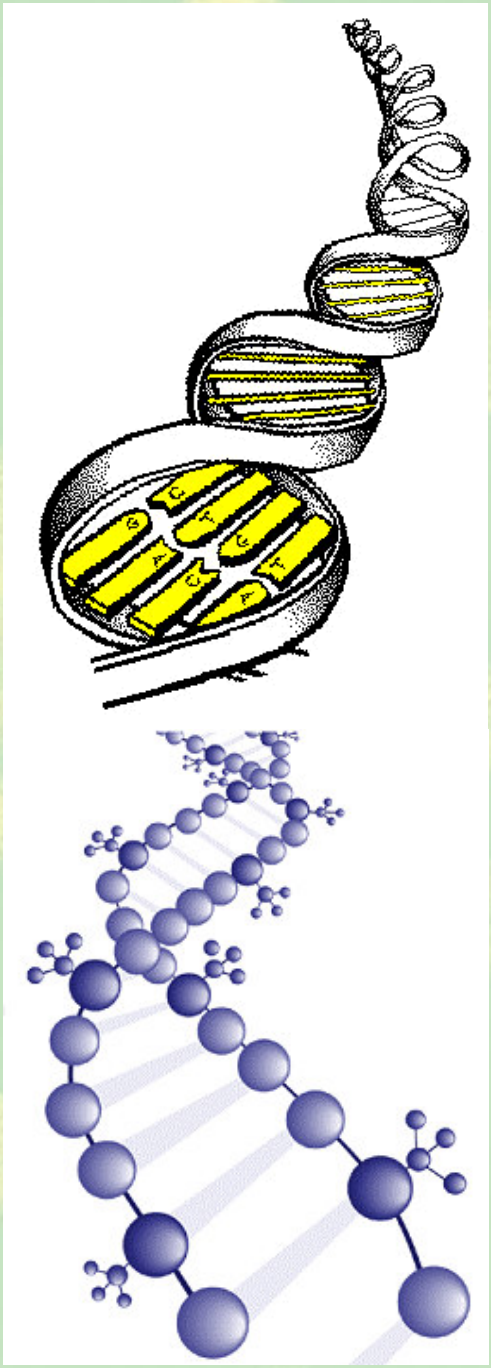
DISOMIA UNIPARENTALE:
Patogenesi e diagnosi di laboratorio

Francesca Romana GRATI, Ph.D.
TOMA, Advanced Biomedical Assays, S.p.A.

Controversie e nuove tecnologie nella diagnosi prenatale del primo trimestre: dal laboratorio alle procedure strumentali

Bologna, 5 Aprile 2008



INFORMAZIONE DEL DNA

Informazione genetica

Istruzioni per la sintesi di proteine e RNA.

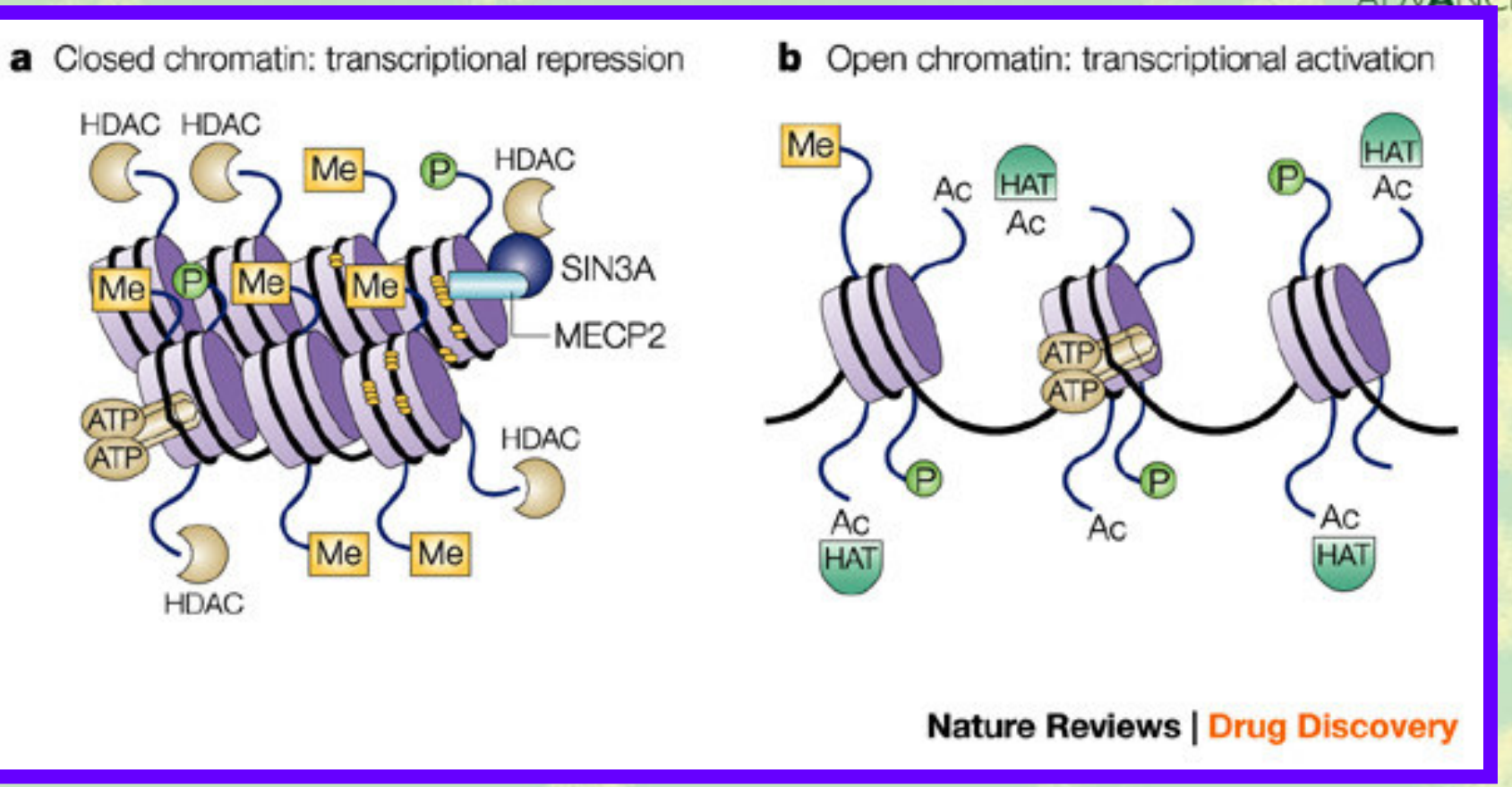
E' "STABILE"

Informazione epigenetica

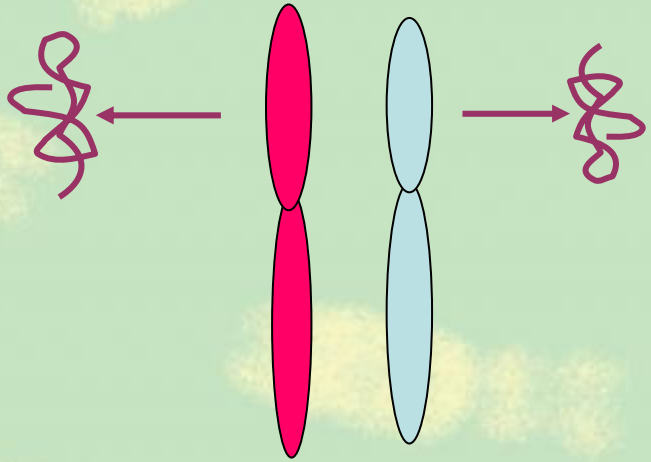
Istruzioni su come, quando e dove deve essere usata l'informazione genetica.

E' "DINAMICA"

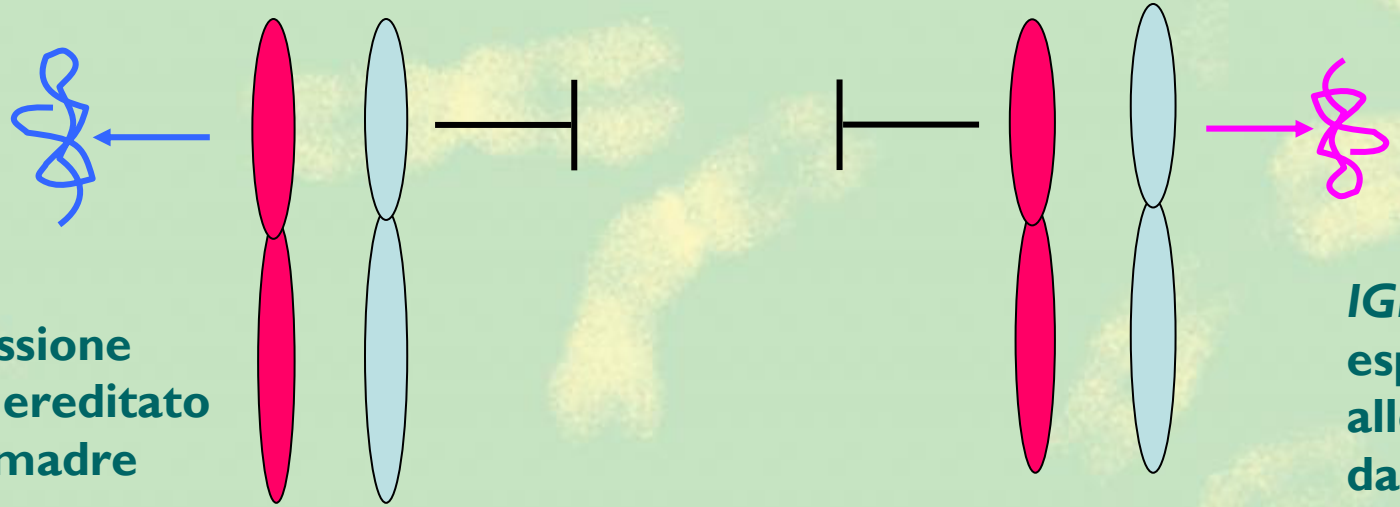
IMPRINTING


a Closed chromatin: transcriptional repression
HDAC
HDAC
Me
P
HDAC
SIN3A
Me
P
Me
Me
MECP2
ATP
ATP
HDAC
Me
Me
HDAC
b Open chromatin: transcriptional activation
Me
Ac
HAT
Ac
P
HAT
Ac
ATP
ATP
P
P
Ac
HAT
Ac
Ac
HAT
Nature Reviews | Drug Discovery

Gene non “imprinted”(98%): espressione biallelica



Gene “imprinted”(2%): espressione monoallelica



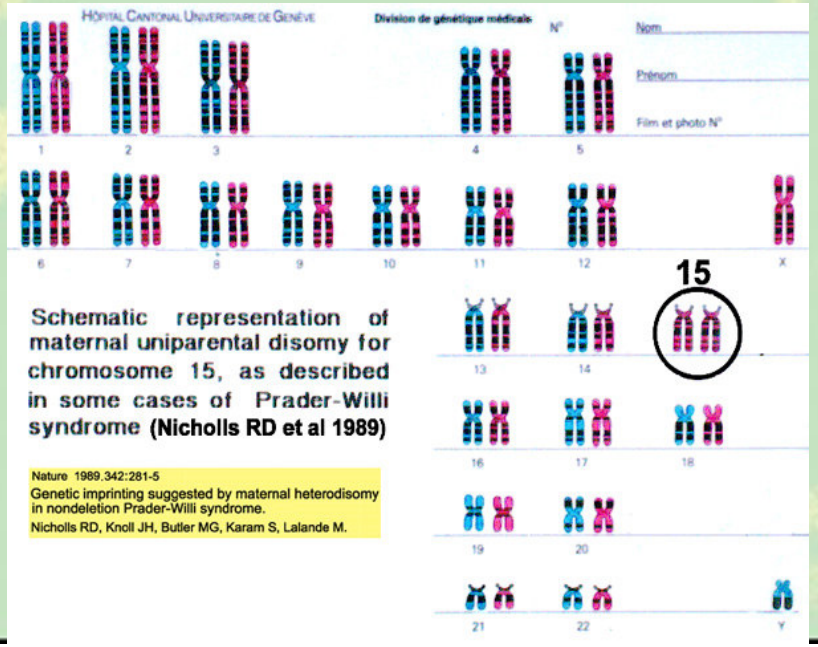
***H19*:**
espressione allele ereditato dalla madre

***IGF2*:**
espressione allele ereditato dal padre

Uniparental Disomy (UPD)

"A new genetic concept: the uniparental disomy and its potential effect, the isodisomy." ENGEL E., Am J Med Genet, 1980

Condizione in cui il numero cromosomico e' normale, ma una coppia di cromosomi omologhi e' ereditata da un solo genitore

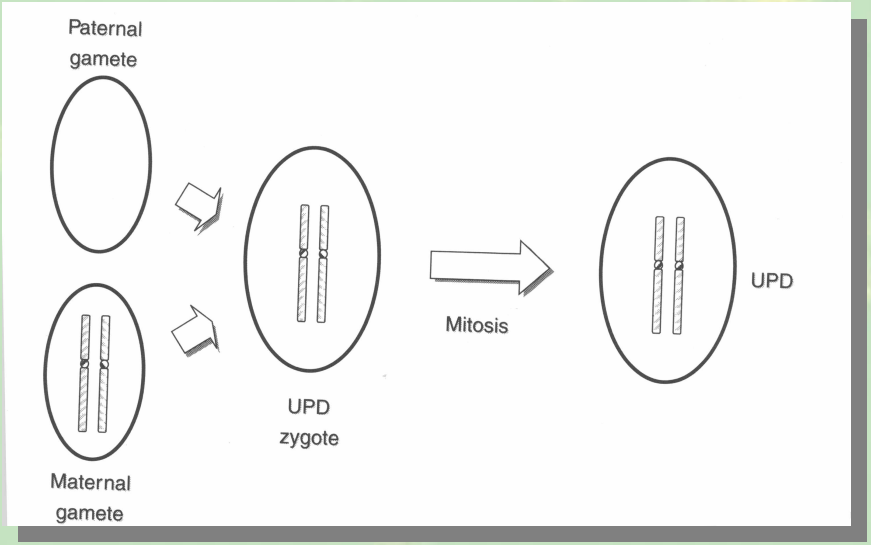


CALCULS DES FREQUENCES POTENTIELLES DE LA DISOMIE UNIPARENTALE

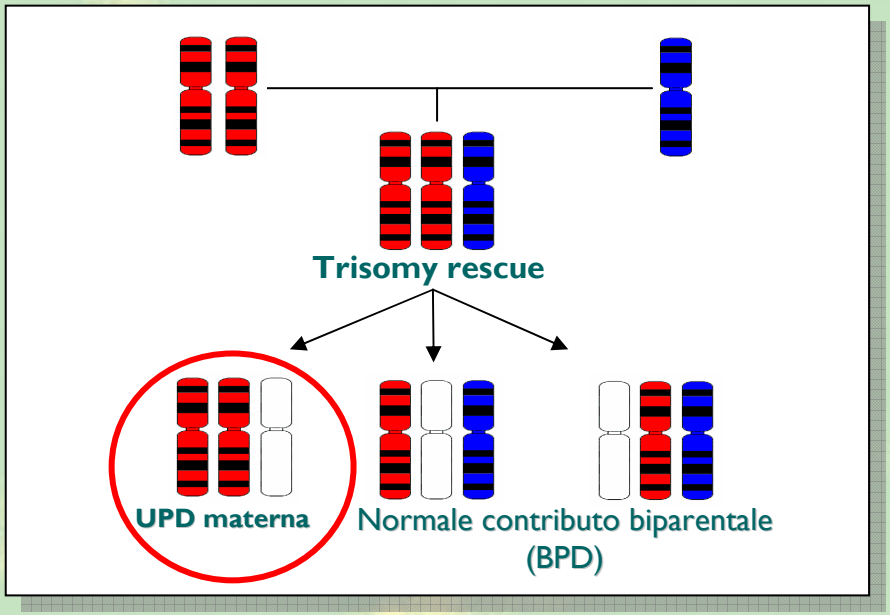
TYPE DE LA DISOMIE (CHROMOSOMES IMPLIQUES)	POUR 20 % D' AVORTEMENTS FREQUENCES / 10.000	POUR 50 % D' AVORTEMENTS FREQUENCES / 10.000
46, (16)	(0,8 %)² - 0,60	(2 %)² - 4,00
46, (21)	(0,3 %)² - 0,09	(0,75 %)² - 0,56
46,(15)	(0,25 %)² - 0,06	(0,60 %)² - 0,36
46,(22)	(0,25 %)² - 0,06	(0,60 %)² - 0,36
46, (XX OU XY)	(1,3 %)² - 1,70	(3,2 %)² - 10,20
TOTAL	2,51	15,48

BIOMEDICAL ADVANCED ASSAYS

GAMETIC COMPLEMENTATION
(fusione di un gamete nullisomico e uno disomico)



UPD and Trisomy rescue



CONFINED PLACENTAL MOSAICISM

G. Simoni and SM Sirchia

Prenatal Diagnosis, 14:1185-1189 (1994)

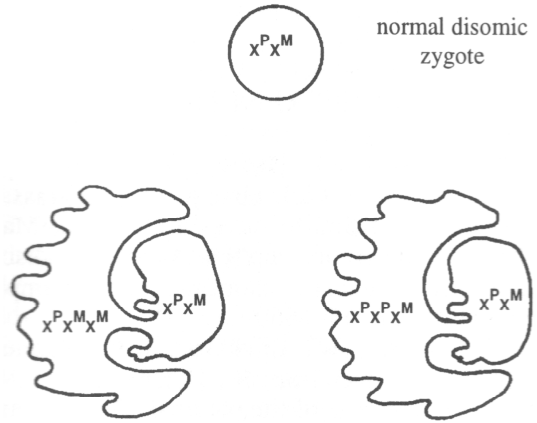


Fig. 1—Possible mechanism by which a confined placental mosaicism could originate from a normal zygote. Chromosomal anaphase lag occurs at a late gestational stage, after fetal and placental compartments have separated. We can assume a different imprinting effect according to the maternal or paternal origin of the supernumerary chromosome. M=Maternal; P=Paternal

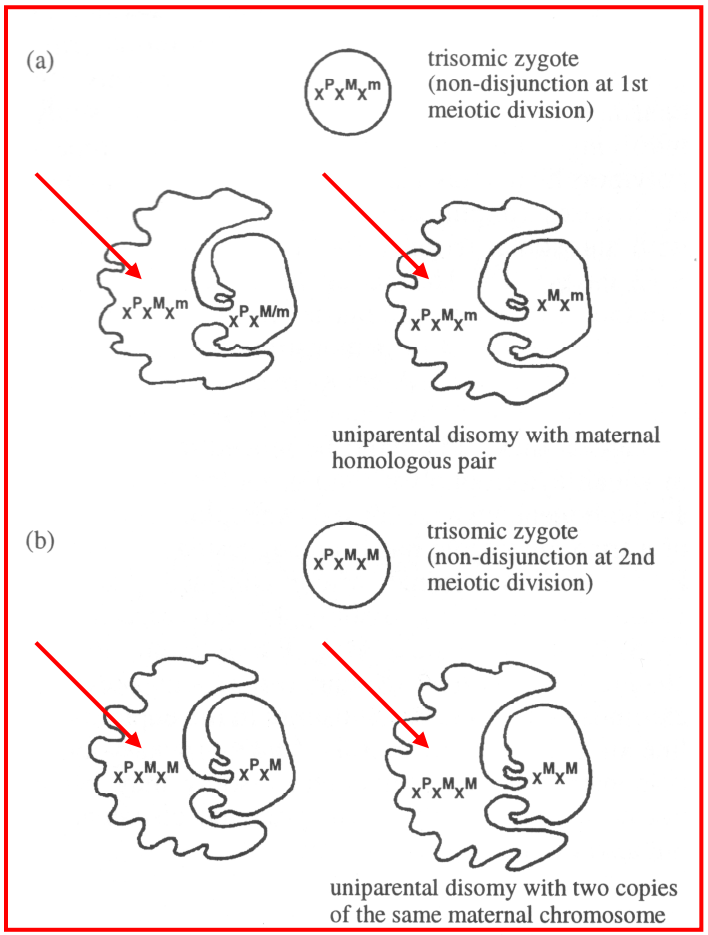


Fig. 2—Possible mechanism by which confined placental mosaicism could originate from a trisomic zygote. Fetal karyotype could be 'normalized' by a chromosome loss. We can assume different events would lead to uniparental heterodisomy (a); or isodisomy (b)

European Journal of Human Genetics (2006) 14, 282–288

www.nature.com/ejhg

ARTICLE

Confirmation of mosaicism and uniparental disomy in amniocytes, after detection of mosaic chromosome abnormalities in chorionic villi

Francesca R Grati*[,1], Beatrice Grimi[1], Giuditia Frascoli[1], Anna Maria Di Meco[1], Rosaria Liuti[1], Silvia Milani[1], Anna Trotta[1], Francesca Dulcetti[1], Enrico Grosso[2], Monica Miozzo[3], Federico Maggi[1] and Giuseppe Simoni[1,3]

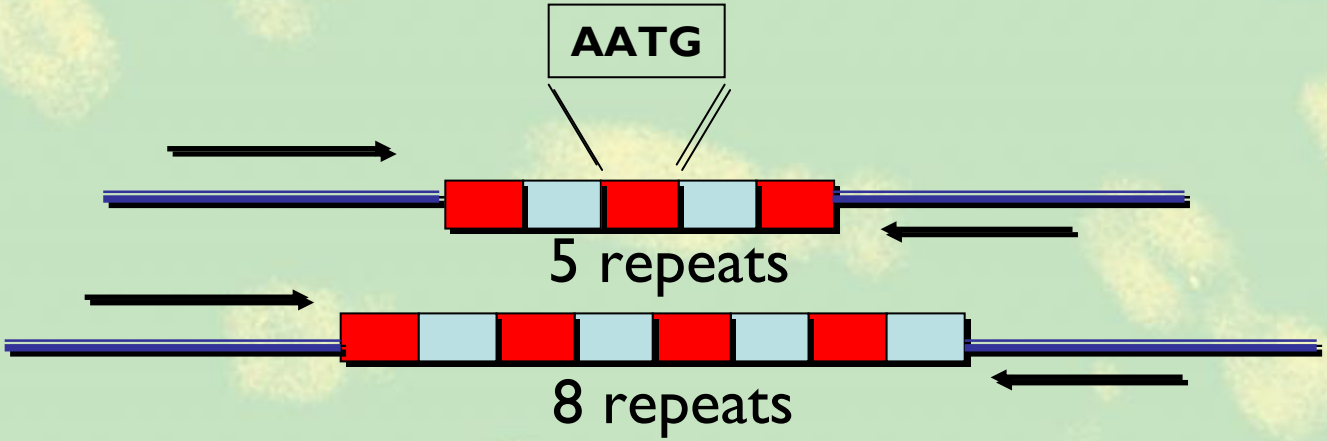
[1]*Units of Cytogenetics and Molecular Biology, TOMA Laboratory, Busto Arsizio, Varese, Italy;* [2]*SCDU Genetica Medica, Azienda Ospedaliera San Giovanni Battista, Turin, Italy;* [3]*Cattedra di Genetica Medica, Dipartimento di Medicina, Chirurgia e Odontoiatria, University of Milan, Milan, Italy*

UPD: 1 case out of 51 (1.96%)

Table 3 Distribution of specific chromosomal alterations in the different types of mosaicisms (CPM and TFM)

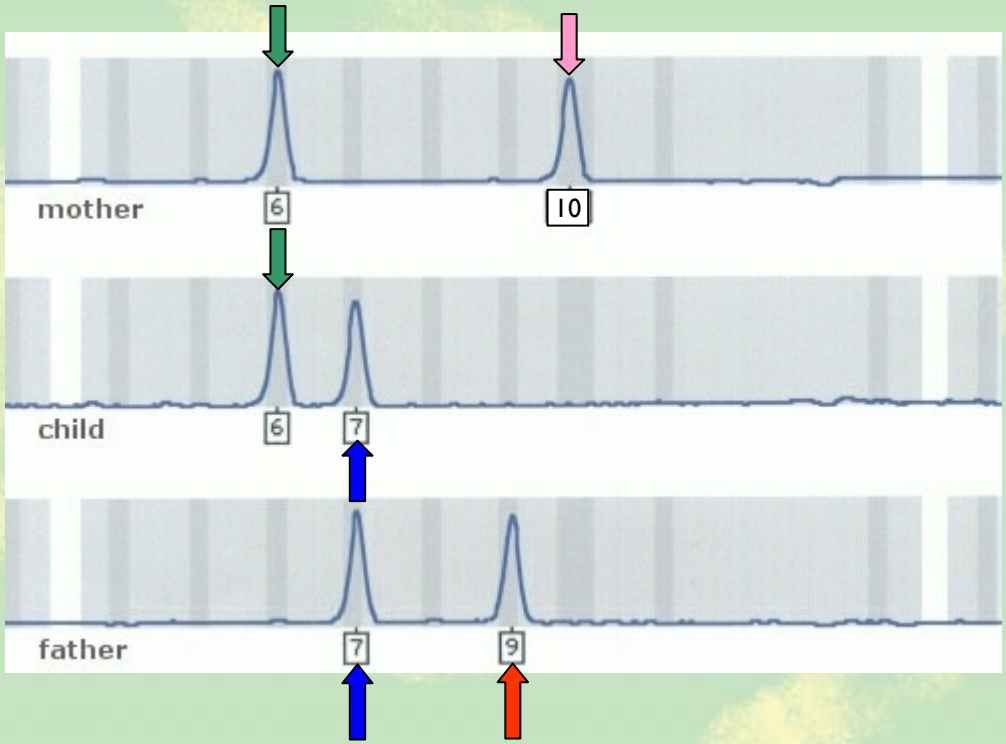
	CPM number of cases			TFM number of cases			
Chr abnormality	*Type I*	*Type II*	*Type III*	*Type IV*	*Type V*	*Type VI*	*Total (203)*
+1		1					1
+2	1	8+2^					11
+3	6		1				7
+4	1						1
+6		1	1^{C}				2
+7	5+1^	9+1^	1				17
+8	1+2^	1	1				5
+9	2	3					5
+10	1	2					3
+11	2						2
+12	1						1
+13	3	3	$1+2^{C}$				9
+14	1+(1*						2
+15	2+1^	1	1^{C}				5
+16	1+1^	1					3
+17		1					1
+18	4	3			(3^)	(1^{M})	11
+20	1	2	2^{C}				5
+21	1	6+1^			1+(2^)	(1^{C})	12
+22			1^{CM}				1
−22		1					1
+X	3 XY+1 XX+1^ XX	1 XY			1 XY	1 XX+(1^{M} XY)	9
+Y						1 XY	1
−X	6+2^	2			2+1^	1	14
−Y	2	3	1		1^	1+(1^{M})	9
X/XX/XXX						$2+1^{M}$	3
47,+mar	4+1^	2+1^		1^+1		1	11
46,rearr	7+5^	14+4^			(1^)		31
Tetraploidy	4+4^	2+2^	2^{C}				14
Multiple trisomy	2	4					6

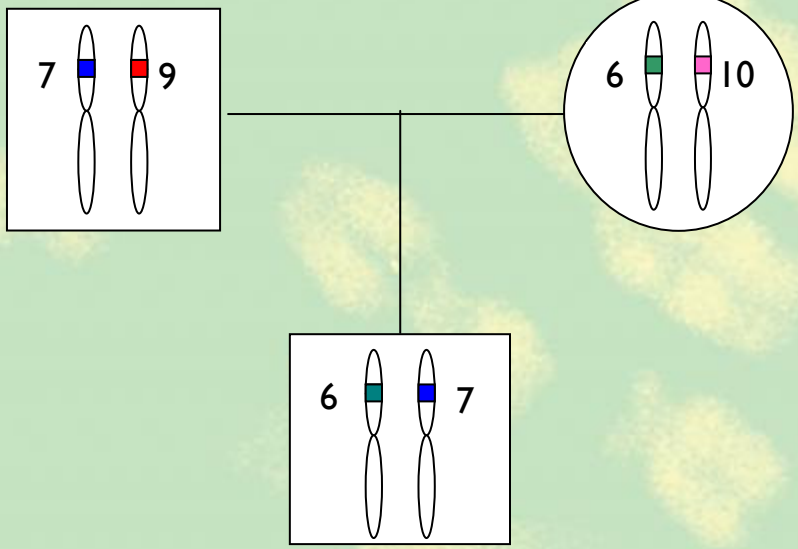
* = UPD case.
( ) = nonmosaic abnormal cell line in the fetus.
^ = nonmosaic abnormal cell line in placenta (in cytotrophoblast (^{C}) or in mesenchyme (^{M})).
XY/XX = normal karyotype associated with the +X/+Y cell line.



Heterozygote = alleles differ and can be resolved from one another

Homozygote = both alleles are the same length


mother
6
10
child
6
7
father
7
9


7
9
6
10
6
7

STR	Map Localization	M	F	P	Result
D14S122*	q11.2	1,2	1,1	3,4	ISOUPD
D14S283*	q11.2	2,3	2,2	1,1	ISOUPD
D14S990*	q11.2	2,3	3,3	1,2	ISOUPD
D14S52	q21	1,1	1,1	2,3	UPD
D14S58	q21	1,1	1,1	2,2	UPD
D14S258	q23-q23.3	1,1	1,1	2,2	UPD
D14S77	q23-q24	2,3	2,3	1,3	NI
D14S43	q24.3	2,3	2,3	1,3	NI
D14S68	q24.3	2,4	2,4	1,3	HETEROUPD
D14S59*	q24.3	1,1	1,1	2,3	UPD
D14S983	q24.3	1,1	1,1	1,1	NI
D14S53	q24.3	1,1	1,1	1,2	NI
D14S42	q24.3	1,2	1,2	1,2	NI
D14S81	q31-q32	1,1	1,1	1,2	NI
D14S51	q32.2-q32.3	1,2	1,2	2,2	NI

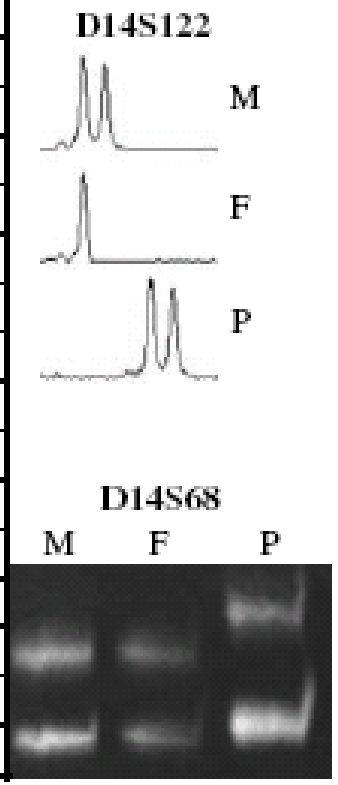


Figure 1 Molecular results of matUPD14 case. For each informative marker, maternal (M), fetal (F) and paternal (P) alleles are indicated. The fetus inherited alleles only from the mother and failed to inherited a paternal allele, consistent with a maternal disomy 14. * Indicate STRs analysed by fluorescent capillary system.

UPD and Robertsonian translocations

PRENATAL DIAGNOSIS
Prenat Diagn 2004; **24**: 997–1000.
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/pd.961

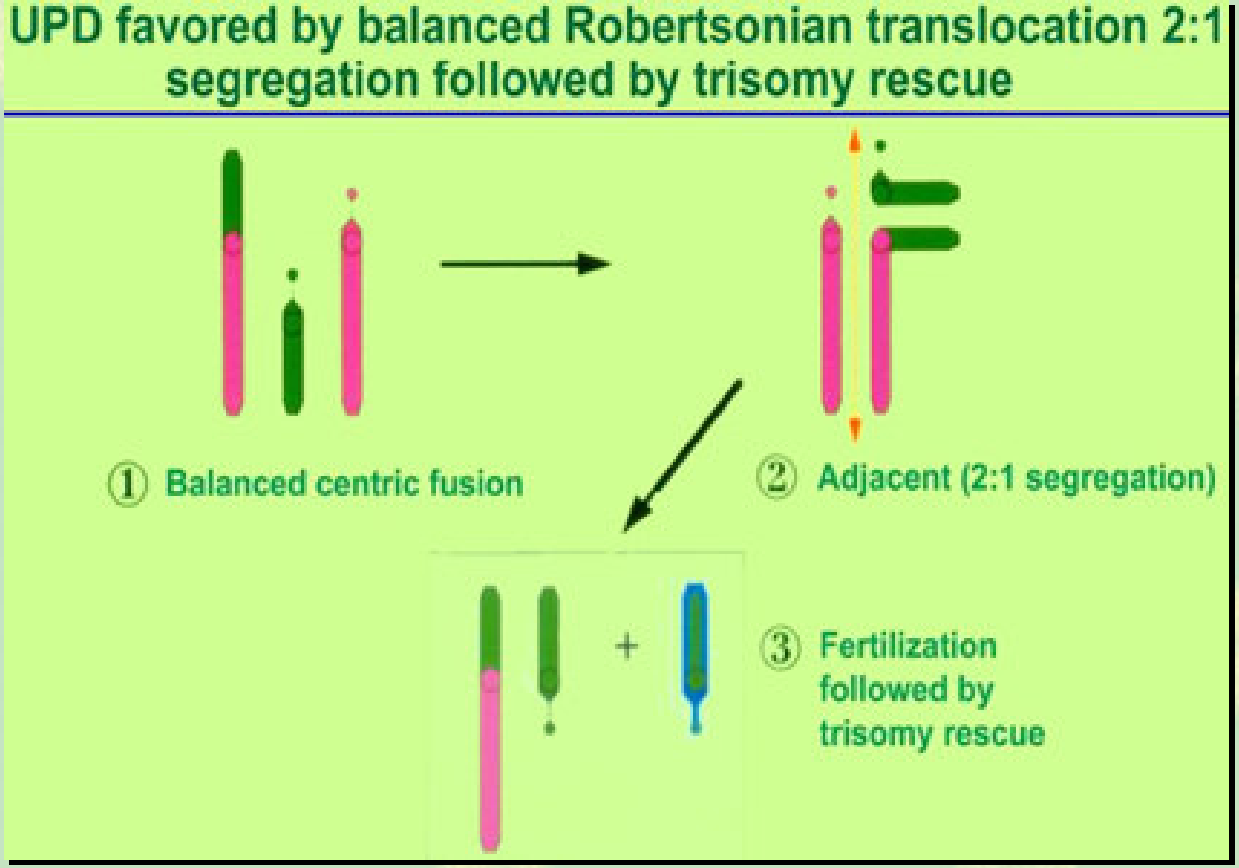
Prenatal search for UPD 14 and UPD 15 in 83 cases of familial and *de novo* heterologous Robertsonian translocations

Anna Ruggeri[1]*, Francesca Dulcetti[1], Monica Miozzo[2], Francesca R. Grati[2], Beatrice Grimi[1], Silvano Bellato[3], Federica Natacci[4], Federico Maggi[1] and Giuseppe Simoni[1,2]

[1]*Units of Cytogenetics and Molecular Biology, TOMA Laboratory, Busto Arsizio, Varese, Italy*
[2]*Laboratory of Medical Genetics, Department of Medicine, Surgery and Dentistry, San Paolo School of Medicine, University of Milan, Milan, Italy*
[3]*Division of Pediatrics, L. Cazzavillan Hospital, Arzignano, Vicenza, Italy*
[4]*Servizio di Genetica Medica, Istituti Clinici di Perfezionamento, Milano, Italy*

TOMA BIOMEDICAL ADVANCED ASSAYS

UPD favored by balanced Robertsonian translocation 2:1 segregation followed by trisomy rescue



STR	M	F	P
D14S49	1,2	2,2	1,2
D14S77	1,2	1,2	3,3
D14S43	2,3	2,3	1,1
D14S68	2,2	2,2	1,1
D14S51	1,4	1,4	2,3

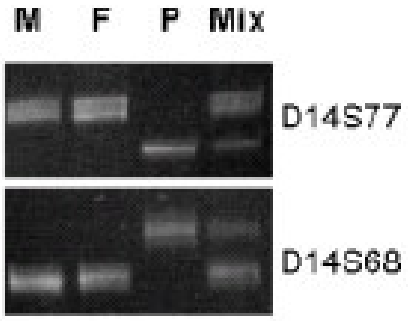


Figure 1—Molecular results of matUPD14 case. For each informative marker, maternal (M), fetal (F) and paternal (P) alleles are indicated (Mix: sample containing M and P alleles). The fetus inherited alleles only from the mother and failed to inherit a paternal allele, consistent with a maternal UPD 14

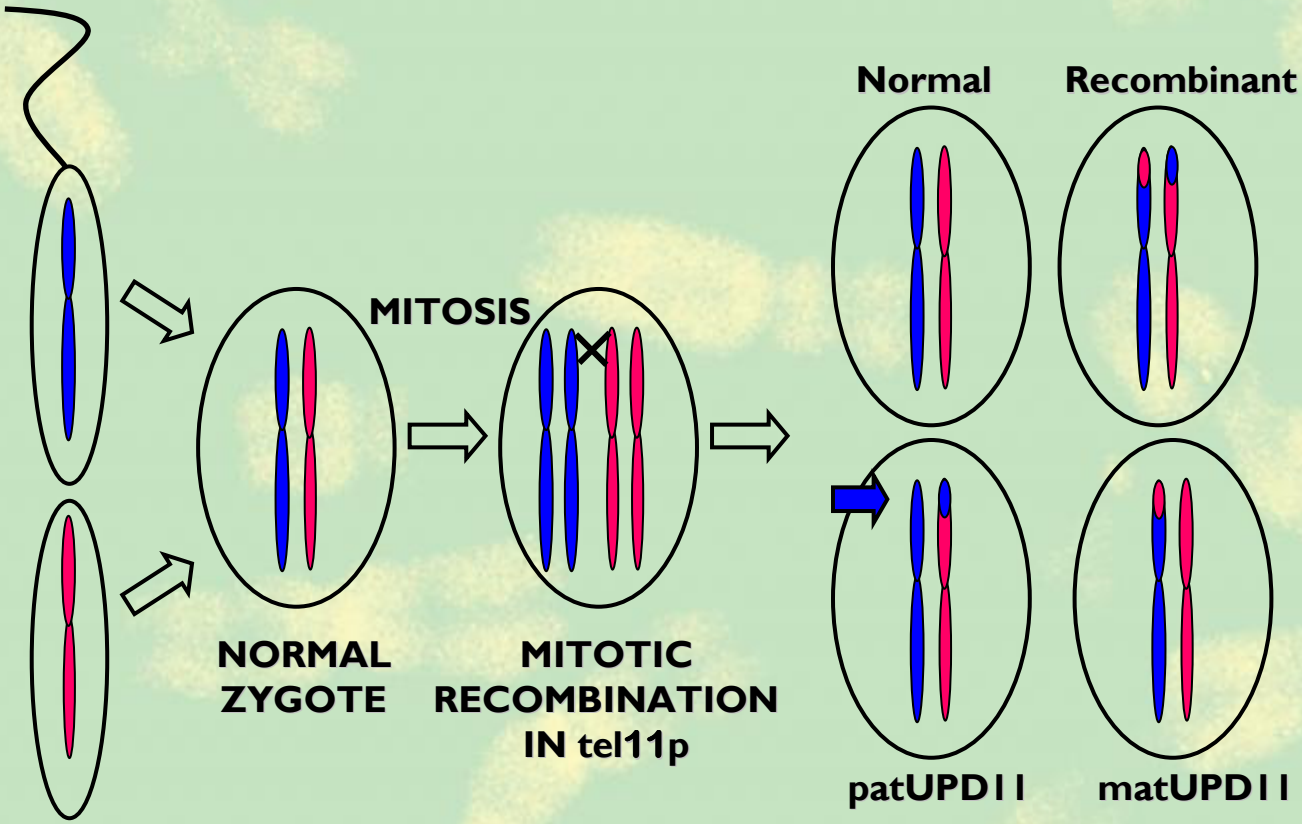
BIOMEDICAL ADVANCED ASSAYS

ROBs	n° cases
13;14	**224
13;15	14
14;15	24
14;21	*42
14;22	13
15;21	3
15;22	5
Subtotal	**425 (0.7%)**
NotDefined	181
TOT	**506 (0.6%)**

Papenhousen et al, 1996
Silverstein et al, 2002
Ruggeri et al, 2004

*=UPD14 *de novo*

patUPD11 and mitotic crossing-over



A MOSAICO!

CORRELAZIONE GENOTIPO - FENOTIPO

ONFALOCELE:

- **MUTAZIONI INATTIVANTI/IPERMETILAZIONE DI CDKN1C**
- **MAI DESCRITTO IN ASSOCIAZIONE CON patUPD11**

J Med Genet 2000;37:921–926

Epigenotype-phenotype correlations in Beckwith-Wiedemann syndrome

Jacqueline R Engel, Alan Smallwood, Antonita Harper, Michael J Higgins, Mitsuo Oshimura, Wolf Reik, Paul N Schofield, Eamonn R Maher

Am. J. Hum. Genet. 70:604–611, 2002

Epigenetic Alterations of *H19* and *LIT1* Distinguish Patients with Beckwith-Wiedemann Syndrome with Cancer and Birth Defects

Michael R. DeBaun,[1,2] Emily L. Niemitz,[3,4] D. Elizabeth McNeil,[2] Sheri A. Brandenburg,[3] Maxwell P. Lee,[3] and Andrew P. Feinberg[3,5]

[1]Division of Pediatric Hematology-Oncology, Department of Pediatrics, Washington University School of Medicine, Saint Louis; [2]Genetic Epidemiology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD; and [3]Institute of Genetic Medicine, [4]Predoctoral Program in Human Genetics, and [5]Departments of Medicine, Oncology, and Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore

TOMA BIOMEDICAL ADVANCED ASSAYS

ORIGINAL ARTICLE

Chromosome 11 segmental paternal isodisomy in amniocytes from two fetuses with omphalocoele: new highlights on phenotype–genotype correlations in Beckwith–Wiedemann syndrome

F R Grati, L Turolla, P D'Ajello, A Ruggeri, M Miozzo, G Bracalente, D Baldo, L Laurino, R Boldorini, E Frate, N Surico, L Larizza, F Maggi, G Simoni

J Med Genet 2007;**44**:257–263. doi: 10.1136/jmg.2006.046854

RICERCA DELLA LINEA CELLULARE CON CONTRIBUTO BIPARENTALE NEI TESSUTI FETALI

M: MADRE

AF: AMNIOCITI

CNS: SISTEMA NERVOSO CENTRALE

Li/C/K: FEGATO, CORDONE OMBELICALE, RENE

H/Lu: CUORE, POLMONE

T: TIMO

P: PADRE

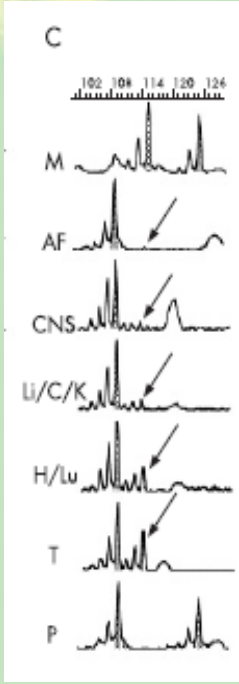


Figure 4 Schematic representation of short tandem repeat (STR) genotyping of case 1. (A) Allelic segregation of the tested polymorphic markers. (B) Electropherograms of D11S1923 and D11S995 STRs included in the uniparental region and of D11S911 mapped in 11q13 showing a normal biparental contribution. (C) Allelic segregation of D11S988 and quantification of the paternal uniparental disomy (pUPD) cell line in different fetal tissues and in alleles from amniotic fluid (AF). The biparental component is represented by the maternal allele (arrow). C, umbilical cord; CNS, central nervous system; H, heart; IsoUPDpat, paternal isodisomy; K, kidney; Li, liver; Lu, lung; M/P, maternal and paternal alleles; NI, not informative; T, thymus.

M: MADRE

AF: AMNIOCITI

S/To/C: SURRENE, LINGUA, CORDONE

B/Pa: INTESTINO, PANCREAS

U/Lu/C/H: UTERO, POLMONE, CORDONE, CUORE

CNS: SISTEMA NERVOSO CENTRALE

P: PADRE

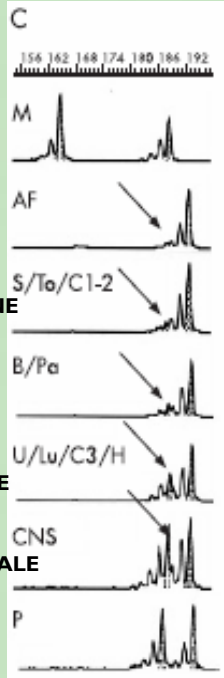


Figure 5 Schematic representation of case 2 short tandem repeat (STR) analysis. (A) Allelic segregation of the tested polymorphic markers. (B) Electropherograms of D11S1923 and D11S986 STRs included in the uniparental region and of D11S4191 mapped in 11q12 showing a normal biparental contribution. (C) Allelic segregation of D11S2071 and quantification of the biparental cell line in different fetal tissues and in alleles from amniotic fluid (AF). The paternal uniparental disomy (pUPD) component is represented by the maternal allele (arrow). B, bowel; C, umbilical cord; CNS, central nervous system; H, heart; isoUPDpat, paternal isodisomy; K, kidney; Lu, lung; M/P, maternal and paternal alleles; NI, not informative; Pa, pancreas; S, adrenal gland; To, tongue; U, uterus.

CONCLUSIONS:

1. the distribution of pUPD cell line is super imposable to that of the organs mainly involved in the omphalocele; the presence at high level of the paternal isodisomic cell line in the abdominal organs could have mimicked the effect of CDKN1C/p57 inactivating mutations or hypermethylation causing the omphalocele.
2. **amniotic fluid cell cultures is the preferential prenatal sample for BWSUPD11 testing: during the AF culture the pUPD11 cell line might have a proliferative advantage due to the autocrine effect of IGF2 getting the upper hand over the normal biparental cell line, leading to the unmasking of the UPD cell line.**

UPD TYPE	SYNDROME	PHENOTYPE
Certain		
Pat 6	Transient Neonatal Diabetes Mellitus (TNDM)(MIM:601410)	IUGR, diabete neonatale
Mat 7/Mat 11	Silver Russell Syndrome (SRS) (MIM:180860)	IUGR/PNGR, dismorfismi
Pat 11	Beckwith-Wiedemann Syndrome (BWS) (MIM:130650)	Overgrowth, dismorfismi, tumori
Mat 14		IUGR, dismorfismi
Pat 14		Nanismo, dismorfismi
Mat 15	Prader Willi Syndrome (PWS) (MIM:176270)	Obesità, dimorfismi,
Mat/Pat 20	Pre- and postnatal growth retardation, dysmorphisms	IUGR/PNGR
Probable		
Mat 2	Growth failure, bronchopulmonary dysplasia	IUGR e displasia broncopolmonare
Mat 16	Growth failure, abnormalities	IUGR e dismorfismi

TOMA LAB

Prof. Giuseppe Simoni
dott. Federico Maggi

CYTOGENETICS
dott.ssa Beatrice Grimi
dott.ssa Giuditta Frascoli
dott.ssa Anna Maria Di Meco
dott.ssa Rosaria Liuti
dott.ssa Silvia Milani
dott.ssa Anna Trotta

BIOMEDICAL TOMA ADVANCED ASSAYS

R&D + BIOLOGIA MOLECOLARE

Simona De Toffol Francesca Dulcetti Anna Ruggeri
Barbara Malvestiti

Chromosome 1

ARH1/NOEY2
p73

Chromosome 5

U2AF1RS1

Chromosome 6

AIR
HYMA1
M6P/IGF2R
SLC22A2
SLC22A3
ZAC/PLAGL1

Chromosome 7

ASB4
COPG2
GRB10
MIT1/LB9
PEG1-AS
PEG1/MEST
PEG10
SGCE

Chromosome 11

> ASCL2/HASH2
> AWT1
BWRT/KCNQ1DN
> CD81
> H19
> IGF2
> IGF2-AS
> INS
> INS2
> KCNQ1
> KCNQ1QT1
> MSUIT1
> NAPIL4
> OBPH1
> SDHD
> SLC22A1L/ITM
> TRPM5
> TSSC3/IPL
> TSSC4
> WT1
> ZNF215
> p57kip2/CDKN1C

Chromosome 12

ATA3
DCN

Chromosome 13

HTR2A

Chromosome 14

DIO3
DLK1/PREF-1
MEG3/GTL2
MEG8/snoRNAs
PEG11

Chromosome 18

ELONGIN A3
IMPACT

Chromosome 19

PEG3/ZIM2
USP29
ZIM1
ZIM3
ZNF264

Chromosome 20

GNAS1
GNAS1-AS
L3MBTL
NNAT

Chromosome 15

ATP10C
GABRB3
HBII-13
HBII-52
IPW
MAGEL2
MKRN3
NDN
PAR-SN
PAR1
PAR5
PEG12
PWCR1
RASGRF1
SNRPN
UBE3A
UBE3A-AS
ZNF127-AS

Chromosome X

XIST

BIOMEDICAL TOMA ADVANCED ASSAYS

www.geneimprint.com

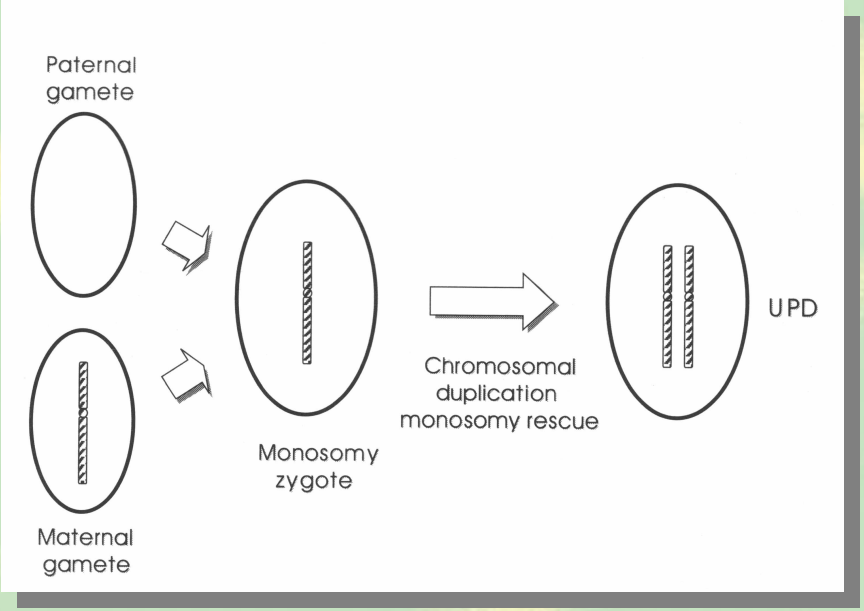
Am. J. hum. Genet. 42: 215-216, 1988
Editorial : Uniparental Disomy: A Rare Consequence of the High Rate of Aneuploidy in Human Gametes
Dorothy Warburton
Department of Genetics and development of Pediatrics, Columbia University. New York

Am. J. hum. Genet. 42: 217-225, 1988
Uniparental Disomy as Mechanism for Human Genetic Disease
J. Edward Spence, Ronald G. Perciccante, Guillian M. Greig. Huntington F. Willard. David H. Ledbetter. J. Fielding Hejtmancik, Marilyn S. Pollack, William E. O'Brien and Arthur L. Baudet
Howard Hughes Medical Institute, Institute of Molecular Genetics and Department of Microbiology and Immunology, Baylor College of Medicine, Houston: Mercy Hospital, Watertown, NY: and Department of Medical Genetics, University of Toronto, Toronto

The first thoroughly analyzed and described case of UPD. It was one involving maternal chromosome 7, responsible for cystic fibrosis in an unusually short girl who carried Gly542Ter mutation in her CFTR gene.

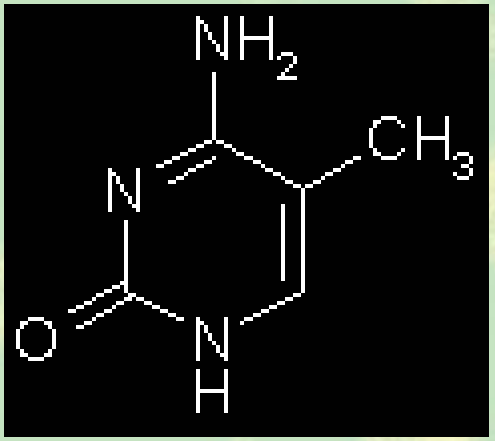
This article, of Beaudet's lab, with Ledbetter among the Authors and Spence as the Senior Author, was not only featuring the first case ever sighted of non-traditional recessive inheritance through reduction to homozygosity of the recessive mutant only carried by one of the two parents. It also offered a most comprehensive review of the possible mechanisms leading to the occurrence of UDP.

MONOSOMY RESCUE (fusione di un gamete nullisomico e uno normale monosomico seguita da duplicazione del cromosoma monosomico)

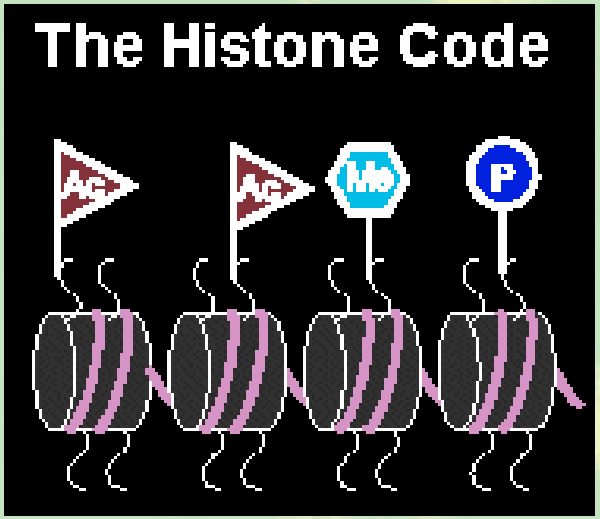


MODIFICAZIONI CHIMICHE DELLA CROMATINA

- **Metilazione della citosina**



- **Metilazione, acetilazione, fosforilazione proteine istoniche**



UPD and supernumarary marker chromosomes

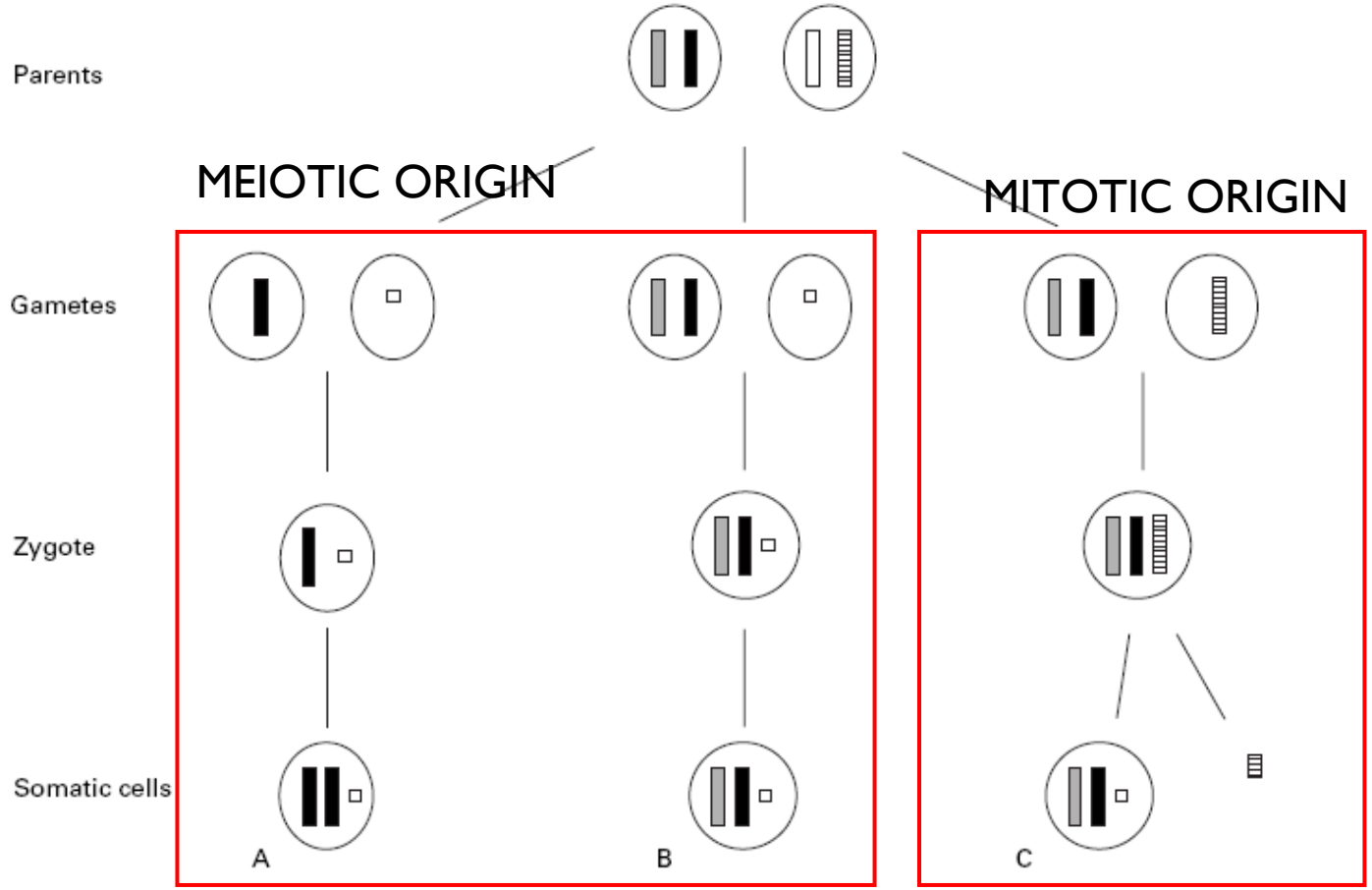
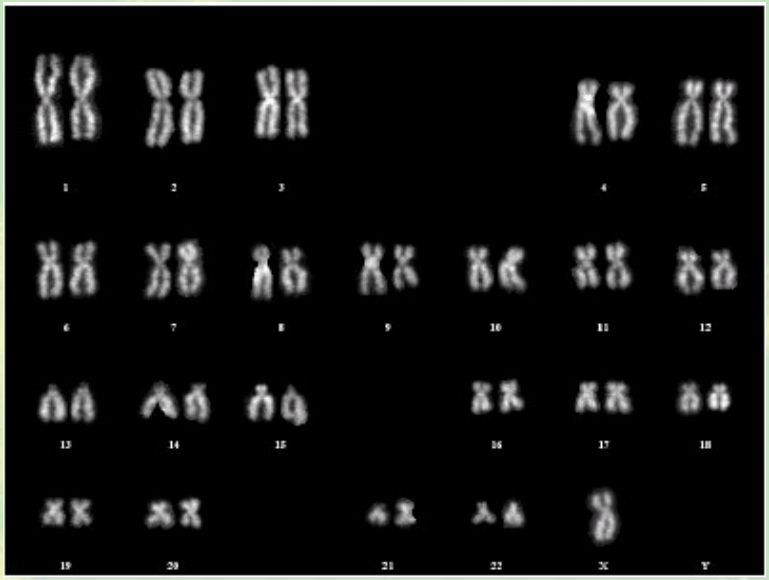
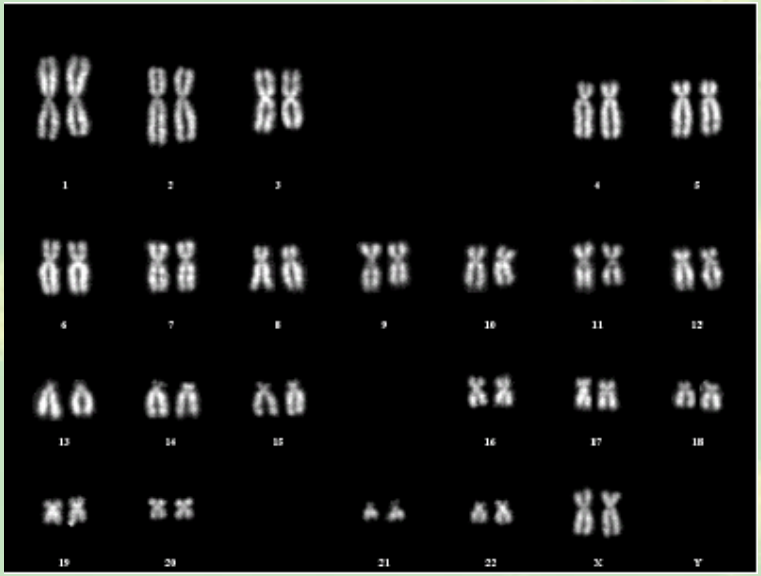


Figure 5 Formation of UPD associated with an additional marker chromosome. (A) The marker chromosome was formed in meiosis and a gamete with the marker chromosome but no normal homologue was fertilised by a normal gamete. (B) A disomic gamete is fertilised by a gamete with a marker chromosome formed in meiosis. (C) A disomic gamete is fertilised by a normal gamete and subsequent mitotic formation of the marker chromosome. In (B) and (C) not only heterodisomy as shown but also isodisomy is possible.

IUGR, diagnosi prenatale (età materna avanzata): 46,XX/45,X





RISULTATO Cariotipo fetale: mos45,X/46,XX

OSSERVAZIONI

L'analisi è stata eseguita su 34 piastre metafasiche derivate da 18 colonie di 3 colture. E' presente una condizione di mosaicismo con due linee cellulari: una con monosomia X (8 metafasi da 4 colonie di 3 colture) e l'altra con corredo cromosomico 46,XX (26 metafasi da 14 colonie di 3 colture). Si consiglia il colloquio con il genetista.

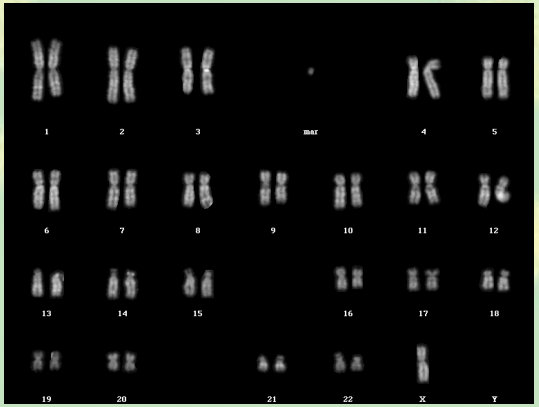
PNGR severo, fronte ampia e bombata (sospetta SRS)

RISULTATO Cariotipo: mos46,X,+mar[25]/45,X[7]/46,XX[68]

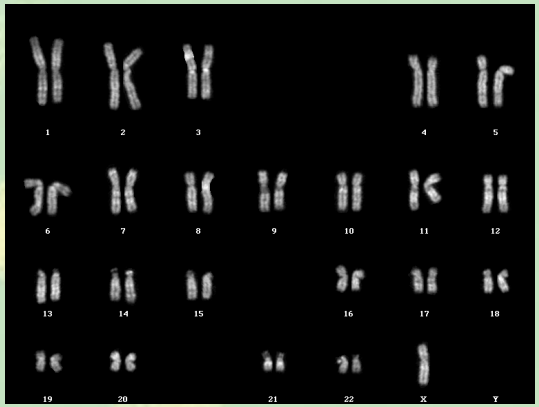
OSSERVAZIONI

L'analisi é stata eseguita su 100 metafasi con risoluzione di 450 bande per set aploide. E' presente una condizione di mosaicismo con tre linee cellulari: una a 46 cromosomi con monosomia X e con presenza di un piccolo marcatore puntiforme (25%), una a 45 cromosomi con monosomia X (7%) e una con corredo cromosomico 46,XX (68%). L'applicazione della tecnica FISH (vedi referto allegato) ha mostrato segnale positivo del marcatore all'ibridazione con sonda centromerica specifica per il cromosoma 7. L'analisi molecolare (vedi referto allegato) ha permesso di identificare la presenza della disomia uniparentale materna del cromosoma 7. Si consiglia il colloquio con il genetista.

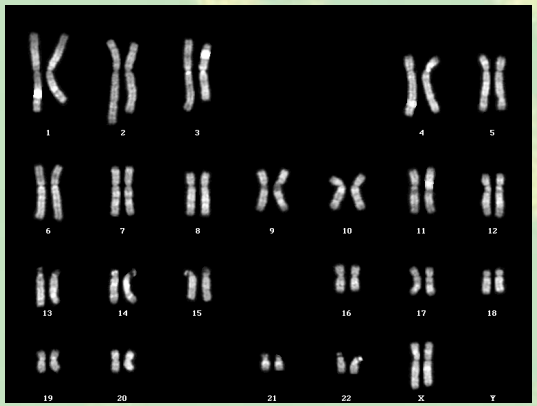
46,X,+mar (25%)



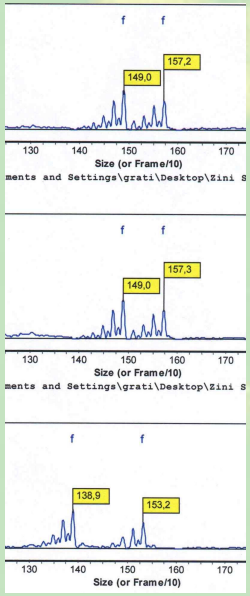
45,X (7%)



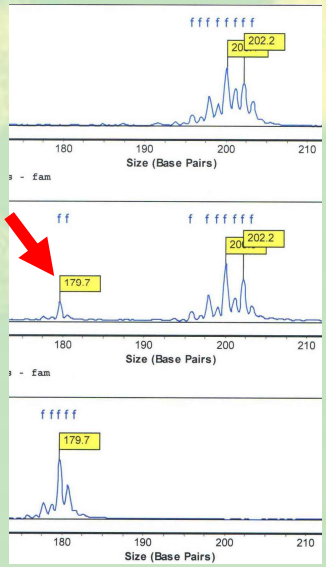
46,XX (68%)



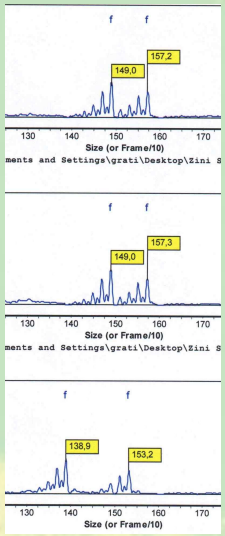
D7S640



D7S801



D7S636

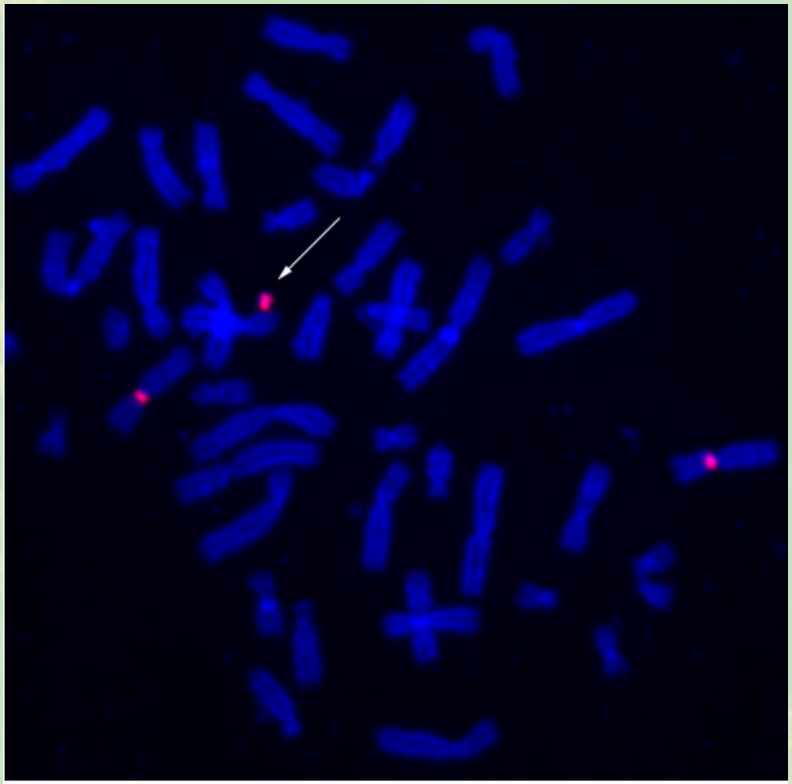


LOCI POLIMORFICI ANALIZZATI	ALLELI			RISULTATO
	MADRE	FIGLIA	PADRE	
D7S3069	1.1	1.1	2.3	UPD MAT
D7S801	2.3	(1).2.3	1.1	ETERO UPD MAT+ LINEA TRISOMICA CON CR7 PAT
D7S663	1.2	1.2	1.3	ESCLUSIONE UPD PAT
D7S480	1.3	1.3	2.4	ETERO UPD MAT
D7S640	1.4	1.4	2.3	ETERO UPD MAT
D7S636	2.4	2.4	1.3	ETERO UPD MAT
D7S483	1.2	1.2	2.2	ESCLUSIONE UPD PAT
NOS3	1.2	1.2	1.3	ESCLUSIONE UPD PAT

Risultato: L'analisi di segregazione allelica di 8 marcatori polimorfici localizzati lungo il cromosoma 7 ha evidenziato nella probanda una eterodisomia pura di entrambi i bracci; a livello del centromero (D7S801) è presente una condizione di mosaicismo tra la linea cellulare con eterodisomia materna e quella con la trisomia.

Il risultato ottenuto indica che è avvenuto un errore di non-disgiunzione alla meiosi I materna con la formazione di uno zigote trisomico per il cromosoma 7. Successivamente è avvenuta una ricombinazione mitotica con la perdita di entrambi i bracci dell'omologo di origine paterna; tale evento ha generato una condizione di UPD materna a livello di queste sequenze e mosaicismo per le regioni centromeriche.

MATERIALE:	sangue periferico
METODO:	IBRIDAZIONE IN SITU A FLUORESCENZA
SONDA UTILIZZATA:	Sonda alfa satellite specifica per i centromeri dei cromosomi X/Y. Sonda alfa satellite specifica per il centromero del cromosoma 7.
COMMENTO:	L'applicazione della tecnica FISH con l'utilizzo delle sonde sopra indicate, ha permesso di caratterizzare il marcatore come un derivativo del cromosoma 7 (vedere foto allegata: la freccia indica il cromosoma marcatore).
	Si consiglia il colloquio con il genetista.



Prot.: BM 14515

Analisi: FISH eseguita con sonda alfa satellite specifica per il centromero del cromosoma 7.