La Genetica: come interrogarla e cosa farsene

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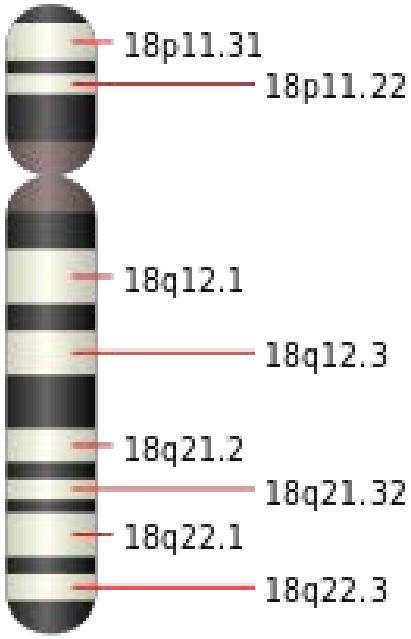
Cromosoma 18

- 76.117.153 paia di basi
- 337 geni
- 2.7% del genoma
- È il cromosoma a più bassa densità genica



Quali domande?

- Esiste IL test genetico?
- Cosa possiamo sapere dalla genetica per la DIAGNOSI?
- Cosa possiamo sapere dalla genetica per l’ASSISTENZA?

MUTAZIONE

Cambiamento nella costituzione genetica di una cellula

1. cellule somatiche non ereditabile
2. cellule germinali ereditabile

Può interessare

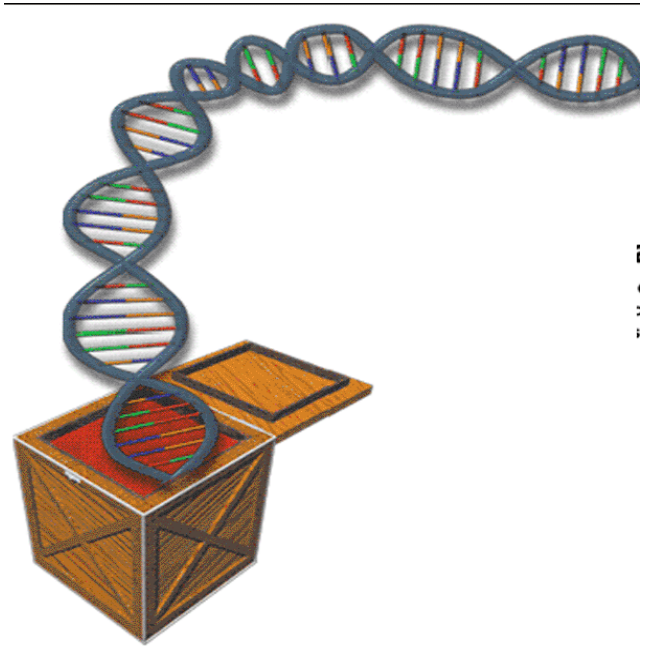
- genoma — **mutazioni genomiche**
- cromosoma — **mutazioni cromosomiche**
- gene — **mutazioni geniche**

COS'E' UN TEST GENETICO?

Analisi a scopo clinico di DNA, RNA, cromosomi, proteine, metaboliti, altri prodotti genici per evidenziare un genotipo, un fenotipo, un cariotipo correlato o meno con una patologia umana ereditabile.

Può essere:

- **DIAGNOSTICO**
- preclinico/presintomatico
- di suscettibilità
- per l'identificazione di eterozigoti
- a scopo medico-legale



Test genetico

Prevede che chi lo richiede abbia:

- conoscenza del risultato che può offrire (possibilità e limiti del test)
- consapevolezza delle ricadute del test su paziente/famiglia (consulenza genetica)

Ricadute di un test genetico

- Conferma o esclusione (parziale/completa) di un'ipotesi diagnostica

- Indicazione o meno all'allargamento dello studio genetico a livello di altri soggetti, spesso sani, all'interno del nucleo familiare per

  a) definire il loro eventuale rischio genetico

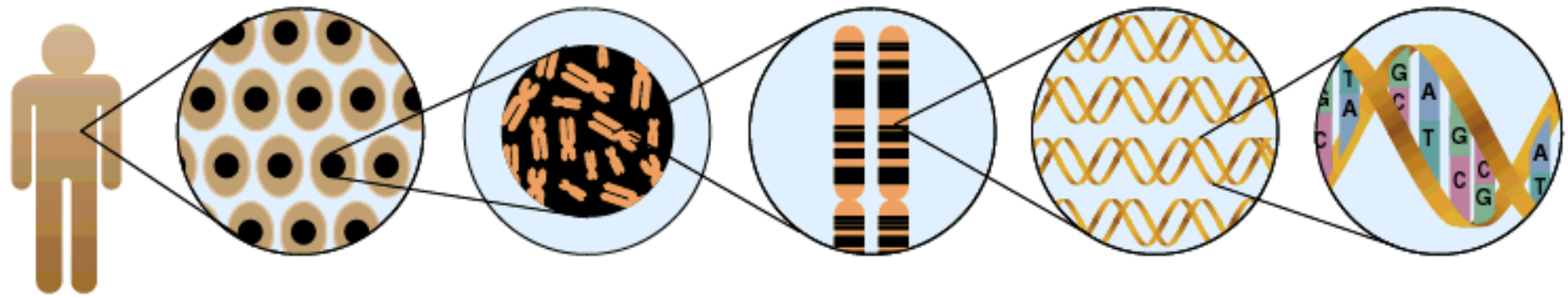
  b) interpretare correttamente il risultato del test genetico stesso sul paziente

INDAGINI DI LABORATORIO

Citogenetiche

Citogenetico-molecolari

Molecolari



INDAGINI DI LABORATORIO

- CARIOTIPO STANDARD/AD ALTA RISOLUZIONE
- ANALISI CITOGENETICO-MOLECOLARE (FISH)
- ARRAY-CGH
- ANALISI MOLECOLARE
- WES (ESOMA)

Anomalie cromosomiche

si distinguono in anomalie:

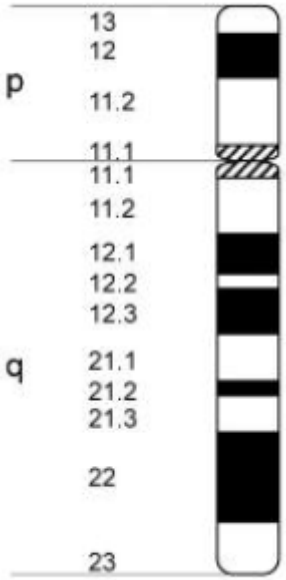
di numero (varia il numero totale dei cromosomi)

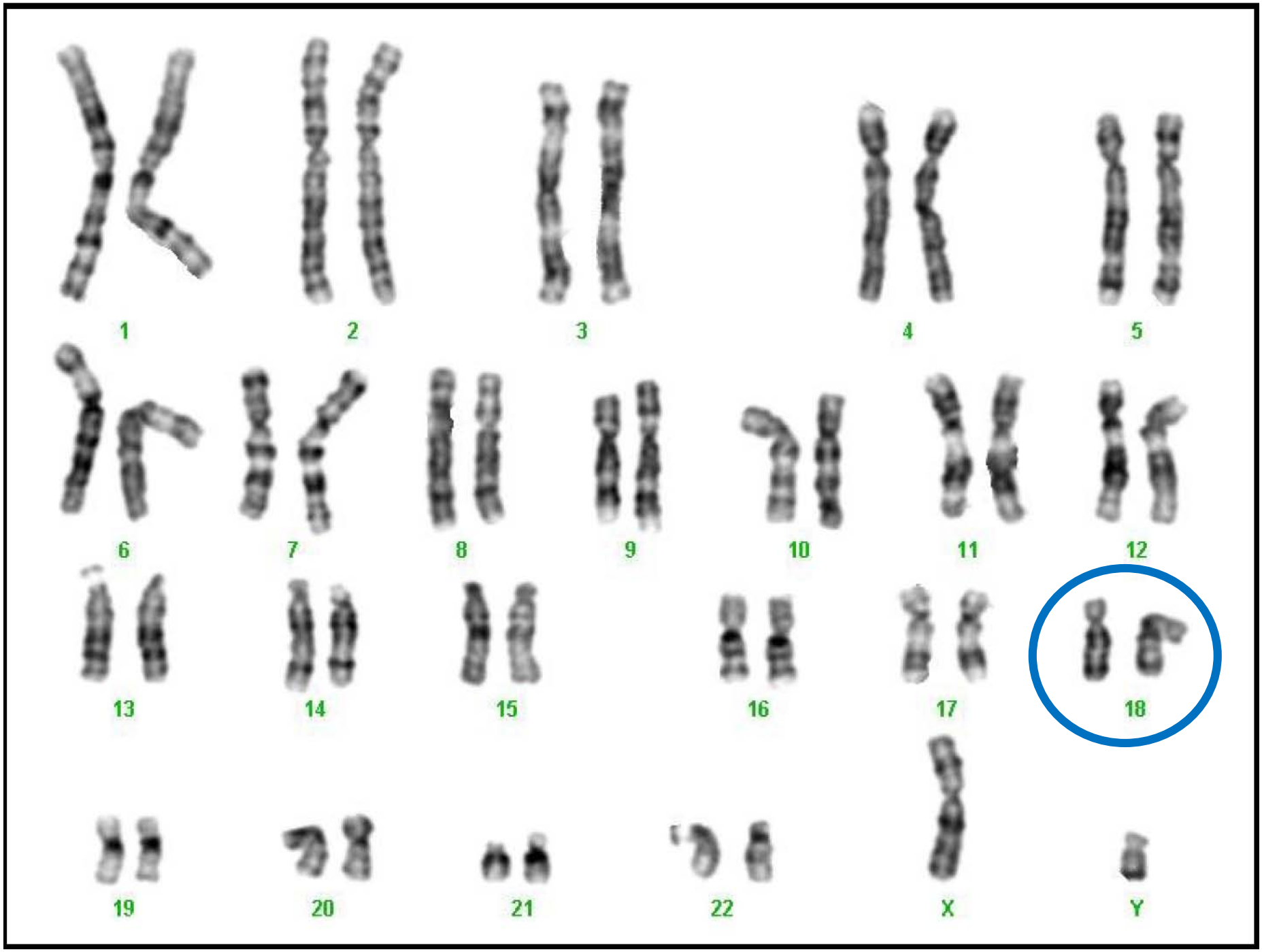
di struttura (varia la struttura del/i cromosoma/i)

Mutazioni cromosomiche di numero.....

Aneuploidie

Uno o pochi cromosomi
in più o in meno rispetto
all'assetto cromosomico normale

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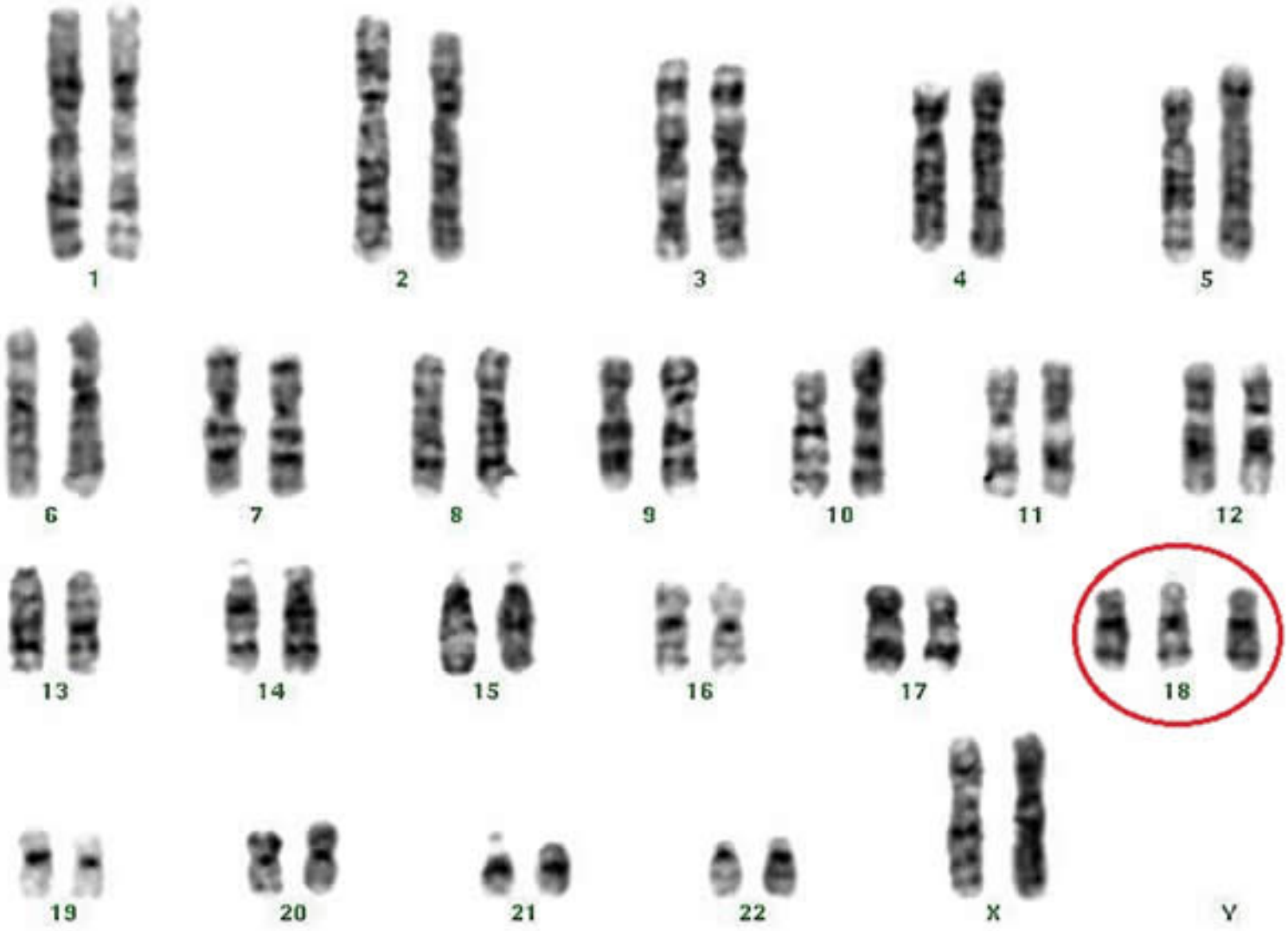
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Trisomia 18 (S.Edwards)

Incidenza 1:4-6000 (seconda trisomia più comune, dopo la S.Down)

- Ritardo di sviluppo grave
- Oloprosencefalia
- Malformazioni craniofacciali
- Malformazioni mani/piedi
- Malformazioni scheletriche
- Malformazioni renali
- CHD 90% (DIV, DIA, displasie valvolari)
- 50% sopravvivenza a 1 settimana, 10% arrivano ad un anno
- Rischio tumorale (tumore di Wilms, epatoblastoma)

REGIONE OCCIPITALE DEL CRANIO PROMINENTE
BOCCA PICCOLA, MICROGNATIA, COLLO CORTO
ORECCHIE DISPLASTICHE E MALFORMATE
PETTO CARENATO
DITO INDICE E MIGNOLO SOVRAPPOSTI, RISPETTIVAMENTE, A MEDIO E ANULARE
ALLUCE RIVOLTO VERSO L'ALTO E CALCAGNO PROMINENTE

An Analysis of Cardiac Defects and Surgical Interventions in 84 Cases with Full Trisomy 18

Am J Med Genet Part A 170A:337–343.

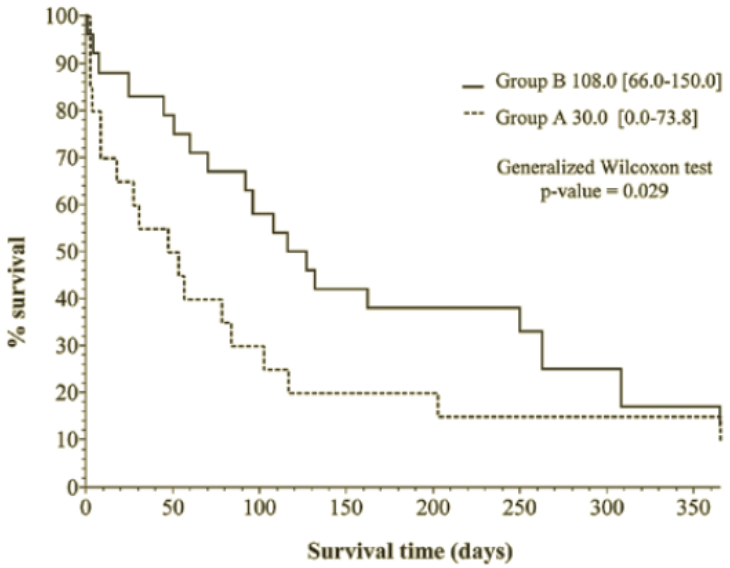
CONCLUSION

The data described here offer multiple points and largely improved outcomes from surgical intervention for cardiac conditions. Although efforts are needed to further delineate characteristics and interventions, it is hoped that these findings will encourage discussion and an increased willingness to consider cardiac surgery for cases with full trisomy 18.

Miglioramento sopravvivenza con intervento!!!

Clinical features and survival in individuals with trisomy 18: A retrospective one-center study of 44 patients who received intensive care treatments

MOLECULAR MEDICINE REPORTS 13: 2457-2466, 2016



Does Medical Intervention Affect Outcome in Infants with Trisomy 18 Or Trisomy 13?

Am J Med Genet Part A 170A:847–849.

While more investigation needs to be accomplished to help address this question including risk stratification of patients with trisomy 18 or trisomy 13 in view of surgical intervention, the body of work indicates that intervention does make a difference in terms of survival in infants with trisomy 13 or trisomy 18 and could be a reasonable option in discussion of care in a prenatal or postnatal session with parents.

JAMA. 2016;316(4):420-428.

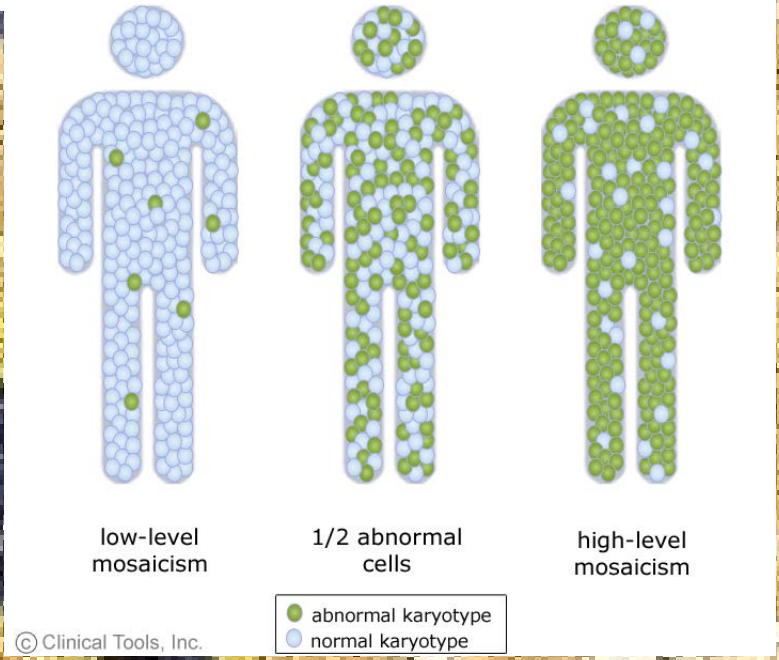
CONCLUSIONS AND RELEVANCE Among children born with trisomy 13 or 18 in Ontario, early mortality was the most common outcome, but 10% to 13% survived for 10 years. Among children who underwent surgical interventions, 1-year survival was high.

E se il fenotipo è più sfumato?

Mosaicismo

mos 46,XX/47,XX,+18

Coesistenza nello stesso individuo di popolazioni cellulari geneticamente distinte.



Nel sospetto di un mosaicismo cromosmico…

- Esplicitare il sospetto clinico affinchè il citogenetista valuti un numero adeguato di metafasi
- Considerare la ripetizione dell'esame se il numero di metafasi studiate è insufficiente ad escludere un mosaicismo a bassa quota
- Valutare se il tessuto studiato è quello più idoneo a confermare il nostro sospetto diagnostico

Mosaicismo per trisomia 18

- Presenza nello stesso individuo sia di una linea cellulare normale, sia di una linea con trisomia 18.
- Circa il 5% dei casi di trisomia 18 sono legati a mosaicismo
- Fenotipo estremamente variabile, da uguale a trisomia 18 completa a fenotipo normale
- QI da ID grave a normale

Assenza di correlazione tra la percentuale di cellule trisomiche nei leucociti e nei fibroblasti e reperti fisici e intellettivi

Trisomy 18 mosaicism: report of two cases

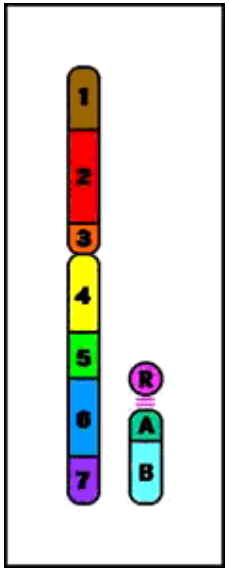
World J Pediatr 2013;9(2):179-181

Tucker et al[3] also showed that there is a significant discrepancy between the levels of trisomy in different tissues. This difference is highlighted by our first case, where 88% (44/50) of the patient's lymphocytes were trisomic for chromosome 18 whereas she had only 30% (15/50) trisomic skin fibroblasts. Routine karyotyping involves examination of only 10 lymphocytes unless mosaicism is suspected. Therefore some cases of mosaicism may be missed, resulting in misdiagnosis.

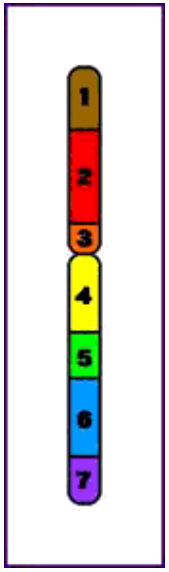
Our second patient suffered from significant feeding problems, hypertrophic cardiomyopathy, multiple cardiac anomalies and bilateral inguinal herniae. Despite these, his overall outcome has been relatively good in terms of his general health and development. This illustrates that the outlook for children with trisomy 18 mosaicism can be good even in the presence of multiple congenital malformations.

Il cariotipo su sangue può non essere sufficiente a dg mosaicismo!!!

Trisomia parziale 18



- Un segmento del cromosoma 18 è presente in quantità tripla (in particolare 18q)
- Spesso risulta da traslocazione bilanciata o inversione parentale
- Rende conto del 2% circa dei casi di trisomia 18.
- Fenotipo molto variabile e fortemente determinato dall'estensione del segmento triplicato e dalla possibile associazione di una delezione di altro materiale genomico, legata a una traslocazione sbilanciata



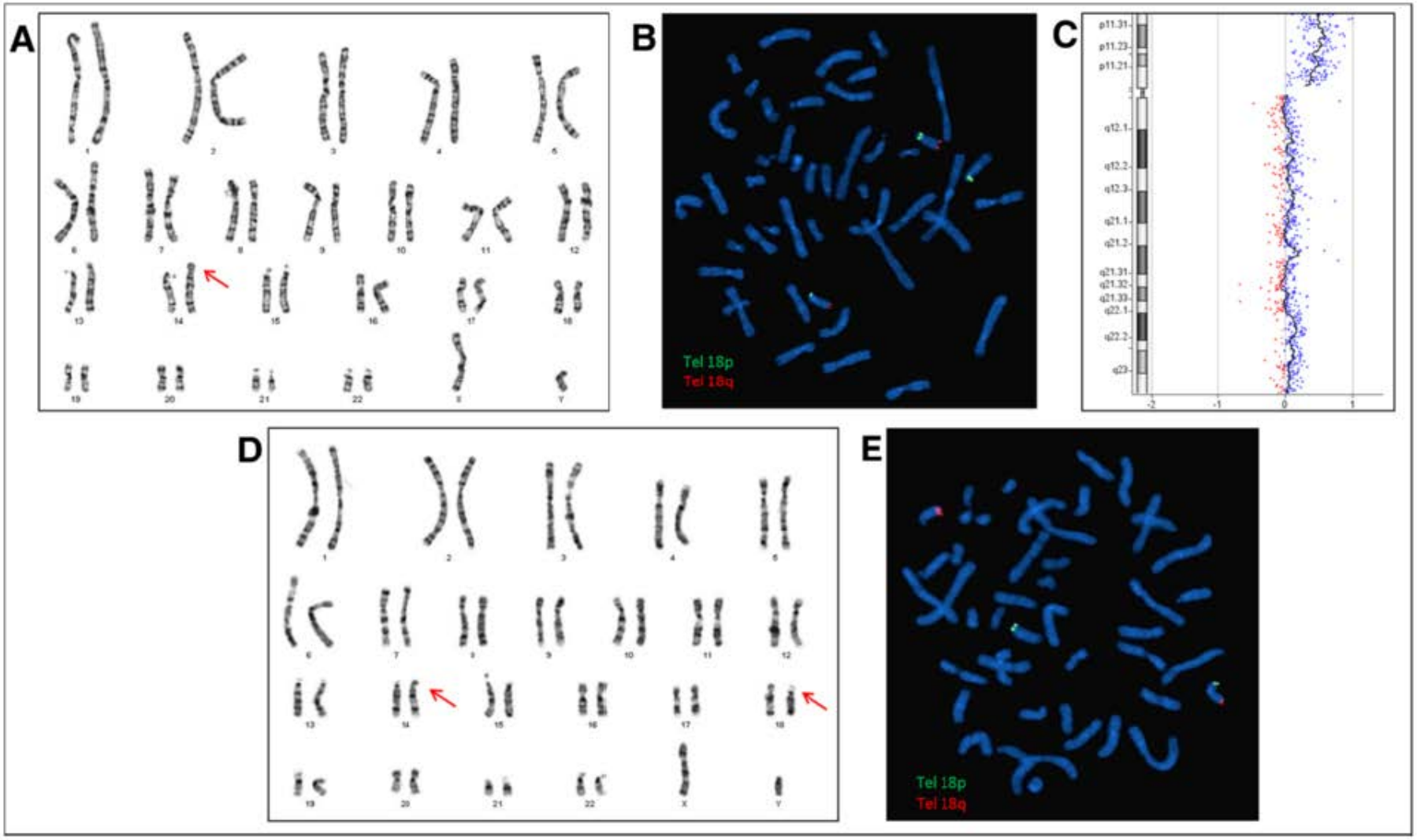
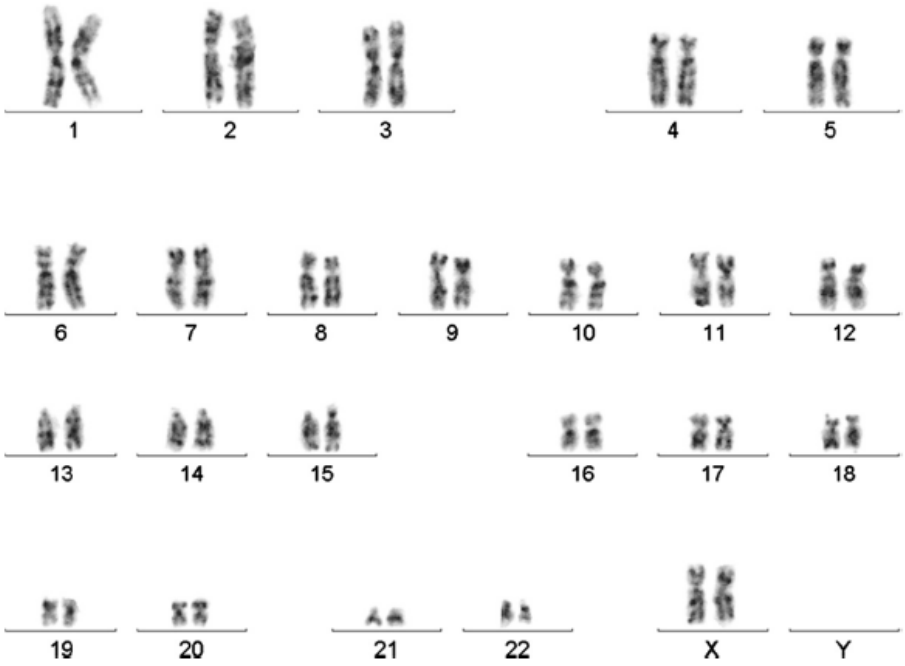


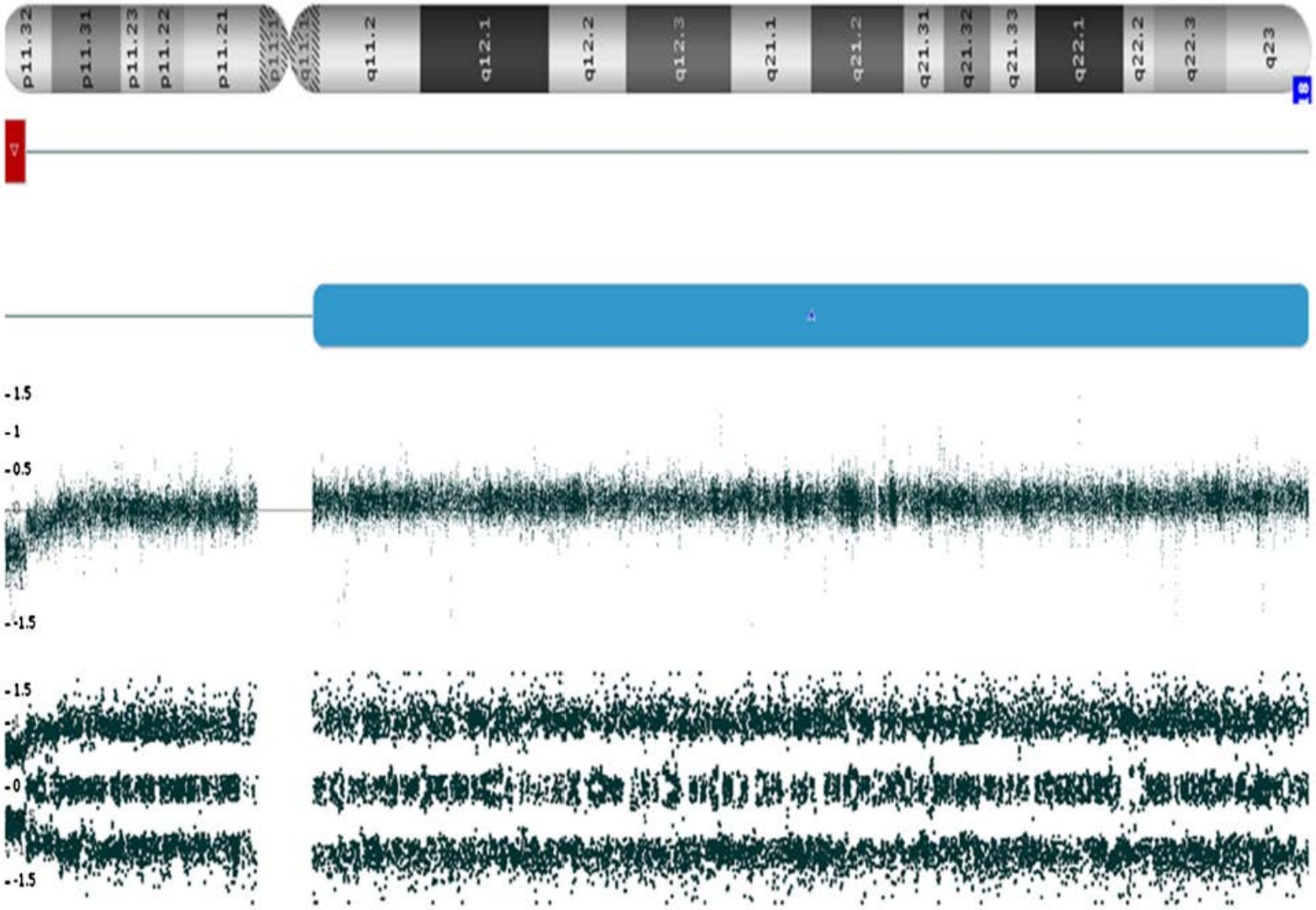
Fig. 2 Cytogenetics analyses. Patient's karyotyping (**a**) revealed an additional segment on the short arm of one chromosome 14 (red arrow), which is labeled by a telomere 18p arm FISH probe (**b**). Array-CGH results showing a pure and complete trisomy 18p (**c**). It is the result of the unbalanced inheritance of a paternal t(14;18)(p10;p10) (**d** and **e**)

A non-syndromic intellectual disability associated with a *de novo* microdeletion at 7q and 18p, microduplication at Xp, and 18q partial trisomy detected using chromosomal microarray analysis approach

Pinto *et al. Molecular Cytogenetics* 2014, **7**:44



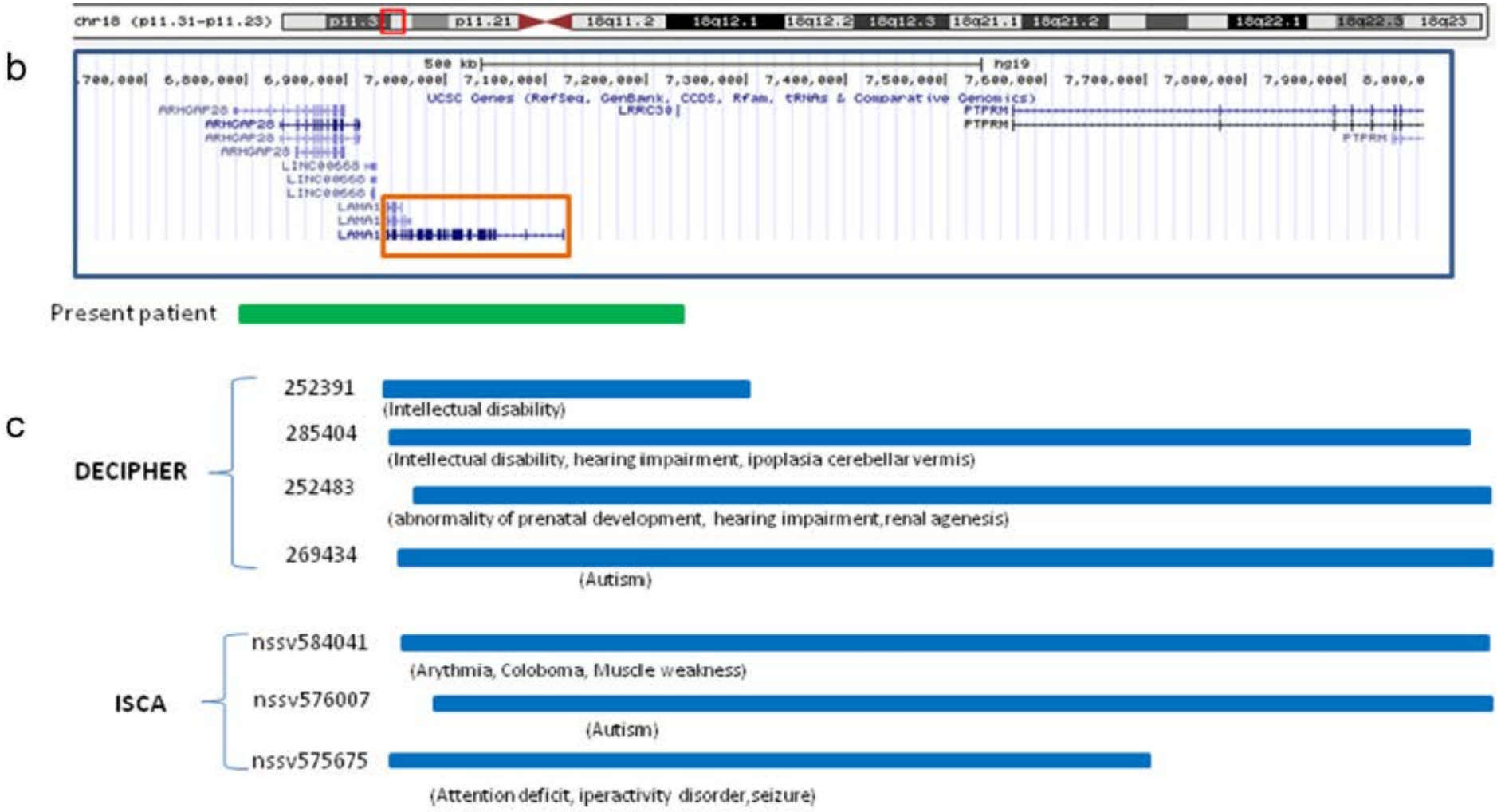
Cariotipo normale


p11.32
p11.31
p11.23
p11.22
p11.21
q11.1
q11.2
q12.1
q12.2
q12.3
q21.1
q21.2
q21.31
q21.32
q21.33
q22.1
q22.2
q22.3
q23
-1.5
-1
-0.5
0
-1.5
-1.5
0
-1.5

A 18p11.23-p11.31 microduplication in a boy with psychomotor delay, cerebellar vermis hypoplasia, chorioretinal coloboma, deafness and GH deficiency

Giordano *et al. Molecular Cytogenetics* (2016) 9:89

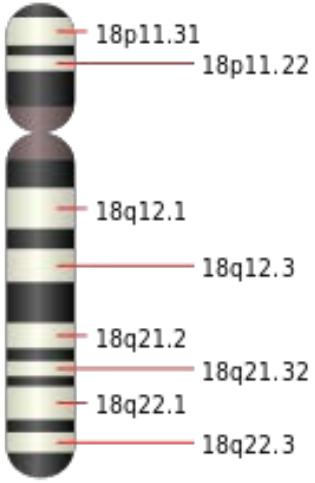
- Microdup molto rare (<dieci casi)
- Dup 400 Kb, tre geni (ARHGAP28, LINC00668, LAMA1)



Inherited duplication of the short arm of chromosome 18p11.32–p11.31 associated with developmental delay/intellectual disability

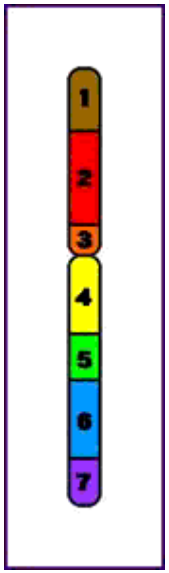
Clinical Dysmorphology 2016, 25:19–22

- Casi familiari
- Dup 2.6 Mb
- Non malformazioni, solo microcefalia relativa e difficoltà scolastiche

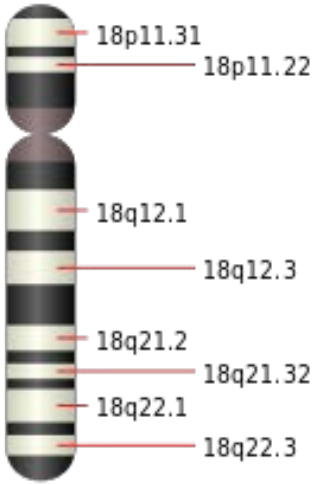


Consequences of Chromsome18q Deletions

American Journal of Medical Genetics Part C (Seminars in Medical Genetics) 169C:265–280 (2015)



- Descritta nel 1964 da De Grouchy
- 1:40.000
- Non delezioni ricorrenti, Del da 60 Kb a 30 Mb
- Del terminali e interstiziali
- Non uniformità del genotipo = fenotipo ampiamente variabile ↖ aCGH
- Non attuabili linee guida per follow up

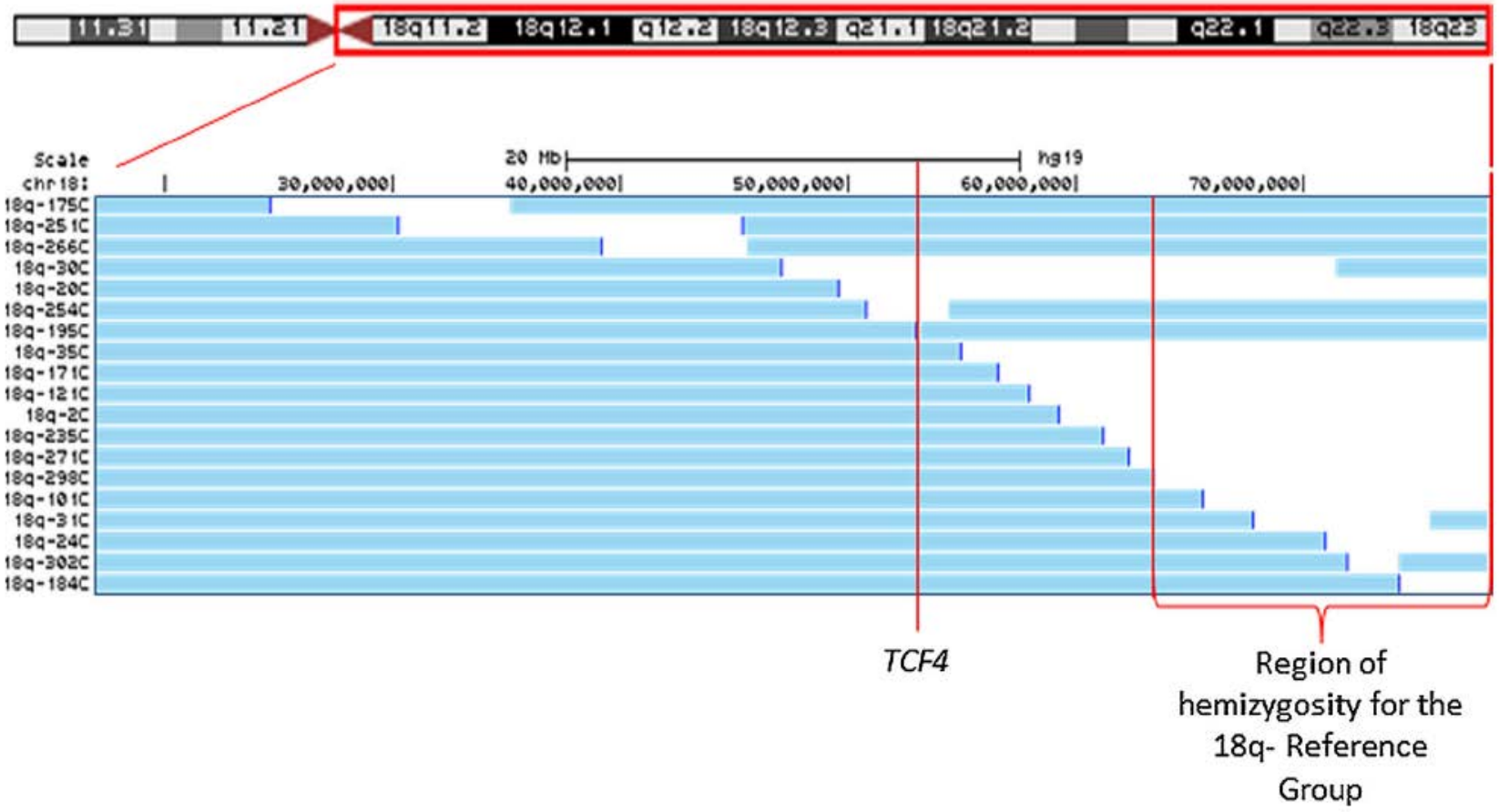


Cosa sappiamo?

- ID, ipotonia, epilessia, nistagmo, tremori
- Anomalie SB, ipoplasia cerebellare
- Bassa statura
- Microcefalia
- Dismorfismi
- Atresia auris senza microtia
- Anomalie piedi

- Meno comuni malformazioni renali, displasia ossea, deficit GH, CHD, manifestazioni cutanee (eczema 30.6%)

- Deficit mielinizzazione legato a difetto gene MBP
- Deficit GH legato ad aploinsufficienza MBP o GALR1


11.31
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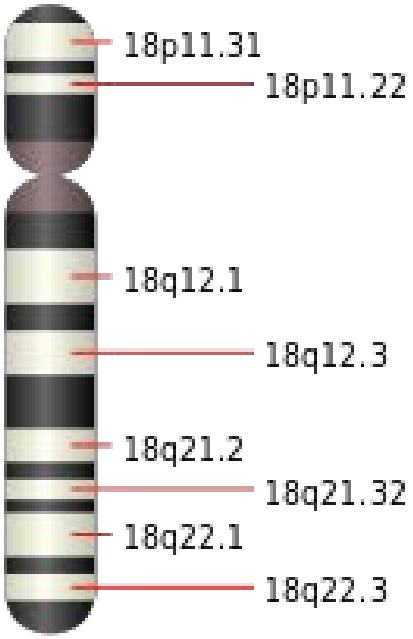
18q12.1
q12.2
18q12.3
q21.1
18q21.2
q22.1
q22.3
18q23
Scale
chr18:
20 Mb
hg19
30,000,000
40,000,000
50,000,000
60,000,000
70,000,000
18q-175C
18q-251C
18q-266C
18q-30C
18q-20C
18q-254C
18q-195C
18q-35C
18q-171C
18q-121C
18q-2C
18q-235C
18q-271C
18q-298C
18q-101C
18q-31C
18q-24C
18q-302C
18q-184C
TCF4
Region of hemizygosity for the 18q- Reference Group

Del 18q prossimali

- Del prossimali interstiziali meno frequenti

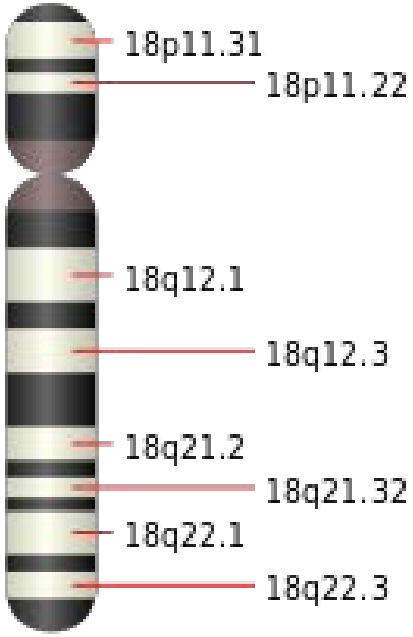


Del(18)(q12.2q21.1) syndrome: a case report and clinical review of the literature

2015; 19: 3241-3245

- Assenza di problematiche cardiache, polmonari, SNC
- Epilessia (2/3)
- Dismorfismi minori (ptosi)
- Strabismo
- RPM/ipotonia
- Obesità
- Bassa statura (1/3)

Del 18q distali



Recently, TSHZ1 (teashirt family zinc finger 1) was linked with the aural atresia phenotype. Aural atresia without microtia is a very rare finding and because it is easily observable and highly penetrant (78%), it is the most characteristic clinical feature of distal 18q-.

Otologic Characteristics of Individuals With Deletions of Distal 18q

Laryngoscope, 124:2606–2609, 2014

- Atresia aurale (senza microtia)
- Ipoacusia trasmissiva e NS
- Palatoschisi/insufficienza velofaringea 18%
- Labioschisi 3.5%

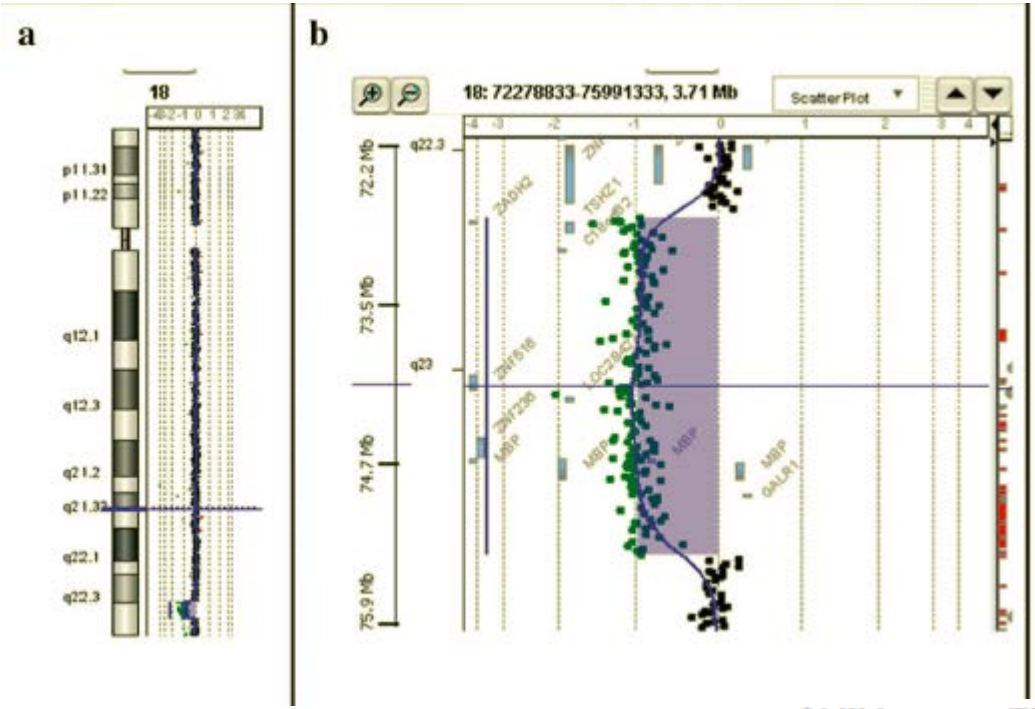
ATRESIA AURIS SENZA MICROTIA È PATOGNOMONICA PER DEL 18q

Congenital aural atresia is uncommon, occurring in one of 10,000 births, and is almost always associated with microtia.[12] Although most cases are sporadic, both autosomal dominant and recessive patterns have been

Interstitial *de novo* 18q22.3q23 deletion: clinical, neuroradiological and molecular characterization of a new case and review of the literature

Tassano *et al. Molecular Cytogenetics* (2016) 9:78

- Del 2.5 Mb



OMIM genes: *TSHZ1*, *MBP*, *ZNF516*, *GALR1*, and *ZNF236*. Among these genes, *TSHZ1* and *MBP* were

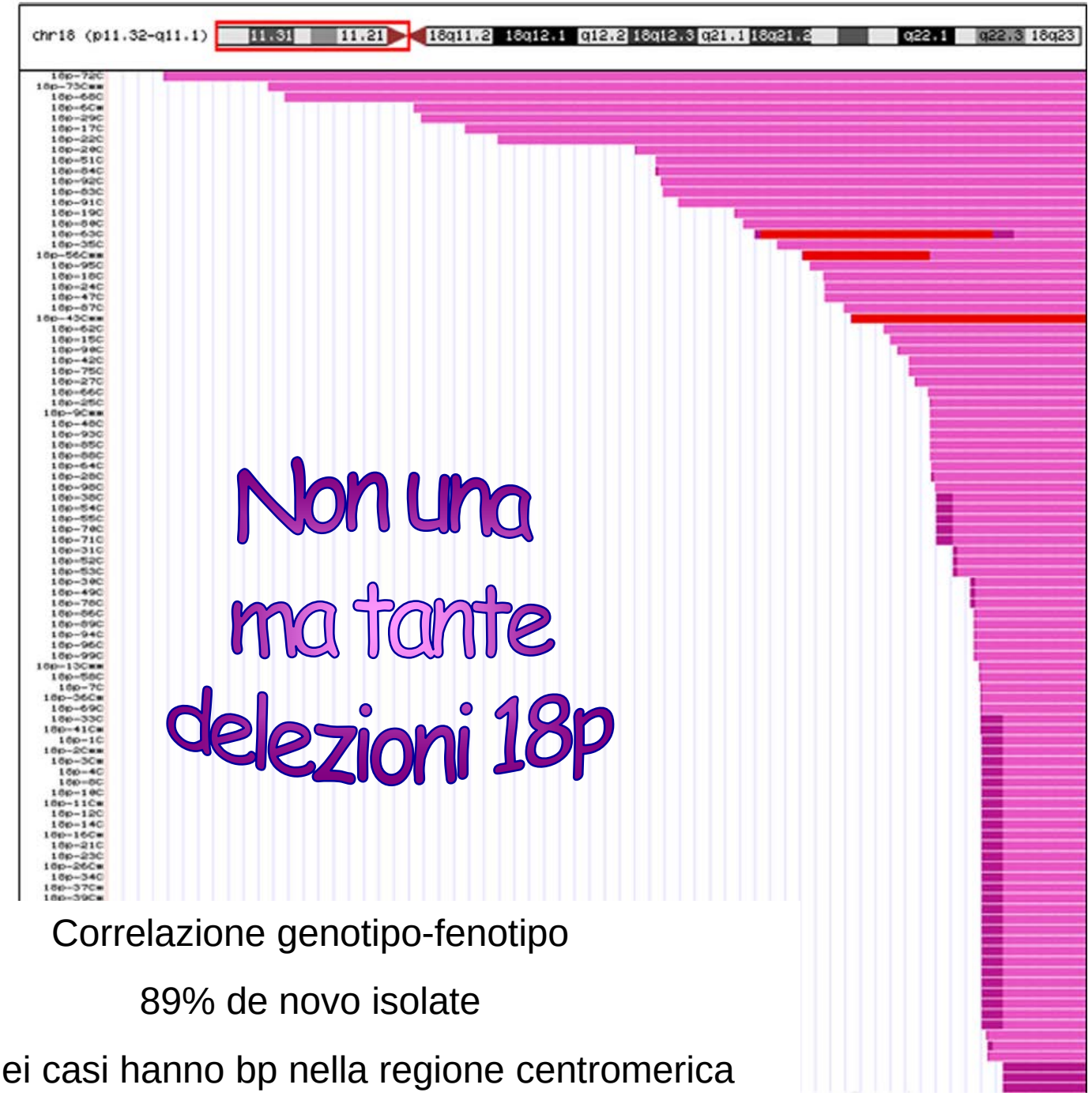
- GATA 6 => CHD
- ZNF521 => anomalie ossee minori
- SS18 => ritardo di crescita
- ZNF24 => dismielinizzazione
- SETBP1 => ritardo linguaggio espressivo
- SLC14A2 => RVU/idronefrosi
- SMAD4 => poliposi
- TCF4 => S.Pitt-Hopkins
- TXNL1 => ID
- CYB5A => ipospadia
- MBP => ipoacusia NS alte frequenze

A Review of 18p Deletions

American Journal of Medical Genetics Part C (Seminars in Medical Genetics) 169C:251–264 (2015)

- Descritta nel 1963 da De Grouchy
- 1:50.000
- ID/distonia → DYT7
- Oloprosencefalia (10%), anomalie ipofisarie → TGIF1
- Bassa statura, deficit GH (20%)
- Microcefalia, DM, ptosi, strabismo → Radice nasale depressa, bocca ampia, micrognazia, orecchie grandi, collo corto
- Cifoscoliosi, pectus excavatum
- Otiti ricorrenti (40%)
- Deficit IgA (20%)/patologie autoimmuni
- Ipotiroidismo (10%)
- CHD (10%)
- Diversi casi di trasmissione familiare

- VARIABILITA' FENOTIPICA



Correlazione genotipo-fenotipo

89% de novo isolate

Metà dei casi hanno bp nella regione centromerica

TABLE I. Features Associated With Centromeric18p- and Their Frequency

Finding	Frequency (%)
Hypotonia/mixed tone abnormalities	84
Neonatal complications (jaundice, respiratory distress, feeding difficulties)	71
MRI anomalies (excluding HPE spectrum)	66
Recurrent otitis media	61
Heart defects	56
Ptosis	55
Refractive errors	52
Strabismus	42
Pectus excavatum	29
Hearing loss	23
Isolated growth hormone deficiency	23
Scoliosis/kyphosis	19
Pes planus	19
Cryptorchidism	14
Panhypopituitarism or hypopituitarism	13
Seizures	13
IgA, IgG, or IgM deficiency	13
Holoprosencephaly or HPE microform	13
Autoimmune disorder	10
Sacral agenesis	6
Optic nerve hypoplasia	6
Congenital cataracts	6
Myelomeningocele	3

QI da 51 a 99 (media 69)

Whole Arm Deletions of 18p: Medical and Developmental Effects

Am J Med Genet Part A 167A:313–323.

delezioni complete 18p

	Number affected (n = 31)	Percentage affected
Neonatal complications	22	71%
Congenital anomalies		
Holoprosencephaly or HPE microform	4	13%
Sacral agenesis	2	6%
Myelomeningocele	1	3%
Heart defects	9 of 16*	56%
Neurologic abnormalities		
Hypotonia/mixed tone abnormalities	26	84%
Non-HPE MRI abnormalities	10 of 15**	66%
Seizures	4	13%
Endocrine abnormalities		
Panhypopituitarism or hypopituitarism	4	13%
Isolated growth hormone deficiency	7	23%
ENT/Vision abnormalities		
Recurrent otitis media	19	61%
Hearing loss	7	23%
Strabismus	13	42%
Ptosis	17	55%
Refractive errors	16	52%
Optic nerve hypoplasia	2	6%
Congenital cataracts	2	6%
Nystagmus	2	6%
Iris coloboma	1	3%
Orthopedic abnormalities		
Pes planus	6	19%
Pectus excavatum	9	29%
Scoliosis/kyphosis	6	19%
Other		
Autoimmune disorder	3	10%
IgA, IgG, or IgM deficiency	4	13%

- DSA 19-38%
- Stipsi 1/3
- Ernie inguinali/ombelicali 1/3
- Criptorchidismo 14%
- QI da ID lieve a normale

Differenze anche in genotipo identico!!!

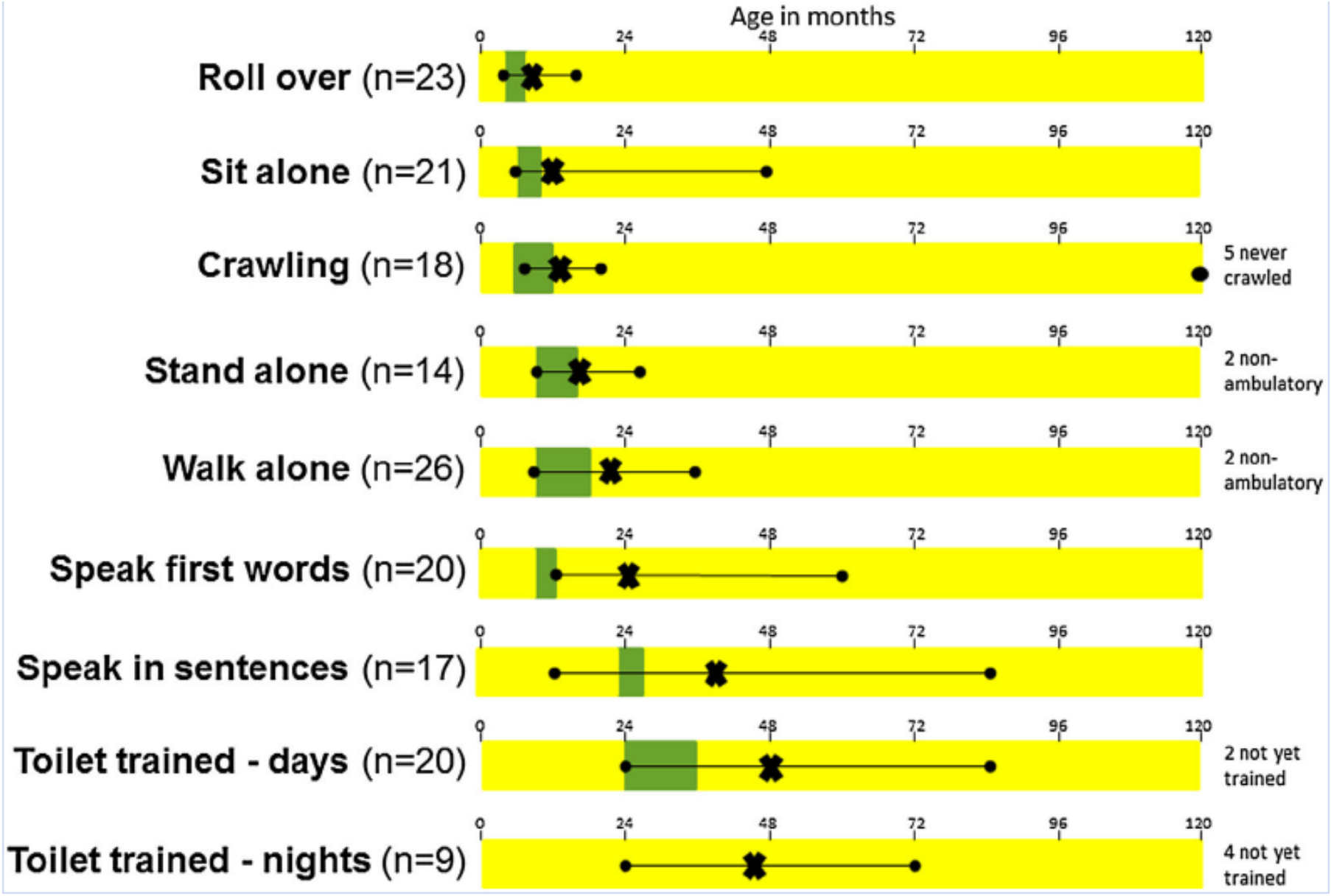


FIG. 2. Age of milestone acquisition in study participants. "X" indicates the average age of milestone acquisition, while the horizontal ball point line indicates range at which this milestone was achieved. The darker (green) segment indicates the average age of milestone acquisition in a typical population.

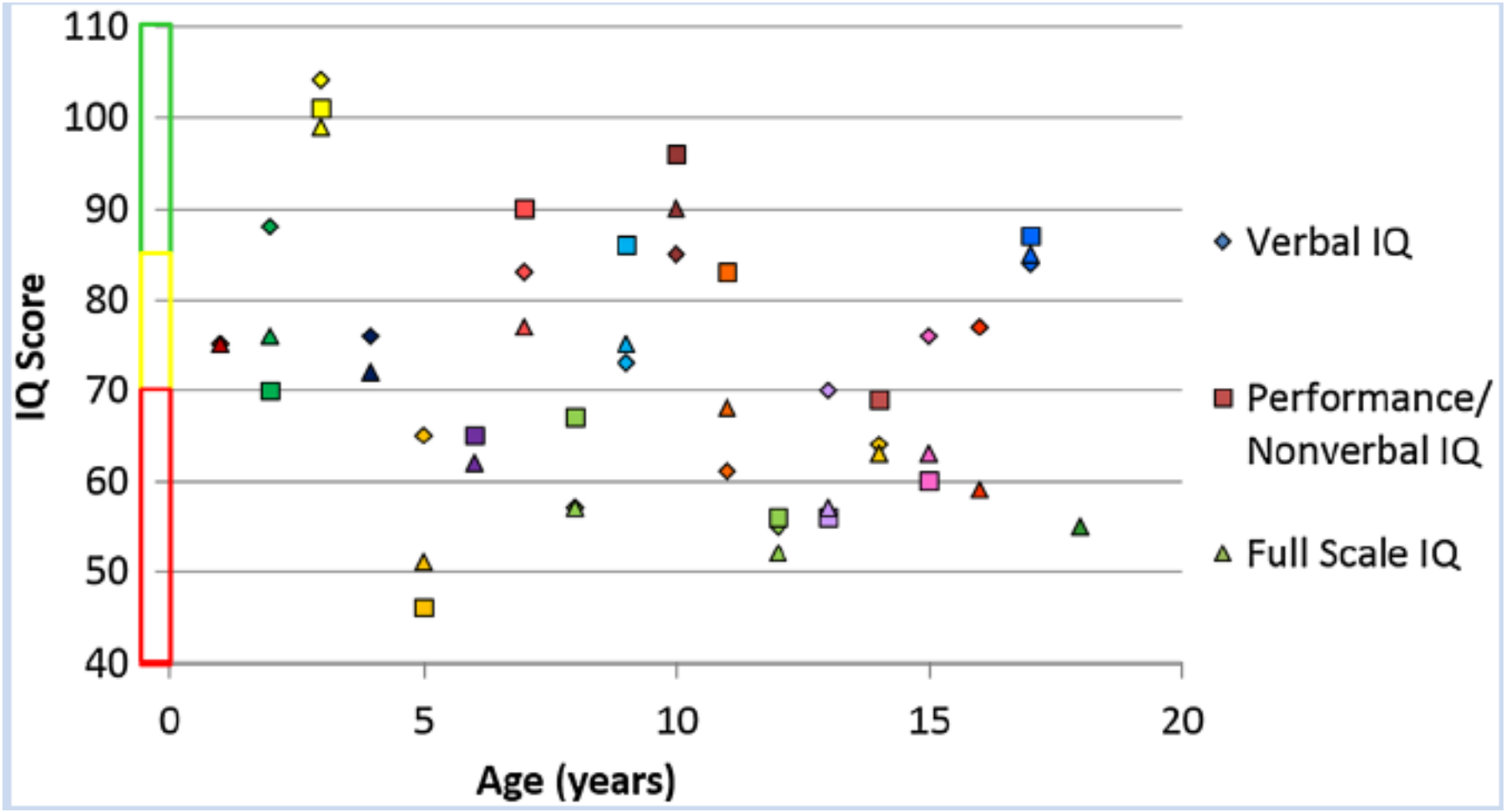


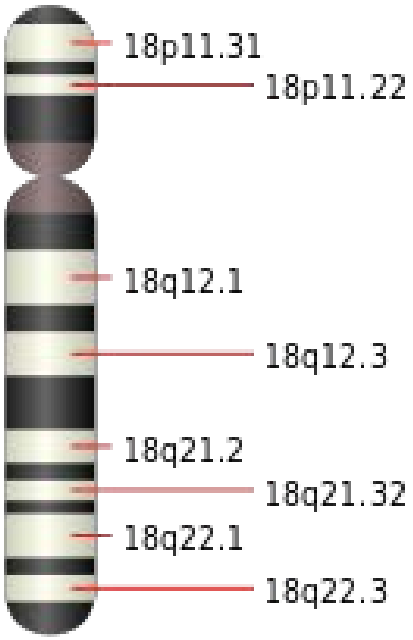
FIG. 3. Cognitive ability in study participants. The normal range is indicated on the vertical scale by the medium grey (green) bar, the borderline range is indicated by a light grey (yellow) bar and the cognitive disability range is indicated by a dark grey (red) bar. Each participant's Verbal IQ, Performance/Nonverbal IQ and Full Scale IQ are indicated where available. Each participant's data is shown at the age at which it was measured allowing the scores from the same individual to be identified.

Deletion 18p11.32p11.31 in a Child with Global Developmental Delay and Atypical, Drug-Resistant Absence Seizures

Cytogenet Genome Res 2015;146:115–119

To date, epilepsy has never been reported in the literature to be associated with this chromosomal alteration.

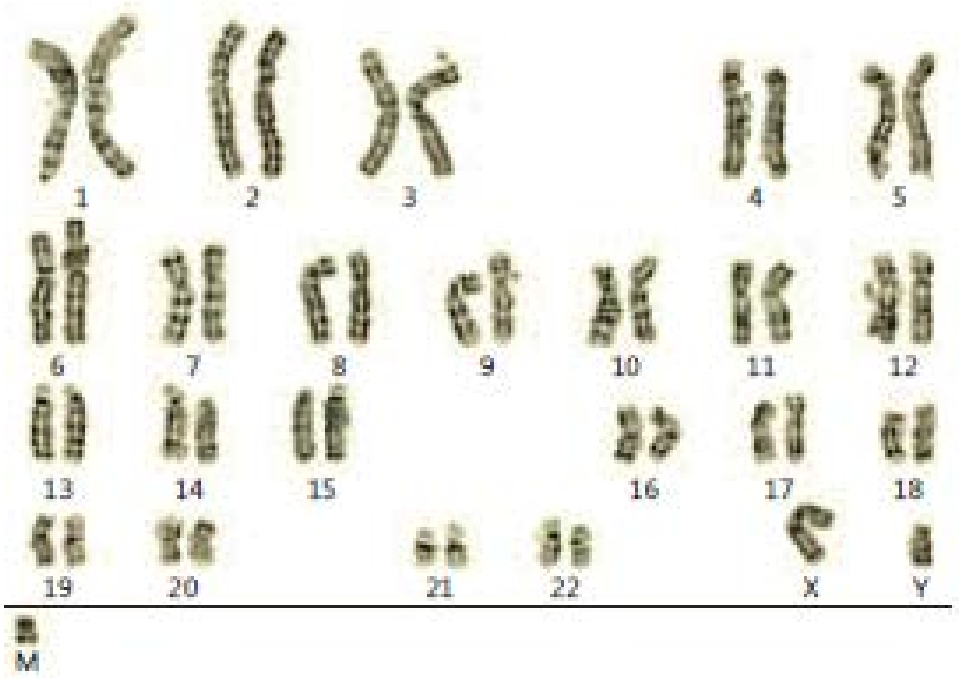
IPOTESI RUOLO TGIF1



Clinical Outcome: A Monosomy 18p is Better than a Tetrasomy 18p

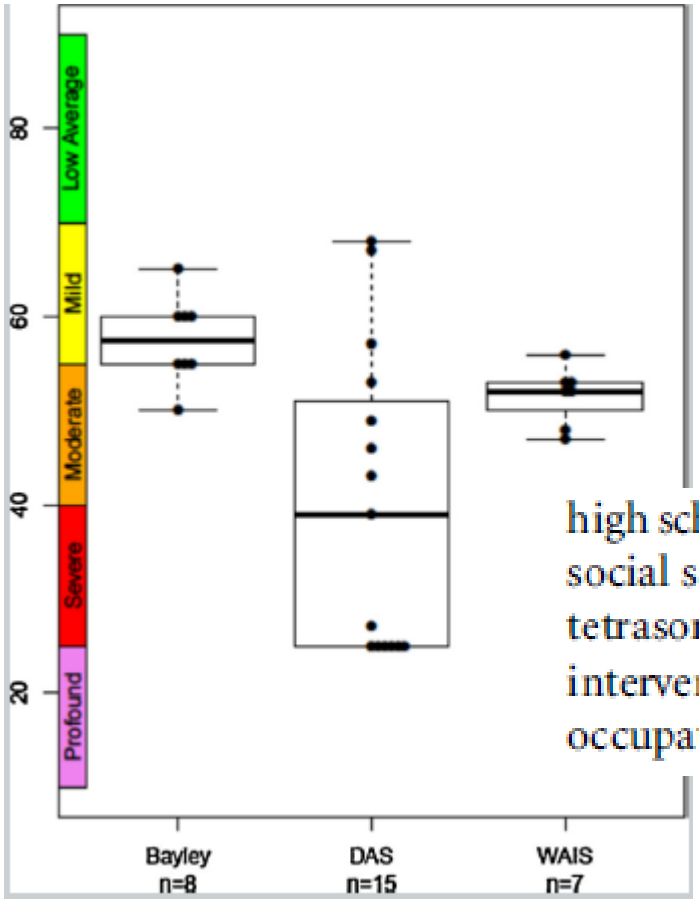
Cytogenet Genome Res 2014;144:294–298

Isocromosoma 18p = tetrasomia 18p
1:40.000
ID moderato-grave
Microcefalia
Dismorfismi
Ipotonia
23% epilessia



Tetrasomy 18p: Report of Cognitive and Behavioral Characteristics

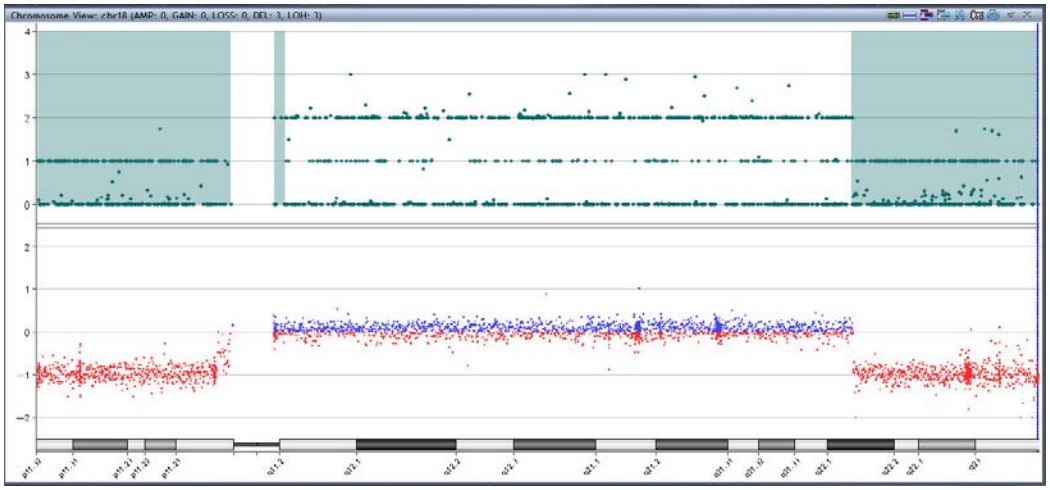
Am J Med Genet Part A 167A:1474–1482.



ID lieve-moderato
Disturbi comportamentali, difficoltà nell'adattamento, organizzazione, pianificazione

high school [Seltzer et al., 2009]. Consequently the development of social skills and behavior regulation capabilities for persons with tetrasomy 18p needs to be one of the targeted goals of early intervention efforts and integrated into academic training and occupational planning.

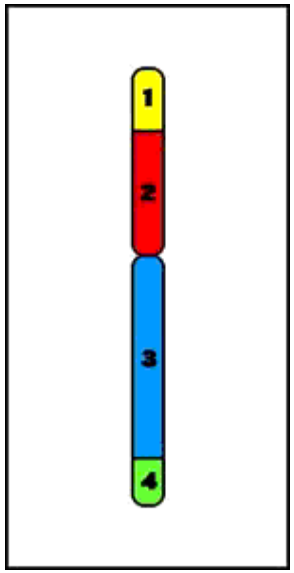
Ring 18



- DD/ID/ipotonia
- Scarso accrescimento
- Dismorfismi
- Microcefalia
- CHD
- Manifestazioni cliniche dipendono dal bp

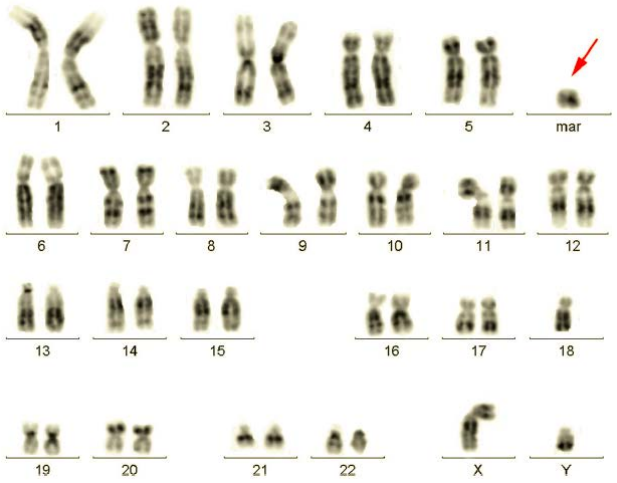
(es.ipomielinzzazione da deficit MBP in 18q23)

Formazione del ring = perdita parziale di p e q

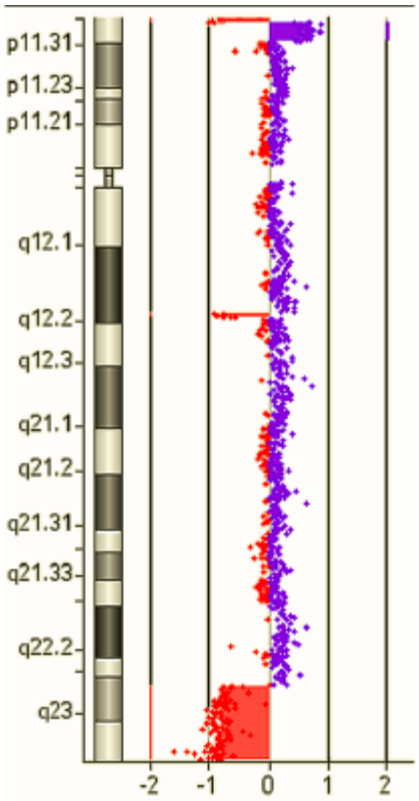


Ring chromosome 18 in combination with 18q12.1 (*DTNA)* interstitial microdeletion in a patient with multiple congenital defects

Zlotina *et al. Molecular Cytogenetics* (2016) 9:18



Caratteristiche r(18) ma stenosi subaortica



Gene DTNA

Here we support the utility of array-CGH analysis for accurate individual diagnosis, disease prognosis and genetic counseling of patients with multiple congenital defects and non-typical clinical picture.

Breakpoints and deleted genes identification of ring chromosome 18 in a Chinese girl by whole-genome low-coverage sequencing: a case report study

Yao *et al. BMC Medical Genetics* (2016) 17:49

Dettaglio della localizzazione dei breakpoint e verifica dei singoli geni presenti nelle aree delete e duplicate

Migliore stima del rischio di singole problematiche

Adults with Chromosome 18 Abnormalities

J Genet Counsel (2015) 24:663–674

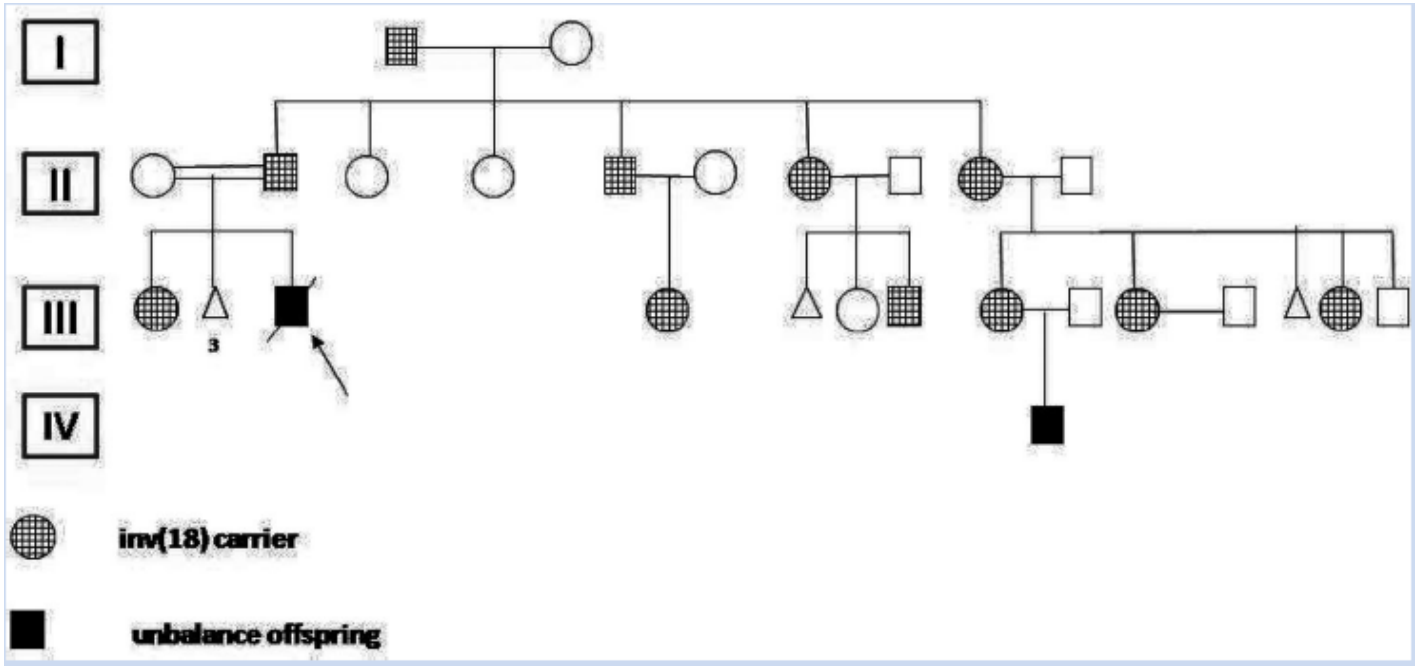
- Non riduzione lifespan per tetrasomia 18p, ring 18, del 18p e del18q, con l'eccezione di del18q comprendenti TCF4 e riarrangiamenti complessi
- La maggioranza completa le scuole dell'obbligo e lavora
- Tetrasomia 18p: iperattività, impulsività, difficoltà a capire le reazioni altrui al proprio comportamento
- Ring18/Del18q: difficoltà socializzazione
- Del18p: abilità cognitive superiori, difficoltà socializzazione

ETEROGENEITA' GENETICA

GENETICA E TRANSIZIONE

Recurrent Proximal 18p Monosomy and 18q Trisomy in a Family due to a Pericentric Inversion

Am J Med Genet Part A 164A:1239–1244.



GENETICA E DEFINIZIONE DEL RISCHIO DI RICORRENZA

Un caso esemplificativo…

- Bitest N
- NIPD: rischio trisomia 18
- Eco fetali: N
- Non eseguita amniocentesi
- Nascita ok
- Ipotonia, RPM
- arrayCGH: tetrasomia 18p
- Cariotipo: isocromosoma 18p

Take home messages

- Non esiste LA sindrome cromosoma 18
- Numerosissimi fenotipi
- arrayCGH ha consentito correlazioni genotipo fenotipo
- Vecchie tecniche genetiche sono tuttora utili per definire correttamente l’alterazione genetica
- Impossibile stilare protocolli generali di follow up

Conseguenze pratiche

- Grande importanza/responsabilità di una corretta DIAGNOSI CLINICA

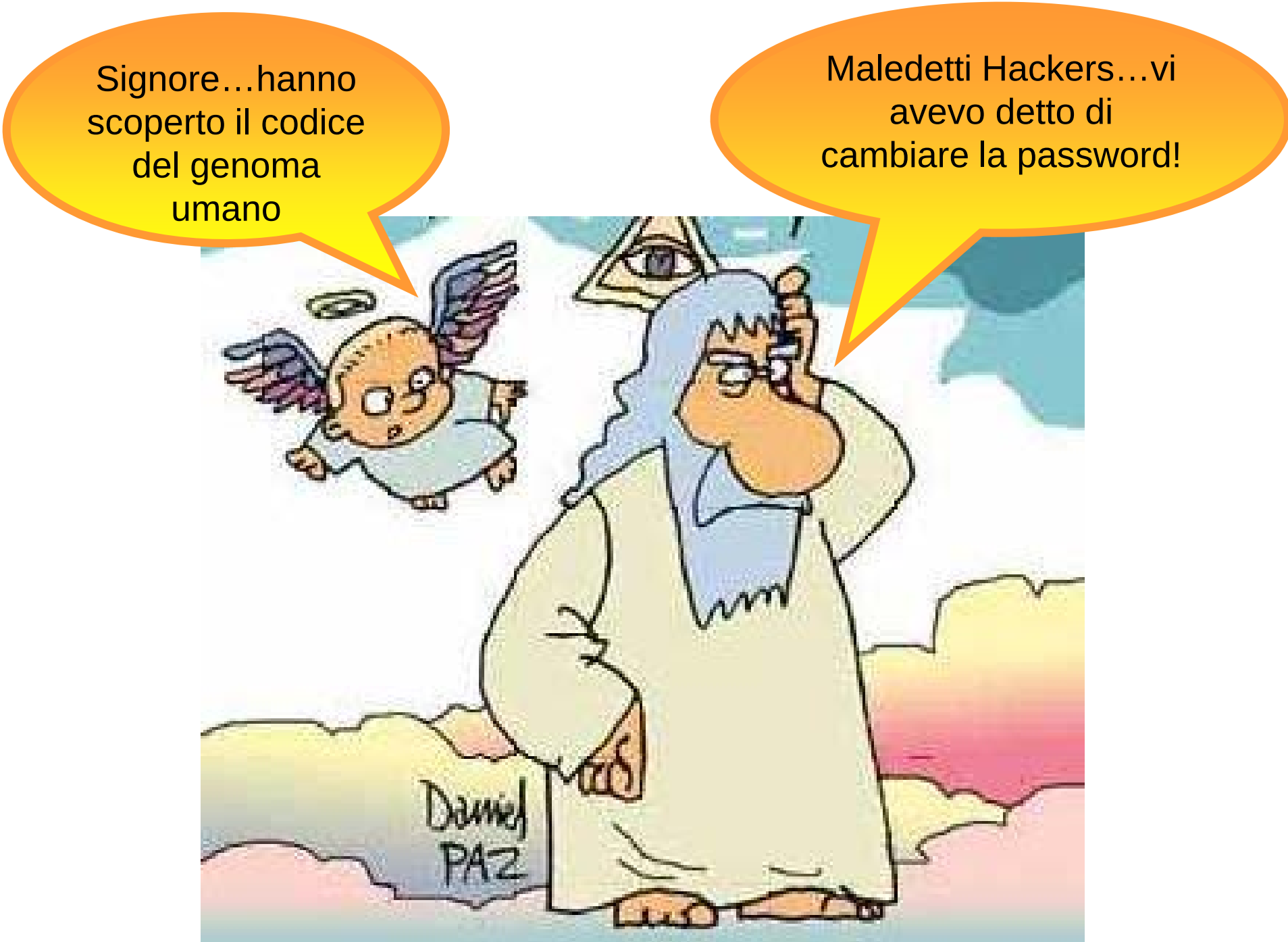
- Assoluta necessità di mantenere nel tempo il contatto con il pz per verificare la correttezza della diagnosi clinica e per mettere a disposizione del pz e della sua famiglia nuovi test diagnostici

Conclusione

E’ indispensabile che:

- ✓ il clinico conosca sempre più nel dettaglio le diverse potenzialità del laboratorio per mirare in modo più accurato e razionale le sue richieste

- ✓ l’interazione tra il clinico ed il biologo sia la più stretta possibile data la complessità delle tematiche in esame


Signore…hanno scoperto il codice del genoma umano
Maledetti Hackers…vi avevo detto di cambiare la password!
Daniel PAZ