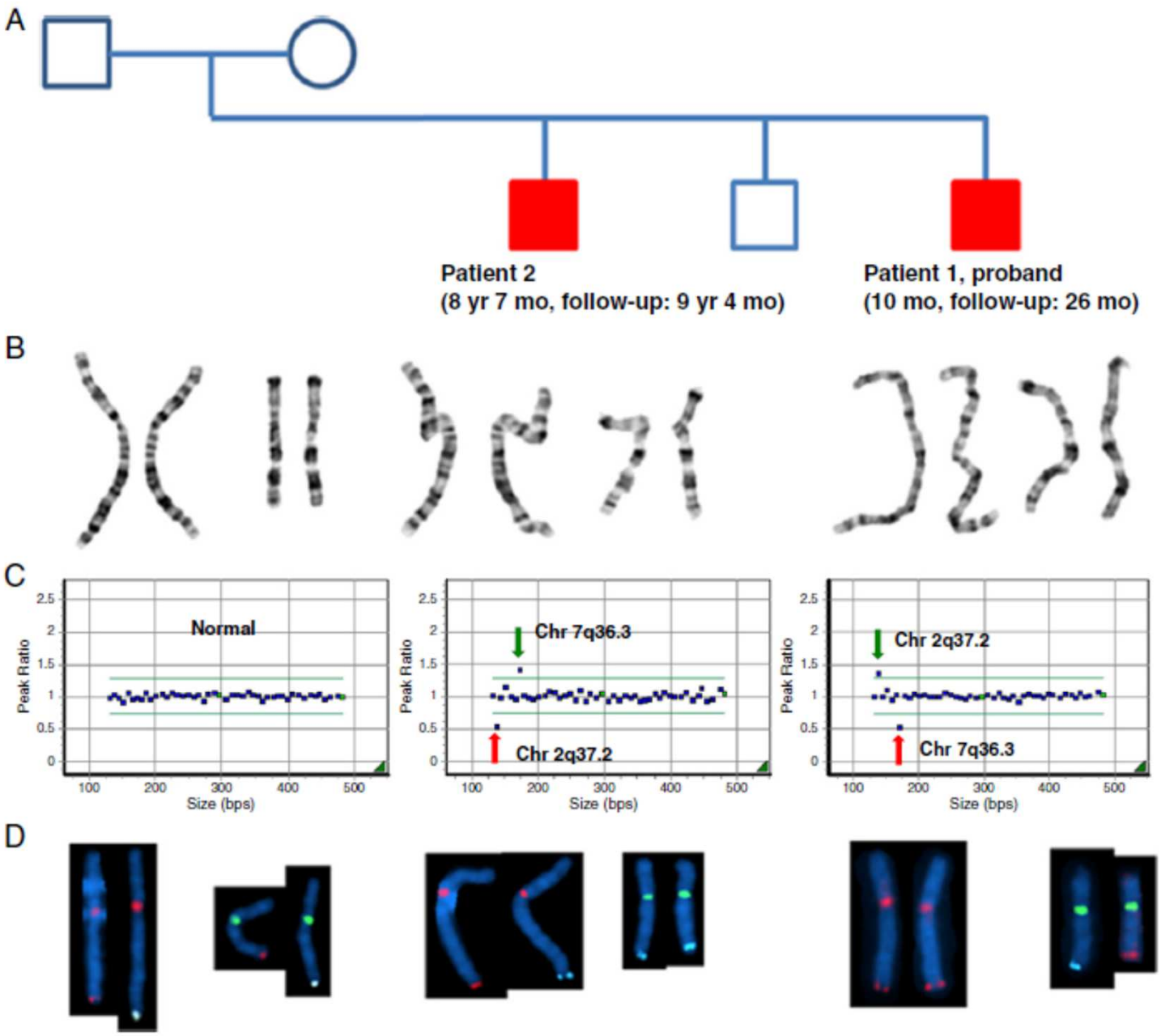
High resolution chromosome analysis → MLPA → FISH → Array CGH

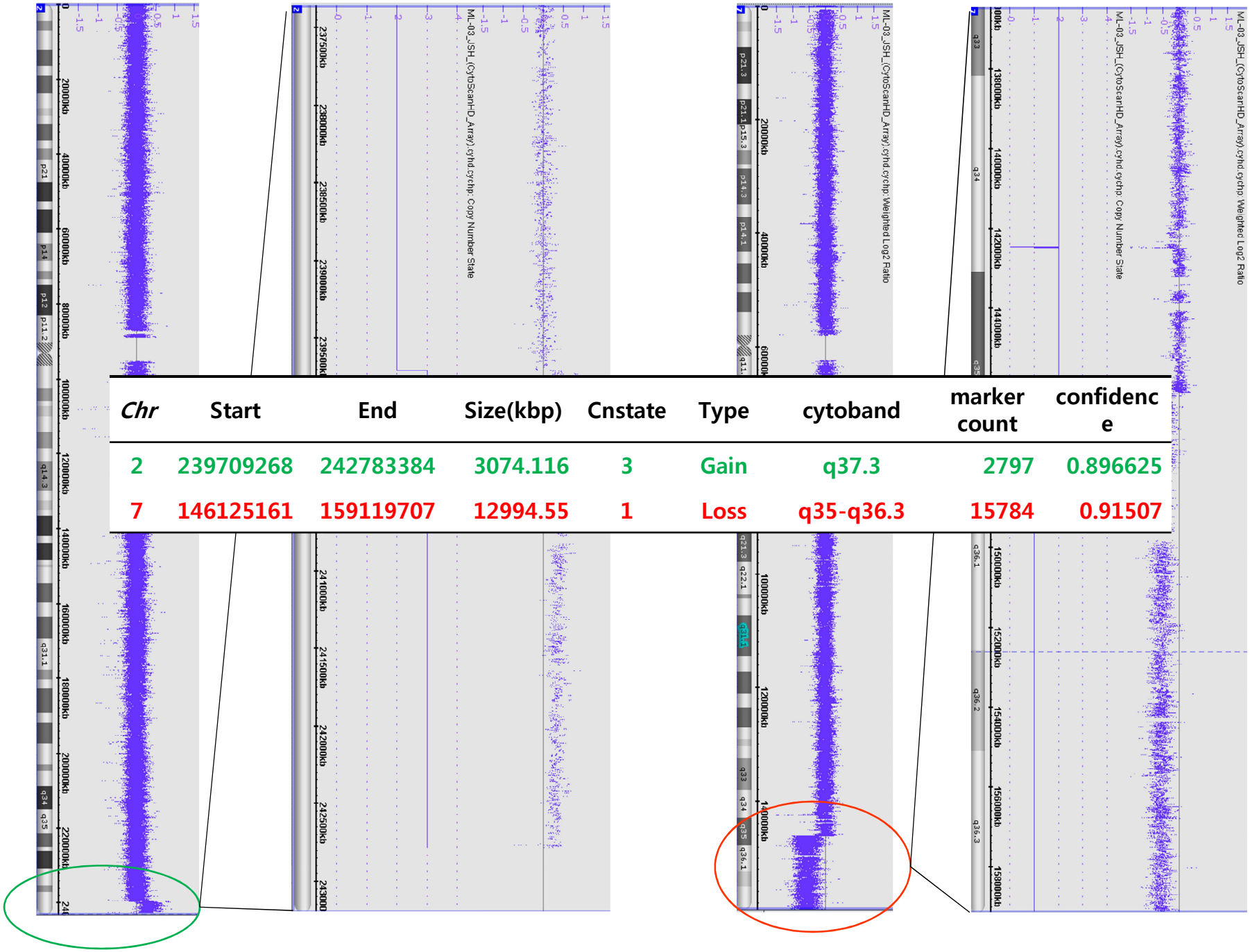


46,XY normal male → 46,XY,der(7)(?)

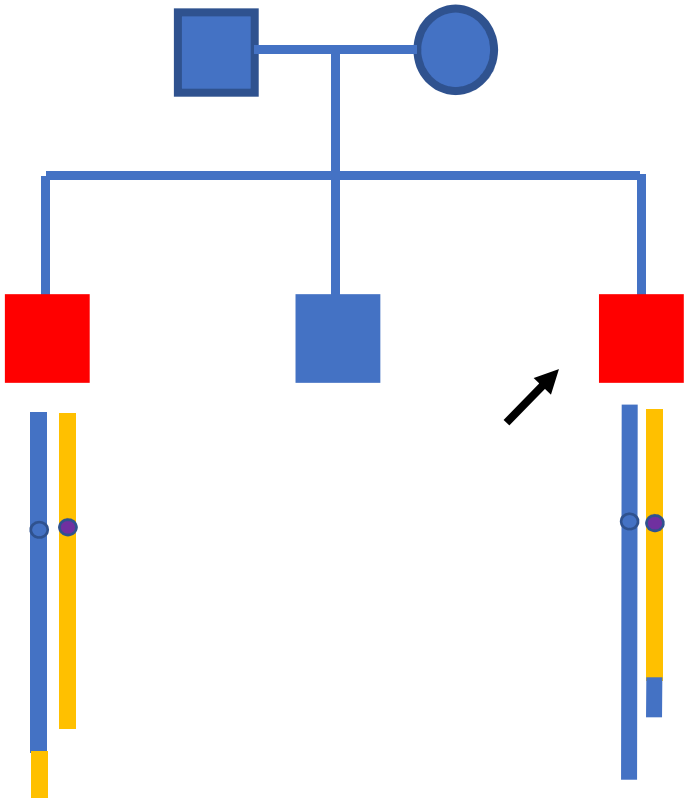


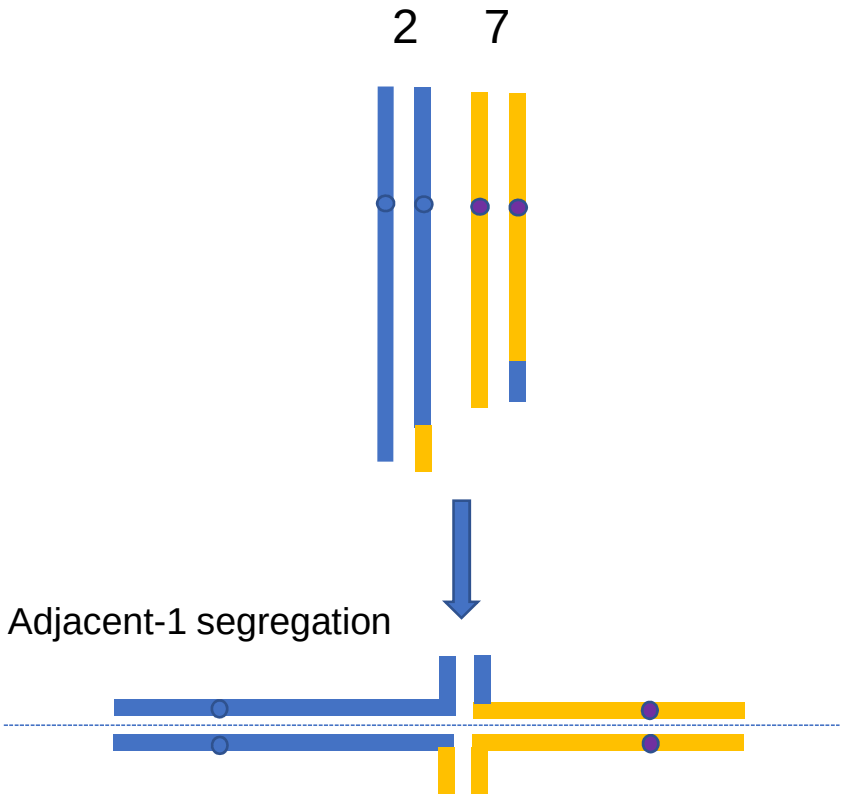
46,XY.rsa 2q37.2(P070)x3, 7q36.3(P070)x1


A
Patient 2
(8 yr 7 mo, follow-up: 9 yr 4 mo)
Patient 1, proband
(10 mo, follow-up: 26 mo)
B
C
Normal
Chr 7q36.3
Chr 2q37.2
Chr 2q37.2
Chr 7q36.3
Peak Ratio
Size (bps)
D



Chr	Start	End	Size(kbp)	Cnstate	Type	cytoband	marker count	confidence
2	239709268	242783384	3074.116	3	Gain	q37.3	2797	0.896625
7	146125161	159119707	12994.55	1	Loss	q35-q36.3	15784	0.91507




2
7
Adjacent-1 segregation

Yon et al. BMC Medical Genetics (2017) 18:30
DOI 10.1186/s12881-017-0394-7

BMC Medical Genetics

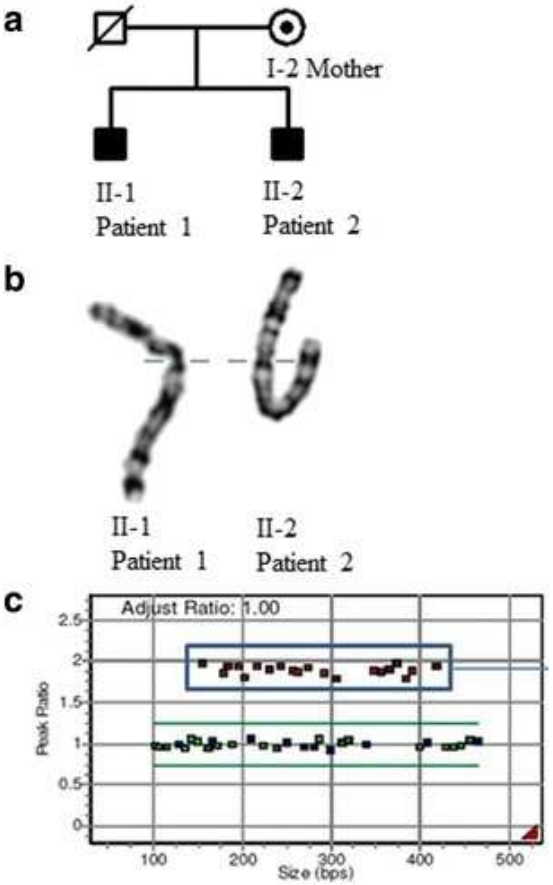
RESEARCH ARTICLE Open Access

A sibship with duplication of Xq28 inherited from the mother; genomic characterization and clinical outcomes

Dong Keon Yon[1], Ji Eun Park[2], Seung Jun Kim[3], Sung Han Shim[2,4*] and Kyu Young Chae[1*]

- A sibship, 11 and 10 years old with MR/DD and others
- Normal male karyotype
- Array CGH result: 411.5kb gain
  arr[GRCh38]Xq28(1530027303_153,438,781)x3

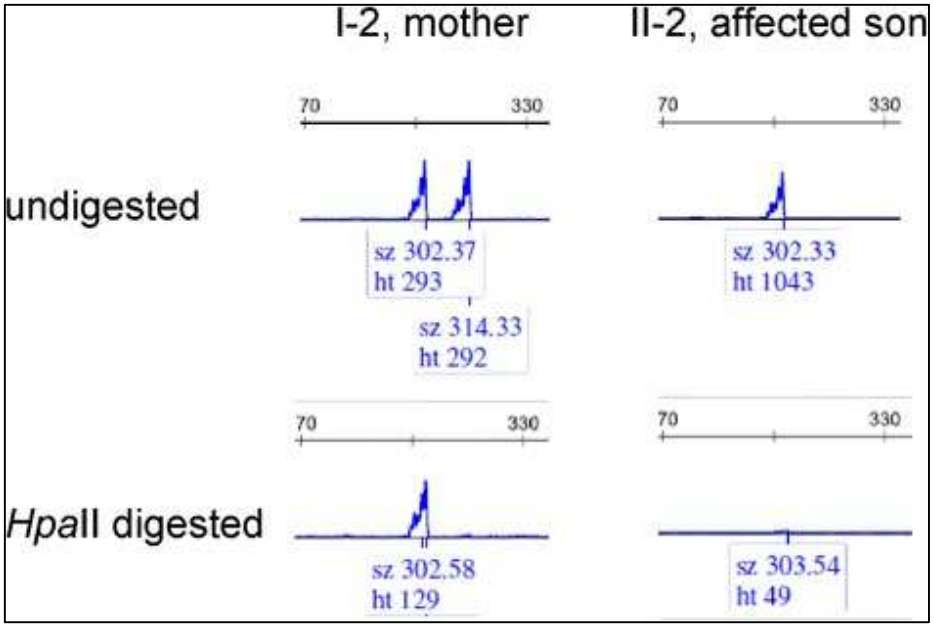
PLXNB3, SRPK3, IDH3G, SSR4, PDZD4, L1CAM, AVPR2, ARHGAP4, NAA10, RENBP, HCFC1, TMEM187, MIR3202-1, MIR3202-2, IRAK1, MIR718, ***MECP2****, OPN1LW*



- **MLPA:** *MECP2* probe mix → duplication of *MECP2* and several other genes

- *MECP2* duplication
  - Severe to profound X-linked intellectual disability
  - Rett syndromic features, progressive spasticity, neonatal or infantile hypotonia, poor speech development, recurrent respiratory infections, epilepsy, and dysmorphic facial features such as large ears, mid-face hypoplasia, brachycephaly, and depressed nasal bridge (Ramocki et al., 2010)

- X inactivation test of the mother: complete skewed of X chromosome with duplication



Research Article

Ophthalmologica

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Published online: July 4, 2018

Clinical and Genetic Characteristics Analysis of Korean Patients with Stargardt Disease Using Targeted Exome Sequencing

Youngje Sung[a] Seung Woo Choi[c] Sung Han Shim[b] Won Kyung Song[a]

[a]Department of Ophthalmology, CHA Bundang Medical Center, CHA University College of Medicine, Seongnam, South Korea; [b]Genetics Laboratory, Fertility Center of CHA Gangnam Medical Center, CHA University College of Medicine, Seoul, South Korea; [c]Department of Health Sciences and Technology, Samsung Advanced Institute for Health Sciences and Technology, Sungkyunkwan University, Suwon, South Korea

- Patients: 24 clinically diagnosed as Stargardt disease
- Targeted exome sequencing design:
  - 39 genes including *ABCA4, ELOVL4, PROM1*
  - Ion Ampliseq™ target selection technology (Thermo Fisher, USA)
  - Target size : 112.72 kb, Amplicon : 958 ea
  - Coverage:

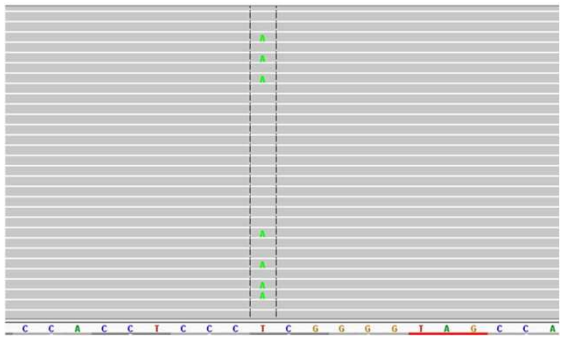
ABCA4	99.7%
ELOVL4	100%
PROM1	100%

Table 2. List of mutations identified from exome sequencing of the *ABCA4* gene

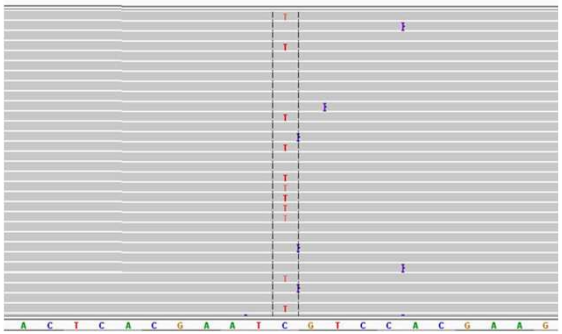
Patient No	Gene	Exon	cDNA sequence change	Amino acid change	Domain	Note	Mutation type	PolyPhen2 prediction	MutationTaster prediction
P001	*ABCA4*	8	c.983A>T	p.Glu328Val	extracellular loop	reported	–	–	–
	ABCA4	13	c.1933G>A	p.Asp645Asn	extracellular loop	reported	–	–	–
	ABCA4	21	c.3106G>A	p.Glu1036Lys	ATP-binding domain	reported	–	–	–
P002	*ABCA4*	19	c.2894A>G	p.Asn965Ser	extracellular loop	reported	–	–	–
	ABCA4	35	c.4972A>C	p.Ser1658Arg	–	novel	missense	possibly damaging	DC
P003	*ABCA4*	20	c.3035_3037delACA	p.Asn1012del	ATP-binding domain	novel	deletion	–	DC
	ABCA4	13	c.1804C>T	p.Arg602Trp	extracellular loop	reported	–	–	–
P004	*ABCA4*	19	c.2974A>C	p.Thr972Pro	ATP-binding domain	novel	missense	probably damaging	DC
	ABCA4	43	c.5929G>A	p.Gly1977Ser	ATP-binding domain	reported	–	–	–
P011	*ABCA4*	10	c.1268A>G	p.His423Arg	extracellular loop	reported	–	–	–
	ABCA4	40	c.5656G>A	p.Gly1886Arg	transmembrane domain	reported	–	–	–
	ABCA4	45	c.6146_6146delA	p.Lys2049Arg	ATP-binding domain	novel	deletion	benign	DC
P012	*ABCA4*	10	c.1268A>G	p.His423Arg	extracellular loop	reported	–	–	–
	ABCA4	10	c.1309C>A	p.Gln437Lys	extracellular loop	novel	missense	benign	DC
P013	*ABCA4*	6	c.635G>A	p.Arg212His	extracellular loop	reported	–	–	–
	ABCA4	12	c.1699G>A	p.Val567Met	extracellular loop	reported	–	–	–
P015	*ABCA4*	6	c.635G>A	p.Arg212His	extracellular loop	reported	–	–	–
	ABCA4	49	c.6764G>T	p.Ser2255Ile	–	reported	–	–	–
P016	*ABCA4*	5	c.560G>A	p.Arg187His	extracellular loop	novel	missense	benign	DC
	ABCA4	6	c.635G>A	p.Arg212His	extracellular loop	reported	–	–	–
	ABCA4	10	c.1268A>G	p.His423Arg	extracellular loop	reported	–	–	–
P017	*ABCA4*	8	c.880C>T	p.Gln294*	extracellular loop	novel	stop-gain	–	DC
	ABCA4	10	c.1294G>A	p.Glu432Lys	extracellular loop	reported	–	–	–
	ABCA4	33	c.4685T>A	p.Ile1562Thr	extracellular loop	reported	–	–	–
P018	*ABCA4*	10	c.1268A>G	p.His423Arg	extracellular loop	reported	–	–	–
	ABCA4	20	c.3035_3037delACA	p.Asn1012Ile	ATP-binding domain	novel	deletion	–	DC
	ABCA4	47	c.6389T>A	p.Met2130Lys	–	novel	missense	probably damaging	DC
P020	*ABCA4*	8	c.880C>T	p.Gln294*	extracellular loop	novel	stop-gain	–	DC
	ABCA4	23	c.3398T>C	p.Ile1133Thr	extracellular loop	novel	missense	benign	DC
P021	*ABCA4*	14	c.1958G>A	p.Arg653His	transmembrane domain	reported	–	–	–
	ABCA4	40	c.5656G>A	p.Gly1886Arg	transmembrane domain	reported	–	–	–
P022	*ABCA4*	13	c.1933G>A	p.Asp645Asn	extracellular loop	reported	–	–	–
	ABCA4	44	c.6146-6146delA	p.Lys2049Arg	ATP-binding domain	novel	deletion	benign	DC
P023	*ABCA4*	20	c.3035_3037delACA	p.Asn1012Ile	ATP-binding domain	novel	deletion	–	DC
	ABCA4	24	c.3547G>T	p.Gly1183Cys	–	reported	–	–	–
	ABCA4	46	c.6289C>T	p.Pro2097Ser	ATP-binding domain	novel	missense	probably damaging	DC
P024	*ABCA4*	10	c.1268A>G	p.His423Arg	extracellular loop	reported	–	–	–
	ABCA4	33	c.4748T>C	p.Leu1583Pro	extracellular loop	reported	–	–	–
P026	*ABCA4*	16	c.2384G>A	p.Ser795Asn	transmembrane domain	novel	missense	possibly damaging	DC
	ABCA4	23(?)	c.3470T>G	p.Leu1157*	–	novel	stop-gain	–	DC

DC, disease causing.

Patient S-001



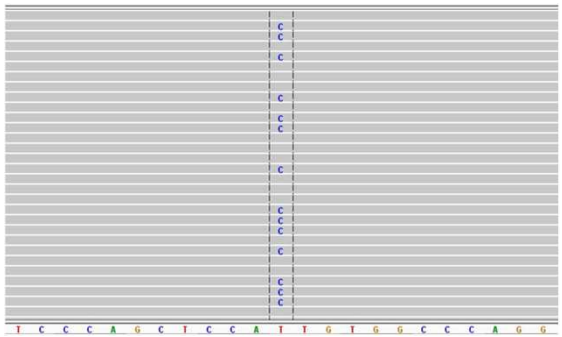
c.983A>T
Glu328Val
(E328V)
Read depth: 1914
T allele (%): 26.8%



c.1933G>A
Asp645Asn
(D645N)
Read depth: 796
A allele (%): 42.9%

c.3106G>A
Glu1036Lys
(E1036K)
Read depth: 4538
A allele (%): 49.6%

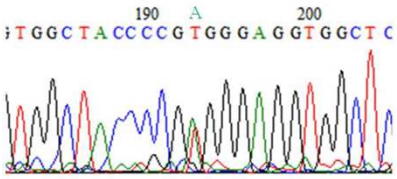
Patient S-002



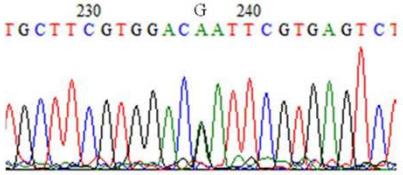
c.2894A>G
Asn965Ser
(N965S)
Read depth: 1964
G allele (%): 49.8%

c.4972A>C
Ser1658Arg
(S1658R)
Read depth: 1658
C allele (%): 50.8%

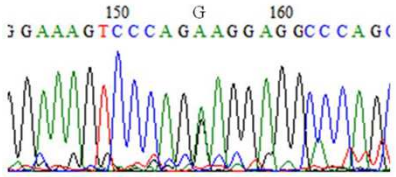
Patient S-001



ABCA4 exon 8
c.983A>T (Glu328Val)

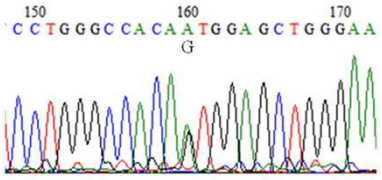


ABCA4 exon 13
c.1933G>A (Asp645Asn)

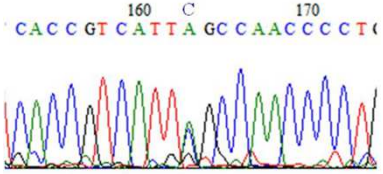


ABCA4 exon 21
c.3106G>A (Glu1036Lys)

Patient S-002



ABCA4 exon 19
c.2894A>G (Asn965Ser)



ABCA4 exon 35
c.4972A>C (Ser1658Arg)

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Original Article

A novel variant of *NPPC* causes abnormal post-translational cleavage: A candidate gene for premature ovarian insufficiency

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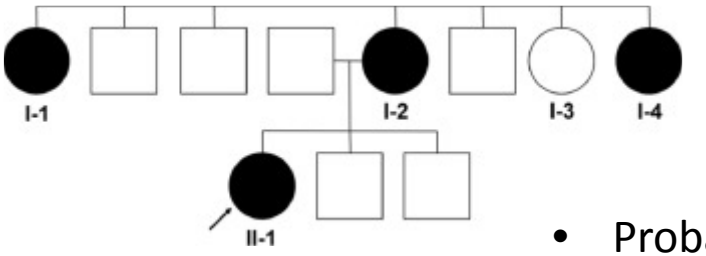
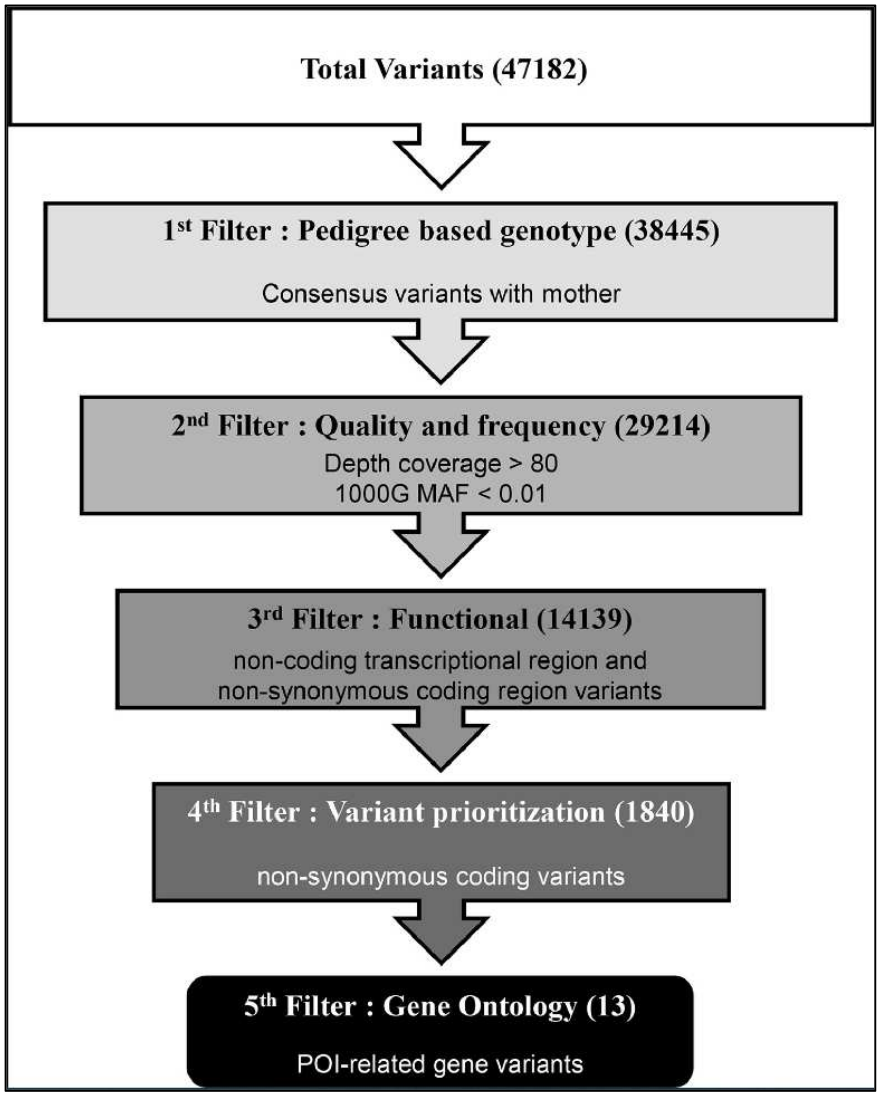


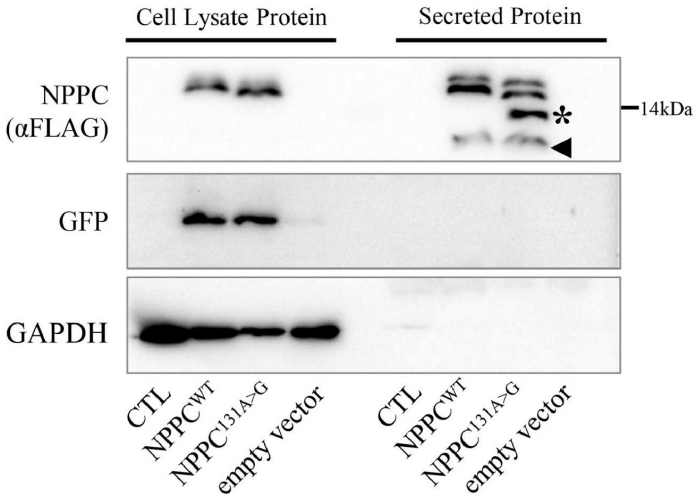
- Proband
  - 36 years old
  - Hypergonadotropic hypogonadism
  - FSH: >100mIU; LH: 32.5mIU; E2: 5.7pg/ml
  - Small uterus, atrophic ovaries, absent antral follicles in both ovaries

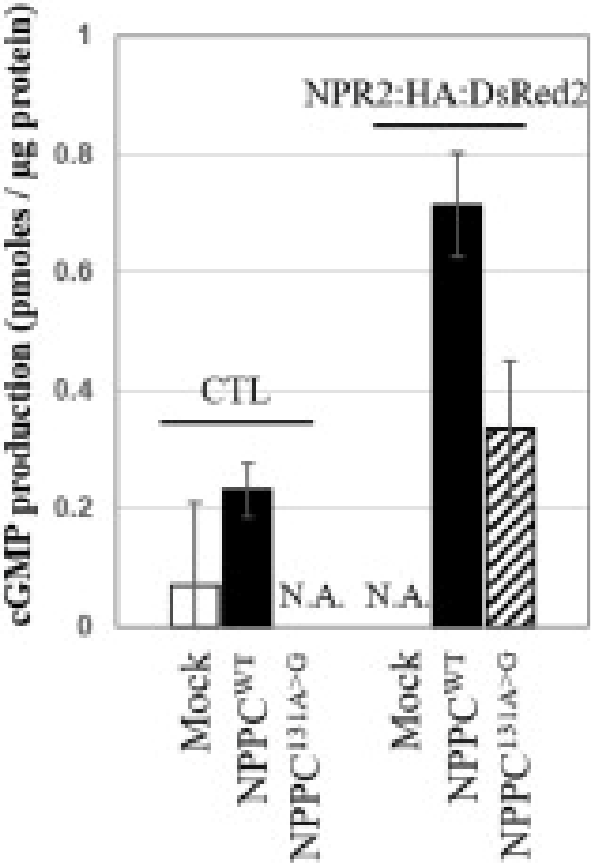
- Whole exome sequencing of the proband and her mother
  - Trusight OneSequencing Panel (Illumina, San Diego, CA, USA)
  - HiSeq500 sequencer (paired-end 2 × 150)
  - Sequencing reads reached 20X coverage or more in 95% of regions in the target exome
  - Alignment to human reference assembly (GRCh37.p13)



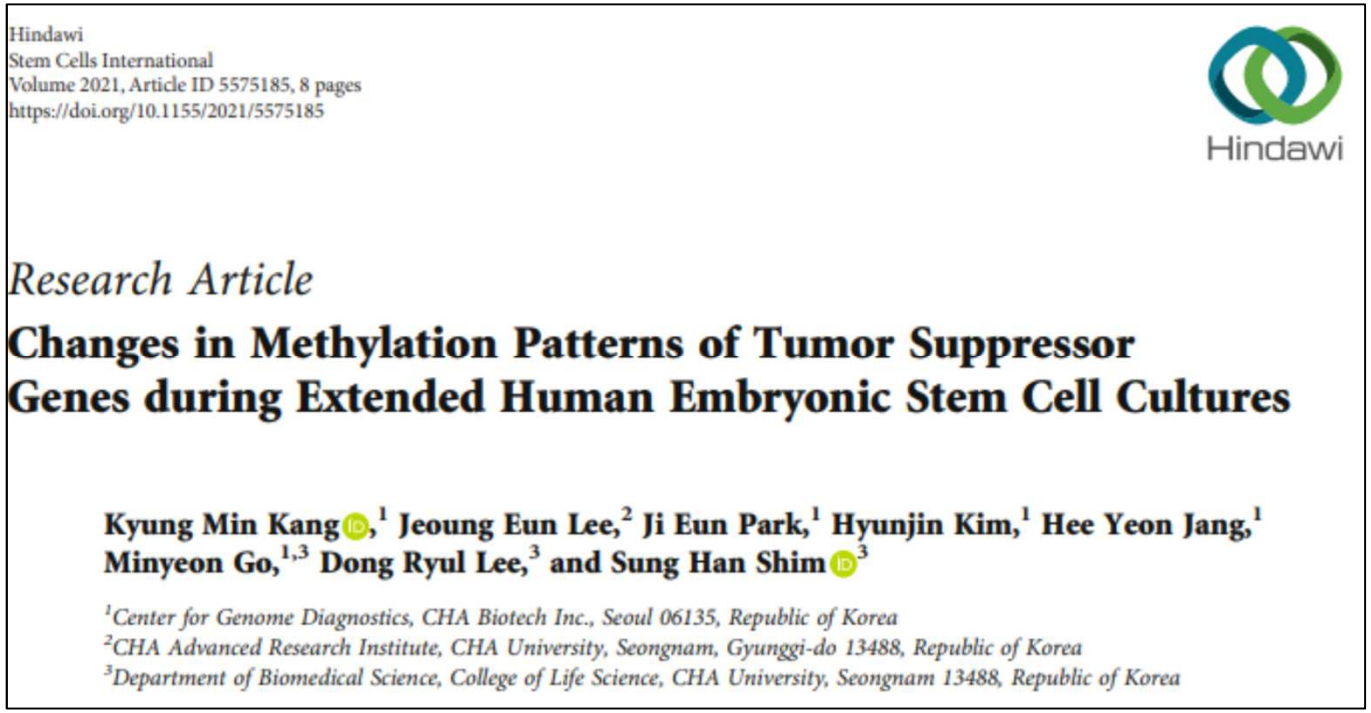
Gene	Position	SNP ID	cDNA change	Protein change	1000 G MAF	KRG	Prediction (Poly Phen2 / SIFT)	Genotype
DDX54	Chr12:-113596897	rs201014565	c.2434G>*A*	p.A812T	0.00092	0.00161	B / T	P(HetAlt) M(Het Alt)
DSG2	Chr18:-29126558	rs149617776	c.3209C>T	p.T1070M	0.00595	0.01045	B / T	
GDF9	Chr5:-132200057	rs138136756	c.169G>*T*	p.D57Y	0.00458	0.03376	B / T	
GPAM	Chr10:-113917134	NA	c.1994A>*G*	p.E665G	NA	0.00046	PD / D	
MDK	Chr11:-46404341	NA	c.449C>G	p.A150G	0.00004	NA	B / NA	
NOTCH1	Chr9:-139391211	rs202065858	c.6980G>*A*	p.R2327Q	0.00092	0.00080	B / T	
NPPC	Chr2:-232790385	rs80022541	c.131A>*G*	p.Q44R	0.00458	0.00402	B / D	
NSD1	Chr5:-176562115	NA	c.11C>A	p.T4N	0.00001	NA	B / D	
PNPLA8	Chr7:-108155171	NA	c.764delC	p.A255 frameshift	NA	NA	NA / NA	
PSPH	Chr7:-56087300	rs75395437	c.268G>*A*	p.G90S	0.00506	0.00804	P / D	
	Chr7:-56087319	rs73343757	c.249A>C	p.Q83H	0.00851	0.00080	B / T	
SSTR3	Chr22:-37602809	rs202051882	c.1034C>T	p.P345L	0.00046	0.00482	B / T	
TMF1	Chr3:-69082833	rs147346094	c.2276G>*A*	p.R759Q	0.00366	0.01125	PD / D	
TNC	Chr9:-117848187	NA	c.1823G>*A*	p.R608H	0.00010	0.00080	B / T	

- *NPPC*
  - C-type natriuretic peptide (CNP)-encoding gene
  - Involved in follicle growth at the preantral stage
  - Prevents precocious resumption of oocyte meiosis





The mutation of *NPPC* leads to abnormal peptide cleavage, resulting in a decrease in cGMP levels and meiotic resumption, it could induce an exhaustion of follicular reserve or failure of follicular development.



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Hindawi

Research Article

Changes in Methylation Patterns of Tumor Suppressor Genes during Extended Human Embryonic Stem Cell Cultures

Kyung Min Kang,[1] Jeoung Eun Lee,[2] Ji Eun Park,[1] Hyunjin Kim,[1] Hee Yeon Jang,[1] Minyeon Go,[1,3] Dong Ryul Lee,[3] and Sung Han Shim[3]

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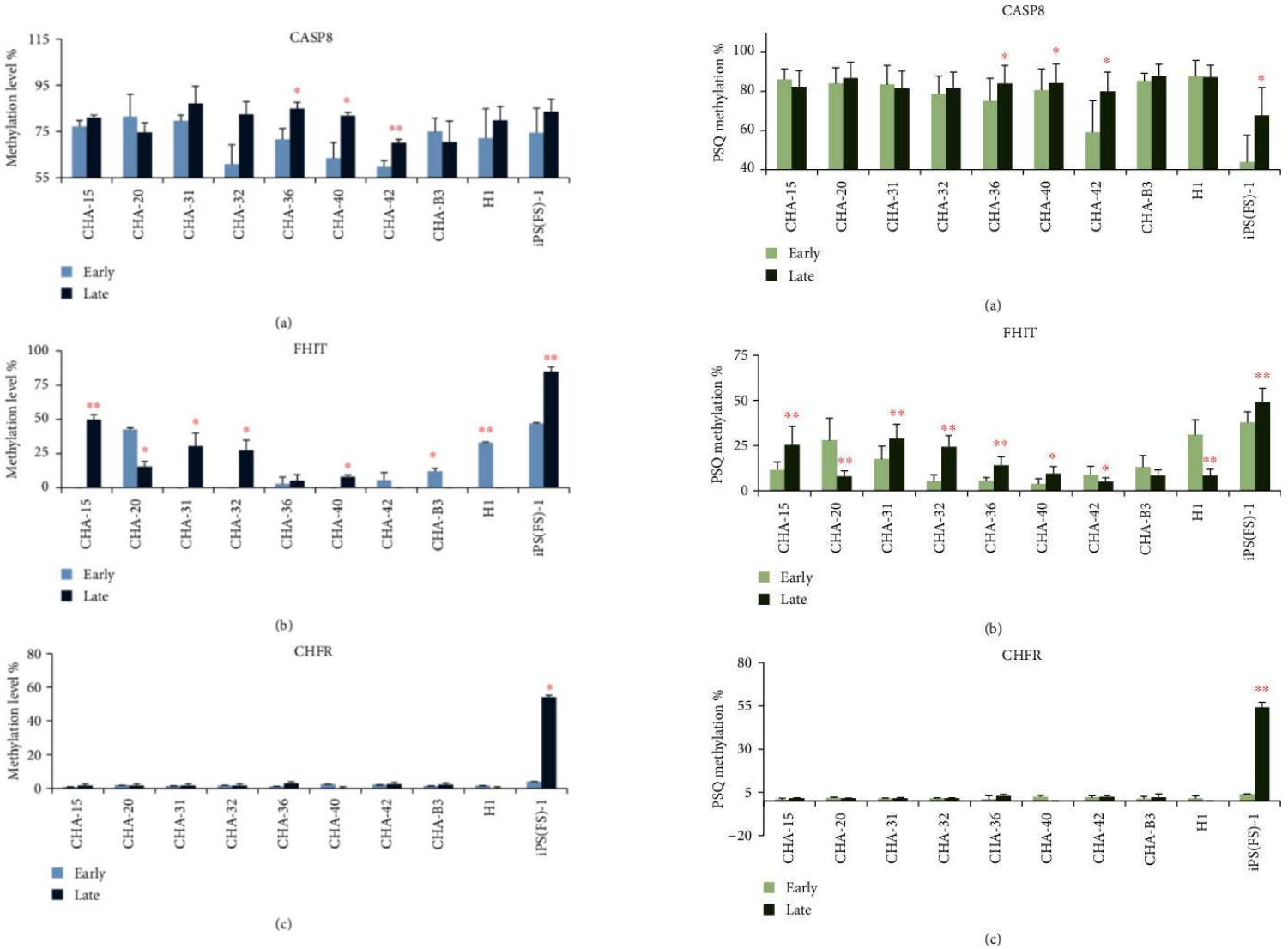
[3]*Department of Biomedical Science, College of Life Science, CHA University, Seongnam 13488, Republic of Korea*

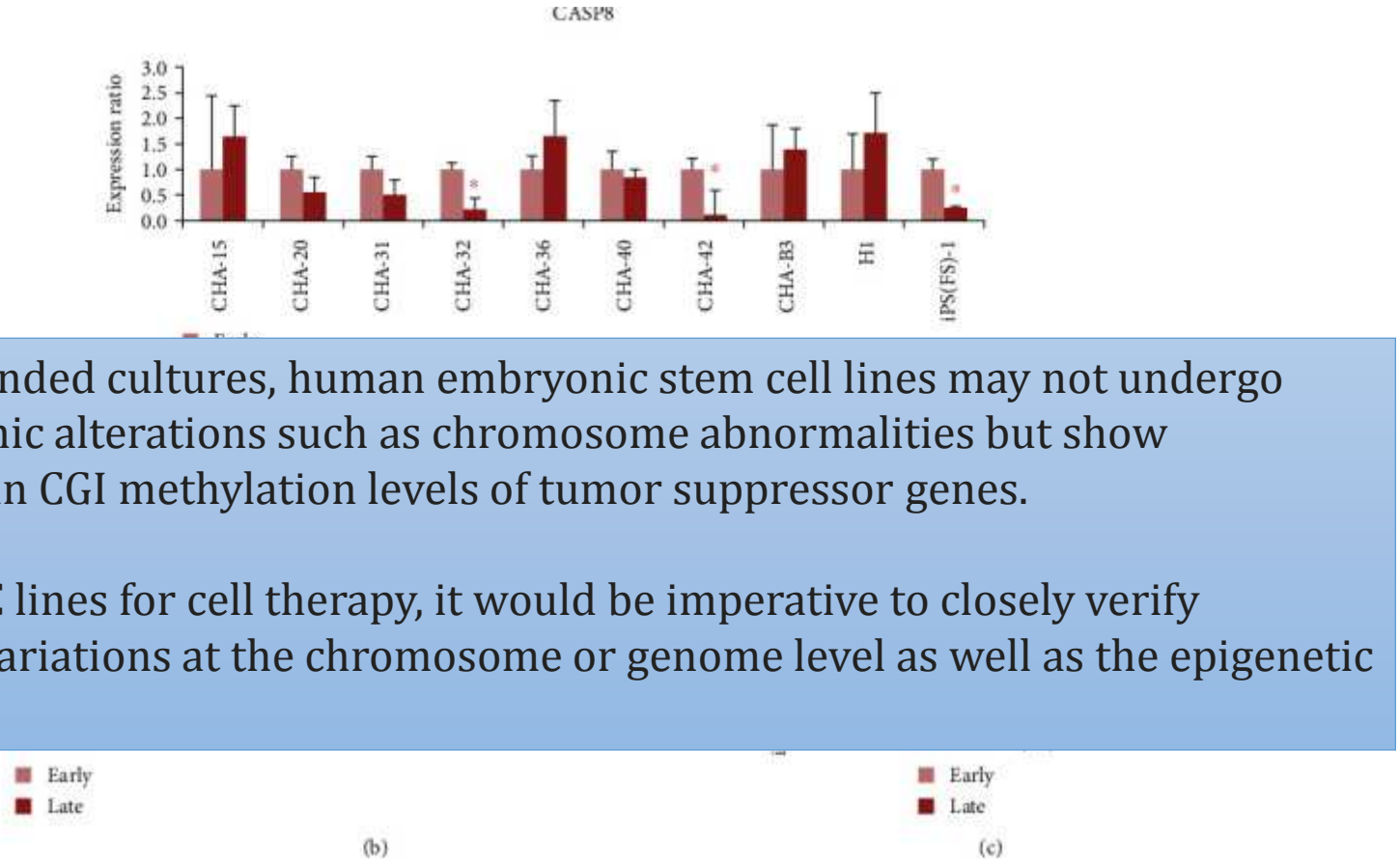
- 10 hESc lines
- CpG island methylation levels of 24 tumor suppressor genes were analyzed
- Methylation specific MLPA, pyrosequencing, real-time PCR



MS-MLPA

Pyrosequencing

Methylation level of the three genes (*CASP8, FHIT, CHFR*) between early passage and late passage of 10 cell lines using MS-MLPA and pyrosequencing.



- During extended cultures, human embryonic stem cell lines may not undergo large genomic alterations such as chromosome abnormalities but show alterations in CGI methylation levels of tumor suppressor genes.

- To use hESC lines for cell therapy, it would be imperative to closely verify structural variations at the chromosome or genome level as well as the epigenetic changes.

Expression level of the three genes between early passage and late passage of 10 cell lines using real-time PCR analysis. Expression ratio of the *CASP8* gene (a), *FHIT* gene (b), and *CHFR* gene in iPS (FS)-1 cell (c).

감사합니다.