약 력

1. 인적사항

성 명	이 수 만
소속기관	포천중문의과대학
직 위	부교수
전자메일	suman@cha.ac.kr

2. 학력/경력

연 도	학교 / 기관	전공 / 직위	학위 / 비고
1991. 8 - 1996. 2	미국 노스캐롤라이나 주립대학 (채플힐 소재) 의과대학		생화학 박사
1996. 3 - 1997. 9	미 국립 라인버거 암 연구소		박사후 과정
1997. 9 - 1999. 9	미 국립 로스 알라모스 연구소	연구원	
1999. 9 - 2004	포천 중문 의대 부설 차 병원	유전학 연구실장	
1999. 9 - 현재	포천 중문 의대 의학과	부교수	

3. 주요연구실적(개조식, 간단하게)

- Cell, Science, Journal of Biological Chemistry,
- Current opinion in structural biology, Fertility and Sterility, BBRC, American Journal of Medical Genetics등 국제 SCI 학술지 30편

4. 발표시 사용 기자재

* LCD projector의 사용을 원칙으로 합니다.
* LCD 사용을 위해 CD나, 저장 매체에 담아 오시는 것을 권장하며, Zip드라이브는 학회에서 준비하지 않습니다.

Gene Expression study of human chromosomal aneuploid

Suman Lee

Functional Genomics Lab, Bundang Campus, College of Medicine, Pochon CHA University, 222 Yatap-Dong, Bundang-Gu, Sungnam-Si, Kyunggi-Do, 463-836, Korea

Chromosomal copy number changes (aneuploidies) are common in human populations. The extra chromosome can affect gene expression by whole-genome level. By gene expression microarray analysis, we want to find aberrant gene expression due to aneuploidies in Klinefelter (+X) and Down syndrome (+21). We have analyzed the inactivation status of X-linked genes in Klinefelter Syndrome (KS) by using X-linked cDNA microarray and cSNP analysis. We analyzed the expression of 190 X-linked genes by cDNA microarray from the lymphocytes of five KS patients and five females (XX) with normal males (XY) controls. cDNA microarray experiments and cSNP analysis showed the differentially expressed genes were similar between KS and XX cases.

To analyze the differential gene expressions in Down Syndrome (DS), Amniotic Fluid (AF)cells were collected from 12 pregnancies at 16~18 weeks of gestation in DS (n=6) and normal (n=6) subjects. We also analysis AF cells for a DNA microarray system and compared the chip data with two dimensional protein gel analysis of amniotic fluid. Our data may provide the basis for a more systematic identification of biological markers of fetal DS, thus leading to an improved understanding of pathogenesis for fetal DS.

Gene expression study of human chromosomal aneuploid

Suman Lee, PhD

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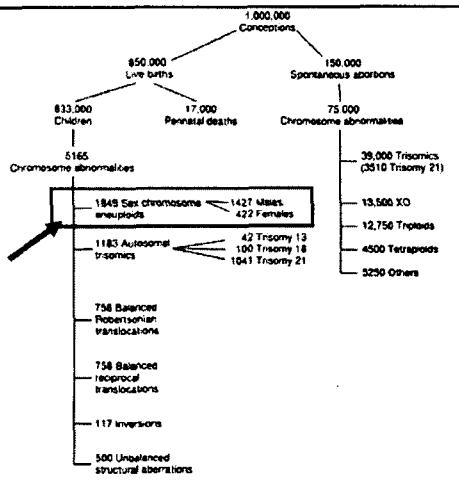
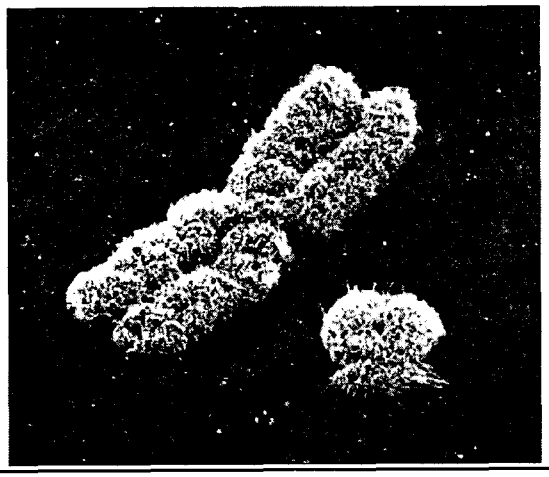
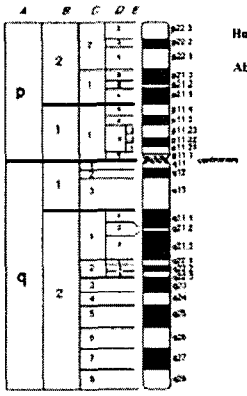




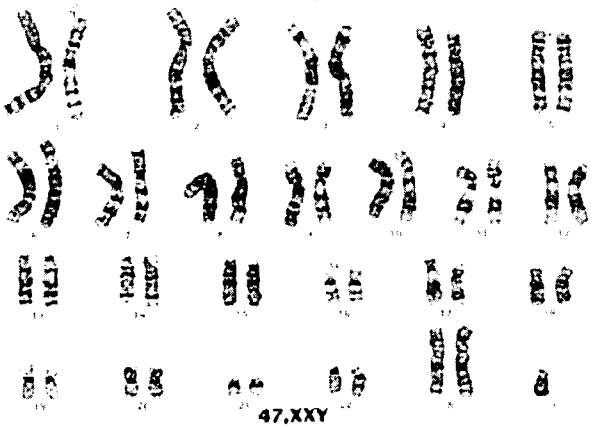
X Chromosome



Human X chromosome (160 Mb)

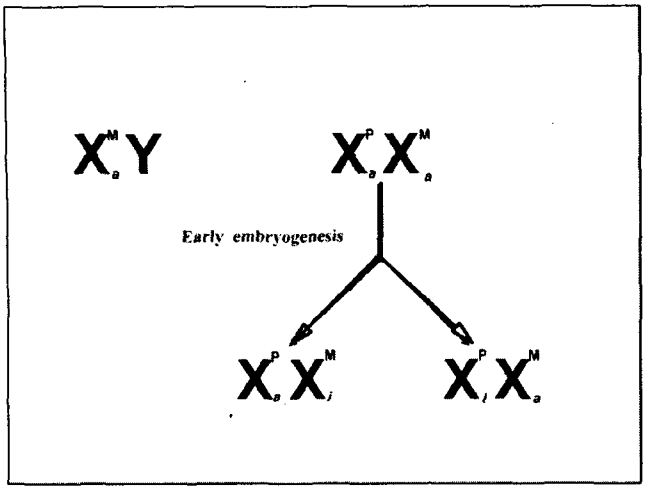
Abnormalities

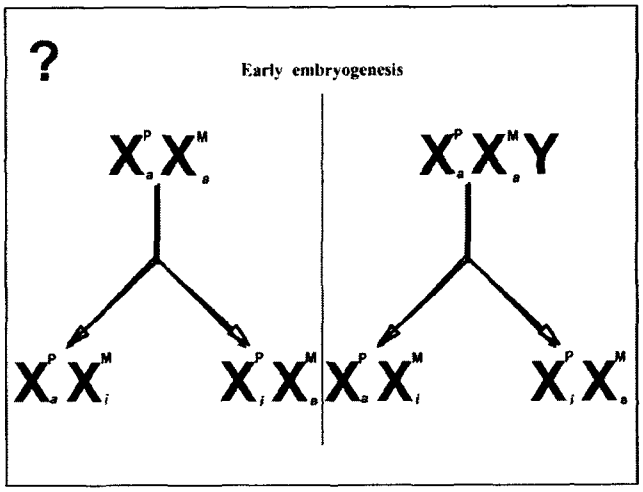
- **Turner's syndrome**: XO, XXX, XXXX, XXXXX
- **Klinefelter's syndrome**:XXY or XXXY, males

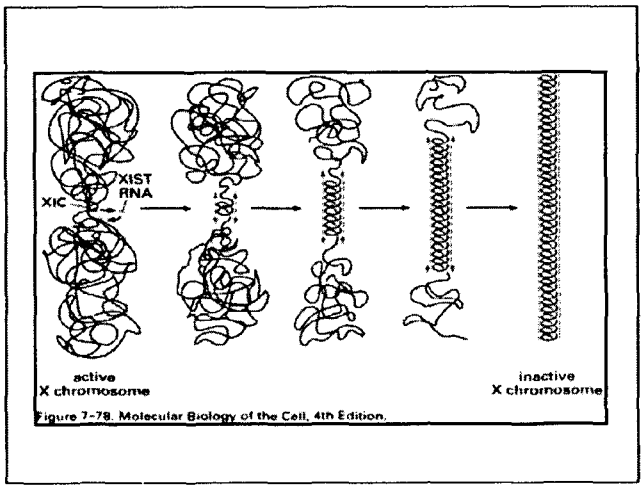


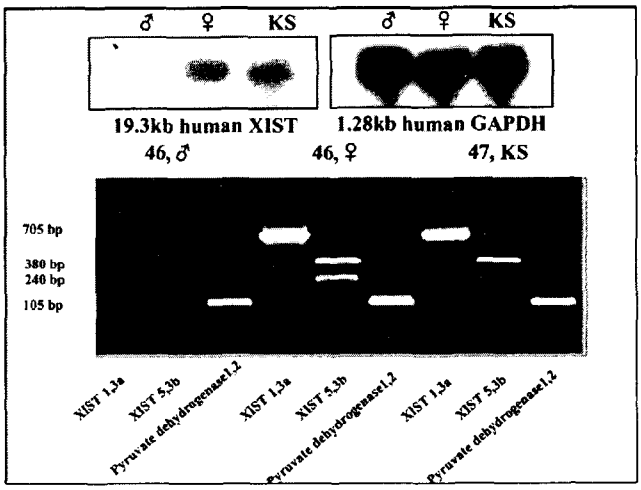
X chromosome polyploidy

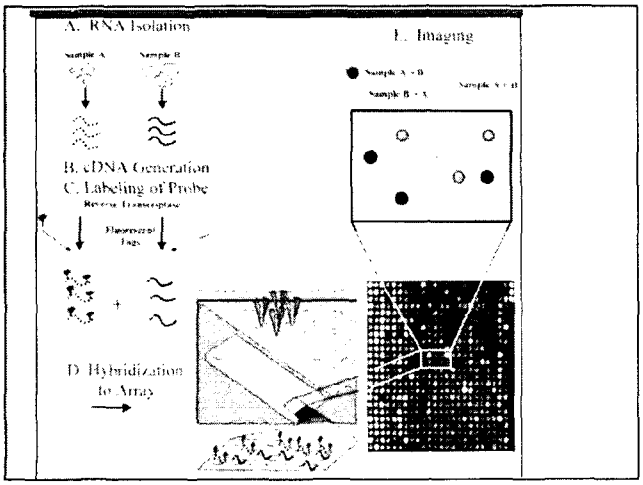
47, XXY (**Klinefelter's syndrome**) extra X chromosomes predispose to azoo and female phenotype development (micropenis, etc.), **small testes and elevated gonadotropin levels.** mental retardation, disproportionate growth of the legs, and other somatic anomalies, with estimated incidence of 1:500 in newborns.

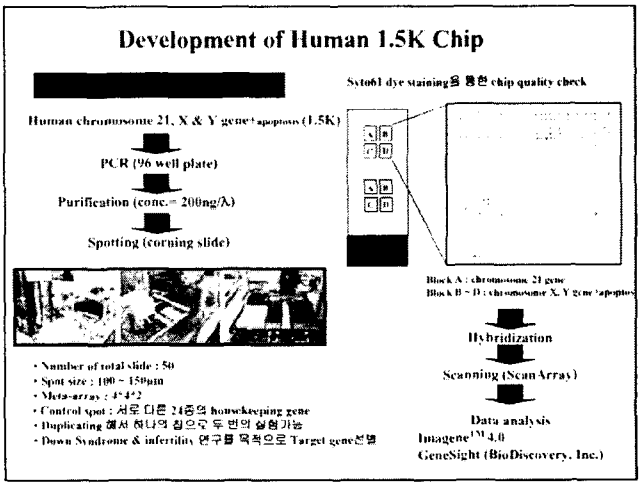

Early embryogenesis


?
Early embryogenesis


XIC
XIST RNA
active X chromosome
inactive X chromosome
Figure 7-78 Molecular Biology of the Cell, 4th Edition.


♂
♀
KS
19.3kb human XIST
1.28kb human GAPDH
46, ♂
46, ♀
47, KS
705 bp
380 bp
240 bp
105 bp
XIST 1.3a
XIST 5.3b
Pyruvate dehydrogenase1.2


A. RNA Isolation
B. cDNA Generation
C. Labeling of Probe
D. Hybridization to Array
E. Imaging


Development of Human 1.5K Chip
Syto61 dye staining을 통한 chip quality check
PCR (96 well plate)
Purification (conc.= 200ng/Λ)
Spotting (corning slide)
Hybridization
Scanning (ScanArray)
Data analysis
Imagene™ 4.0
GeneSight (BioDiscovery, Inc.)

I. Cytogenetic and Y Chromosome Microdeletion Assay

⇩

II. Cell and Cell culture

→ We obtained EBV-lymphoblstoid cell lines.

→ Cells were grown in a CO_2 incubator (37℃, 5% CO_2) in RPMI 1640 medium

⇩

III. RNA Extraction and DNAse treatment

⇩

IV. Semi-quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)

→ RT-PCR for expression of each up- and down-regulated genes in cells of each sample were examined by RT-PCR that confirm the *results of cDNA chip.*

⇩

VII. Probe Preparation and Hybridization to cDNA microarray

→ RNA reverse transcription, Cy3 (male and female) and Cy5 (KS) labelling, purify, prehybridizaton, hybridization (58℃, 16hr) and washing

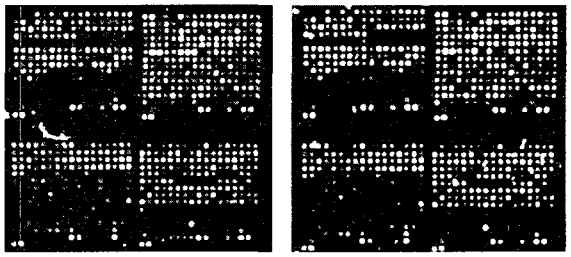
⇩

VIII. Analysis of Gene Expression

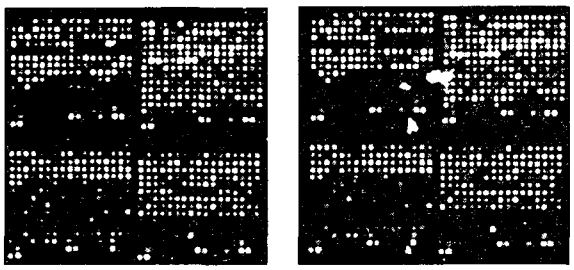
→ scanned on ScanArray 4000XL system and analyzed with GeneSight 3.2, normalized with global normalization method on GeneSight , classified category by GeneSpling

Chip Analysis

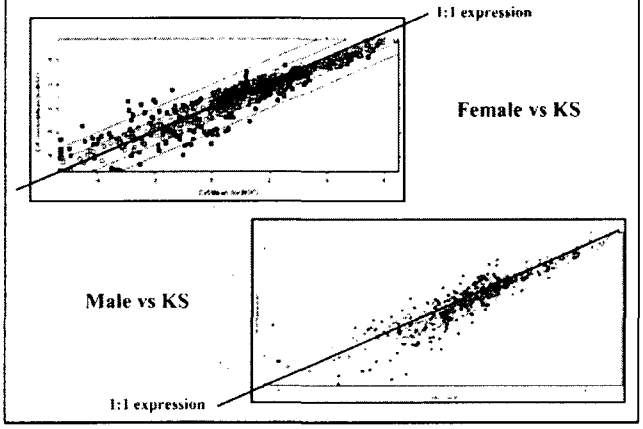
XXY (n=5)-XY (n=5)

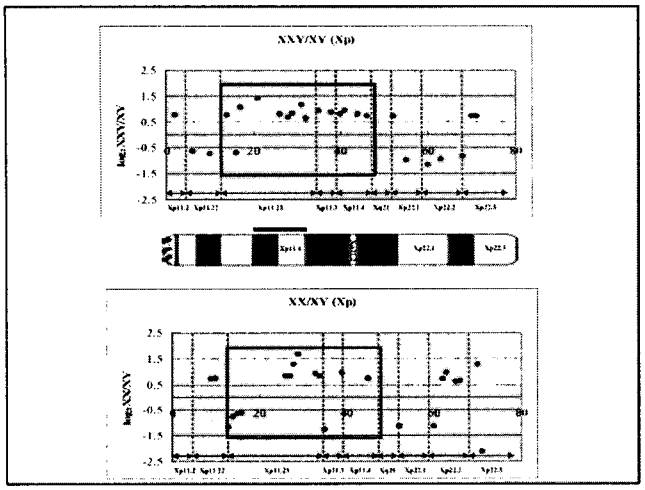


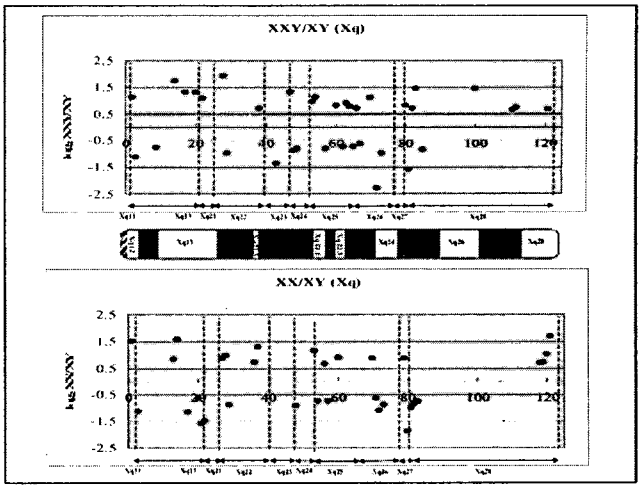
XX (n=5)- XY (n=5)



RESULTS





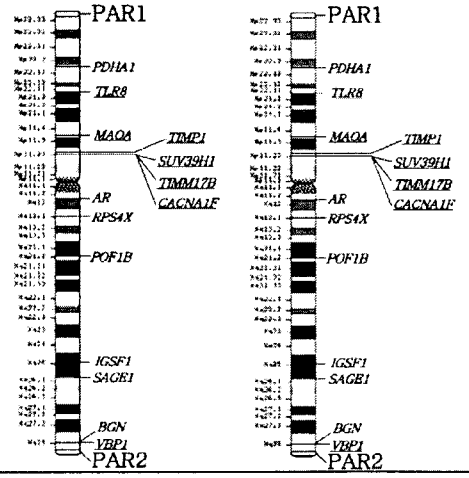


Identification of randomly selected genes with differential expression from male, female and KS through cDNA microarray

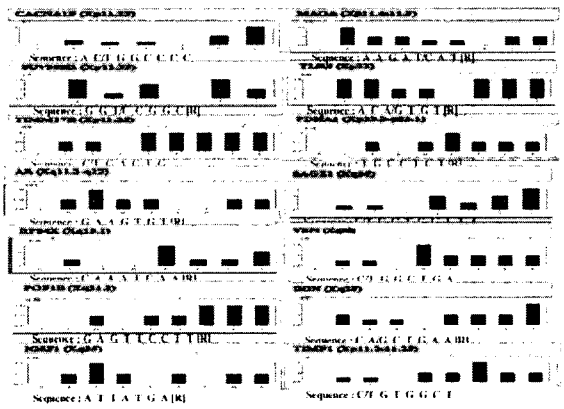
Gene ID	Gene Name	Cytogenic band	XX	XXY
Genes up-regulated in KS patient than in normal female				
NM_024539	RNF 128	Xq 22.3		
NM_000169	GLA	Xq 22		
NM_001008	RPS4Y	Yp 11.3		
NM_022052	NXF3	Xq 22-q 23		
NM_004726	REPS2	Xp22.22		
Genes up-regulated in normal female than in KS patient				
NM_006875	PIM2	Xp 11.23		
NM_005120	TNRC11	Xq13		
NM_002046.2	GAPDH			

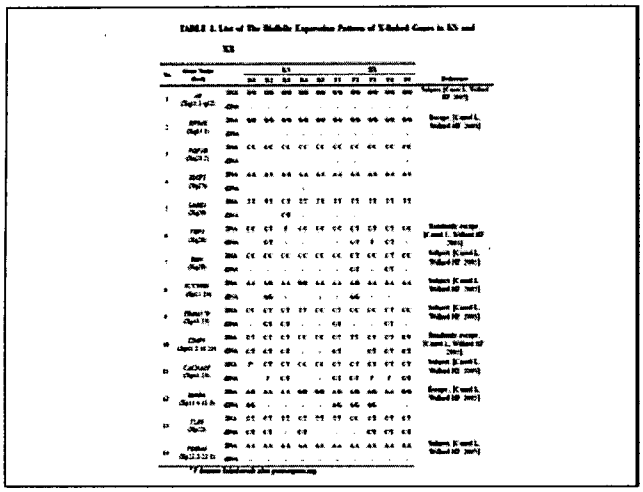
Gene ID	Gene Name	Cytogenic band	XY	XXY
Genes up-regulated in KS patient than in normal male				
NM_024539	RNF 128	Xq 22.3		
NM_022052	NXF3	Xq 22-q 23		
NM_001666	ARHGAP4	Xq 28		
NM_012280	FTSJ1	Xp 11.23		
Genes up-regulated in normal male than in KS patient				
NM_002668	PLP2	Xp 11.23		
NM_002046.2	GAPDH			

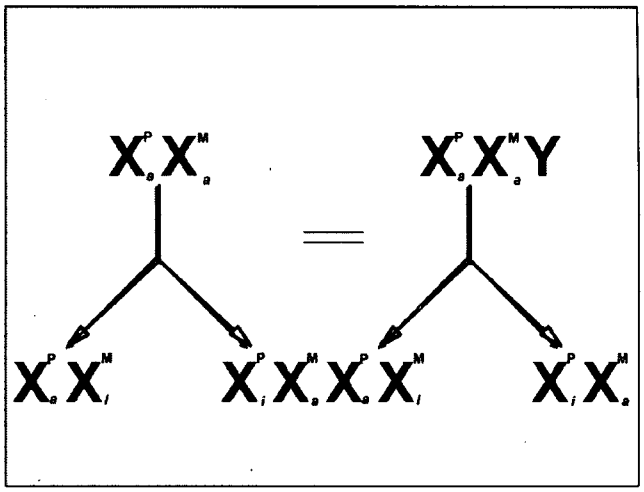
Comparison of gSNP and cSNP on X-linked genes

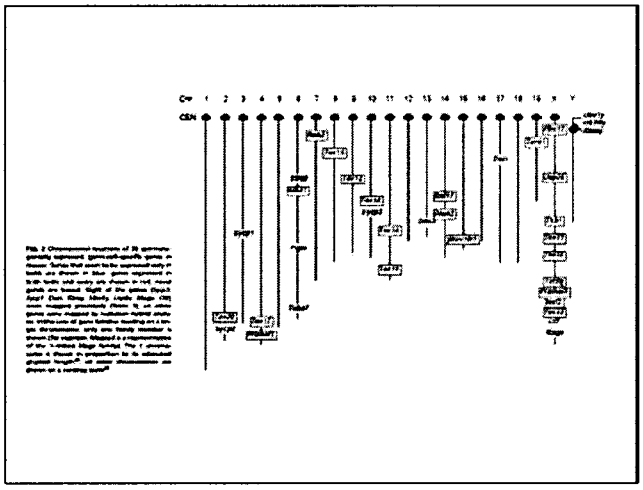


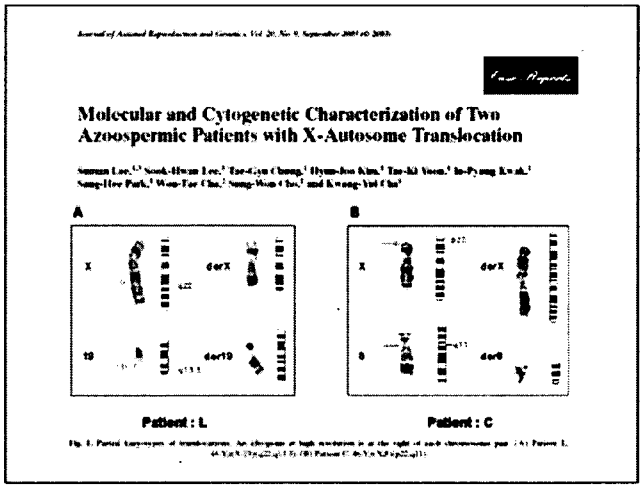
Comparison of gSNP and cSNP on X-linked genes

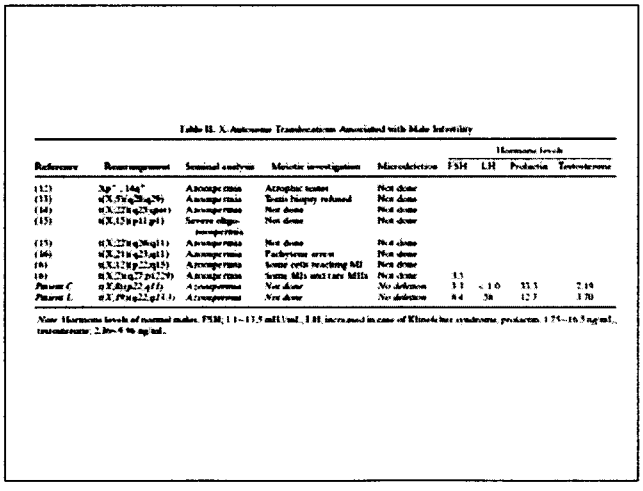


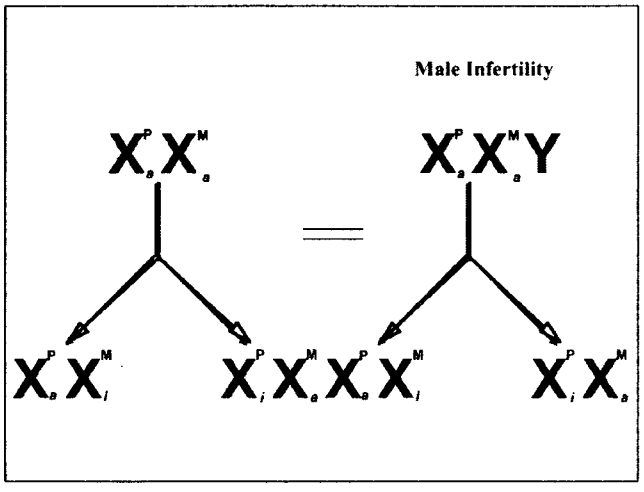


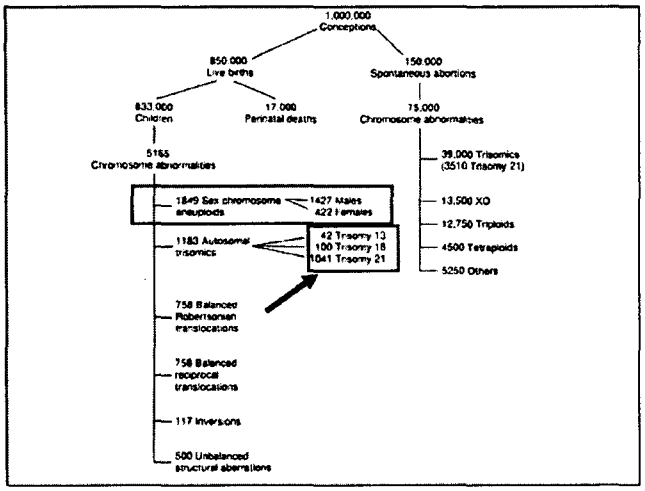


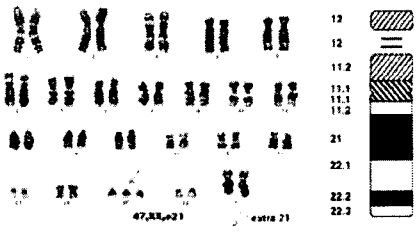



Molecular and Cytogenetic Characterization of Two Azoospermic Patients with X-Autosome Translocation
Patient : L
Patient : C




Male Infertility





Trisomy +21 hC 21

The physical features of Down Syndrome

- flat facial profile
- upward slant to the eye
- short neck
- abnormally shaped ears
- white spots on the iris of the eye (called Brushfield spots)
- single, deep transverse crease on the palm of the hand

- Trisomy 21 is the most common autosomal aneuploidy and this complex disease cause developmental and neuronal defects.
- Three copies of chromosome 21 genes results in disruption of normal patterns of development.
- DS occurs 1 out of 700 live births.
- Maternal-age passes 35 years is an important reason for occurrence of Down Syndrome.
- DS can affect many aspects of development, producing variable clinical features in DS patients.
- DS patients shows many clinical features, which are mental retardation, congenital heart disease, short stature, defects of glucose metabolism and so on.

RELATIONSHIP OF DOWN SYNDROME INCIDENCE TO MOTHERS' AGE

Mothers Age	*Incidence of Down Syndrome*
Under 30	Less than 1 in 1,000
30	1 in 900
35	1 in 400
36	1 in 300
37	1 in 230
38	1 in 180
39	1 in 135
40	1 in 105
42	1 in 60
44	1 in 35
46	1 in 20
48	1 in 16
49	1 in 12

Source: Hook, E.G., Lindsjo, A. Down Syndrome in Live Births by Single Year Maternal Age.

Down Syndrome and Associated Medical Disorders

- Hearing loss
- Vision disorders
- Seizure disorders
- Congenital heart disease
- Congenital hypothyroidism
- Mental retardation

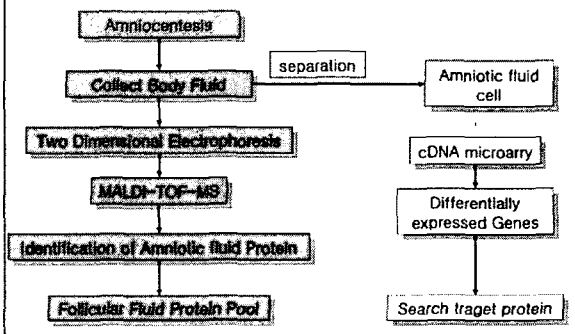
The Chromosomal Basis of Down Syndrome

1. Trisomy 21 : Three copies of chromosome 21 are present in all cells of the individual (92%)
2. Mosaicism : The extra chromosome 21 is present in some, but not all, cells of the individual (2-4%)
3. Translocation : material from one chromosome 21 gets stuck or translocated onto another chromosome, either prior to or at conception (3-4%)

- *Newborns* – Hypotonia (decreased fetal activity)
- *Infants and Preschool Children* – Leukemia, Infectious diseases
- *Adolescence* - Males with Down syndrome generally have a reduced sperm count and rarely father children. Females with Down syndrome have regular menstrual periods and are capable of becoming pregnant and carrying a baby to term.
- *Adults* - Premature aging is a characteristic of adults with Down syndrome. In addition, dementia, or memory loss and impaired judgment similar to that occurring in Alzheimer disease patients, may appear in adults with Down syndrome.

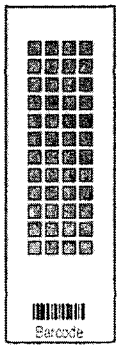
- ***The purpose of this study was to find biomarker in DS for diagnosis and drug targetting***
- ***Cytogenetic analysis is expensive, time consuming. is nearly dangerous for sampling.***

Down syndrome and normal cells



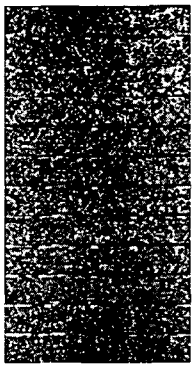
34k Oligo Chip

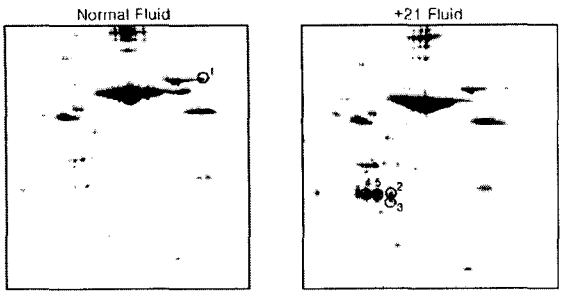
- Gene : 34,580
- Regular spot size : 100㎛, Oligo size : 70mer
- Format : 4 block * 16 block) * 1 array, (23*23 spots)/block
- Positive control : 12 * 16 repeat
- Negative control : 12 * 16 repeat



33K OligoDNA Chip

>>Report for DC Oligo clone grouping by list	list.txt
>signal_transduction	1142 / 19357
>cell_cycle	347 / 19357
>cell_growth_and_maintenance	1615 / 19357
>cell_death	211 / 19357
>response_to_stress	354 / 19357
>transcription	823 / 19357
>immune_response	287 / 19357
>apoptosis	202 / 19357





Protein and mRNA pattern

- S1
  - DNA chip Data
    - 0.3624134 → 1.28556 increase
  - 2D Data
    - 6.96526 increase
  - Function
    - Progesteron secretion regulation
    - Late pregnancy disorders.
    - Pregnancy and pregnancy disorders

- S2
  - DNA chip Data
    - 0.566472 → 1.48089771 increase
  - 2D Data
    - New
  - Function
    - *Chronic disease*

- S3
  - DNA chip Data
    - 0.0956 → 1.068509
  - 2D Data
    - New
    - Study not yet

Further Study

- 2DE : Blood sample
- RT-PCR and Real-time PCR
- Western blotting
- Statistical analysis