"Quale citogenetica nel futuro prossimo"

Roma, 23 Ottobre 2013

Centro di Formazione dell'Ordine Nazionale dei Biologi

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CITOGENETICA
LA SITUAZIONE
ATTUALE

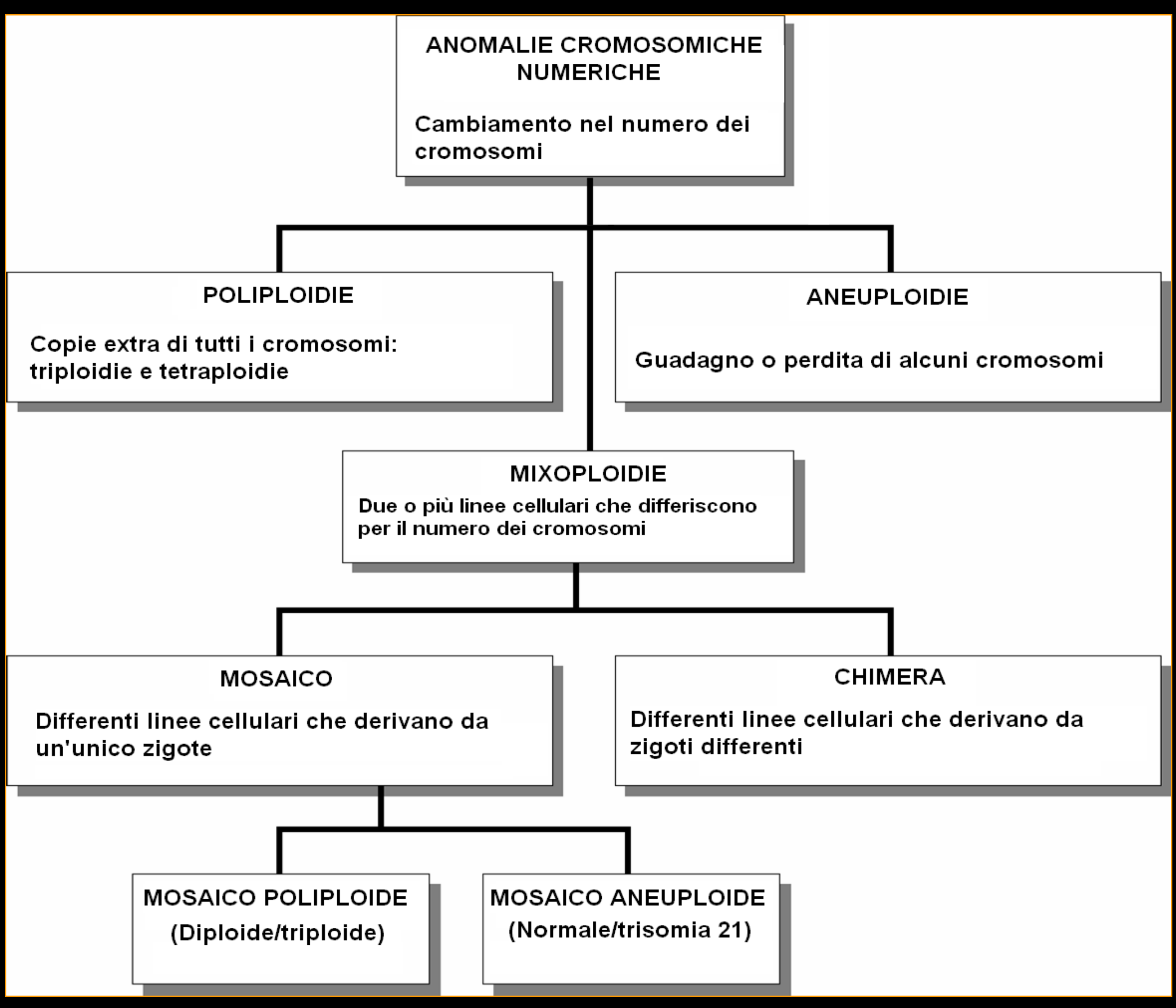
ABERRAZIONI CROMOSOMICHE

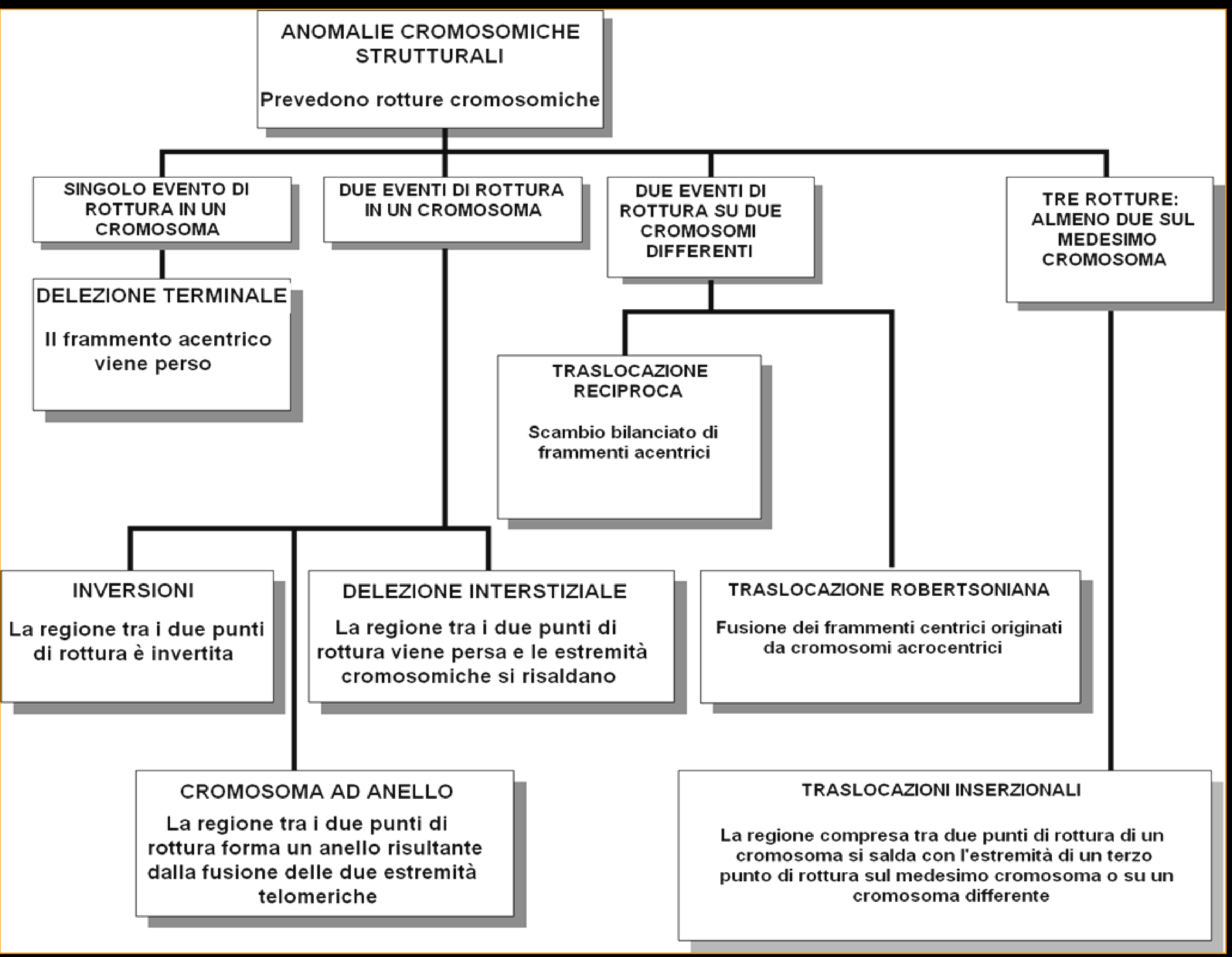
Le anomalie cromosomiche interessano alla nascita una persona ogni 150

comprendono:

ANOMALIE DI NUMERO
ANOMALIE DI STRUTTURA

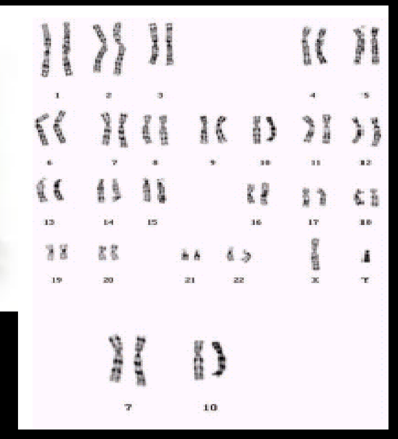
I CORREDI EUPLOIDI hanno un numero cromosomico corrispondente ad un multiplo esatto del corredo aploide presente nei gameti (diploide, triploide, tetraploide, ecc…)


ANOMALIE CROMOSOMICHE NUMERICHE
Cambiamento nel numero dei cromosomi
POLIPLOIDIE
Copie extra di tutti i cromosomi: triploidie e tetraploidie
ANEUPLOIDIE
Guadagno o perdita di alcuni cromosomi
MIXOPLOIDIE
Due o più linee cellulari che differiscono per il numero dei cromosomi
MOSAICO
Differenti linee cellulari che derivano da un'unico zigote
CHIMERA
Differenti linee cellulari che derivano da zigoti differenti
MOSAICO POLIPLOIDE
(Diploide/triploide)
MOSAICO ANEUPLOIDE
(Normale/trisomia 21)


ANOMALIE CROMOSOMICHE STRUTTURALI
Prevedono rotture cromosomiche
SINGOLO EVENTO DI ROTTURA IN UN CROMOSOMA
DUE EVENTI DI ROTTURA IN UN CROMOSOMA
DUE EVENTI DI ROTTURA SU DUE CROMOSOMI DIFFERENTI
TRE ROTTURE: ALMENO DUE SUL MEDESIMO CROMOSOMA
DELEZIONE TERMINALE
Il frammento acentrico viene perso
TRASLOCAZIONE RECIPROCA
Scambio bilanciato di frammenti acentrici
INVERSIONI
La regione tra i due punti di rottura è invertita
DELEZIONE INTERSTIZIALE
La regione tra i due punti di rottura viene persa e le estremità cromosomiche si risaldano
TRASLOCAZIONE ROBERTSONIANA
Fusione dei frammenti centrici originati da cromosomi acrocentrici
CROMOSOMA AD ANELLO
La regione tra i due punti di rottura forma un anello risultante dalla fusione delle due estremità telomeriche
TRASLOCAZIONI INSERZIONALI
La regione compresa tra due punti di rottura di un cromosoma si salda con l'estremità di un terzo punto di rottura sul medesimo cromosoma o su un cromosoma differente

Consulenza PRE-TEST

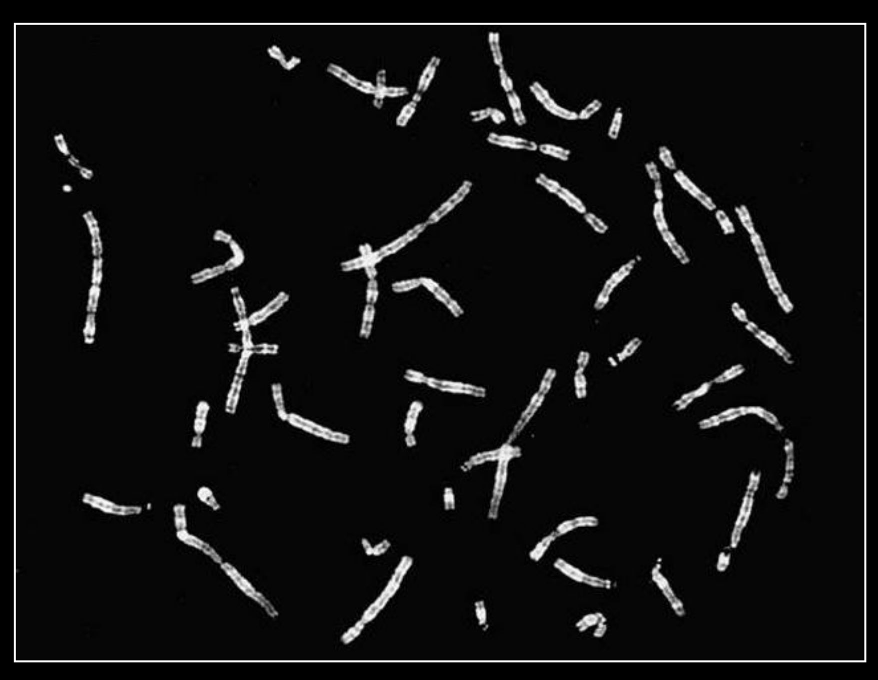
ANALISI di LABORATORIO e MEDICHE



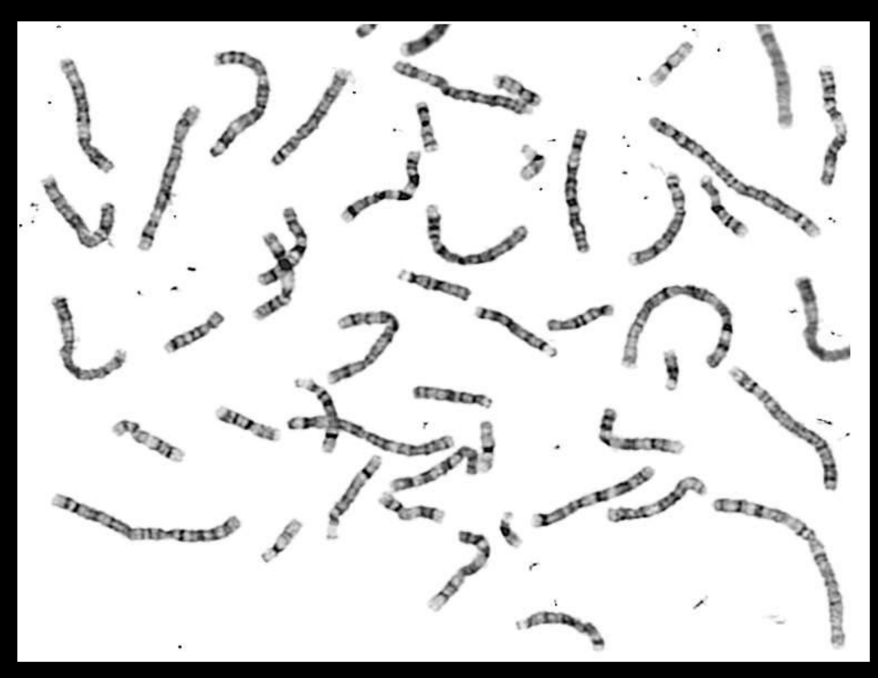
Consulenza POST-TEST

TECNICHE DI BANDEGGIO

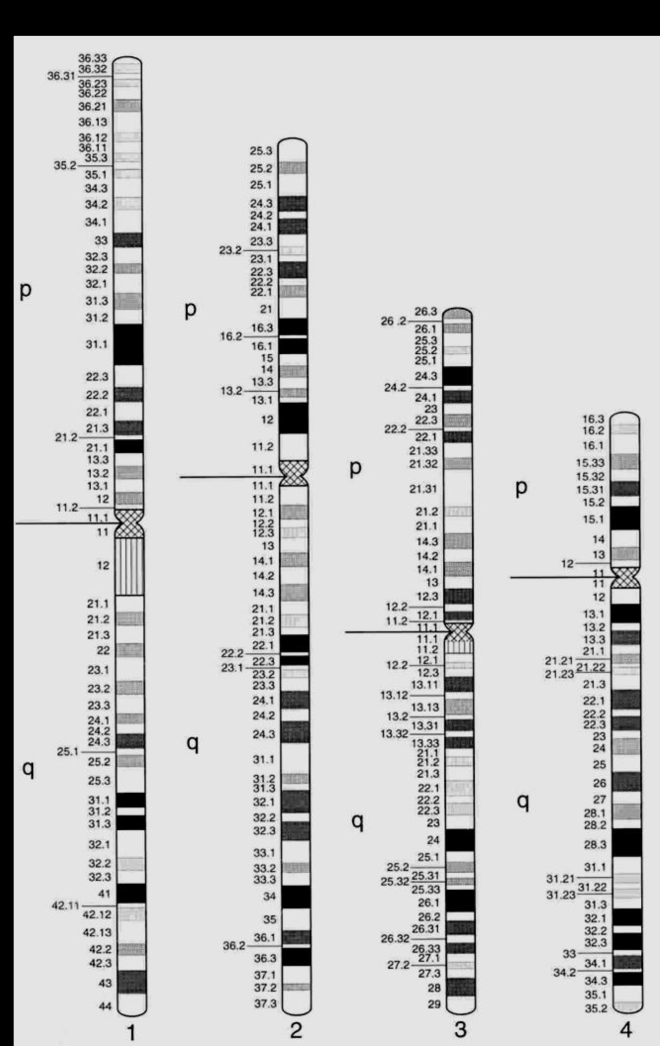
QFQ, Caspersson (1970)



GTG, Seabright (1971)



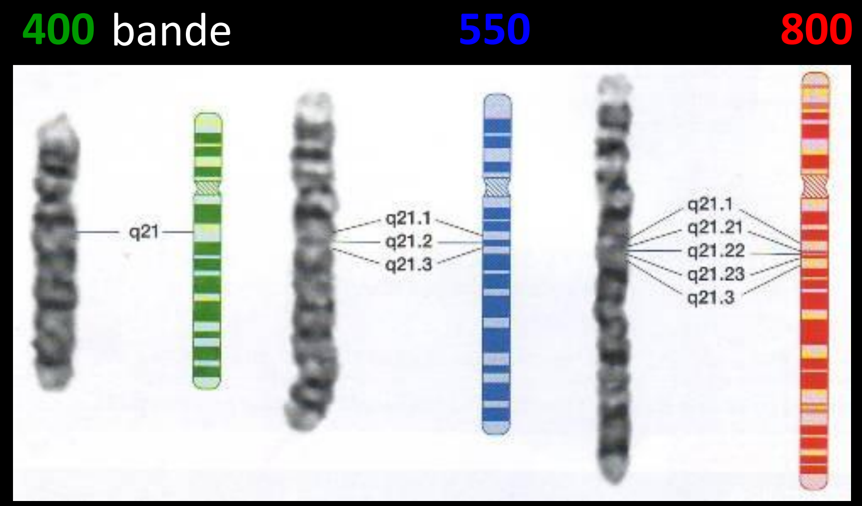
Risoluzione del preparato



"Modern banding allows precise identification of each chromosome and missing or addtional material of 4000 kb or greater can be visualized on routine chromosome analysis"

JM Connor, MA Ferguson-Smith "Essential Medical Genetics" Blackwell Scientific Publications, Oxford, 1993, p. 39

Risoluzione cromosomica

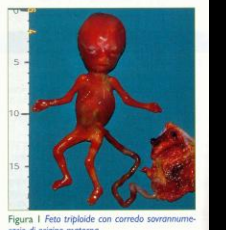


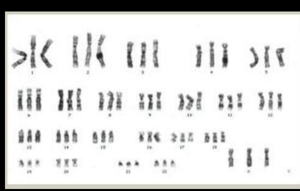
differente

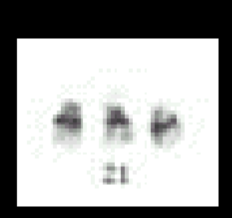
RISOLUZIONE

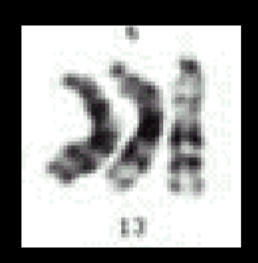
citogenetica

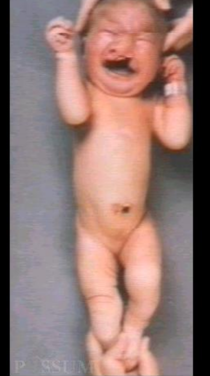
Il cariotipo: una sola analisi per diverse patologie

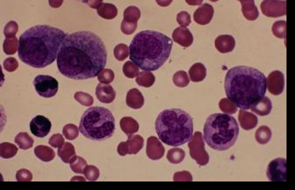


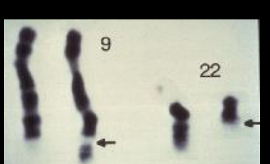


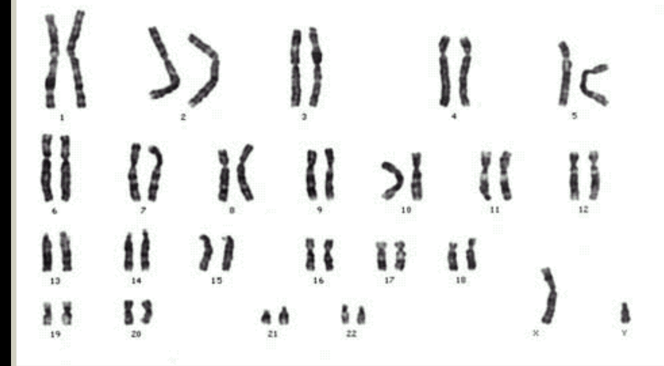


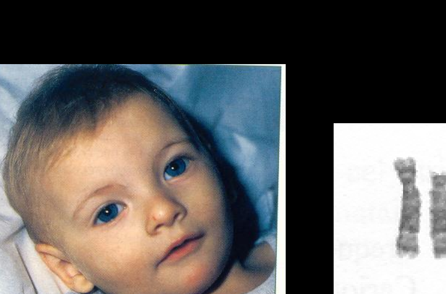


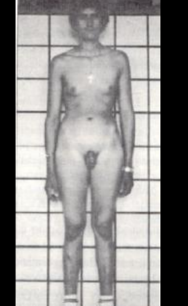


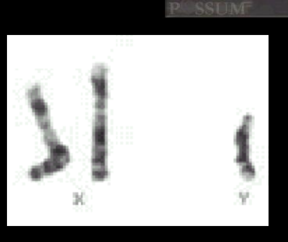


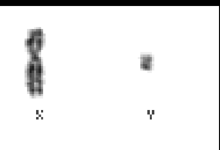


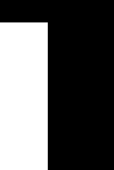




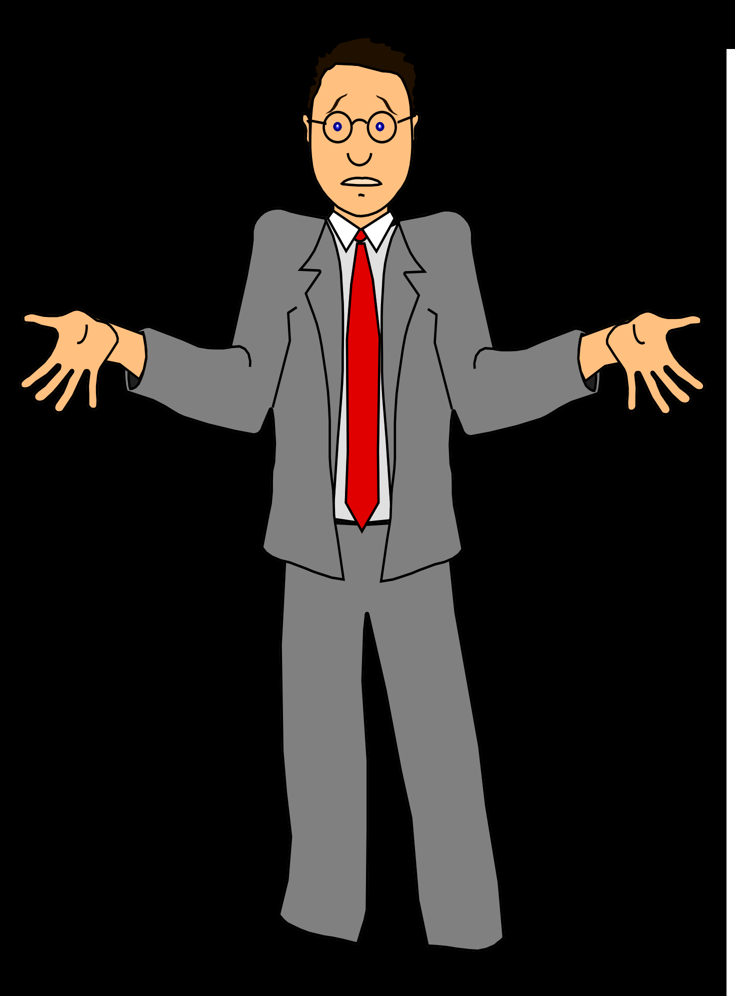








Quando il cariotipo non ce la fa



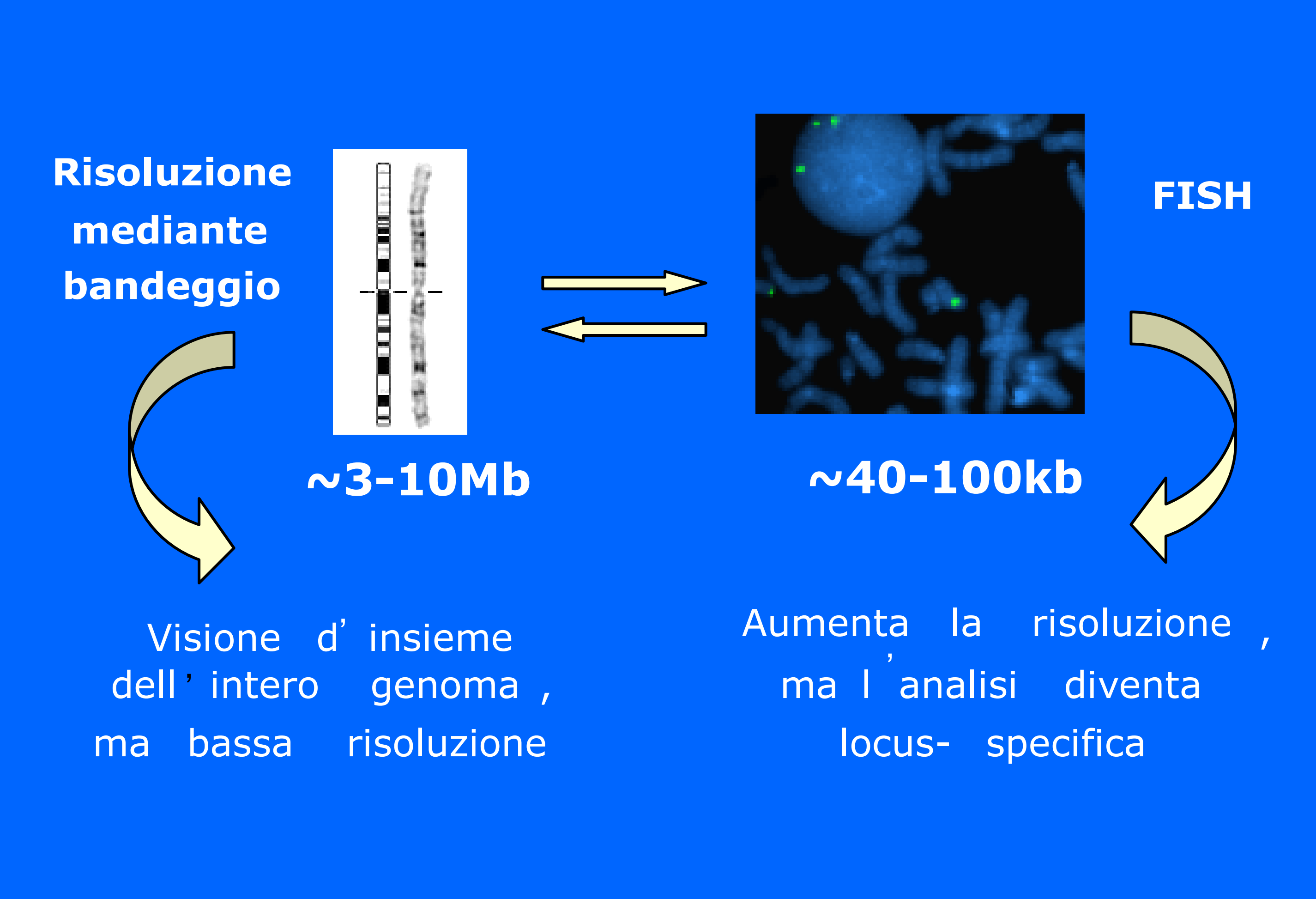
1 2 3 4 5

6 7 8 9 10 11 12

13 14 15 16 17 18

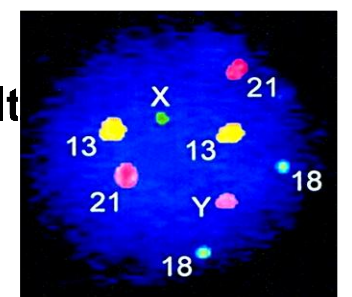
19 20 21 22 X

DALLA CITOGENETICA CLASSICA ALLA CITOGENETICA MOLECOLARE



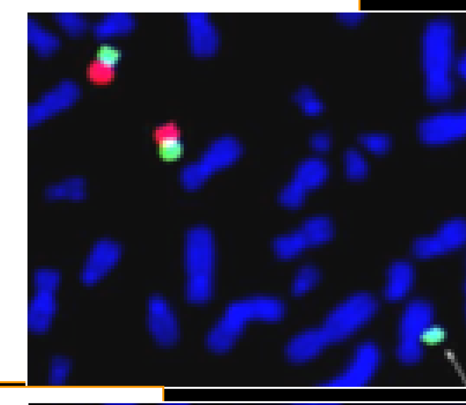
Quando utilizzare tecniche di citogenetica molecolare

- Per ampliare il numero delle cellule analizzate in caso di mosaici sui nuclei in interfase)



- Per definire la ploidia sugli amniociti non colt

- Per definire la natura di un extra cromosoma

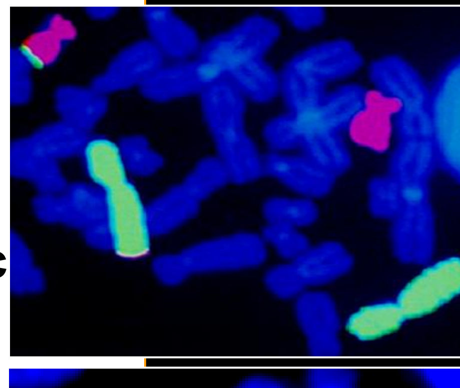


- Per definire lo stato di metilazione di un marcatore (ad es. piccolo X ad ane

Chr. X

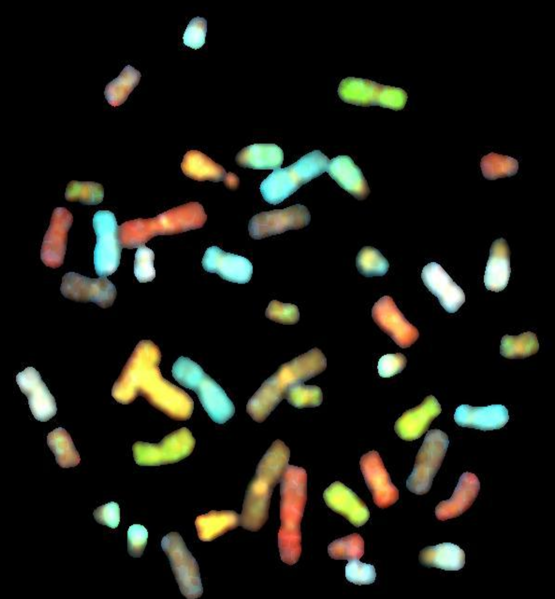
r(X)

- Per analizzare la segregazione di un riarrangiamento criptic



- Per caratterizzare un'anomalia di struttura, dopo avere studi i genitori

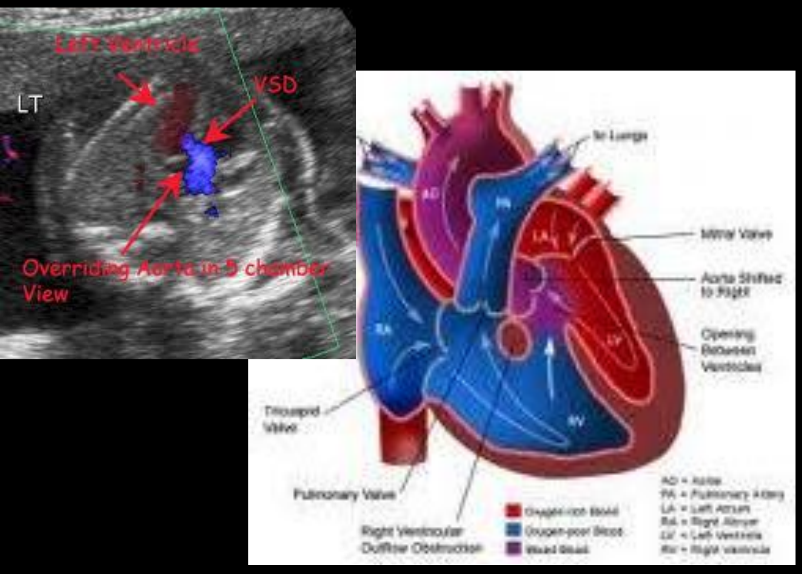
ESAC autosomico ad anello



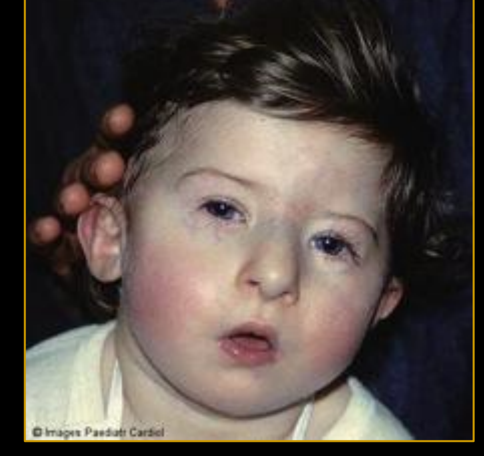
47,XY,r(9)

DP: approccio locus-specifico

Tetralogia di Fallot



Delezione 22q11.2

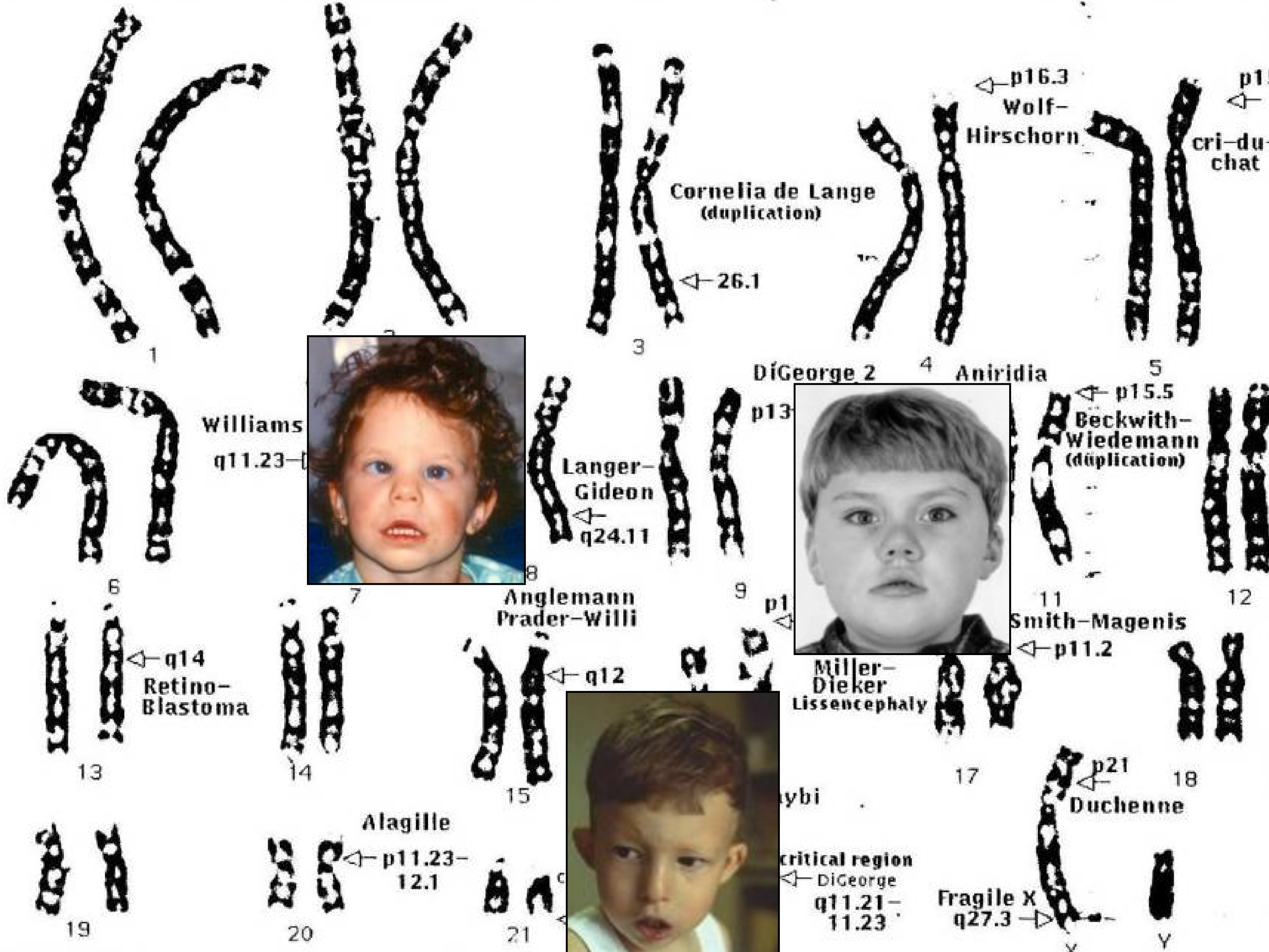


Sindrome Di George / Velocardiofaciale

Tetralogia di Fallot con atresia della polmonare
27%

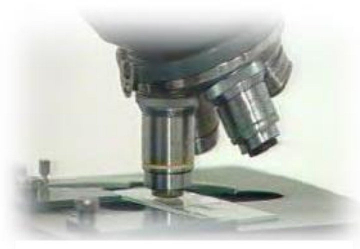
Tetralogia di Fallot classica
26%

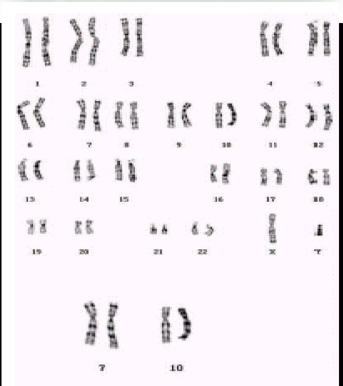
altre cardiopatie



p16.3
Wolf-Hirschorn
cri-du-chat
Cornelia de Lange (duplication)
26.1
1
2
3
4
5
Williams
q11.23
Langer-Gideon
q24.11
DiGeorge 2
p13
Aniridia
p15.5
Beckwith-Wiedemann (duplication)
6
7
8
9
11
12
Anglemann Prader-Willi
q14
Retino-Blastoma
q12
Miller-Dieker Lissencephaly
Smith-Magenis
p11.2
13
14
15
17
18
p21
Duchenne
Alagille
p11.23-12.1
critical region
DiGeorge
q11.21-11.23
Fragile X
q27.3
19
20
21
X
Y

E(RI)VOLUZIONE DELLA CITOGENETICA: DAL CROMOSOMA ALL'ARRAY





10 Mb

Visione d'insieme dell'intero genoma, ma bassa risoluzione

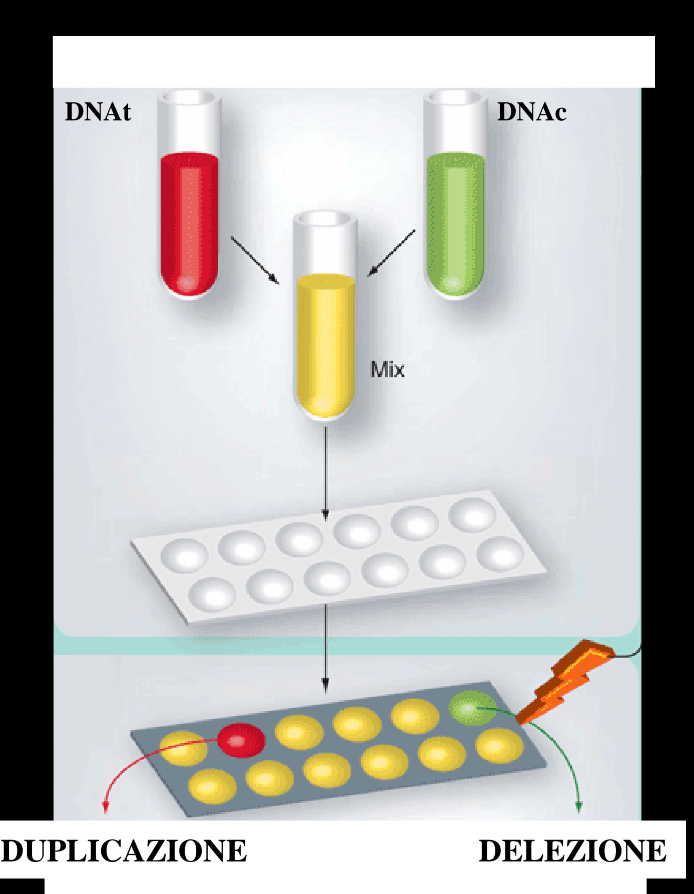
40-100Kb

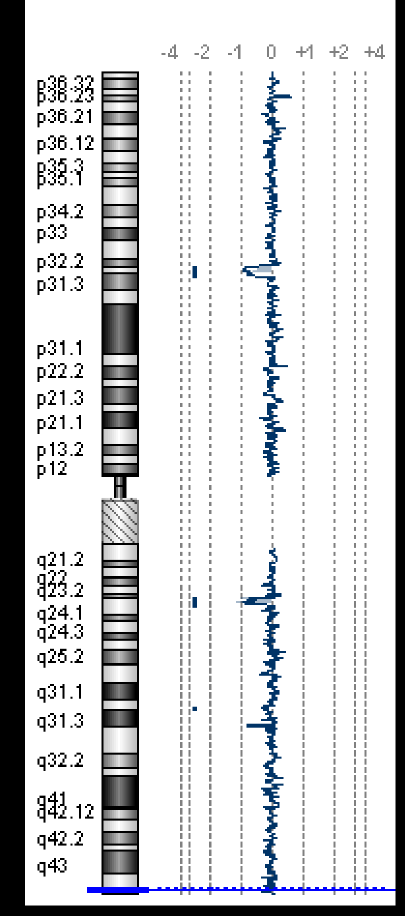
Aumenta la risoluzione, ma l'analisi diventa *locus*-specifica

Array-CGH:
Alta risoluzione (10-12Kb) e analisi dell'intero genoma in un unico esperimento

CGH -ARRAY

si basa sul principio della “ibridazione competitiva” di due campioni di DNA della stessa specie marcati con fluorocromi diversi, utilizzando come base di riferimento migliaia di sequenze di DNA (spot) ordinatamente fissate su un vetrino (microarray). Per ogni cromosoma i rapporti di fluorescenza vengono trasformati su scala logaritmica e visualizzati su un grafico rispetto alla posizione sul cromosoma.





valore = 0→ normale

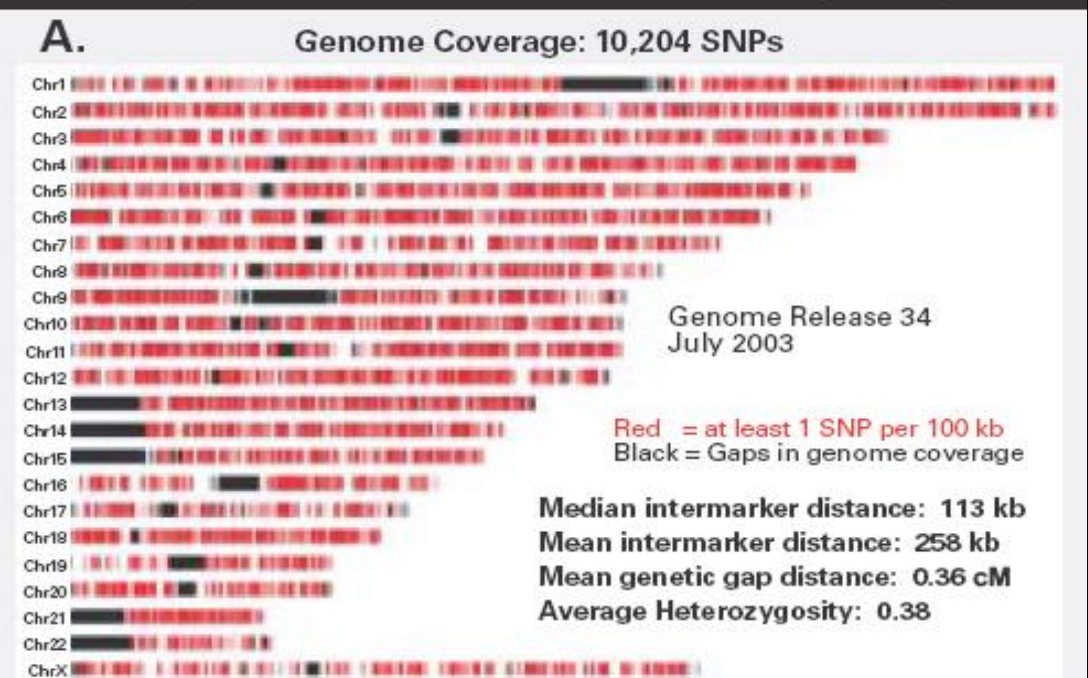
valore < 0→ delezione

valore > 0→ duplicazione

Una “rivoluzione” in corso

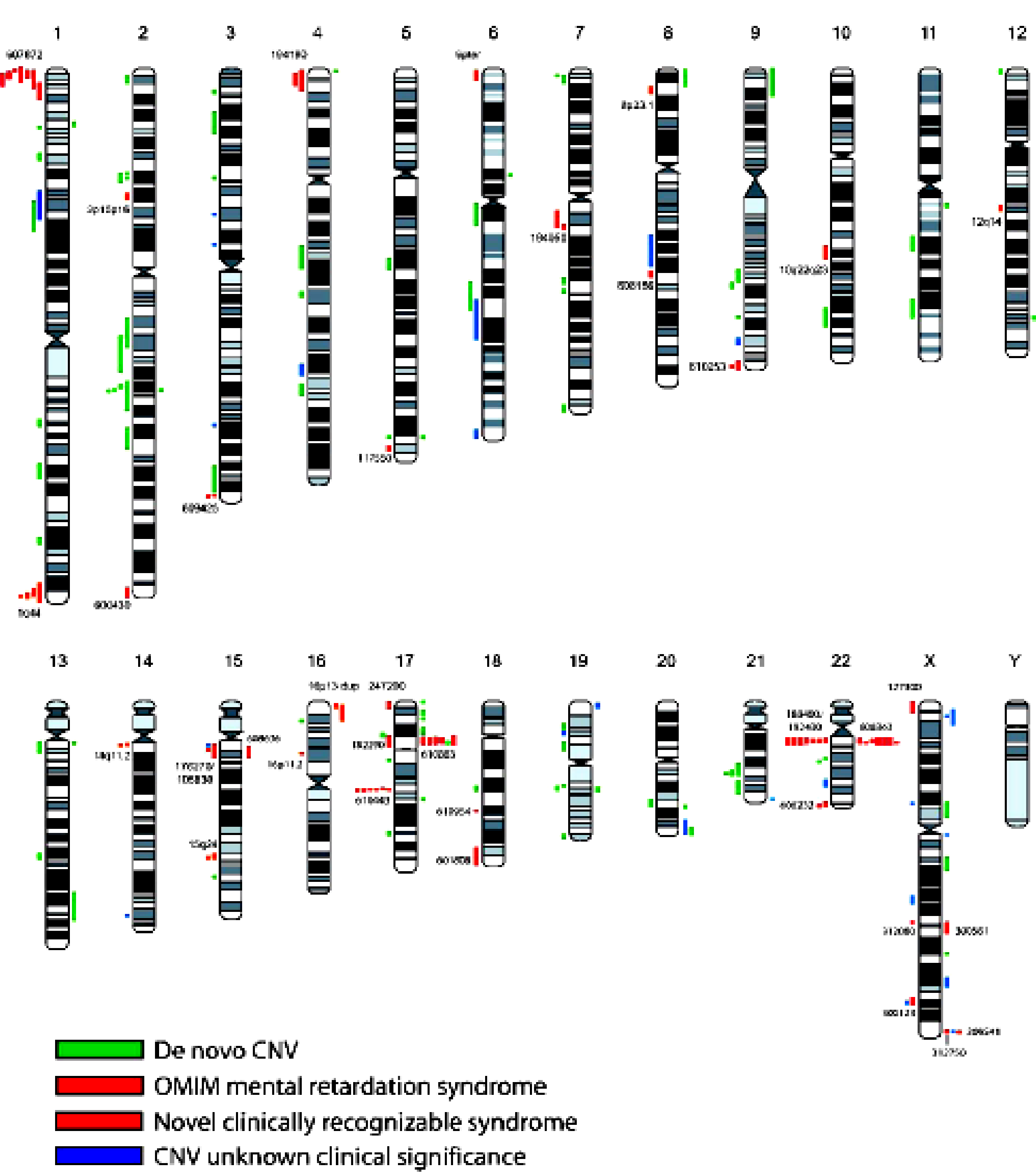
Table 1 Overview of the latest generation of commercially available microarrays for copy number variation (CNV) detection

Microarray vendor	Catalogue whole genome array	Technology	Total number of copy number markers
Affymetrix	Cytogenetics Whole Genome 2.7M array	Single colour hybridisation, CNV and genotyping	2 761 979 oligonucleotides, of which 400 103 are single nucleotide polymorphism (SNP) specific
Agilent	Human High-Resolution Discovery 1*1M	Two-colour hybridisation, CNV only	963 331 oligonucleotides
Illumina	Human 1M-duo BeadChip	Single-colour hybridisation, CNV and genotyping	1 199 187 oligonucleotides, majority SNP specific
NimbleGen	Human CGH 2.1M	Two-colour hybridisation, CNV only	2 100 000 oligonucleotides



Array da 1 milione di SNPs

Crescita esponenziale delle malattie genomiche nella patologia umana



UTILIZZO DEI MICROARRAY GENOMICI

- Possibilità di individuare sbilanciamenti di piccole dimensioni (poche Kb).

CNVs: Segmenti di DNA maggiori di 1 kb che variano per numero di copie da individuo a individuo

- Ruolo delle CNVs (Copy Number Variations) in:
  - Variazioni fenotipiche
  - Patogenesi di un ampio spettro di patologie umane mendeliane e multigeniche

Risoluzione: dipende dalla grandezza e dal numero delle sonde utilizzate
Aumentare la risoluzione -> incrementare il numero degli spot e ridurre la lunghezza delle sonde

(NON E' POSSIBILE SPOTTARE PIU' DI 60,000 SEQUENZE)

TIPI DI SONDE ARRAY:

_ **cloni BAC**: lunghezza 80-200 kb. Buona copertura, forte segnale di ibridazione

_ **fosmidi e cosmidi**: lunghezza 40 kb

_ **cloni di cDNA**: individuazione di CNVs che coprono un singolo gene o parte di esso.
Copertura genomica non omogenea

_ **prodotti di PCR**: risoluzione elevata. Segnale di ibridazione debole.
Produzione per copertura genomica costosa

_ OLIGONUCLEOTIDE-ARRAY:
fino a 244,000 sonde *DIRETTAMENTE SINTETIZZATE SUL SUBSTRATO*

SNPs array: Altissima risoluzione. Copertura non omogenea.
Possibilita' di individuare omodisomie
Debole segnale di ibridazione:
Necessità di riduzione della complessità genomica tramite PCR

SFIDA MAGGIORE: difficoltà interpretative

Come distinguere
tra **CNV benigna** e
CNV effettivamente
implicata in un **fenotipo patologico**

2) UTILIZZO DELLE BANCHE DATI
(Pubbliche e Private)

Valutazione del contenuto genico,
confronto con pazienti,
confronto con controlli,
studio della letteratura

3) VERIFICA DELL'INSORGENZA
(studi familiari)

Valutazione del pattern di insorgenza,
valutazione del rischio di ricorrenza,
valutazione di sintomi minori

4) VERIFICA DELLA PRESENZA DI RIARRANGIAMENTI STRUTTURALI

migliore comprensione della CNV
valutazione del rischio di ricorrenza,

Come procedere se la CNV non e' descritta né in associazione a fenotipi patologici né come variante benigna?

- **Confermare il dato con altre tecniche**

(**FISH**, RT-PCR, MLPA)

Verifica della localizzazione di una CNVs

(duplicazione in tandem, marker sovrannumerario, inserzione, traslocazione)

- **Verifica del pattern di segregazione**

➢ Verifica del pattern di segregazione

tenendo conto del fatto che

una CNV ereditata non sempre è benigna

_ difetti di penetranza ed espressività
_ fattori epigenetici
_ effetti diversi di CNVs del chr X tra maschi e femmine e tra femmine con pattern di inattivazione della X differenti

una CNV insorta *de novo* non necessariamente e' patologica

La verifica pattern di segregazione

aiuta nell'**interpretazione di una CNV** e

permette la valutazione del **rischio di ricorrenza**

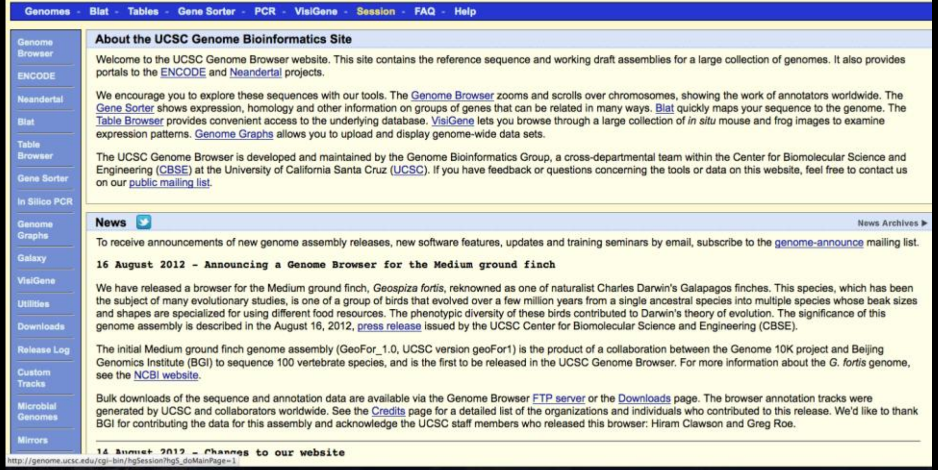
Database Pubblici:

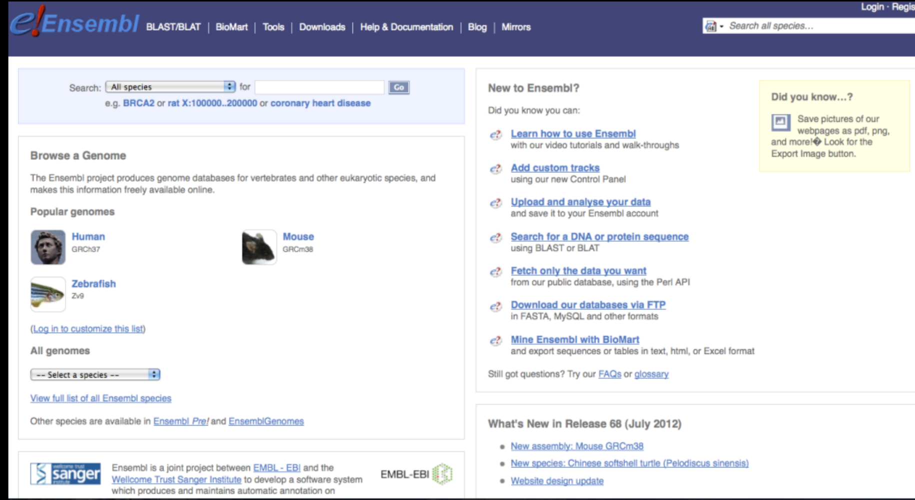
UCSC
http://genome.ucsc.edu/index.html

Ensembl
http://www.ensembl.org/index.html

} Genome browsers

Valutazione del contenuto genico e della natura della regione in esame



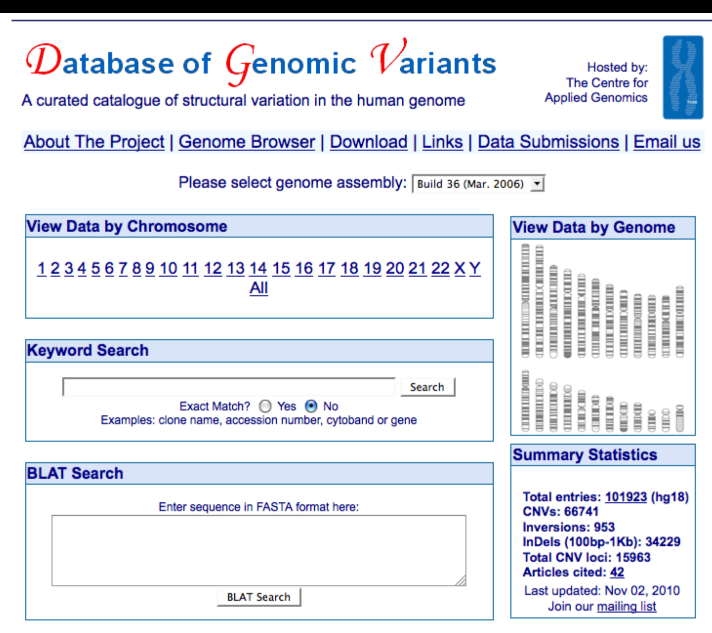


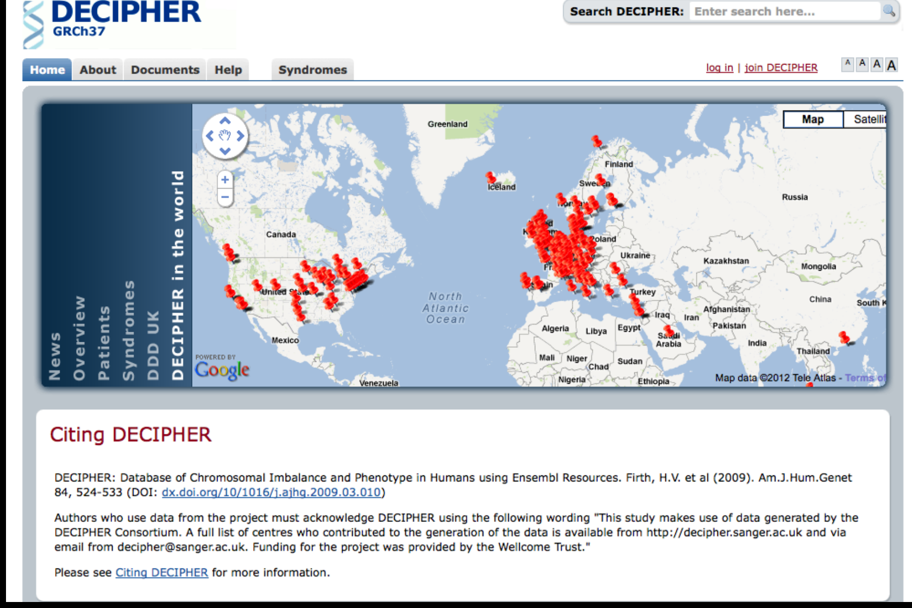
Database Pubblici:

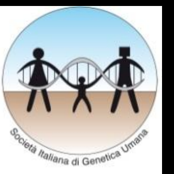
Database of Genomic Variants
http://projects.tcag.ca/variation/

Dechipher
http://decipher.sanger.ac.uk/

} Confronti Fenotipici





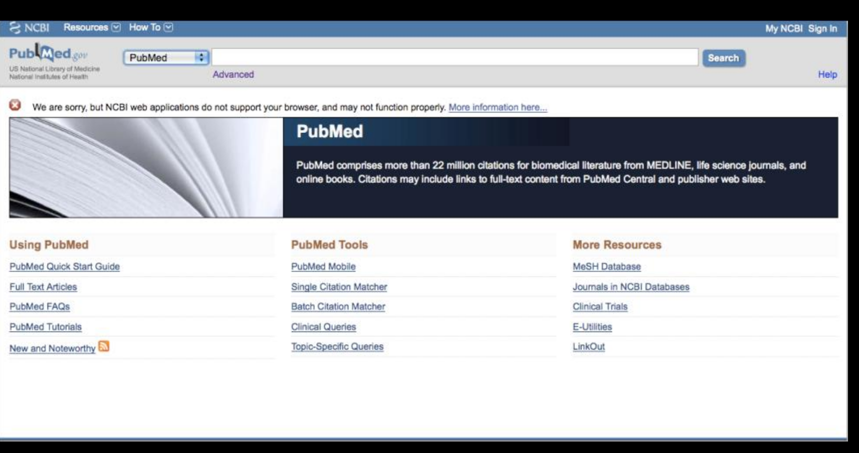


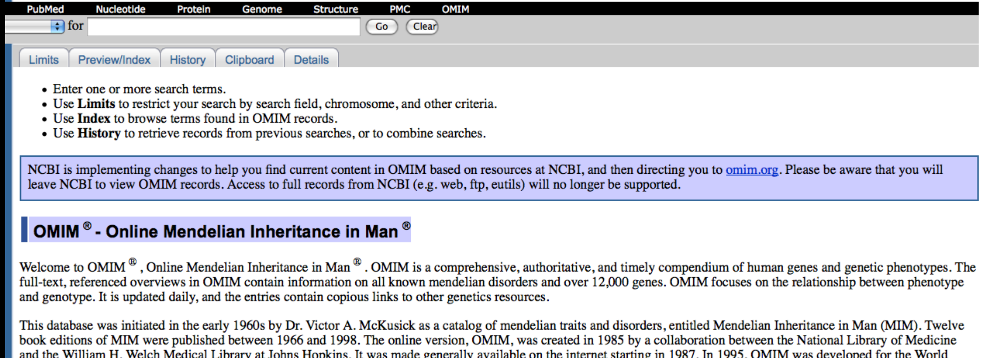
Database Pubblici:

Pubmed
http://www.ncbi.nlm.nih.gov/pubmed/

OMIM
http://www.ncbi.nlm.nih.gov/omim

} Letteratura scientifica





Database Pubblici Nazionali:

Database di Troina
(http://dbcnv.oasi.en.it/gvarianti/index.php Dott. M. Fichera)

} Varianti Popolazione-specifiche

Database Interni:

confronto con pazienti e controlli interni
individuazione di artefatti sperimentali

DOI: 10.1002/pd.3945 PRENATAL DIAGNOSIS

ORIGINAL ARTICLE

Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies

Lisa G. Shaffer[1]*, Mindy P. Dabell[1], Allan J. Fisher[2], Justine Coppinger[1], Anne M. Bandholz[1], Jay W. Ellison[1], J. Britt Ravnan[1], Beth S. Torchia[1], Blake C. Ballif[1] and Jill A. Rosenfeld[1]

Prenat Diagn. 2012 Oct;32(10):976-85. doi: 10.1002/pd.3945. Epub 2012 Aug 2.

Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies.

Shaffer LG, Dabell MP, Fisher AJ, Coppinger J, Bandholz AM, Ellison JW, Ravnan JB, Torchia BS, Ballif BC, Rosenfeld JA.

Signature Genomic Laboratories, PerkinElmer, Inc., Spokane, WA, USA. lisa.shaffer@perkinelmer.com

Abstract

OBJECTIVE: To demonstrate the usefulness of microarray testing in prenatal diagnosis based on our laboratory experience.

METHODS: Prenatal samples received from 2004 to 2011 for a variety of indications (n = 5003) were tested using comparative genomic hybridization-based microarrays targeted to known chromosomal syndromes with later versions of the microarrays providing backbone coverage of the entire genome.

RESULTS: The overall detection rate of clinically significant copy number alterations (CNAs) among unbiased, nondemise cases was 5.3%. Detection rates were 6.5% and 8.2% for cases referred with abnormal ultrasounds and fetal demise, respectively. The overall rate of findings with unclear clinical significance was 4.2% but would reduce to 0.39% if only de novo CNAs were considered. In cases with known chromosomal rearrangements in the fetus or parent, 41.1% showed CNAs related to the rearrangements, whereas 1.3% showed clinically significant CNAs unrelated to the karyotype. Finally, 71% of the clinically significant CNAs found by microarray were below the resolution of conventional karyotyping of fetal chromosomes.

CONCLUSIONS: Microarray analysis has advantages over conventional cytogenetics, including the ability to more precisely characterize CNAs associated with abnormal karyotypes. Moreover, a significant proportion of cases studied by array will show a clinically significant CNA even with apparently normal karyotypes.

Cases with Abnormal Ultrasound Findings

- 2004–2011, 5,003 prenatal cases were analyzed by microarray
- 2,859 cases had an indication of abnormal ultrasound findings
- 6.5% of cases with a structural anomaly had an abnormal microarray result
- 72% (2053/2859) had known normal fetal karyotypes; the remaining cases had concurrent karyotyping. All known abnormal karyotypes were removed
- Cases of fetal demise were removed to not bias the detection rates

Nuchal Translucency

Increased NT	Isolated	Other findings	Total Abnormal
< 4mm	1/113 (0.9%)	1/7 (14.3%)	2/120 (1.7%)
≥4mm	6/96 (6.3%)	2/12 (16.7%)	8/108 (7.4%)
Total	10/303 (3.3%)	6/49 (12.2%)	16/352 (4.5%)

Detection rates in addition to those found by karyotyping

Anomalies in Isolation or with Multiple Findings

Anomaly	Detection Rate
Holoprosencephaly	9/85 (10.6%)
Posterior fossa defects	21/144 (14.6%)
Skeletal anomalies	15/140 (10.7%)
Ventricular septal defect	14/132 (10.6%)
Hypoplastic left heart	11/68 (16.2%)
Club feet/hands	19/194 (9.8%)
Cleft lip/palate	14/136 (10.3%)

Detection rates in addition to those found by karyotyping

Anomalies in a Single Organ System or Single Anomaly

Organ System or Single Anomaly	Detection Rate
CNS	25/381 (6.6%)
Heart	6/237 (2.5%)
Facies (dysmorphism)	6/88 (6.8%)
Diaphragmatic hernia	4/48 (8.3%)
Omphalocele	4/49 (8.2%)
Musculoskeletal	18/203 (8.9%)
Genitourinary	7/115 (6.1%)
Nuchal/other body fluid accumulation	27/628 (4.3%)

Detection rates in addition to those found by karyotyping

Original Article

Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis

Ronald J. Wapner, M.D., Christa Lese Martin, Ph.D., Brynn Levy, M.Sc.(Med.), Ph.D., Blake C. Ballif, Ph.D., Christine M. Eng, M.D., Julia M. Zachary, Melissa Savage, M.S., Lawrence D. Platt, M.D., Daniel Saltzman, M.D., William A. Grobman, M.D., M.B.A., Susan Klugman, M.D., Thomas Scholl, Ph.D., Joe Leigh Simpson, M.D., Kimberly McCall, B.S., Vimla S. Aggarwal, M.B., B.S., Brian Bunke, B.S., Odelia Nahum, M.Sc., Ankita Patel, Ph.D., Allen N. Lamb, Ph.D., Elizabeth A. Thom, Ph.D., Arthur L. Beaudet, M.D., David H. Ledbetter, Ph.D., Lisa G. Shaffer, Ph.D., and Laird Jackson, M.D.

N Engl J Med
Volume 367(23):2175-2184
December 6, 2012

Frequency and Clinical Interpretation of Microdeletions and Duplications on Chromosomal Microarray in the 3822 Samples with a Normal Karyotype, According to Indication for Prenatal Testing.

Table 3. Frequency and Clinical Interpretation of Microdeletions and Duplications on Chromosomal Microarray in the 3822 Samples with a Normal Karyotype, According to Indication for Prenatal Testing.

Indication for Prenatal Diagnosis	Normal Karyotype	Common Benign	Pathogenic	Uncertain Clinical Significance (N=130)		Total Known Pathogenic and Potential for Clinical Significance*
				Likely to Be Benign	Potential for Clinical Significance	
	no.	*no. (%)*				*no. (%) [95% CI]†*
Any	3822	1234 (32.3)	35 (0.9)	69 (1.8)‡	61 (1.6)	96 (2.5) [2.1–3.1]
Advanced maternal age	1966	628 (31.9)	9 (0.5)	37 (1.9)	25 (1.3)	34 (1.7) [1.2–2.4]
Positive on Down's syndrome screening	729	247 (33.9)	3 (0.4)	13 (1.8)	9 (1.2)	12 (1.6) [0.9–2.9]
Anomaly on ultrasonography	755	247 (32.7)	21 (2.8)	16 (2.1)	24 (3.2)	45 (6.0) [4.5–7.9]
Other§	372	112 (30.1)	2 (0.5)	3 (0.8)	3 (0.8)	5 (1.3) [0.6–3.1]

* Total includes those predetermined as known to be pathogenic and those classified by the clinical advisory committee as clinically relevant.
† CI denotes confidence interval.
‡ Includes 36 samples determined likely to be benign by the study geneticist and 33 determined by the independent clinical advisory committee on the basis of size, gene content, inheritance, the literature, and ultrasonography findings.
§ Other indications include family history, previous pregnancy with chromosomal abnormalities, and elective decision.

Clinical Indications for Investigation by Genome-Wide Array

Postnatal

Patients with:

- clinically significant abnormal growth, for example, short stature, excessive growth, microcephaly, macrocephaly, and dysmorphism;
- multiple congenital abnormalities;
- intellectual disability, developmental delay, autistic spectrum disorder;
- suspected deletion/duplication syndrome;
- X-linked recessive disorder in a female.

Raccomandazioni sull'uso delle analisi d *Chromosomal Microarrays* (CGH/SNP-array) applicate alla diagnosi genetica del deficit intellettivo, dei disturbi comportamentali e dei difetti congeniti.

Documento del Gruppo di Lavoro di Genetica Clinica della Società Italiana di Genetica Umana

1. ritardo psico-motorio/deficit intellettivo idiopatico
2. malattie dello spettro autistico idiopatico per la sensibile frequenza di CNV a ruolo francamente causativo o predisponente (R. Toro, 2010)
3. due o più malformazioni maggiori da causa non nota (Miller DT, 2010), soprattutto coinvolgenti mani e cuore (UK Genetic Testing Network, 2009)
4. riarrangiamenti cromosomici apparentemente bilanciati con anomalie fenotipiche
5. costituiscono una indicazione per l'analisi tramite CMA se si associano a ritardo psicomotorio o a anomalie fenotipiche maggiori o minori le seguenti patologie:

- disturbi comportamentali,
- deficit di attenzione-iperattività (N.M. Williams, 2010),
- epilessia (E. Ezugha, 2010),
- microcefalia,
- malformazione maggiore,
- anomalie di crescita (deficit o eccesso)

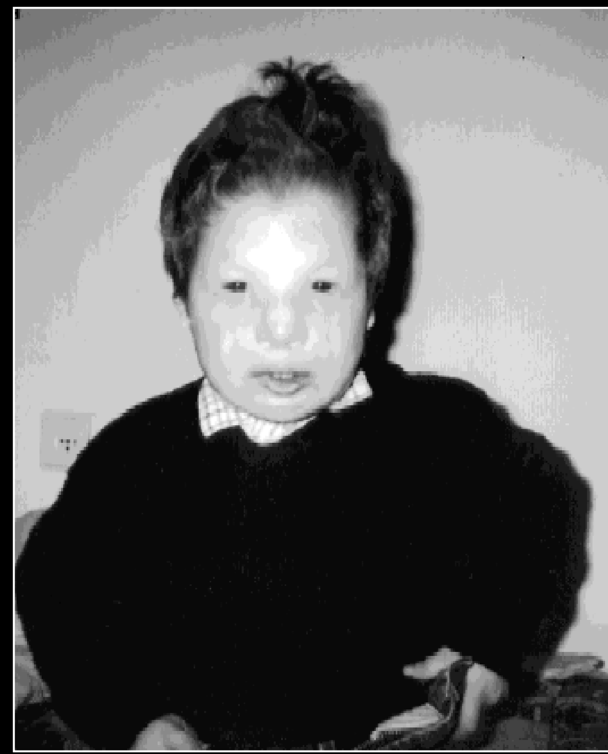
CMA as diagnostic tool for ID/CD in Italy. SIGU consensus statement (clinical branch). Vers. 8.2.2.1, 29 Giugno 2011

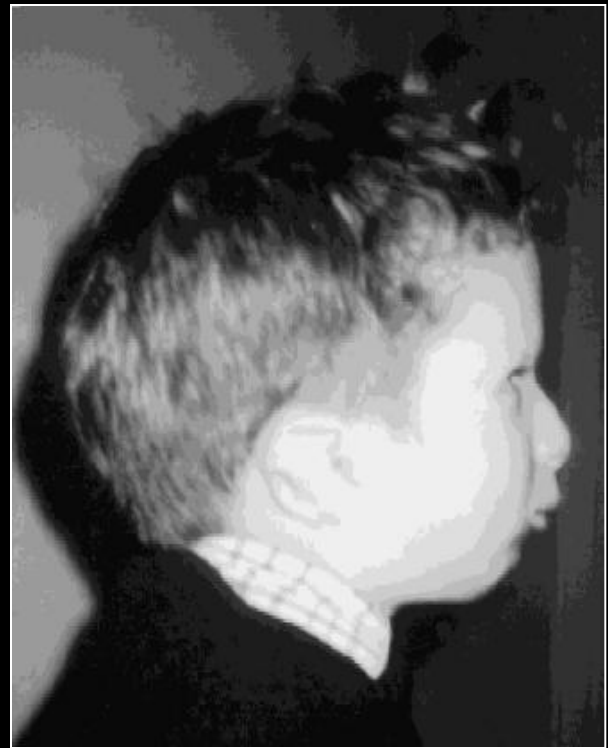
2006: aCGH (1 Mb) Definizione nuove sindromi da geni contigui

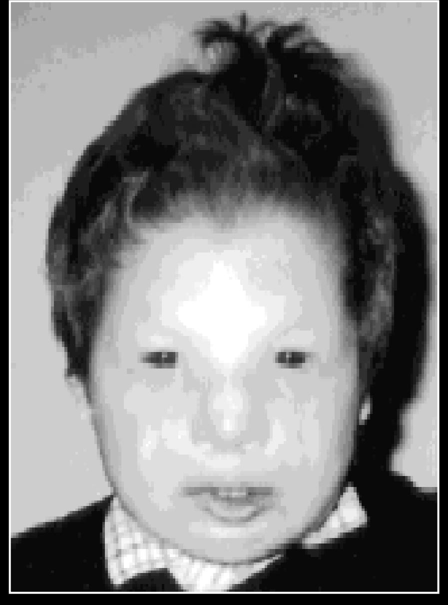
Letter to the Editor

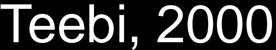
Nablus Mask-Like Facial Syndrome

Teebi AS

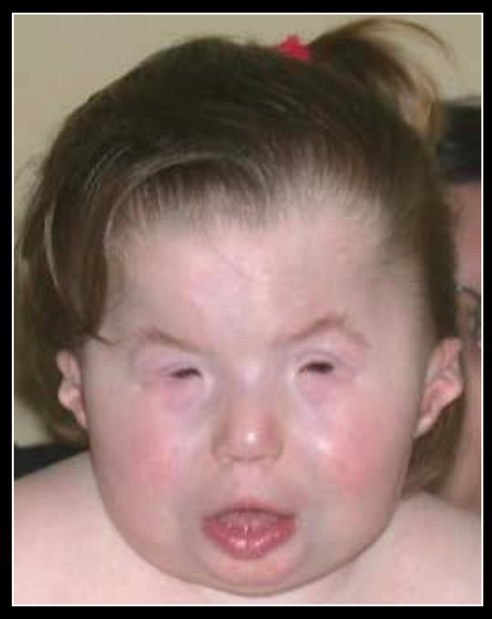








Teebi, 2000



Salpietro et al. 2003

Conferma della Sindrome di Nablus come distinta entita' clinica

Eziologia non nota

- ✓ **cariotipo alta risoluzione: NORMALE**
- ✓ **Analisi delle regioni subtelomeriche: NEGATIVA**

- ✓ **Possibile modello di trasmissione: autosomico recessivo probabile consanguineita'**

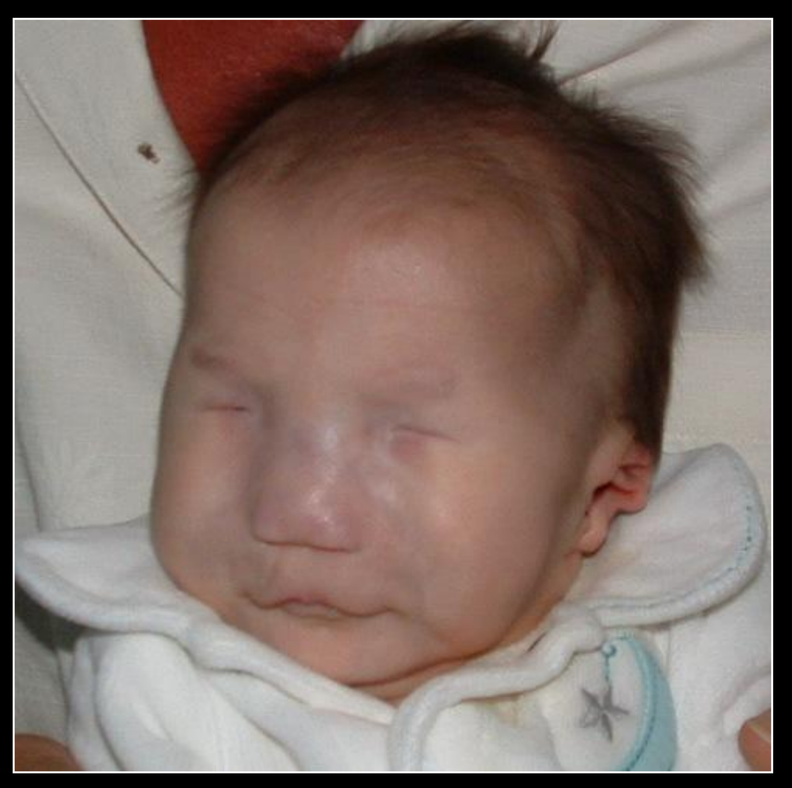
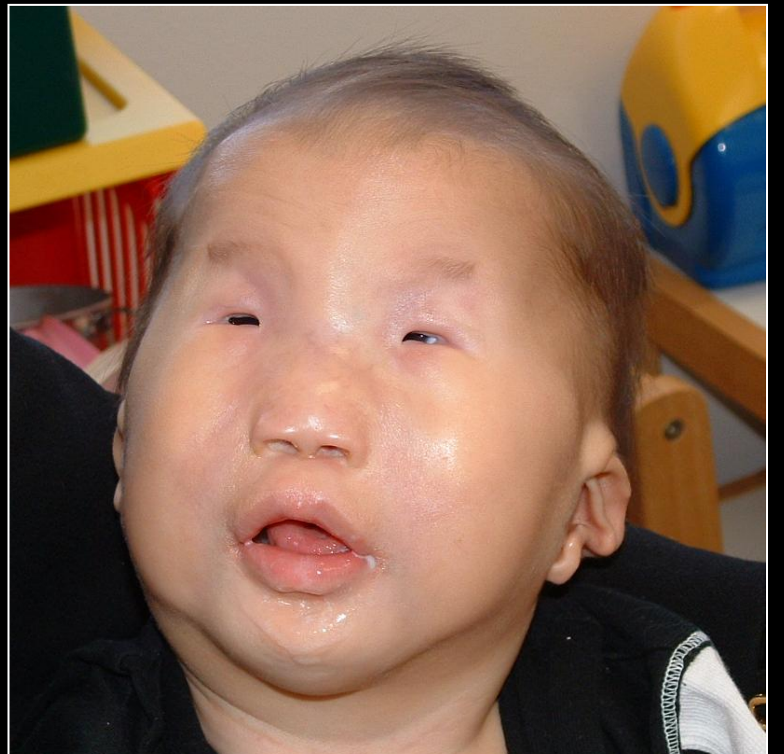
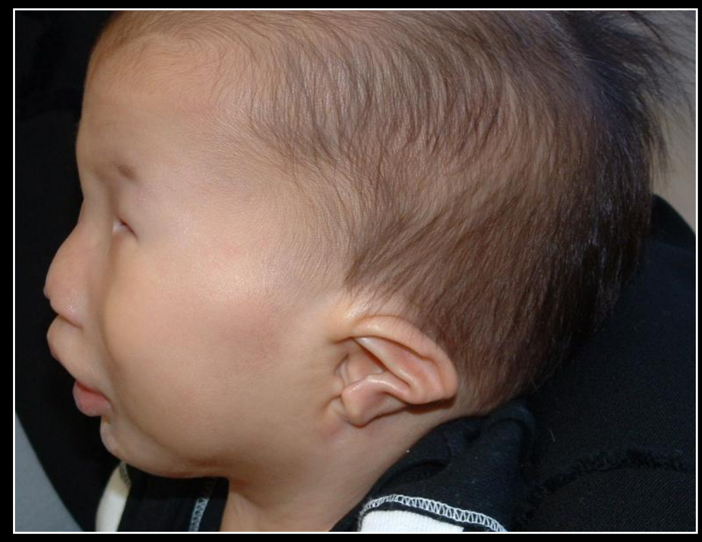
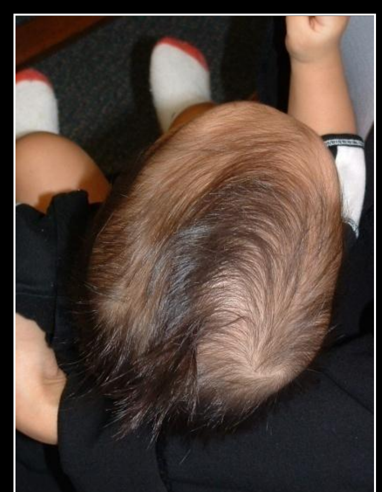
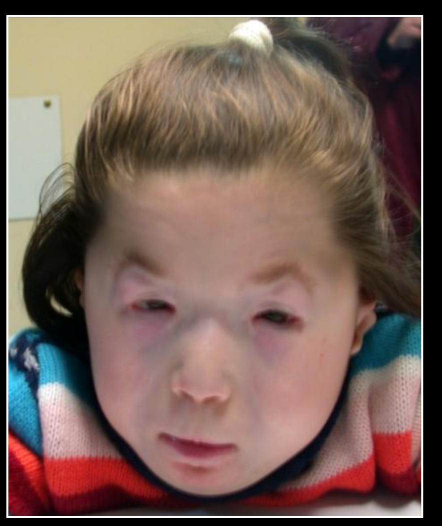





TABLE I. Comparison of Clinical Features of Teebi's Patient and Present Two Patients

Clinical finding	Teebi, 2000	Patient 1	Patient 2
Tight glistening facial skin	+	+	+
Abnormal hair patterning	+	+	+
Blepharophimosis	+	+	+
Sparse eyebrows	+	+	+
Abnormal ear configuration	+	+	+
Maxillary hypoplasia	+	+	+
Abnormal dentition	−	+	+
Long philtrum	+	+	+
Flat nasal tip	+	+	+
Acquired microcephaly	−	+	+
Hypoplastic genitalia	−	+	+
Submucous cleft palate	−	−	+
Displaced nipples	−	−	+
Contractures	−	+	−
Developmental delay	−	+	+

Nablus Mask-Like Facial Syndrome

Shieh JTC, Aradhya S., Novelli A., Manning MA, Cherry AM, Brumblay J., Salpietro CD, Bernardini L., Dallapiccola B., Hoyme H.E. (2006) "Nablus mask-like facial syndrome is caused by a microdeletion of 8q detected by array-based comparative genomic hybridization" American Journal Medical Genetics Part A 140: 1267-1273



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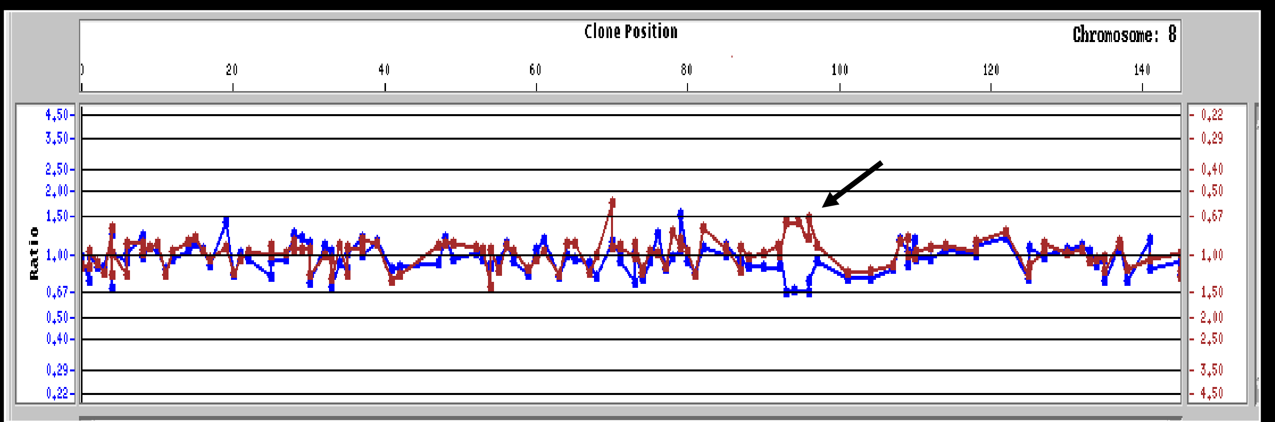

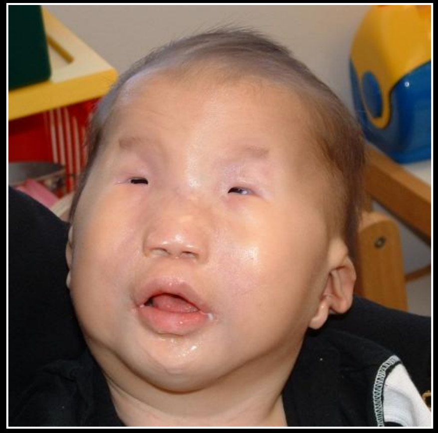

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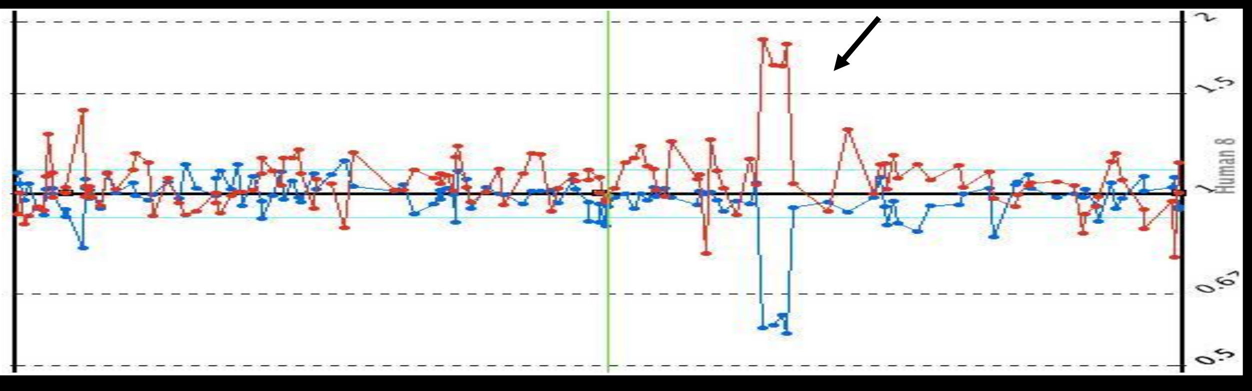
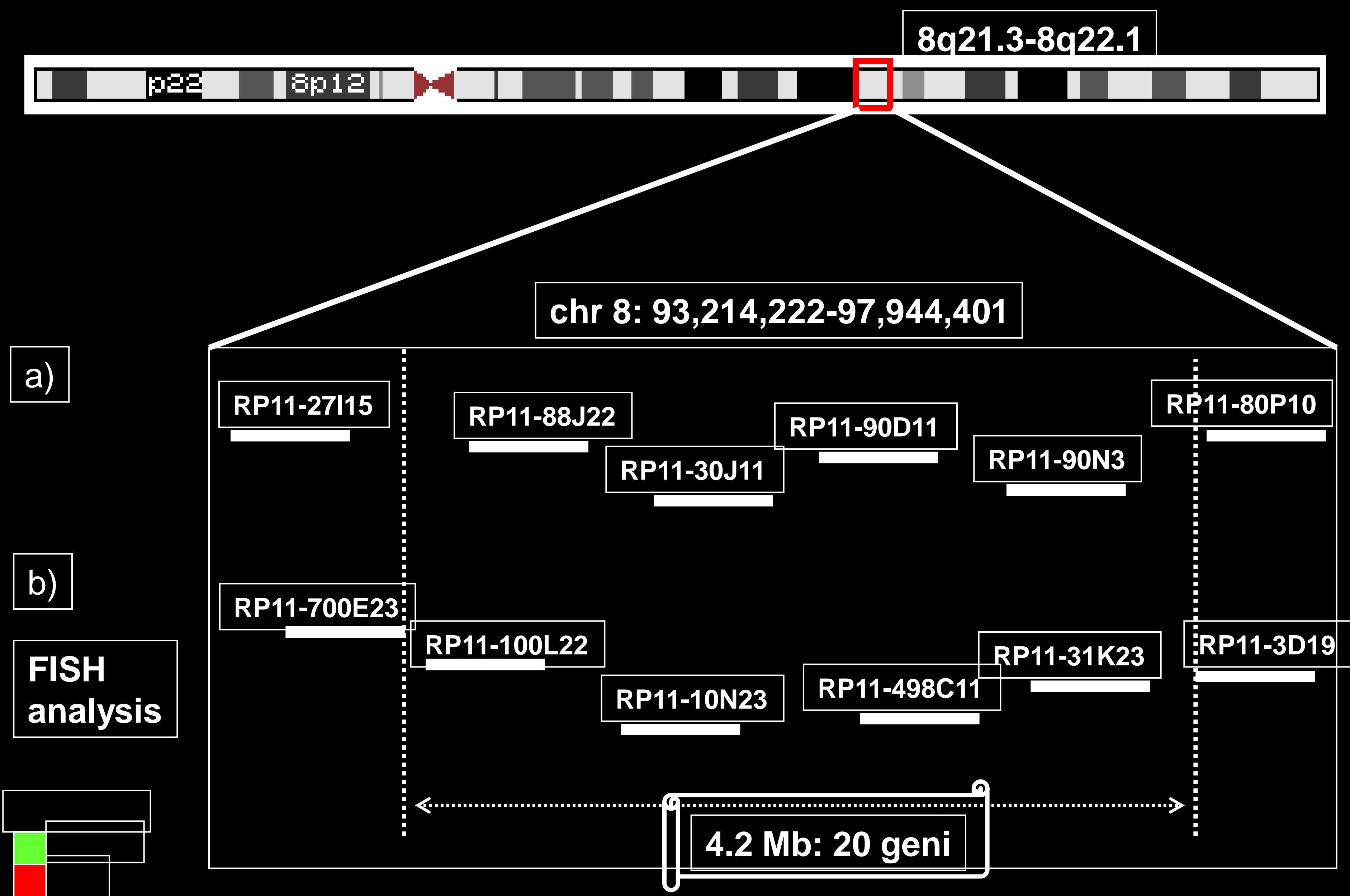


(1 Mb array-CGH; Spectral Genomics)



Shieh JTC, Aradhya S., Novelli A., Manning MA, Cherry AM, Brumblay J., Salpietro CD, Bernardini L., Dallapiccola B., Hoyme H.E. (2006) "Nablus mask-like facial syndrome is caused by a microdeletion of 8q detected by array-based comparative genomic hybridization" American Journal Medical Genetics Part A 140: 1267-1273

European Journal of Medical Genetics 52 (2009) 140–144

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

Original article

The 8q22.1 microdeletion syndrome or Nablus mask-like facial syndrome: Report on two patients and review of the literature

Annick Raas-Rothschild [a,*], Trijnie Dijkhuizen [b], Birgit Sikkema-Raddatz [b], Marion Werner [a], Judith Dagan [a], Devorah Abeliovich [a], Israela Lerer [a]

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CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

A New Case of 8q22.1 Microdeletion Restricts the Critical Region for Nablus Mask-Like Facial Syndrome

Anne Debost-Legrand,[1] Eleonore Eymard-Pierre,[2,3] Céline Pebrel-Richard,[2,3] Laetitia Gouas,[2,3] Carole Goumy,[2,3] Michel Giollant,[2,3] Wiem Ayed,[2,3] Andreï Tchirkov,[2,3] Christine Francannet,[1] and Philippe Vago[2,3]*

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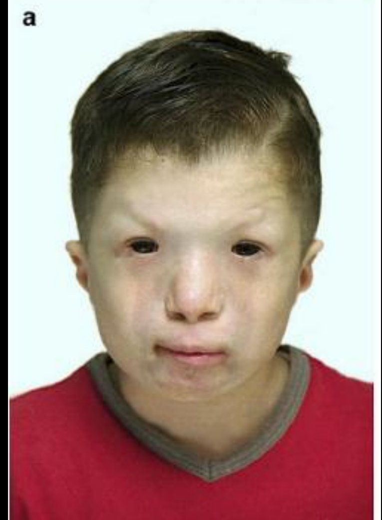
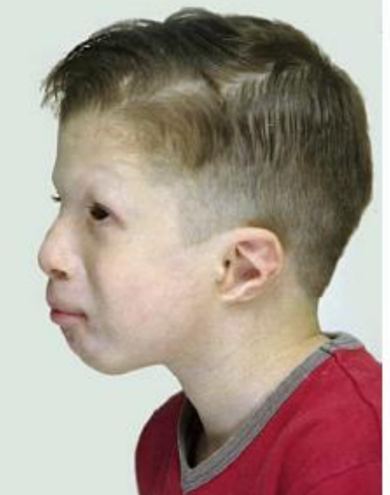
[3]ERTICa, EA 4677, Univ Clermont1, UFR Médecine, F-63000, France

Manuscript Received: 11 May 2012; Manuscript Accepted: 17 July 2012





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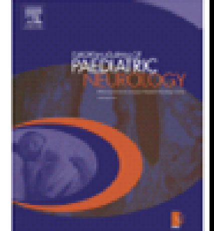
EUROPEAN JOURNAL OF PAEDIATRIC NEUROLOGY XXX (2013) 1–11

Official Journal of the European Paediatric Neurology Society



Original article

Confirmation of chromosomal microarray as a first-tier clinical diagnostic test for individuals with developmental delay, intellectual disability, autism spectrum disorders and dysmorphic features☆,☆☆

Agatino Battaglia [a,*], *Viola Doccini* [a], *Laura Bernardini* [b], *Antonio Novelli* [b], *Sara Loddo* [b], *Anna Capalbo* [b], *Tiziana Filippi* [a], *John C. Carey* [c]

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ARTICLE INFO

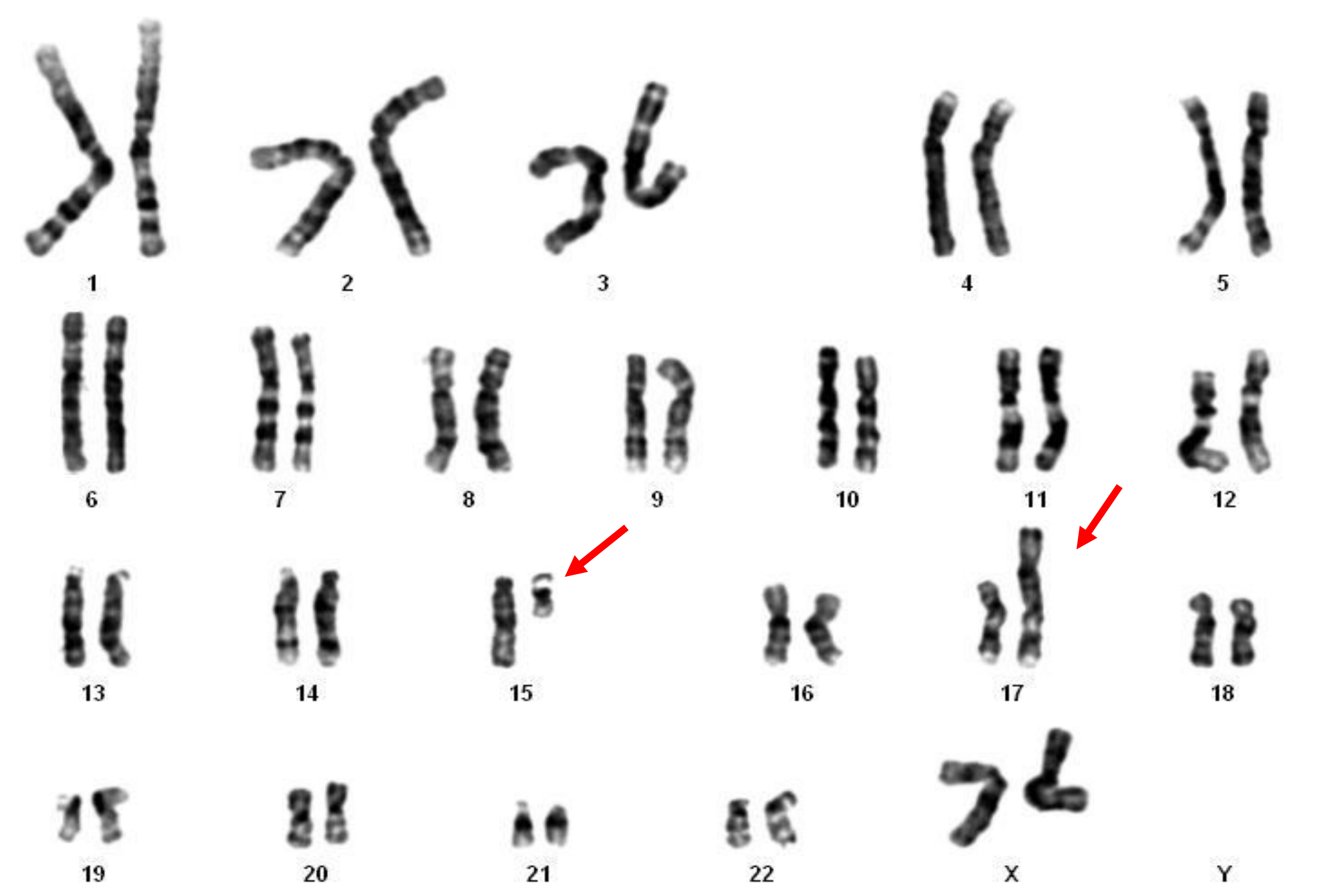
Article history:
Received 30 October 2012
Received in revised form
28 February 2013
Accepted 28 April 2013

Keywords:
Chromosomal microarray (CMA)

ABSTRACT

Background and objectives: Submicroscopic chromosomal rearrangements are the most common identifiable causes of intellectual disability and autism spectrum disorders associated with dysmorphic features. Chromosomal microarray (CMA) can detect copy number variants <1 Mb and identifies size and presence of known genes. The aim of this study was to demonstrate the usefulness of CMA, as a first-tier tool in detecting the etiology of unexplained intellectual disability/autism spectrum disorders (ID/ASDs) associated with dysmorphic features in a large cohort of pediatric patients.

Patients and methods: We studied 349 individuals; 223 males, 126 females, aged 5 months-19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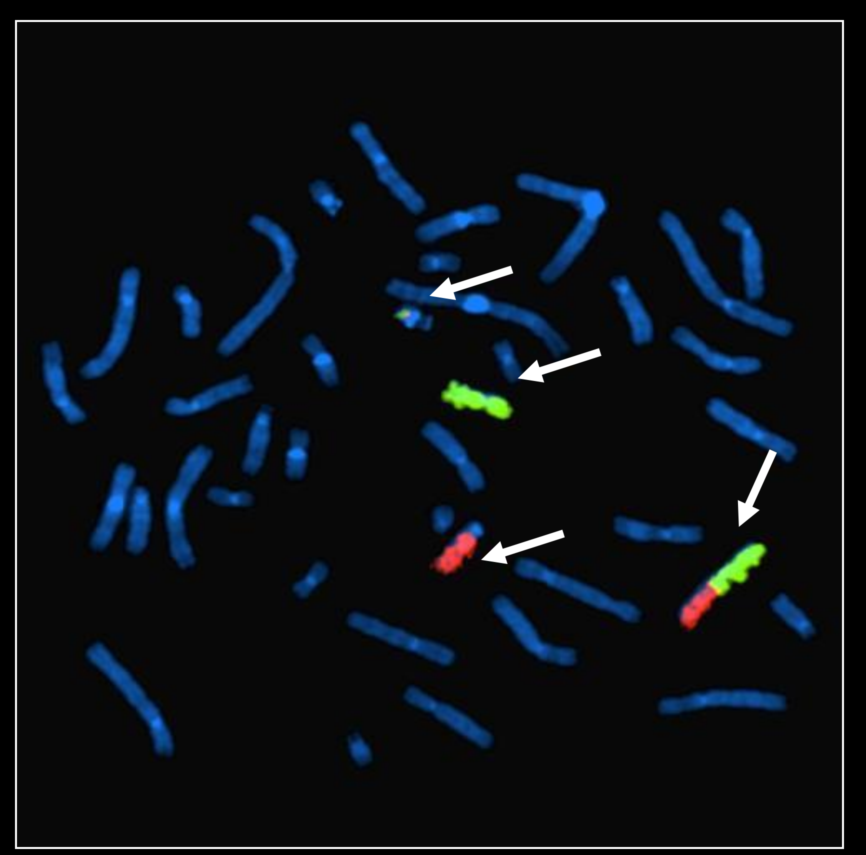
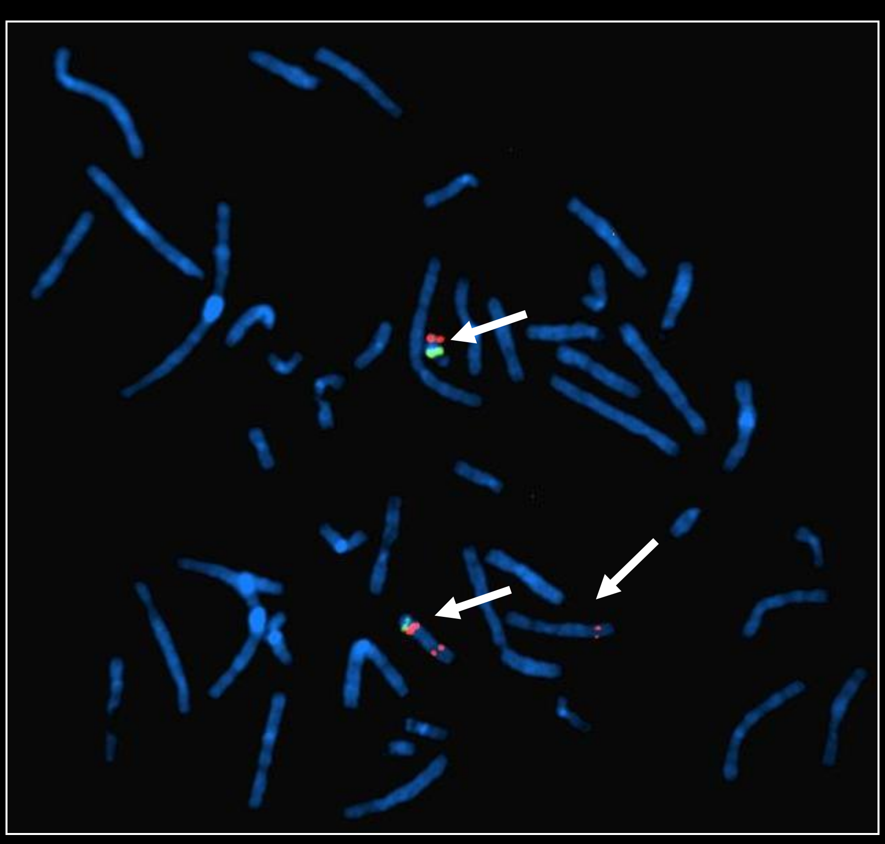


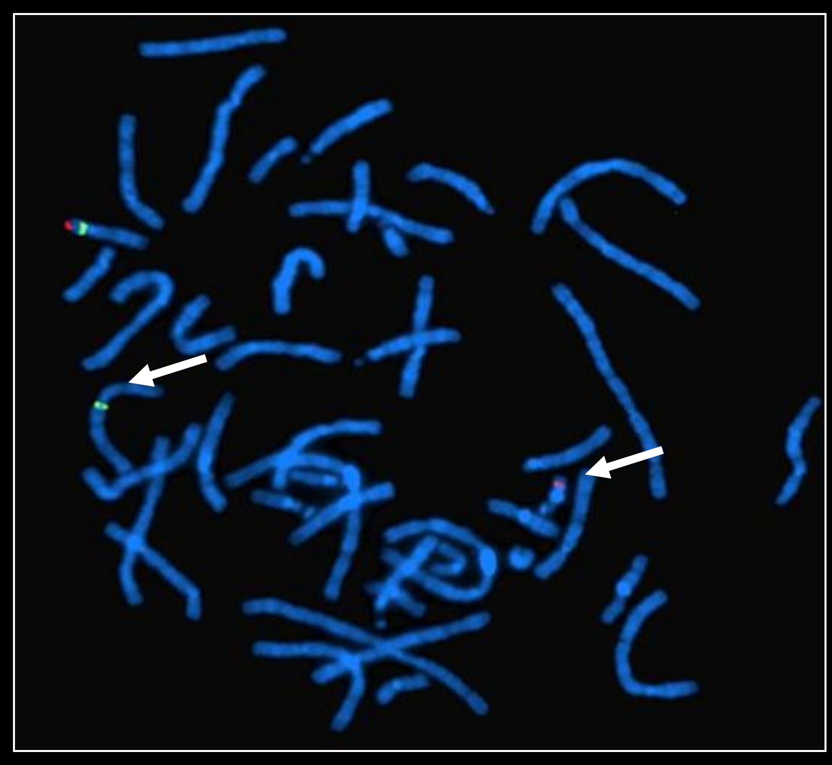


WCP 17

WCP 15

15p11.2 D15Z1

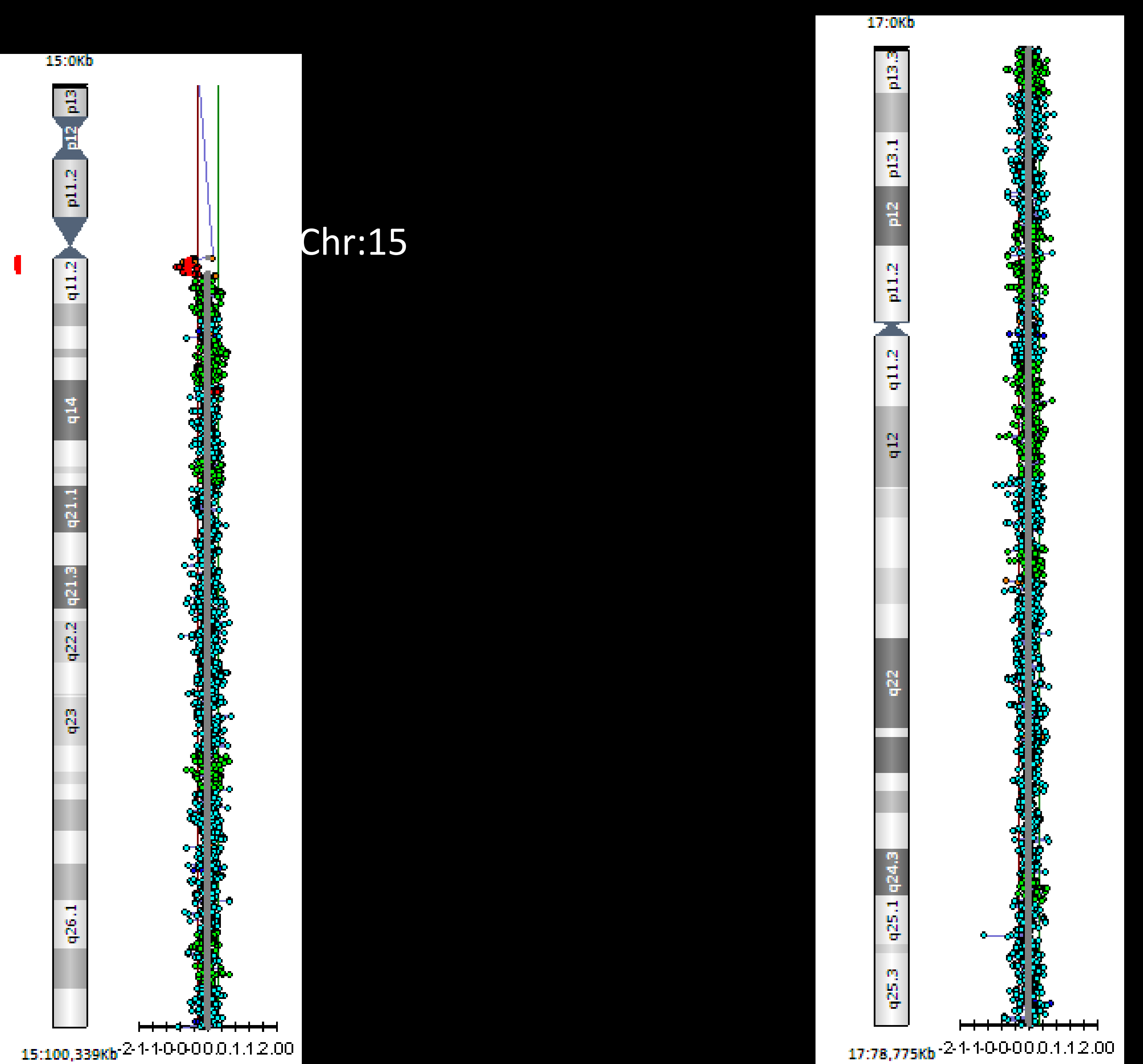
15q11-q13 / 15q22 PML

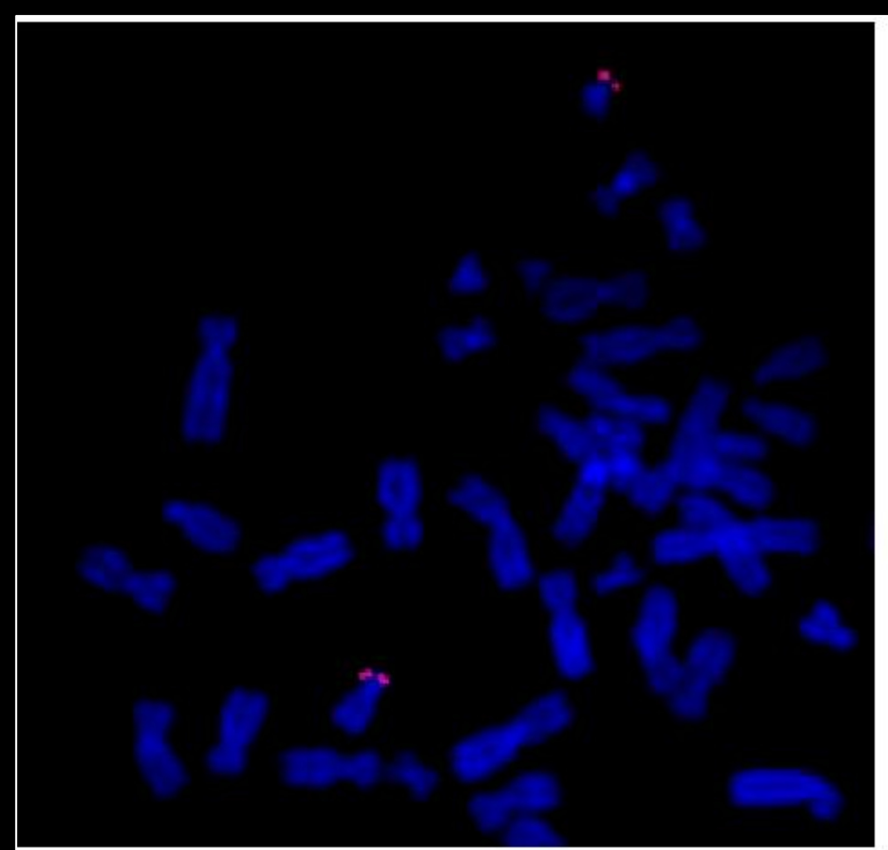


17p11.2 SMS (RAI1)

17p13.3 MDS (LIS1)

Array-CGH piattaforma Oligo 2x105K





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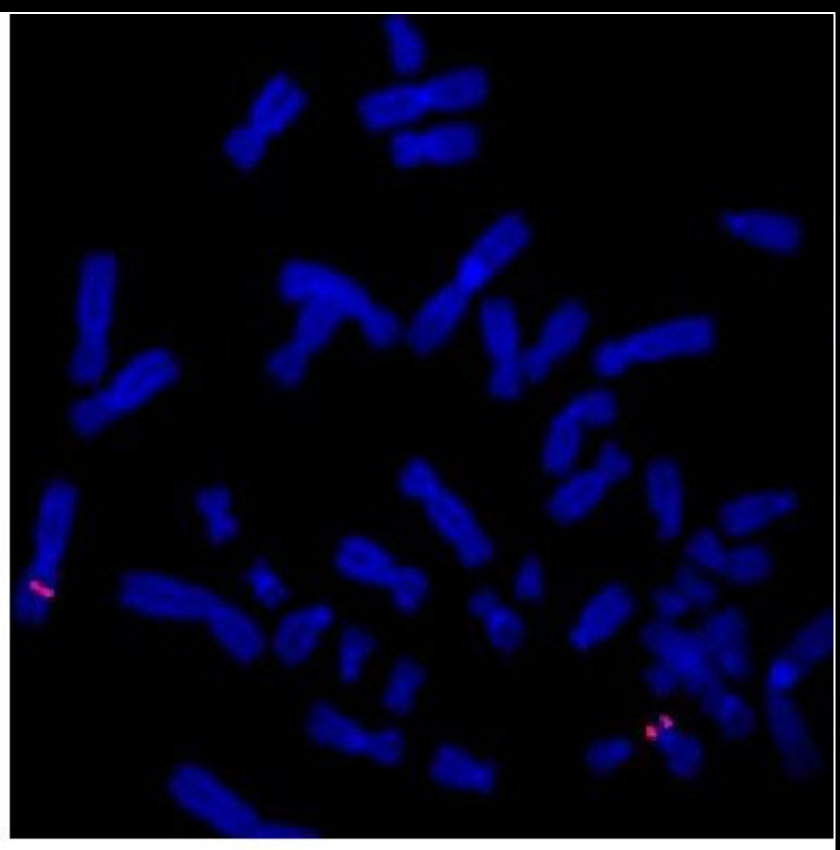
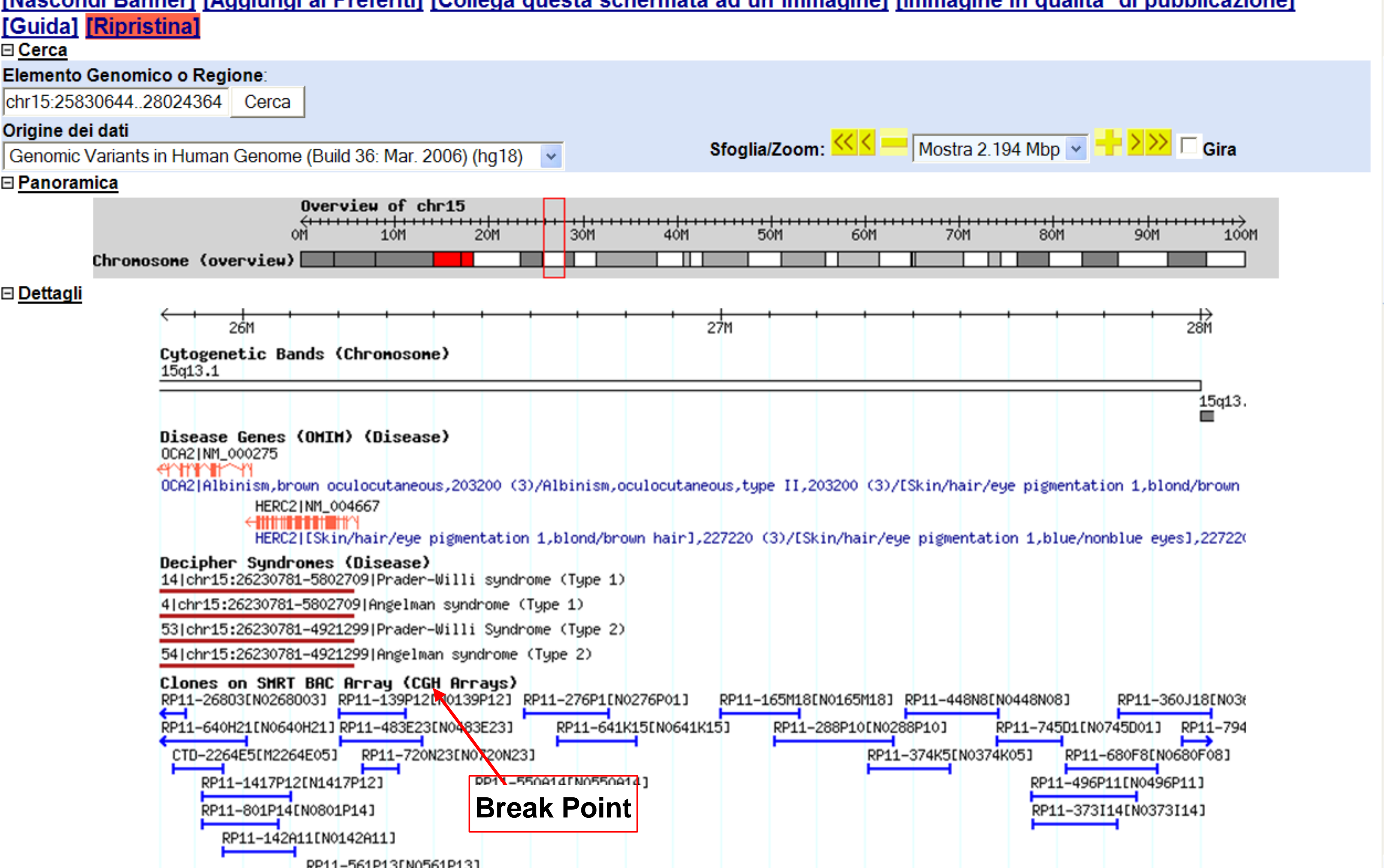


RP11-78N21 (17p13.1)



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Cerca
Elemento Genomico o Regione:
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Cerca
Origine dei dati
Genomic Variants in Human Genome (Build 36: Mar. 2006) (hg18)
Sfoglia/Zoom:
Mostra 2.194 Mbp
Gira
Panoramica
Overview of chr15
Chromosome (overview)
Dettagli
Cytogenetic Bands (Chromosome)
15q13.1
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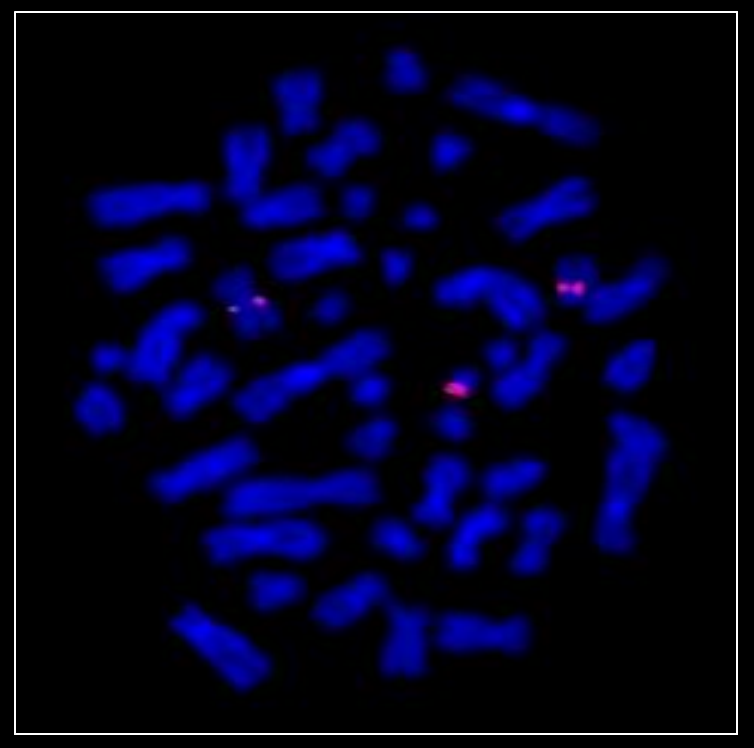
OCA2|Albinism,brown oculocutaneous,203200 (3)/Albinism,oculocutaneous,type II,203200 (3)/[Skin/hair/eye pigmentation 1,blond/brown
HERC2|NM_004667
HERC2|[Skin/hair/eye pigmentation 1,blond/brown hair],227220 (3)/[Skin/hair/eye pigmentation 1,blue/nonblue eyes],22722
Decipher Syndromes (Disease)
14|chr15:26230781-5802709|Prader-Willi syndrome (Type 1)
4|chr15:26230781-5802709|Angelman syndrome (Type 1)
53|chr15:26230781-4921299|Prader-Willi Syndrome (Type 2)
54|chr15:26230781-4921299|Angelman syndrome (Type 2)
Clones on SMRT BAC Array (CGH Arrays)
Break Point



RP11-483E23 (15q13.1)

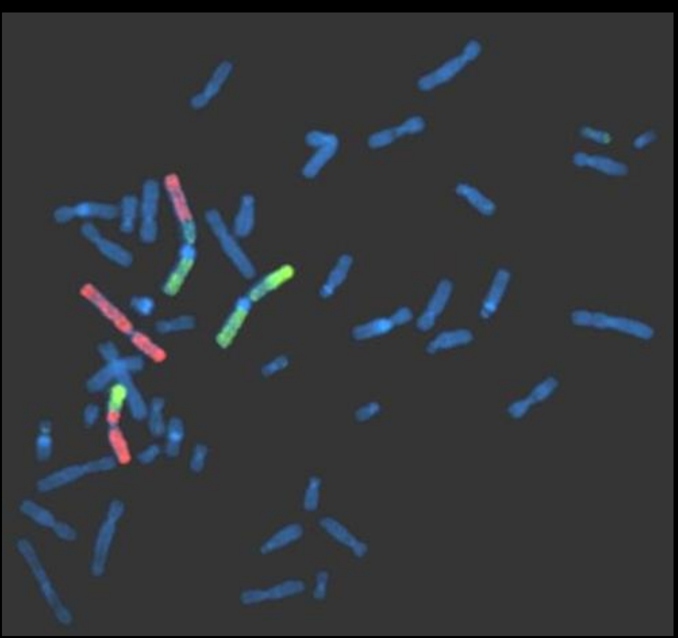
IPOTESI:

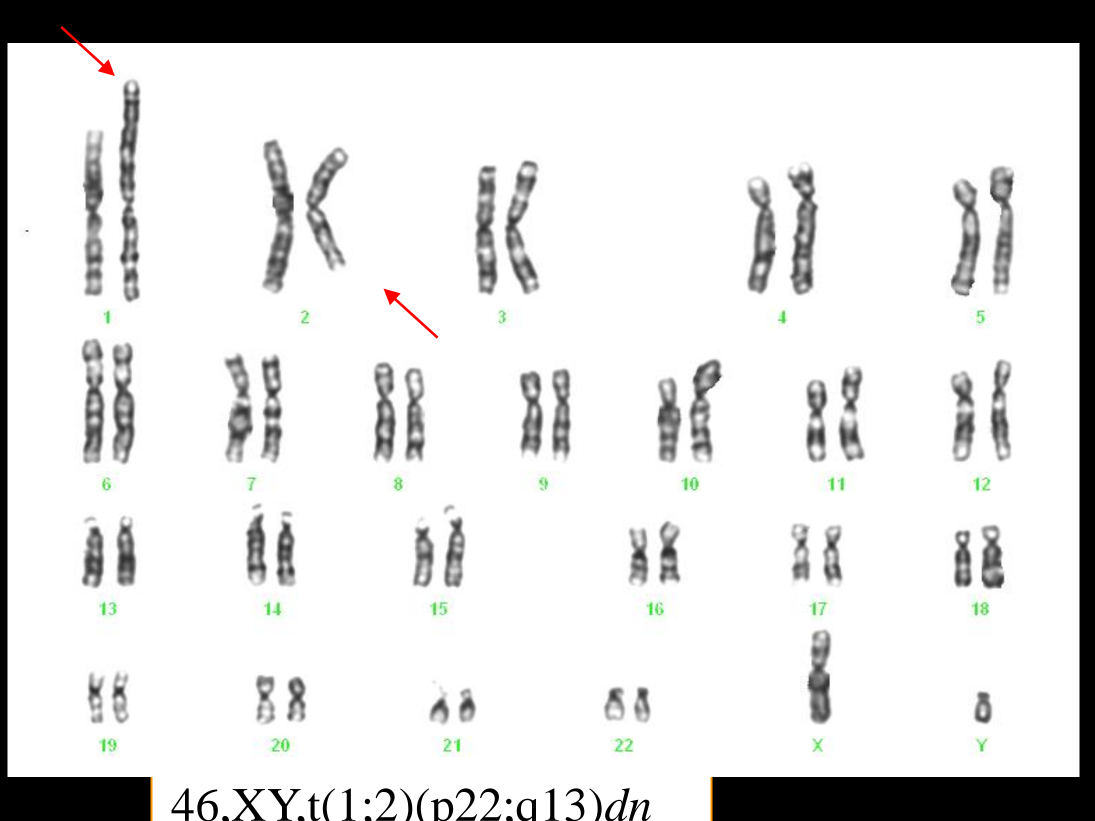
1) Interruzione genica in corrispondenza di uno dei due punti di rottura della traslocazione

2) Effetto di posizione di geni localizzati nelle vicinanze del punto di rottura e traslocati in un locus non proprio

individuare anomalie cromosomiche criptiche non evidenziabili con le tecniche di citogenetica standard: utilizzo aCGH

- liquido amniotico
- cariotipo parentale normale

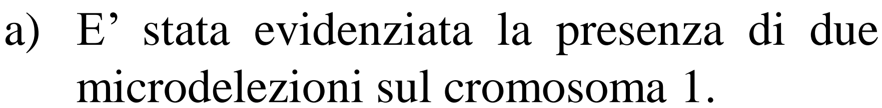




46,XY,t(1;2)(p22;q13)*dn*

- rischio empirico 6% di malformazioni congenite
- alla nascita: ritardo di crescita, dismorfismi

individuare anomalie cromosomiche criptiche non evidenziabili con le tecniche di citogenetica standard: utilizzo aCGH

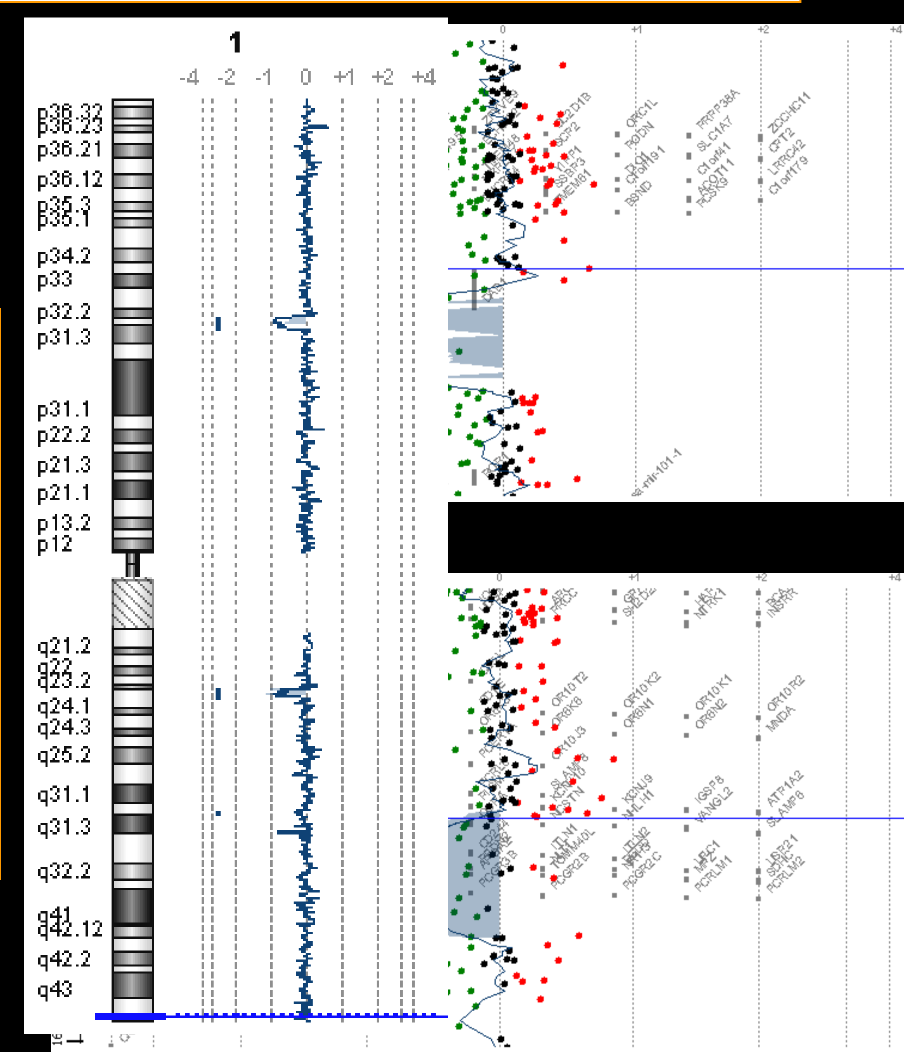


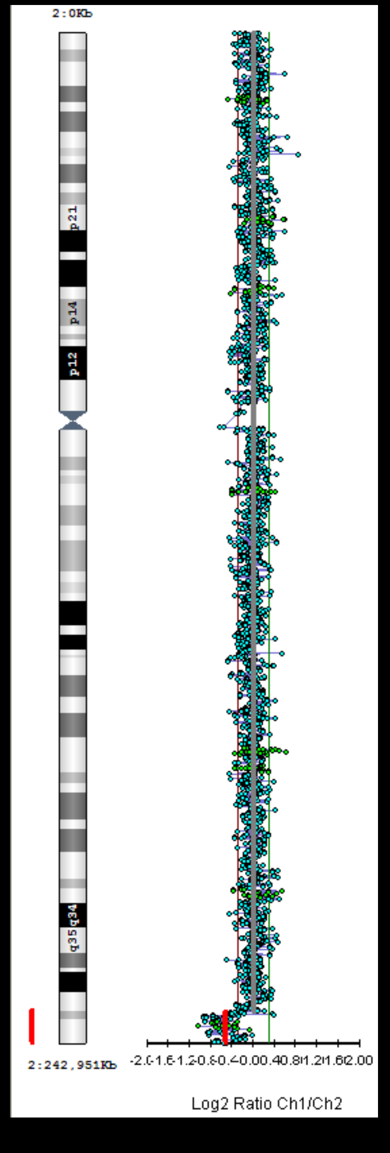
a) E’ stata evidenziata la presenza di due microdelezioni sul cromosoma 1.

b) parziale della regione 1p32p31.3(~3 Mb)

c) parziale della regione 1q23.3(~1,9 Mb)

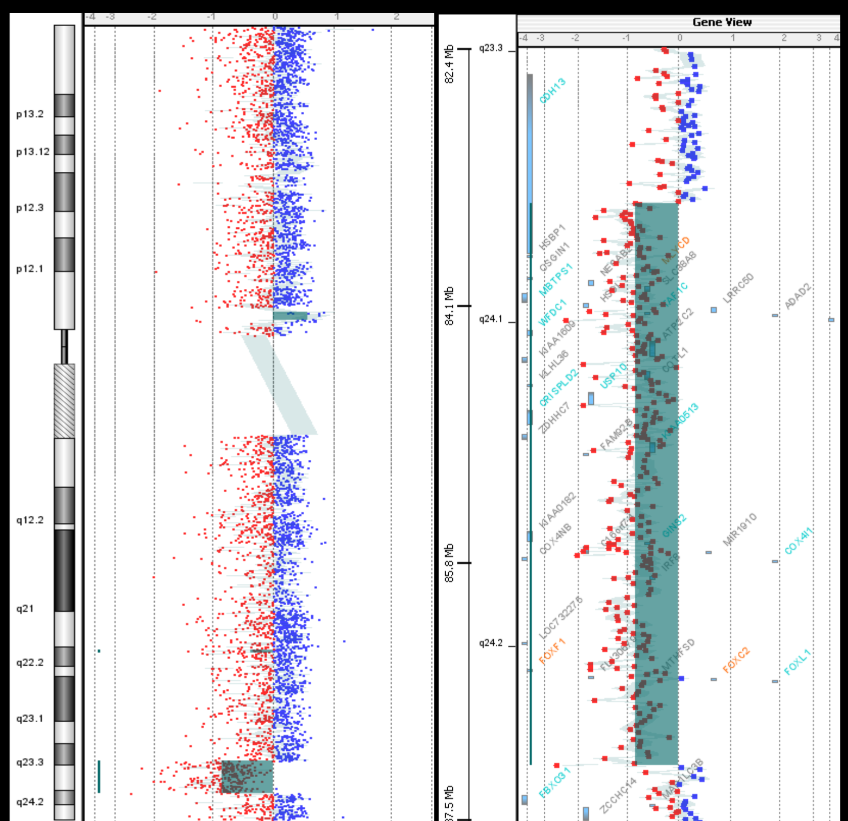
.





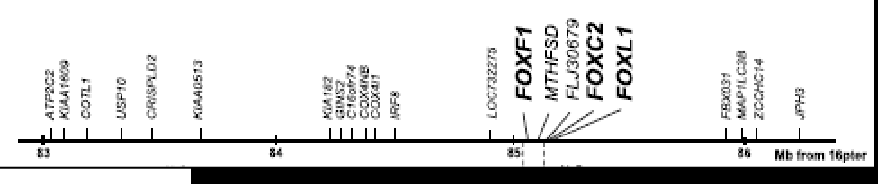
Oligo 4x44K	del 2q37.1-q37.3	dn	7.6 Mb	235164577.5	242717042.5

arr 16q23.3q24.2(82,061,060-85,725,948)x1



All'ecografia: NT >95 percentile, osso nasale assente, sindrome del cuore sinistro ipoplasico, distensione anse intestinali, lieve pielectasia monolaterale

La delezione include i geni *FOXF1* E *FOXC2*



ARTICLE

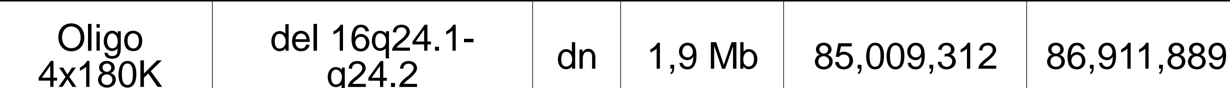
Genomic and Genic Deletions of the FOX Gene Cluster on 16q24.1 and Inactivating Mutations of *FOXF1* Cause Alveolar Capillary Dysplasia and Other Malformations

Paweł Stankiewicz,[1,2,15,*] Partha Sen,[3,15] Samarth S. Bhatt,[1] Mekayla Storer,[4,5] Zhilian Xia,[1] Bassem A. Bejjani,[6] Zhishuo Ou,[1] Joanna Wiszniewska,[1] Daniel J. Driscoll,[7] Juan Bolivar,[8] Mislen Bauer,[9] Elaine H. Zackai,[10] Donna McDonald-McGinn,[10] Małgorzata M.J. Nowaczyk,[11] Mitzi Murray,[12] Tamim H. Shaikh,[10] Vicki Martin,[4,5] Matthew Tyreman,[13] Ingrid Simonic,[13] Lionel Willatt,[13] Joan Paterson,[13] Sarju Mehta,[13] Diana Rajan,[5] Tomas Fitzgerald,[5] Susan Gribble,[5] Elena Prigmore,[5] Ankita Patel,[1] Lisa G. Shaffer,[6] Nigel P. Carter,[5] Sau Wai Cheung,[1] Claire Langston,[14] and Charles Shaw-Smith[4,5]

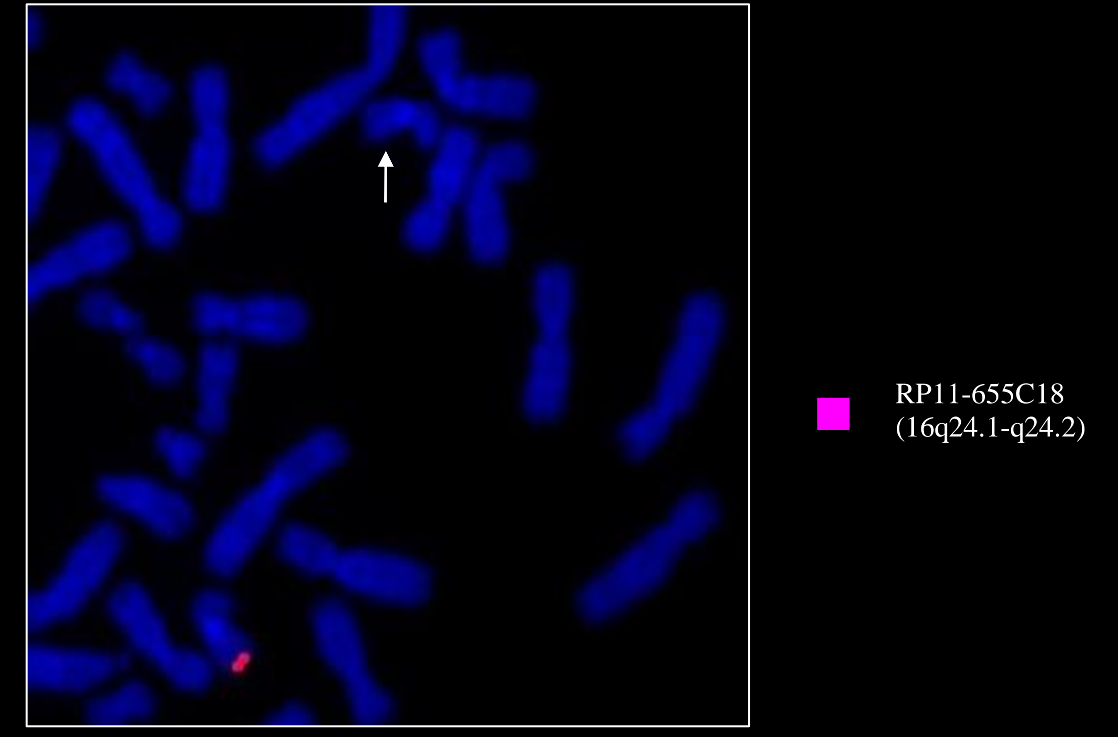
Sindrome del cuore sinistro ipoplasico, atresia gastrointestinale e malformazioni del tratto urinario

19 S.G. Amnio: 46,XY

Ecografia:igroma cistico,onfalocele,idronefrosi

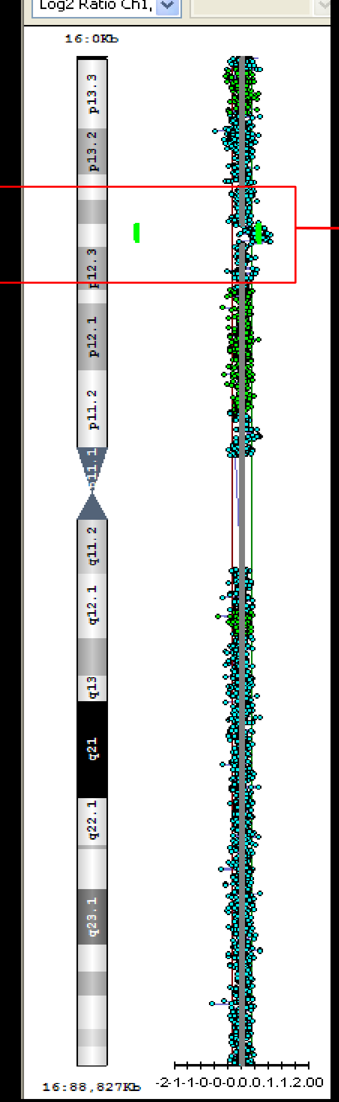


Oligo 4x180K	del 16q24.1-q24.2	dn	1,9 Mb	85,009,312	86,911,889



Gene FOXC2; *Yu et al 2010 idronefrosi congenita*

ALTERAZIONE PATOLOGICA MA NON ASSOCIATO AL FENOTIPO				
RIS	**ORIGINE**	**Mb**	**START**	**END**
dup 16p13.12-p13.11	pat	1,5	14,687,665	16,218,541
dup 15q13.2-q13.3	mat	1,7	28,801,829	30,494,145



Locus di suscettibilità all'autismo e r.m.

Fenotipo variabile con penetranza incompleta:

21 SG: 46,XX

Ecografia: cardiopatia congenita complessa tipo troncoconale

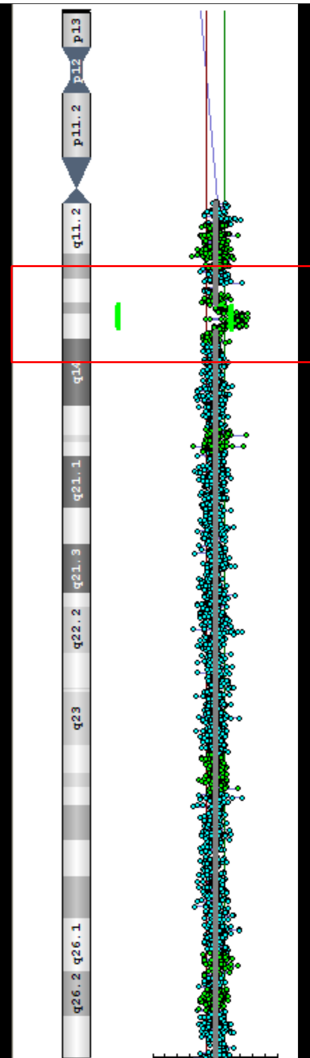
Consulenza: ? Variante normale o locus suscettibilita'?

Gravidanza interrotta per malformazione fetale

Eccesso di info

50% di trasmissione prossima gravidanza ?

ALTERAZIONE PATOLOGICA MA NON ASSOCIATO AL FENOTIPO				
RIS	**ORIGINE**	**Mb**	**START**	**END**
dup 16p13.12-p13.11	pat	1,5	14,687,665	16,218,541
dup 15q13.2-q13.3	mat	1,7	28,801,829	30,494,145

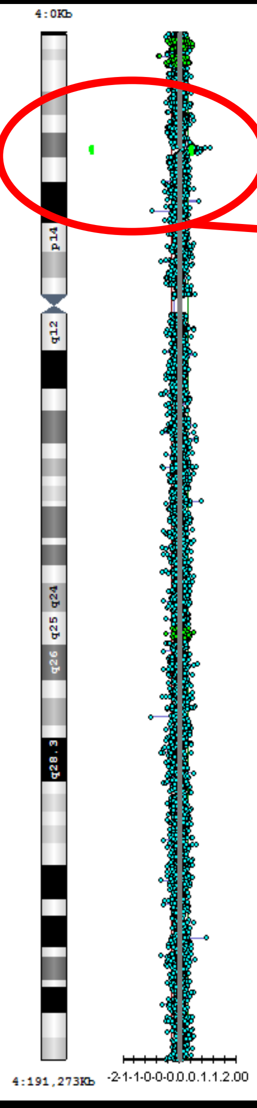


Locus di sucettibilità
alla schizofrenia

Fenotipo variabile con penetranza incompleta:

20 SG: 46,XY
Ecografia: NT alterata
Consulenza: ? Variante normale o locus suscettibilita'?

Informazione non richiesta sulla madre cosa comporta?
Insorgeneza tardiva



L'analisi ha evidenziato due riarrangiamenti:

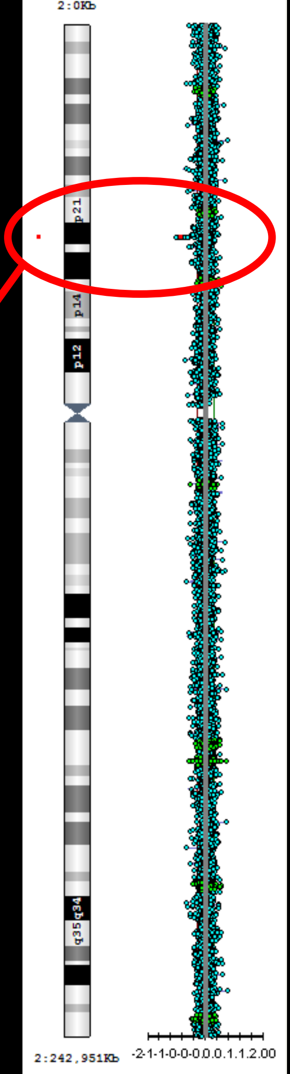
Una microduplicazione di 1,2Mb in posizione 4p15.31 di origine paterna

+

Una microdelezione di 86,8Kb su un cromosoma 2 in posizione 2p16.3 di origine materna

=

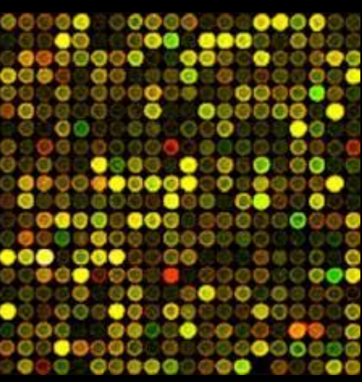
?



Array-CGH

Vantaggi

- Non ha bisogno di coltura cellulare
- Capacità di analizzare l'intero genoma in un esperimento
- Elevata specificità, sensibilità e risoluzione
- Rapidità



Svantaggi

- Incapacità di rilevare riarrangiamenti bilanciati e poliploidie
- Limitata abilità di individuare mosaicismi.
- Presenza di polimorfismi del numero di copie (CNV), di difficile interpretazione

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The future of prenatal diagnosis: karyotype, microarray or both? Technical and ethical considerations

Expert Rev. Proteomics 10(2), 131–134 (2013)

Antonio Novelli*[1], Pietro Cavalli[2] and Laura Bernardini[1]

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[2]Azienda Istituti Ospitalieri – Medical Genetics, Cremona, Italy
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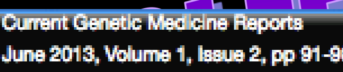
Fax: +39 6 4416 0548
a.novelli@css-mendel.it

Evaluation of: Wapner RJ, Martin CL, Levy B *et al.* Chromosomal microarray versus karyotyping for prenatal diagnosis. *N. Engl. J. Med.* 367(23), 2175–2184 (2012).

Prenatal diagnosis is now offered to high-risk pregnancies, including advanced maternal age, ultrasound anomalies and positive Down's syndrome screening, and karyotype on cultured fetal material is the test of choice to screen these pregnancies. However, microscope analysis can only detect gross chromosome abnormalities, highlighting the need for more sensitive techniques. It has recently been established that the higher resolution of microarray-based platforms can increase the diagnostic yield, offering more information to couples, and it is being discussed as a replacement to the standard karyotype. Conversely, the very high sensitivity of microarray-based analysis allows us to detect small microdeletions/microduplications (copy number variations) with unknown functional role and difficult genotype/phenotype correlation. In addition, the new copy number variation syndromes are often associated with variable outcomes, ranging from normal to severely affected individuals. This means that the microarray-based analysis introduced routinely in prenatal diagnosis needs to answer the question: are laboratory staff, clinical geneticists and counselors really experienced enough to manage these new scenarios?

Keywords: copy number variations • genetic test • karyotype • microarray-based analysis • prenatal diagnosis • uncertain clinical significance

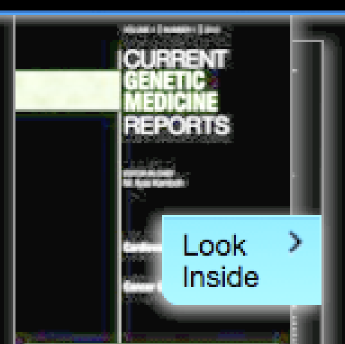
DIAG



Current Genetic Medicine Reports
June 2013, Volume 1, Issue 2, pp 91-98

The Future of Prenatal Cytogenetics: From Copy Number Variations to Non-invasive Prenatal Testing

Paul Brady, Simon Ardui, Joris Robert Vermeesch



BREAKING NEWS

Abstract

The conventional methods of prenatal diagnosis are being challenged by recent technologies. Chromosomal microarrays already in mainstream use for postnatal genetic diagnosis are increasingly used for prenatal diagnosis, mainly in pregnancies with sonographic anomalies but also for routine screening after any invasive procedure. Arrays have demonstrated the ability to detect submicroscopic copy number variations, providing an approximately 2.1 % increase in the detection rate of pathogenic copy number variations regardless of the referral indication, and rising to an approximately 5.3 % increase above conventional karyotyping in the presence of sonographic anomalies. More recently, novel technologies and methods of non-invasive prenatal testing are reaching clinical applications beyond fetal sex determination and rhesus blood group genotyping. Massively parallel sequencing methods have been shown to confidently detect trisomy 21 from cell-free DNA isolated from a maternal plasma sample and are rapidly entering clinical use. Targeted methods including epigenetic differences between the fetal and maternal genomes such as differential methylation are also being applied for non-invasive aneuploidy detection. It can be anticipated that very soon chromosomal microarrays will become the first-tier test for invasive prenatal diagnosis. In addition, we believe that non-invasive prenatal testing will gradually replace the need for invasive prenatal diagnosis with the associated risk of pregnancy loss.

Within this Article

- Introduction
- Chromosomal Microarrays
- Non-invasive Prenatal Testing
- Conclusion
- References
- References

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Review

Trends in Genetics February 2013, Vol. 29, No. 2

Recent advances in the prenatal interrogation of the human fetal genome

Lisa Hui[1,2] and Diana W. Bianchi[1]

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[2] Discipline of Obstetrics, Gynecology and Neonatology, Faculty of Medicine, University of Sydney, Sydney, NSW 2006, Australia

The amount of genetic and genomic information obtainable from the human fetus during pregnancy is accelerating at an unprecedented rate. Two themes have dominated recent technological advances in prenatal diagnosis: interrogation of the fetal genome in increasingly high resolution and the development of non-invasive methods of fetal testing using cell-free DNA in maternal plasma. These two areas of advancement have now converged with several recent reports of non-invasive assessment of the entire fetal genome from maternal blood. However, technological progress is outpacing the ability of the healthcare providers and patients to incorporate these new tests into existing clinical care, and further complicates many of the economic and ethical dilemmas in prenatal diagnosis. This review summarizes recent work in this field and discusses the integration of these new technologies into the clinic and society.

A new era of prenatal genomic diagnosis: deeper, faster, and risk free

was shortly followed by the first prenatal diagnosis of trisomy 21 (Down syndrome) in 1968 [3]. For both clinical and technical reasons, prenatal diagnosis has historically focused on fetal chromosome abnormalities, which are an important cause of perinatal morbidity and mortality, and are relatively easily detected from cultured fetal cells using standard cytogenetic techniques. Diagnosis during early pregnancy gives women the choice of terminating an affected pregnancy, or continuing to birth with better preparation for the postnatal needs of the child.

Classical cytogenetics (i.e., manual microscopic examination of banded metaphase chromosomes) is now rapidly being superseded as the gold standard of chromosomal assessment. A 2010 international consensus statement recommended that chromosome microarrays (CMAs) replace metaphase karyotyping as the first-tier test for children with unexplained multiple congenital anomalies or developmental delay [4]. CMAs detect genomic gains and losses by hybridizing fluorescently labeled sample DNA onto targets with known genomic coordinates that are fixed

The European Society of Human Genetics concluded that arrays were of proven value for investigation of fetal abnormalities and encouraged the establishment of local guidelines for the use of genome-wide array analysis in the prenatal setting [9]. They also recommended that pretest counseling, including written information and parental consent, were essential components of such a diagnostic service. In its 2012 position statement, the Italian Society of Human Genetics recommended that CMAs only be used for specific diagnostic purposes in selected pregnancies and 'never' as a substitute for conventional karyotyping [10]. This is in line with the recommendations from the American College of Obstetricians and Gynecologists, which has not yet altered its 2009 recommendation that conventional karyotyping remain the principal cytogenetic tool in prenatal diagnosis [11].

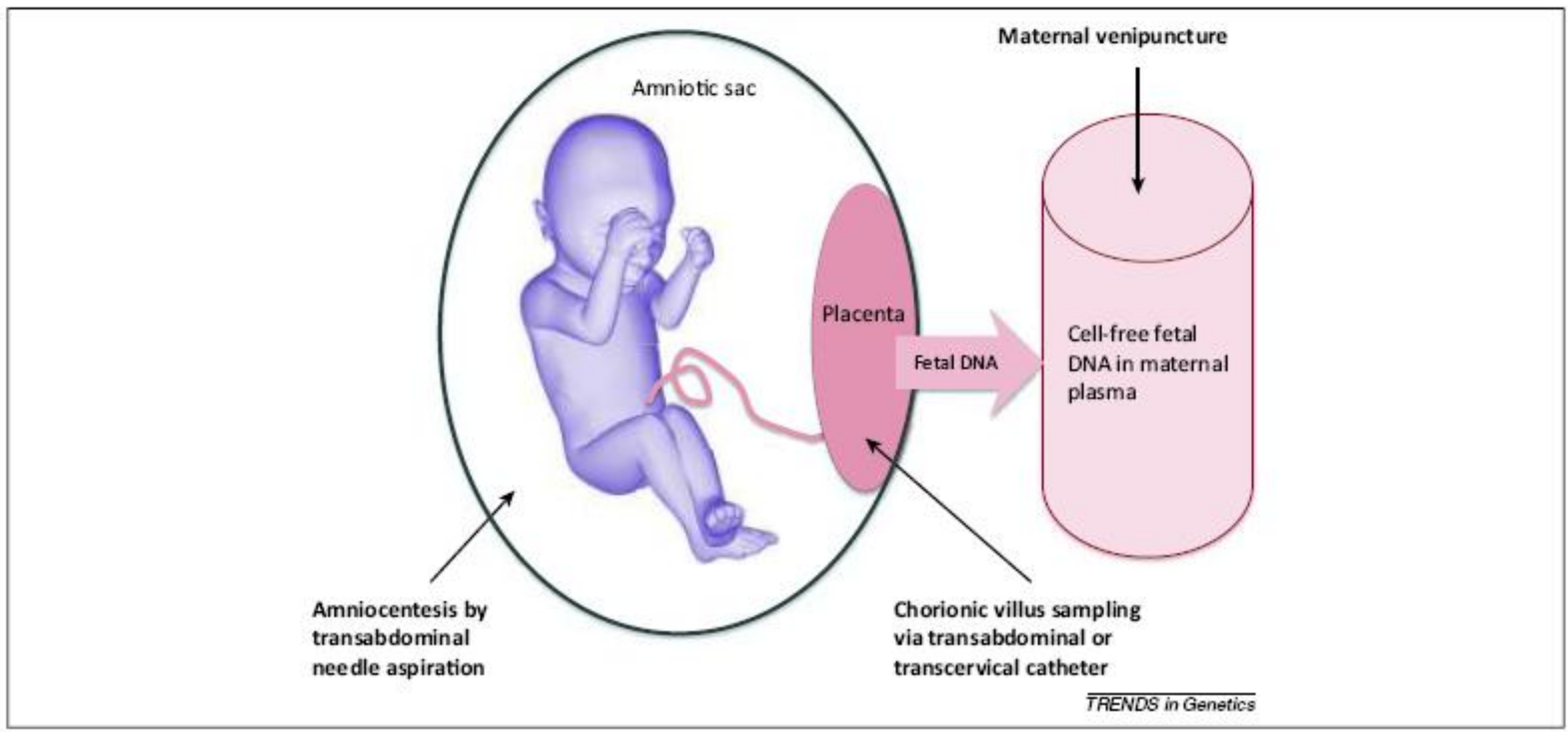


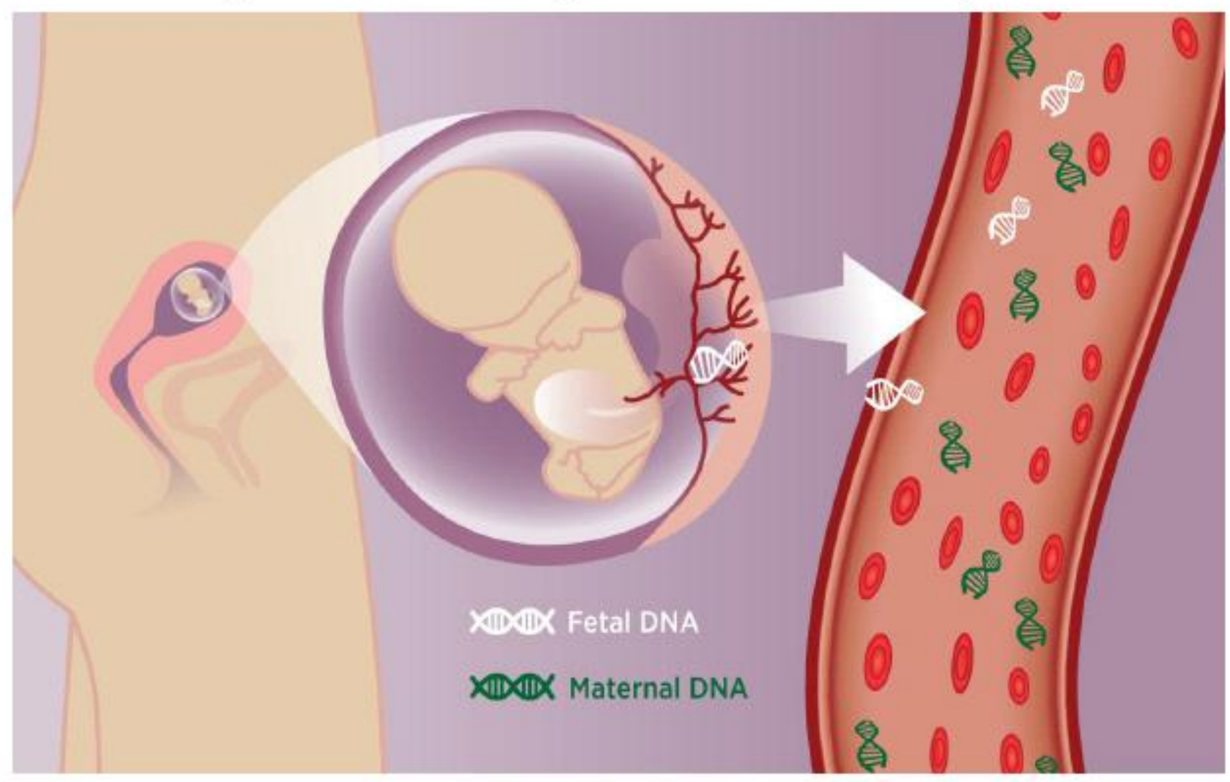
Figure 1. Sampling methods for genetic assessment of the fetus. Invasive methods carrying a risk of miscarriage include amniocentesis and chorionic villus sampling. Sampling of cell-free fetal DNA in maternal plasma is non-invasive and risk free to the fetus.

NEXT-GENERATION SEQUENCING (NGS)

È possibile leggere l'intero patrimonio genetico di un individuo in un esperimento

DNA Libero nel Sangue Materno

- Il DNA libero (cfDNA) é costituito da piccoli frammenti di DNA
- In gravidanza, il cfDNA sia della mamma che del feto é presente nel sangue materno
- La quantità di cfDNA fetale presente é una piccola frazione rispetto al cfDNA materno



WHAT ARE THE CURRENT LIMITATIONS OF NIPS?

1. Risk assessment is limited to specific fetal aneuploidies (trisomy 13, 18, and 21) at this time. Some platforms also screen for sex chromosome abnormalities. Approximately 50% of cytogenetic abnormalities routinely identified by amniocentesis will not be detected when trisomy 21, 18, and 13 are the only aneuploidies being screened. When patients <35 years or >35 years are considered separately, 75 and 43% of cytogenetic abnormalities will be missed, respectively.[11,12]
2. Chromosomal abnormalities such as unbalanced translocations, deletions, and duplications will not be detected by NIPS. Therefore, when fetal anomalies are detected, invasive diagnostic testing and cytogenomic microarray analysis are more likely to detect chromosomal imbalances than NIPS and may be a better testing option.[13]
3. NIPS is not able to distinguish specific forms of aneuploidy. For example, NIPS cannot determine if Down syndrome is due to the presence of an extra chromosome (trisomy 21), a Robertsonian translocation involving chromosome 21, or high-level mosaicism. Identification of the mechanism of aneuploidy is important for recurrence risk counseling and emphasizes the importance of diagnostic testing following NIPS.
4. NIPS does not screen for single-gene mutations.
5. Uninformative test results due to insufficient isolation of cell-free fetal DNA could lead to a delay in diagnosis or eliminate the availability of information for risk assessment. Biologic factors associated with reduced available cell-free fetal DNA include a high body mass index and early gestational age (<10 weeks gestation).[14,15]
6. Q5] Currently, it takes longer for NIPS test results to be returned than for test results on maternal serum analytes. Providers should keep this in mind when offering patients NIPS if timing is important for reproductive decision making. In most cases, NIPS is offered between 10 and 20 weeks gestation, which allows time for follow-up of positive test results. It is reasonable to offer NIPS after 20 weeks if an expectant women desires information regarding risk, reassurance, or knowledge in order to inform obstetrical management and/or preparation for birth.
7. NIPS does not screen for open neural tube defects. Maternal serum α-fetoprotein testing should still be offered at 15–20 weeks gestation to screen for open neural tube defects even when NIPS is performed.[1]
8. NIPS does not replace the utility of a first-trimester ultrasound examination, which has been proven to be useful for accurate gestational dating, assessment of the nuchal translucency region to identify a fetus at increased risk for a chromosome abnormality, identification of twins and higher-order pregnancies, placental abnormalities, and congenital anomalies.[16–19]
9. Limited data are currently available on the use of NIPS in twins and higher-order pregnancies. Utilization in these clinical settings may depend on specific laboratory platforms, proprietary bioinformatics, and clinical validation studies.
10. NIPS has no role in predicting late-pregnancy complications.

SHOULD PRETEST OR POSTTEST GENETIC COUNSELING ABOUT ANEUPLOIDY SCREENING BE PERFORMED?

DOI: 10.1002/pd.4139

POSITION STATEMENT

Position statement from the Aneuploidy Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis†

Peter Benn[1]*, Antoni Borell[2], Rossa Chiu[3], Howard Cuckle[4], Lorraine Dugoff[5], Brigitte Faas[6], Susan Gross[7], Joann Johnson[8], Ron Maymon[9], Mary Norton[10], Anthony Odibo[11], Peter Schielen[12], Kevin Spencer[13], Tianhua Huang[14], Dave Wright[15] and Yuval Yaron[16]

[1]Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA
[2]Prenatal Diagnosis Unit, Institute of Gynecology, Obstetrics and Neonatology, Hospital Clinic, Maternitat Campus, University of Barcelona Medical School, Catalonia, Spain

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Best Ethical Practices for Commercial Test Providers

Companies offering non-invasive prenatal testing should:

1. Offer testing only through licensed clinicians and not directly to consumers.
2. Seek oversight to validate the safety and effectiveness of genetic tests from relevant regulatory agencies.
3. Do their best to comply with national and international regulations and laws regarding the results that can legally be returned to patients.
4. Implement proficiency testing procedures verified independently by a third party to ensure analytic validity. Set transparent standards for data interpretation and error rates.
5. Require verification of comprehensive informed consent from clinicians before testing is conducted. Companies may wish to provide clinicians with appropriate informed consent forms in order to facilitate this process.
6. Obtain written consent for the storage of samples and genetic data and any research conducted using samples or test results. Samples should not be used for research without explicit consent separate from consent obtained to use samples for clinical purposes, and samples destroyed after clinical testing unless specific consent for future use has been obtained. .
7. Provide the capacity to return selected results based on the wishes of the patient.
8. Provide genetic counseling resources to assist clinicians in facilitating the informed consent process .
9. Design marketing and advertising materials to promote values-based decision-making and avoid advocating for specific actions on the basis of test results.
10. Design intellectual property and licensing regimes to facilitate access to and enhance quality of prenatal testing. To maximize equality of access and care, data from tests should be available in the public domain.

Best Ethical Practices for Clinicians and Laboratories in the Provision of Non-Invasive Prenatal Testing

Word Count: 3,569
Figure Count: 2

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ACMG POLICY STATEMENT | Genetics inMedicine

ACMG statement on noninvasive prenatal screening for fetal aneuploidy

Anthony R. Gregg, MD[1], S.J. Gross[2], R.G. Best[3], K.G. Monaghan[4], K. Bajaj[2], B.G. Skotko[5],
[Q1] B.H. Thompson[6] and M.S. Watson[6]; The Noninvasive Prenatal Screening Work Group of the American
[Q2] College of Medical Genetics and Genomics

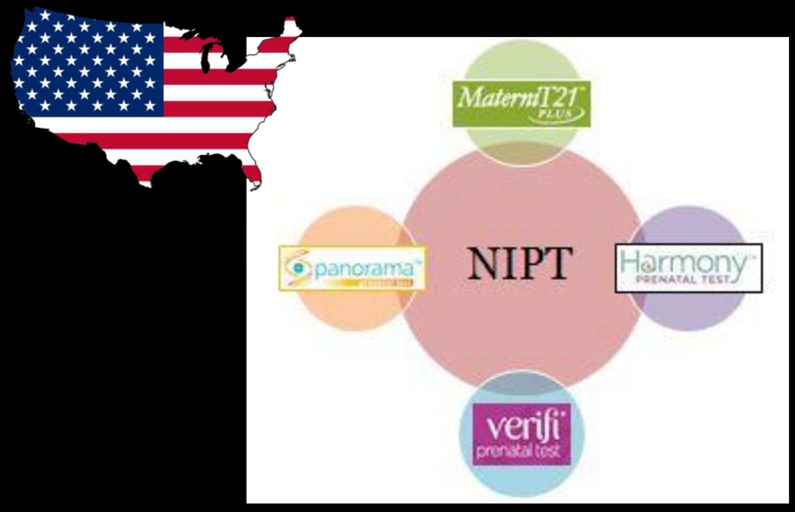
Disclaimer: ACMG position statements are developed primarily as educational resources for medical geneticists to help them provide quality clinical laboratory genetic services. Adherence to this statement is voluntary and does not necessarily assure a successful medical outcome. This position statement should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical laboratory geneticist should apply his or her own professional judgment to the specific circumstances presented by the individual patient or specimen. Clinical laboratory geneticists are encouraged to document in the patient's record the rationale for the use of a particular procedure or test, whether or not it is in conformance with this position statement. They also are advised to take notice of the date any particular positions statement was adopted and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

Noninvasive assessment of the fetal genome is now possible using next-generation sequencing technologies. The isolation of fetal DNA fragments from maternal circulation in sufficient quantity and sizes, together with proprietary bioinformatics tools, now allows patients [Q3] the option of noninvasive fetal aneuploidy screening. However, obstetric care providers must become familiar with the advantages and disadvantages of the utilization of this approach as analysis of results contain key elements and that laboratories adhere to established quality control and proficiency testing standards. The analysis of cell-free fetal DNA in maternal circulation for fetal aneuploidy screening is likely the first of major steps toward the eventual application of whole fetal genome/whole fetal exome sequencing.

Genet Med advance online publication 00 Month 2013

NIPS

Non Invasive Prenatal Screening



	Natera's *Panorama*	Verinata's *verifi*	Sequenom's *MaterniT21 PLUS*	Ariosa's *Harmony*
Trisomies tested	13, 18, 21	13, 18, 21, sex chromosomes	13, 18, 21, sex chromosomes	13, 18, 21
Monosomy tested	X	X	X	
Genetic testing method	Single nucleotide polymorphism	Massively parallel sequencing	Massively parallel sequencing	Chromosome-selective sequencing
Sensitivity	92-99%	87-99%	92%-99%	80-99%
Accuracy	100%	100%	>99%	>99%
Earliest gestational age	9 weeks	10 weeks	10 weeks	10 weeks
Price	$1,495	$1,500	$2,762	$795

OPEN ACCESS Freely available online

Noninvasive Prenatal Molecular Karyotyping from Maternal Plasma

Stephanie C. Y. Yu[1,2], Peiyong Jiang[1,2], Kwong W. Choy[3], Kwan Chee Allen Chan[1,2], Hye-Sung Won[4], Wing C. Leung[5], Elizabeth T. Lau[6], Mary H. Y. Tang[6], Tak Y. Leung[3], Yuk Ming Dennis Lo[1,2], Rossa W. K. Chiu[1,2*]

1 Centre for Research into Circulating Fetal Nucleic Acids, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China, **2** Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China, **3** Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China, **4** Department of Obstetrics and Gynecology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea, **5** Kwong Wah Hospital, Kowloon, Hong Kong SAR, China, **6** Tsan Yuk Hospital, Department of Obstetrics and Gynaecology, University of Hong Kong, Hong Kong SAR, Hong Kong

Abstract

Fetal DNA is present in the plasma of pregnant women. Massively parallel sequencing of maternal plasma DNA has been used to detect fetal trisomies 21, 18, 13 and selected sex chromosomal aneuploidies noninvasively. Case reports describing the detection of fetal microdeletions from maternal plasma using massively parallel sequencing have been reported. However, these previous reports were either polymorphism-dependent or used statistical analyses which were confined to one or a small number of selected parts of the genome. In this report, we reported a procedure for performing noninvasive prenatal karyotyping at 3 Mb resolution across the whole genome through the massively parallel sequencing of maternal plasma DNA. This method has been used to analyze the plasma obtained from 6 cases. In three cases, fetal microdeletions have been detected successfully from maternal plasma. In two cases, fetal microduplications have been detected successfully from maternal plasma. In the remaining case, the plasma DNA sequencing result was consistent with the pregnant mother being a carrier of a microduplication. Simulation analyses were performed for determining the number of plasma DNA molecules that would need to be sequenced and aligned for enhancing the diagnostic resolution of noninvasive prenatal karyotyping to 2 Mb and 1 Mb. In conclusion, noninvasive prenatal molecular karyotyping from maternal plasma by massively parallel sequencing is feasible and would enhance the diagnostic spectrum of noninvasive prenatal testing.

Citation: Yu SCY, Jiang P, Choy KW, Chan KCA, Won H-S, et al. (2013) Noninvasive Prenatal Molecular Karyotyping from Maternal Plasma. PLoS ONE 8(4): e60968. doi:10.1371/journal.pone.0060968

Editor: Noam Shomron, Tel Aviv University, Israel

Received January 3, 2013; **Accepted** March 5, 2013; **Published** April 17, 2013

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Funding: This work was supported by University Grants Committee of the Government of the Hong Kong Special Administrative Region, under the Areas of Excellence Scheme (AoE/M-04/06); Hong Kong Research Grants Council General Research Fund (CUHK 463710); Sponsored Research Agreement from Sequenom Inc. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: Stephanie C.Y. Yu, Peiyong Jiang, K.C. Allen Chan, Y.M. Dennis Lo and Rossa W.K. Chiu have filed a United States patent application 61/751,213 "Noninvasive prenatal molecular karyotyping from maternal plasma". Y.M. Dennis Lo and Rossa W.K. Chiu are consultants to, receive research support from, and hold equities in Sequenom. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: rossachiu@cuhk.edu.hk

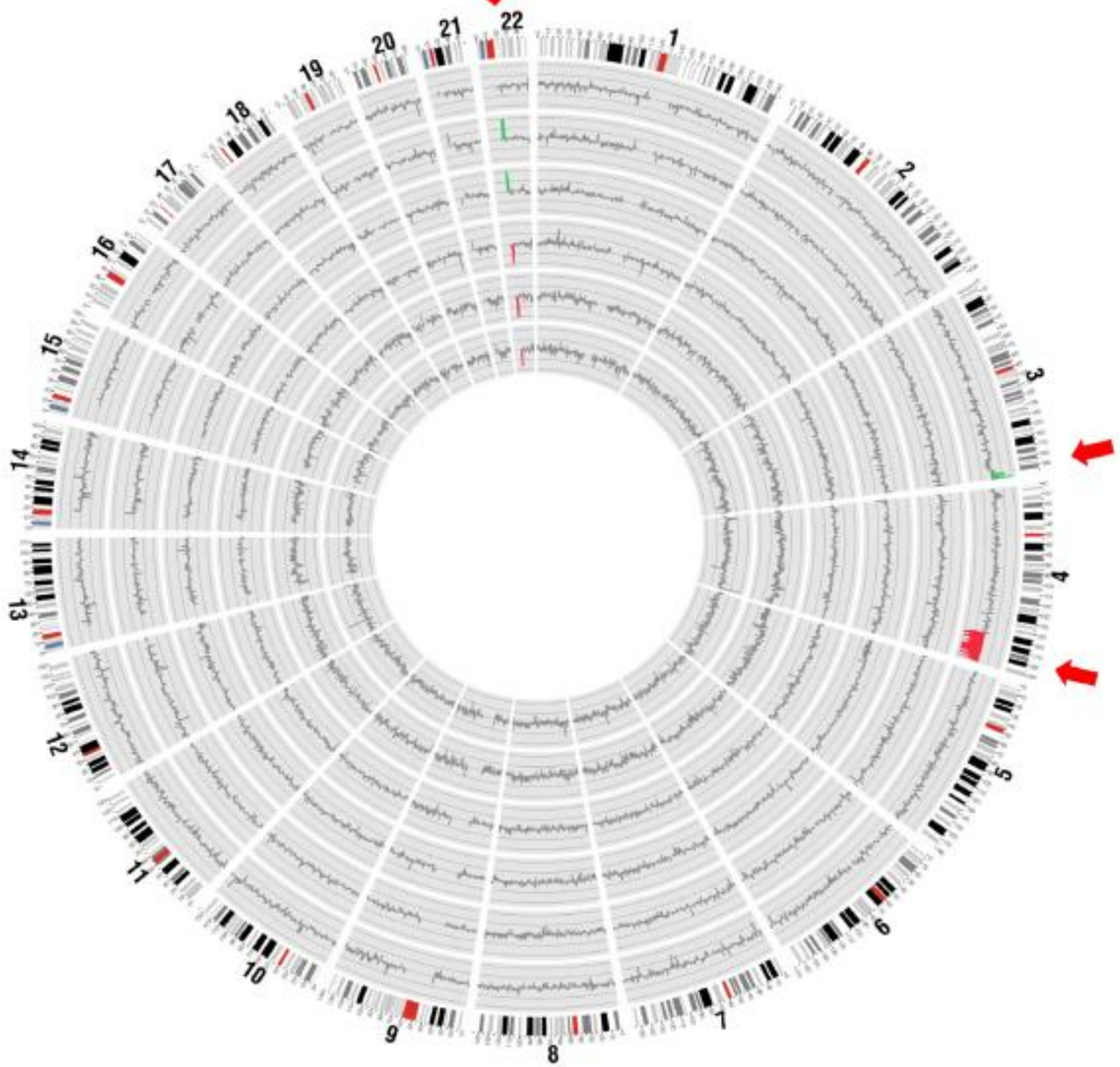
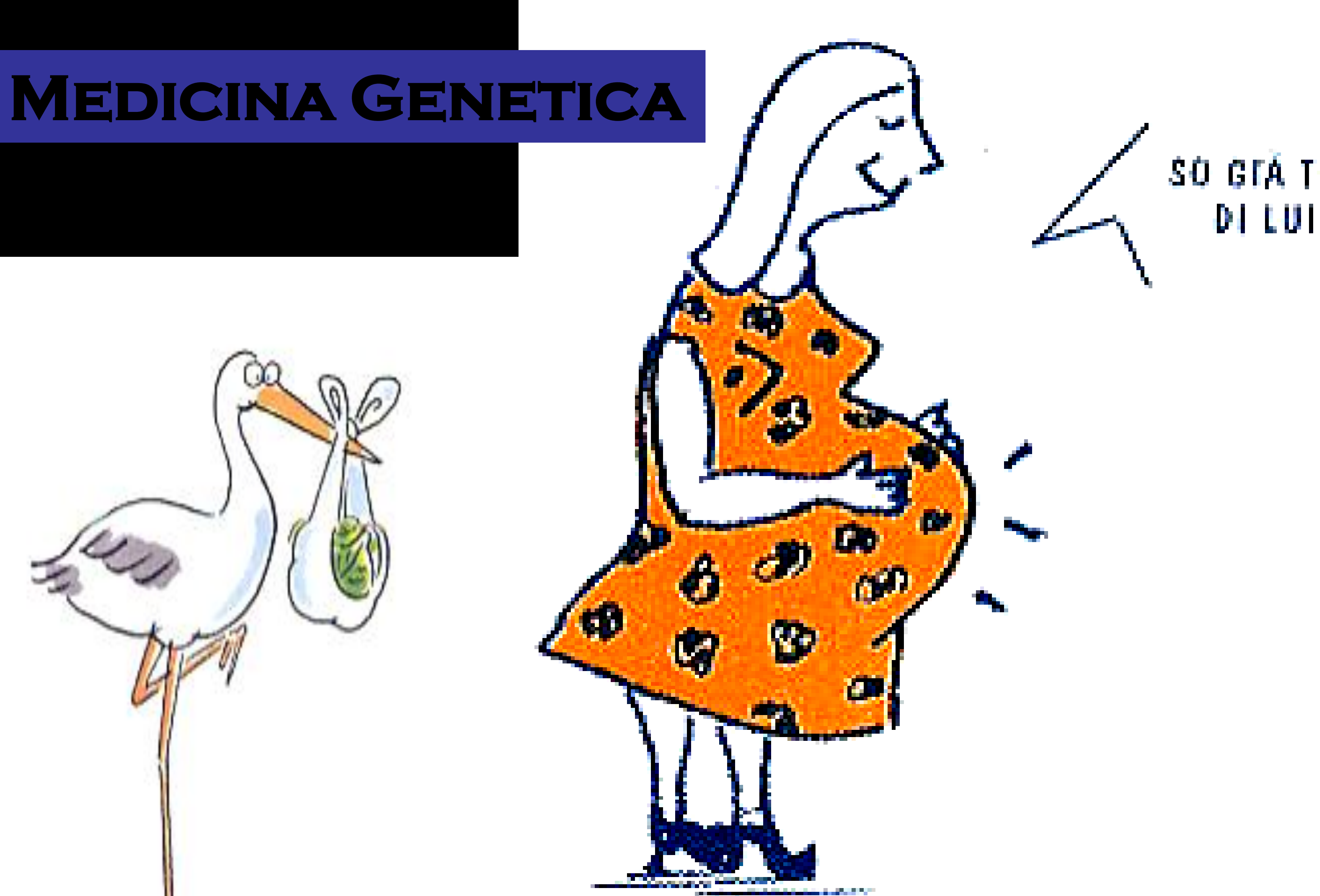


Figure 1. Circos plot of the detected copy number aberrations across the genome in maternal plasma. From inside to outside: cases 01 to 06. Chromosome ideograms (outermost ring) are oriented pter to qter in a clockwise direction. Each bar represents a 1-Mb window. Regions with three or more consecutive 1-Mb bins of increased or reduced representation in plasma are indicated by green and red bars, respectively. Red arrows highlight the approximate chromosomal locations on these aberrant regions.
doi:10.1371/journal.pone.0060968.g001

Lo scenario futuro

- Il genoma umano è stato in pratica decifrato
- Le nanotecnologie potranno consentire lo studio di decine di migliaia di geni
- La diagnosi prenatale potrà essere disponibile per tutte le malattie genetiche

Medicina Genetica



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Take Home Message

Genetics in Medicine:
May 2008 - Volume 10 - Issue 5 - pp 337-342
doi: 10.1097/GIM.0b013e31817283a5
Article

Molecular testing: improving patient care through partnering with laboratory genetic counselors

To maximize the quality of the service, it is important to establish an understanding of what can be expected of both the practitioner and the laboratory genetic counselor. Although some complications in the laboratory cannot be anticipated, discussing the case with the laboratory genetic counselors beforehand may avert certain problems. This article discusses real cases from laboratory genetic counselors to illustrate issues that arise due to technical difficulties and the inherent limitations of molecular testing. The summary describes practical ways in which clinicians and laboratory personnel can work together to either avoid or, when unavoidable, better manage problems and delays. The responsibilities of genetic counselors working in molecular diagnostics are discussed.

Un analisi genetica

deve essere

INTEGRATA in una

consulenza

genetica

e NON essere un

test di laboratorio

conclusioni

- Un test di laboratorio deve essere sottoposto ad una valutazione clinica prima di venire trasferito nella pratica.
- ogni test medico viene definito in base ad alcune caratteristiche, tra le quali sensibilità, specificità, valore predittivo positivo e negativo.-
- non esiste un test migliore: esiste il test appropriato per la specifica condizione clinica, discussa dal paziente e dal medico nell'ambito di ogni singola e specifica situazione.
- Nel caso dei test genetici la consulenza genetica deve essere inserita nel percorso diagnostico.