APORTES DE LA GENÉTICA MOLECULAR A LA CLÍNICA de losdefectoscongénitos

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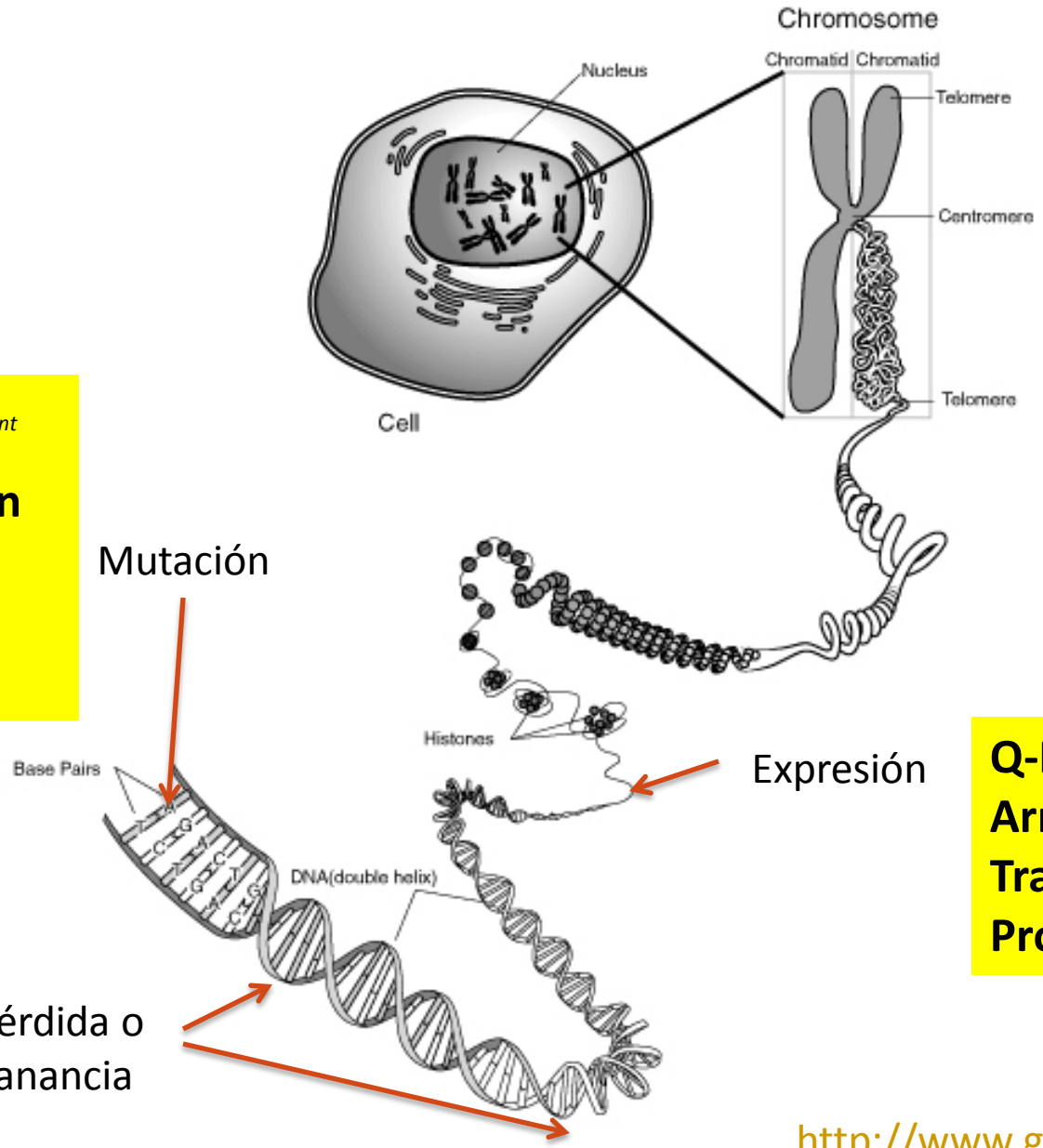
2012

RFLP *restriction fragment length polymorphism*
Sequenciación
DNA array
Exoma
Genoma



Cariótipo
FISH *Fluorescence In Situ Hybridization*
CGH *Comparative genomic hybridization*
DNA Array
MLPA *Multiplex Ligation-dependent Probe Amplification*

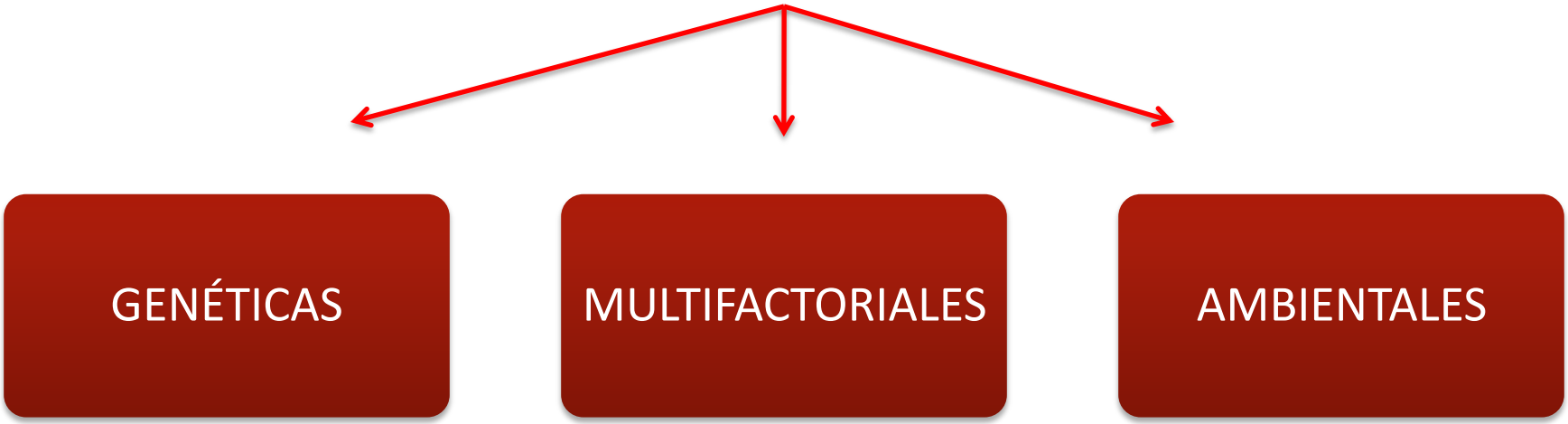
Q-PCR Real-Time *quantitative PCR*
Array de metilación
Transcriptoma
Proteoma

http://www.geneclinics.org

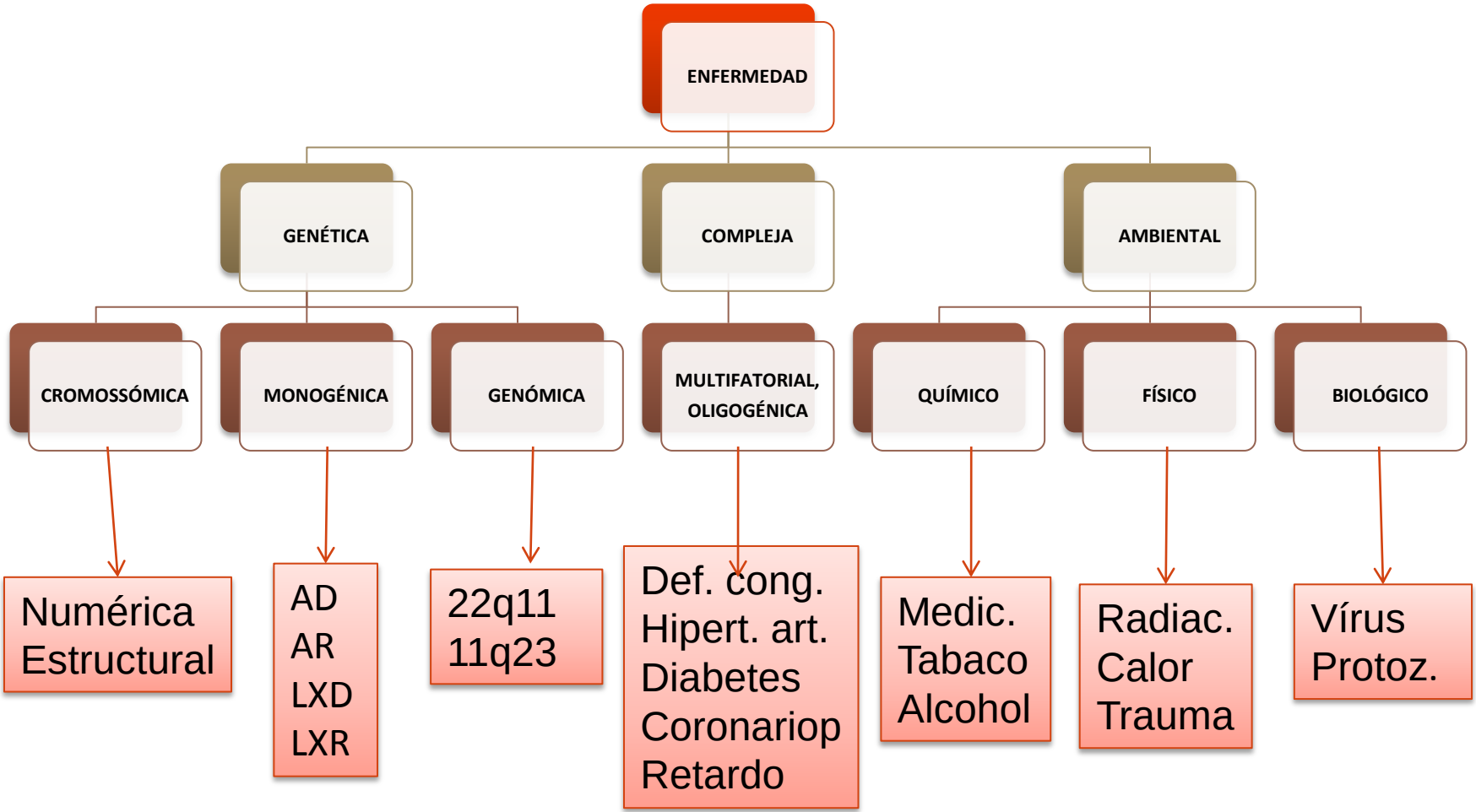

DIAGNÓSTICO
PRONÓSTICO
TRATAMIENTO
PREVENCIÓN
FAMILIAR
INDIVIDUAL
PRIMÁRIA
SECUNDÁRIA
TERCIÁRIA
EVITA
TRATA
REHABILITA


DIFICULTAD EN HACER EL DIAGNÓSTICO
HETEROGENEIDAD ETIOLÓGICA
EXPRESIVIDAD VARIABLE

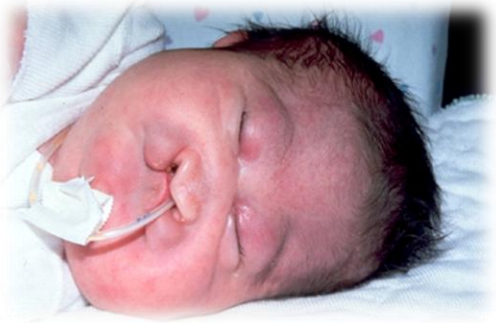
CAUSAS DE ENFERMEDAD
o de defecto congénito



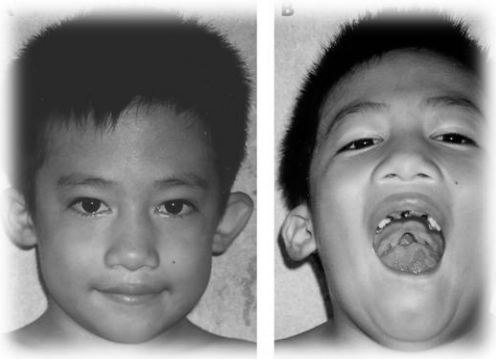
CAUSAS DE ENFERMEDAD
o de defecto congénito



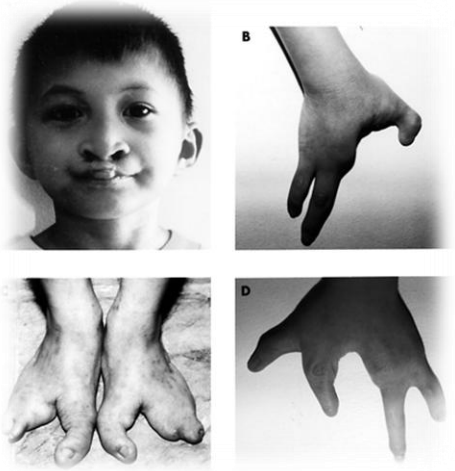
HETEROGENEIDAD ETIOLÓGICA: FISURAS ORALES



ANOMALIAS CROMOSÓMICAS
Trisomia 13



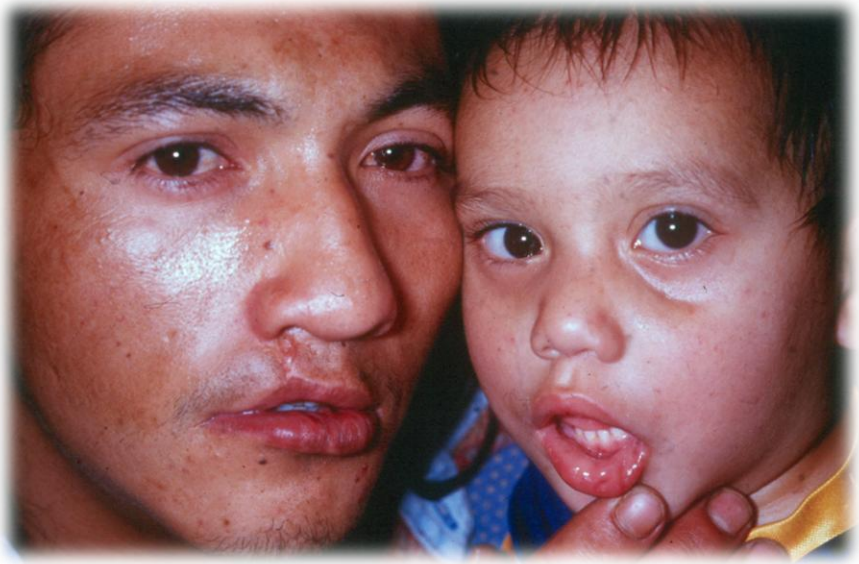
ANOMALIAS GENÓMICAS
Síndrome VCF (22q21-)

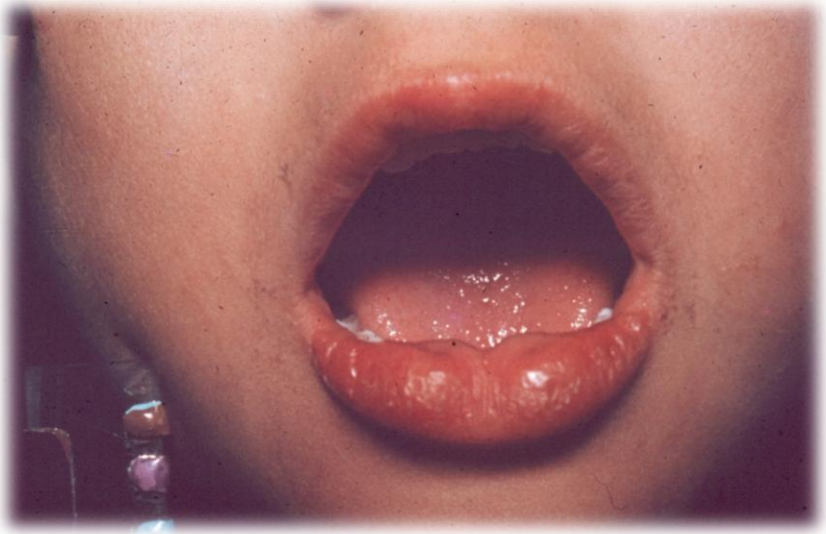


ANOMALIAS MONOGÉNICAS
Síndrome EEC (P63)

ANOMALIAS AMBIENTALES
Embriopatia por isotretinoina

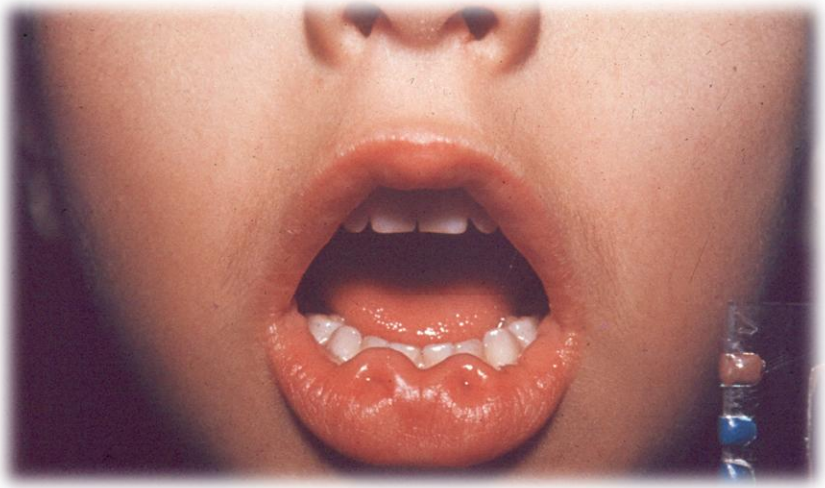
FLP AUTOSÓMICA DOMINANTE





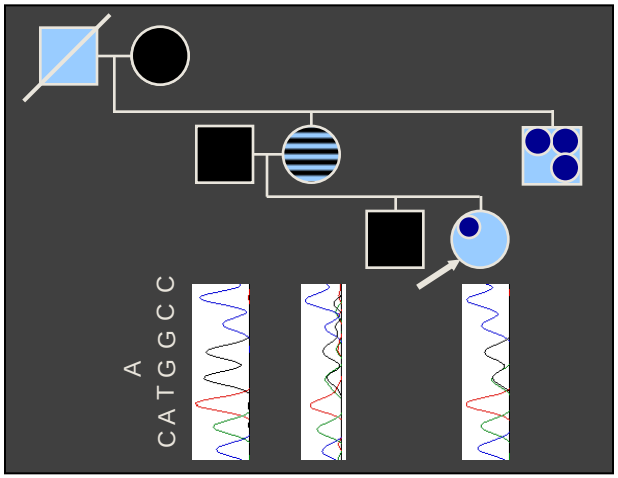
SÍNDROME DE VAN DERWOUDE

FLP + FP + “PITS” LABIALES

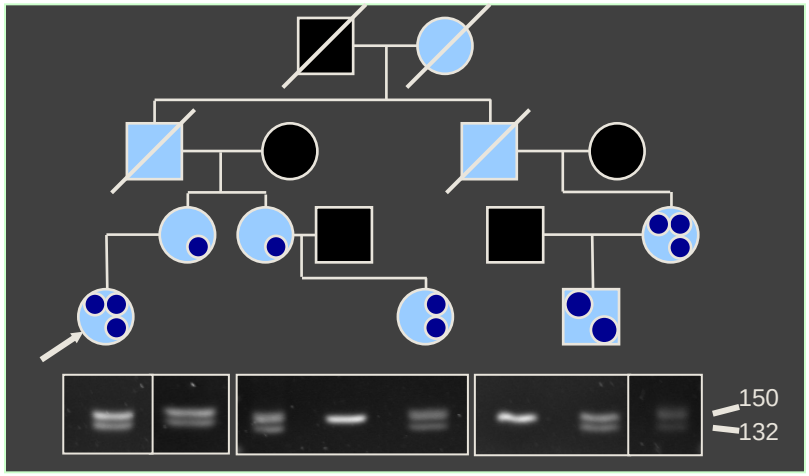


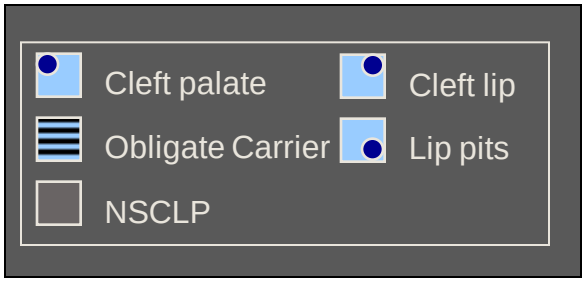
PENETRANCIA Y EXPRESIVIDAD VARIABLE: SVW

NO PENETRANCIA



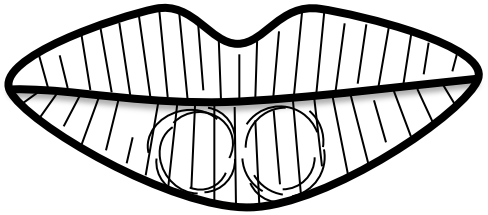
FISURAS MISTAS

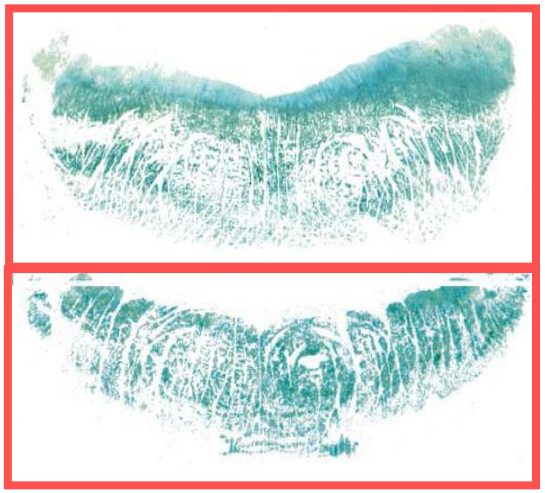




PATRÓN DE IMPRESIÓN LABIAL—VERTICILOS

VERTICILOS LABIALES

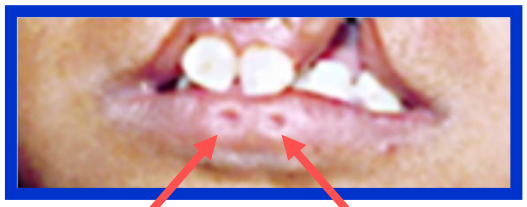




RELATOS DE MÁS VERTICILOS EN FAMÍLIAS CON FLP

INDIVÍDUOS CON FLP	[Pruszewicz et al., 1988]
PADRES	[Hirth et al., 1976]
PARES DE GEMELOS	[Hirth et al., 1978]

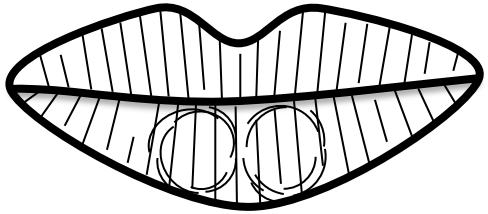
PATRÓN DE IMPRESIÓN LABIAL—VERTICILOS

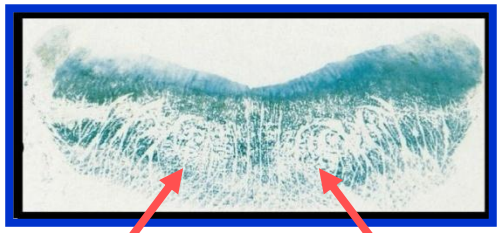


Síndrome Van derWoude

Mutaciones en IRF6

Verticiloslabiales





FL/P no sindrómicas

IRF6 variantes?

H: Verticilos de labio inferior son expresión de riesgo para FL/P asociadas con el gene IRF6

HOLOPROSENCEFALIA

OTRO EJEMPLO DE DEFECTO CONGÉNITO CON

- ALTA HETEROGENEIDAD ETIOLÓGICA Y
- EXPRESIVIDAD ALTAMENTE VARIABLE

HOLOPROSENCEPHALY

ambiental

- madre diabética
- etanol
- ácido retinoico
- cyclopamine (*Veratum californicum*)

genético

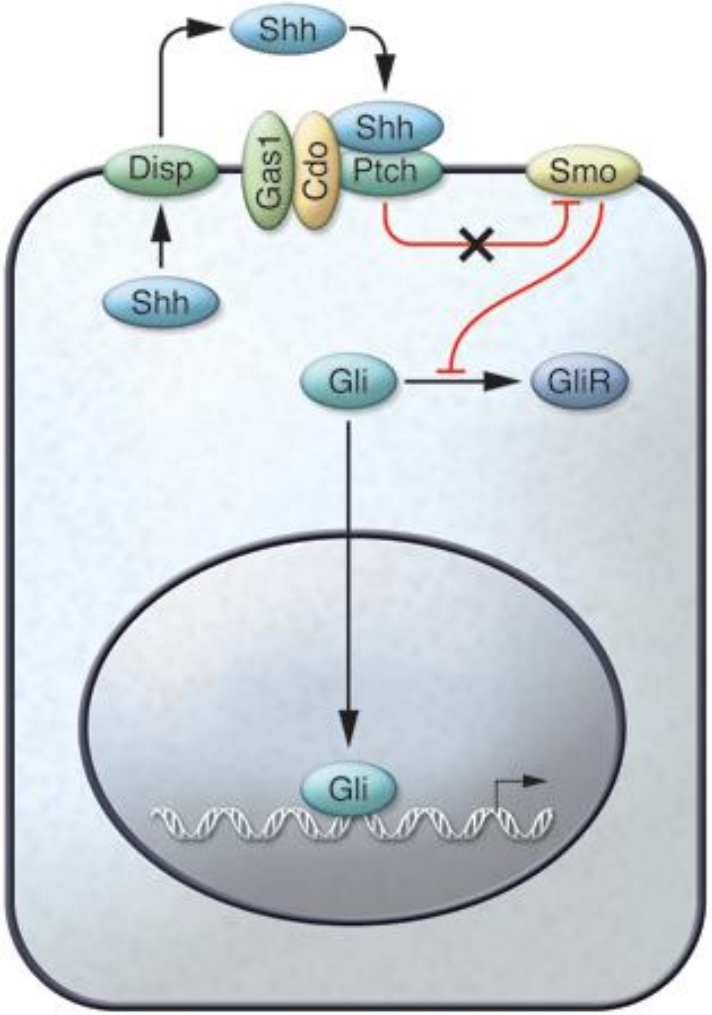
- HPE familiar
  - autosomal dominant with incomplete penetrance (70%)
- MCA (25%)
  - pseudotrisomy 13, Smith-Lemli-Opitz
  - Pallister-Hall, Meckel, velocardiofacial
  - Genoa (craniosynostosis)
  - Lambotte (microcephaly, PGR, hypertelorism)
  - Martin (clubfoot, spinal anomalies)
  - Steinfeld (CHD, abs. gallbladder, renal dysplasia, radial defects) with ectrodactyly and hypertelorism)
- anomalía cromosómica (20- 40%)
  - 21q22.3(HPE1) 2p21(HPE2) 7q36(HPE3) 18p11.3(HPE4)
  - 13q32(HPE5) 3p24-pter(HPE6)13q12-14(HPE7)14q13(HPE8)
  - 20p13(HPE9) 1q42-qter(HPE10) 5p(HPE11) 6q26-qter(HPE12)

AUTOSÓMICA DOMINANTE CON PENETRANCIA INCOMPLETA

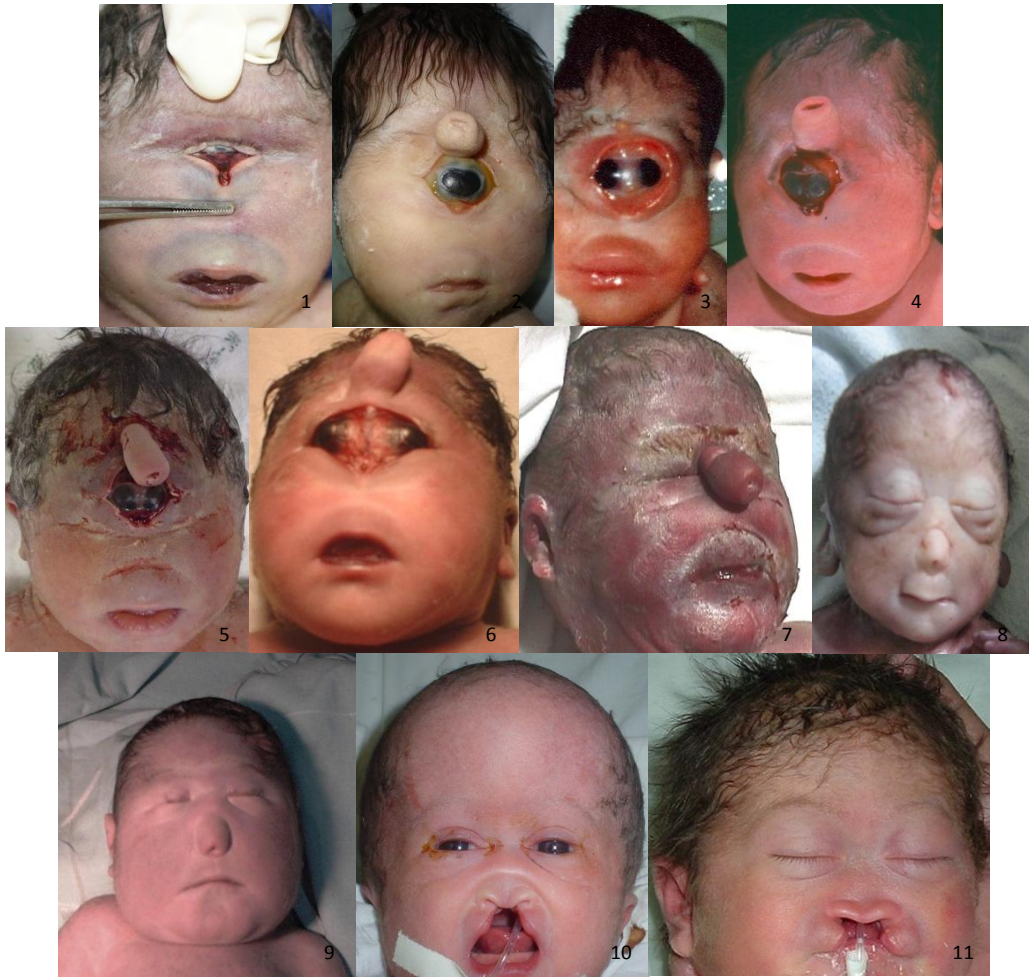
Genes HPE:

- *SHH* (7q36)
- *ZIC2* (13q32)
- *SIX3* (2p21)
- *TGIF* (18p11.3)
- *PTCH* (9q22.3)
- *TDGF1* (3p21.3)
- *GLI2* (2q14)
- *DHCR7*(11q13)
- *FAST1/FOXH1* (8q24.3)
- *DISP1* (1q42)
- *FGF8* (10q24)

VIA DE SEÑALIZACIÓN DEL GENE SHH

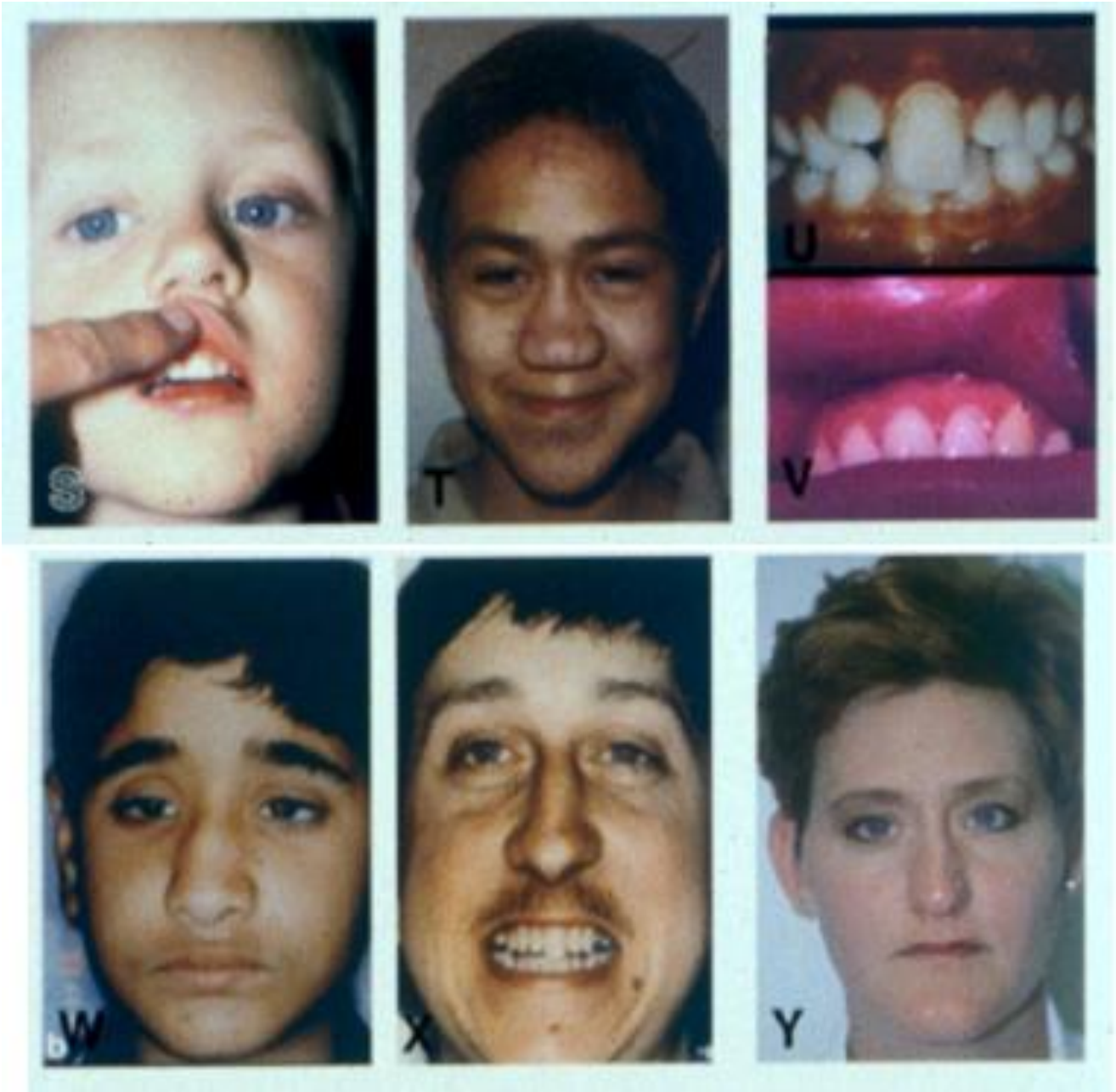


ESPECTRO DE ANOMALÍAS FACIALES EN LA HOLOPROSENCEFALIA



1. Ciclopía
2. Ciclopía
3. Ciclopía con sinoftalmos
4. Ciclopía con sinoftalmos
5. Ciclopía con sinoftalmos
6. Ciclopía con sinoftalmos
7. Etmocefalia
8. Cebocefalia
9. Cebocefalia
10. Agenesia de la pre maxila
11. Agenesia de la pre maxila

PORTADORES DE MUTACIONES EN GENES HPE



Mutation screening

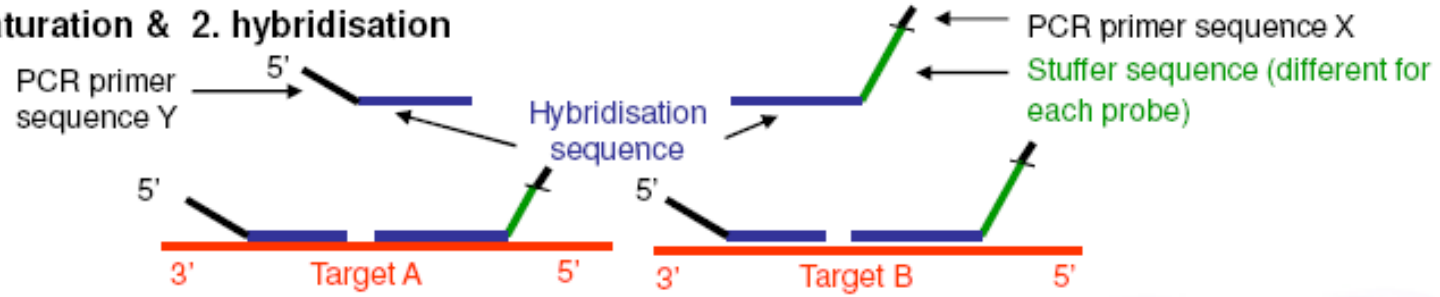
- Extraction
- PCR Amplification
  - *SHH* – 3 exons, 4 amplicons
  - *ZIC2* – 3 exons, 4 amplicons
  - *SIX3* – 2 exons, 3 amplicons
  - *TGIF* – 3 exons, 3 amplicons
- Purification
- Sequencing

MLPA (Multiplex Ligation-Dependent Probe Amplification):

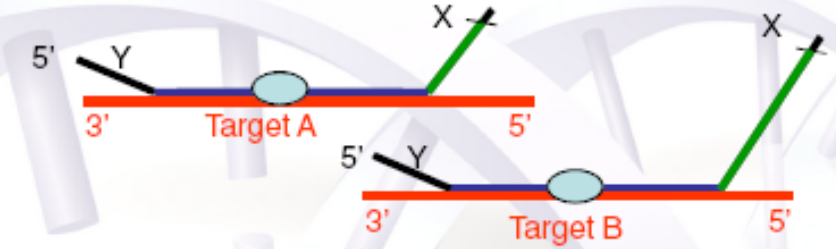
- Multiplex PCR for copy number quantification
- Probes of target regions are amplified in the case of hybridization
- Analysis is performed by capillary electrophoresis
- Cases are compared with controls of the same type of extraction

MLPA

1. Denaturation & 2. hybridisation



3. Ligation

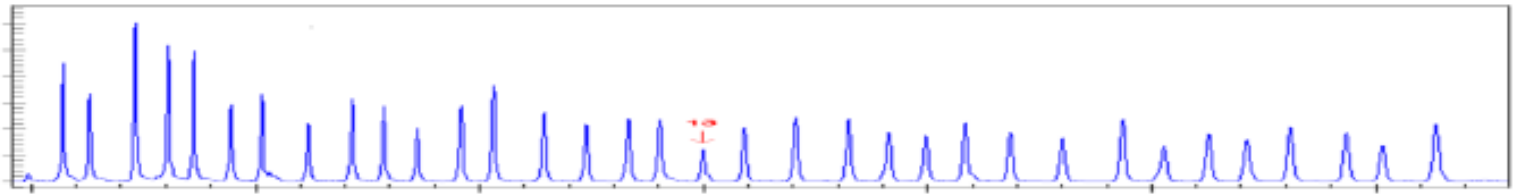


The two parts of each probe hybridize to adjacent target sequences and are ligated by a thermostable ligase.

4. PCR: All probe ligation products are amplified by PCR using only one primer pair.

The amplification product of each probe has a unique length (130-480 nt).

5. Separation of amplification products by electrophoresis: Amplification products are separated by electrophoresis. Relative amounts of probe amplification products, as compared to a control DNA sample, reflect the relative copy number of target sequences.



Schouten, J.P. et al. Nucl. Acid Res. 30, e57.

MRC-Holland MLPA®

MLPA (Multiplex Ligation-Dependent Probe Amplification):

- Kit P187 Holoprosencephaly:
  - 41 probes.
  - Indicates deletion and duplication of exons in the four main genes associated with HPE, and still *PTCH*, *FBXW11,* and *TRAPPC10*.

- Kit P187-B1 Holoprosencephaly:
  - 48 probes.
  - Indicates deletion and duplication of exons in the four main genes associated with HPE, and still GLI2, *PTCH*, *FBXW11,* and *TRAPPC10*.

Screening for trisomies by QF-PCR:

- QF-PCR (*quantitativefluorescentpolymerasechainreaction*) - in the early exponential phase of amplification, the quantity of product is proportional to the amount of target sequences present in the initial sample.

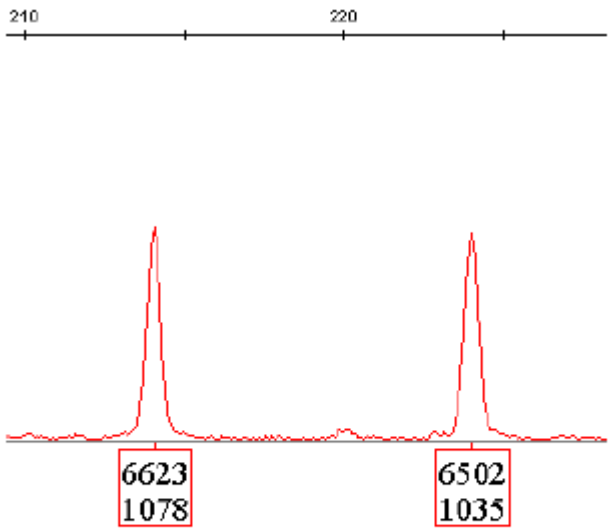
- The kit uses STR markers to determine the incidence of trisomies of chromosomes 13, 18, 21 and the sex chromosomes aneuploidies.

Screening for trisomies by QF-PCR:

- STR markers are amplified by PCR with primers labeled with fluorescence.
  - 4 markers for each chromosome
  - Specific kits for each chromosome are used to confirm the results
- The alleles are determined by capillary electrophoresis.
- The amount of fluorescent PCR product is a numerical value which corresponds to the peak area viewed in the electropherogram.

Screening for trisomies by QF-PCR:

In a normal heterozygous individual, the same amount of fluorescence is generated for both alleles, and the relationship between the areas (and height) of each fluorescence peak is 1:1

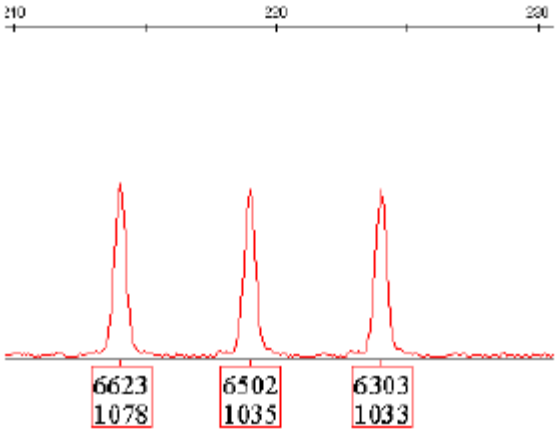


Normal Heterozygous

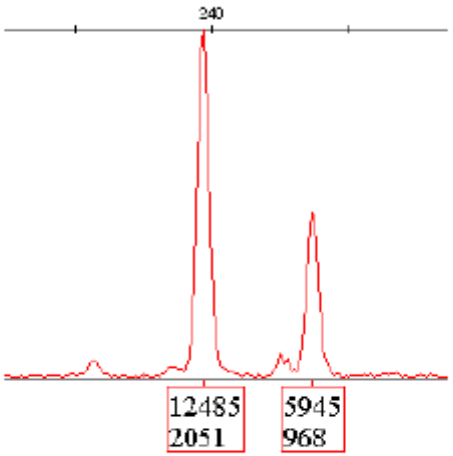
1:1

Screening for trisomies by QF-PCR:

In trisomy: three peaks with same fluorescence intensity and a ratio between the peak areas (or heights) of 1:1:1 (in the case of trissomictriallelic), or two fluorescence peaks with an unbalanced area/height ratio of 2:1 (trissomicdiallelic).



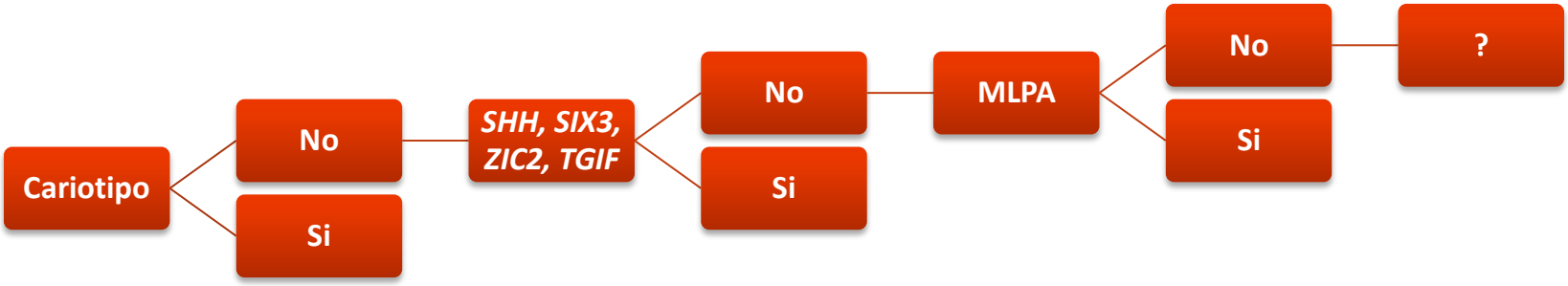
Trisomic Triallelic
1:1:1



Trisomic Diallelic
2:1

Estudio de Holoprosencefalía

Methodologia	Numero de casos estudiados	Numero de casos estudiados del ECLAMC
Mutation screening of *SHH*, *ZIC2*, *SIX3,* and *TGIF* gene	111	56
Screening of microdeletions by MLPA	50	17
Screening for trisomiesby QF-PCR	52	36



Screening para trisomía: ANEUFAST QF-PCR kit

-Only 32% of HPE cases receivedby MOLECLAMC hasinformedkaryotype

-25% of cases tested with the kit showed trisomy of chromosomes 13, 18 or 21

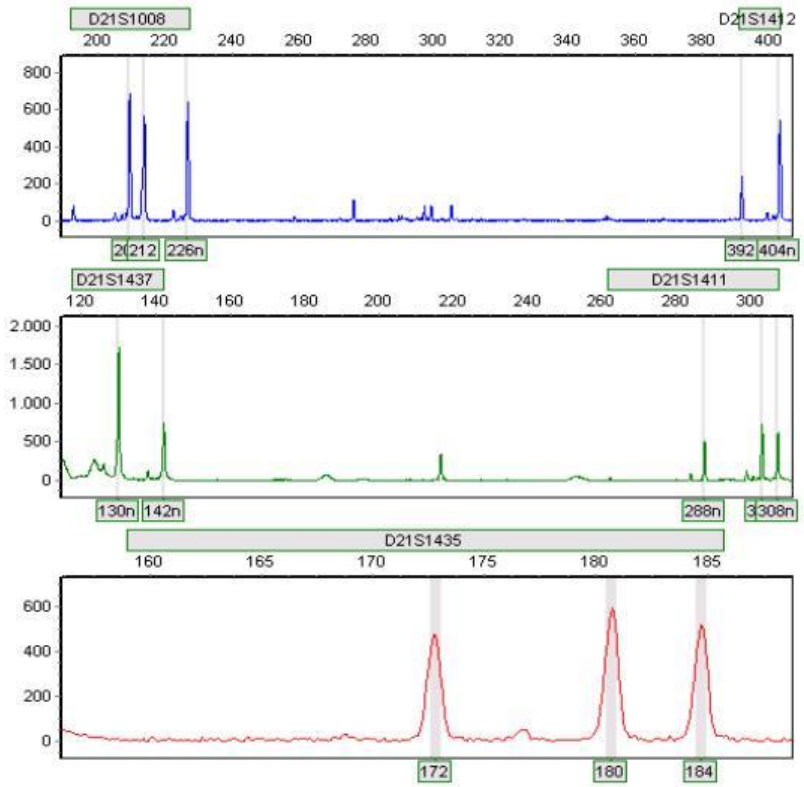


Figura 1 QF-PCR eletropherogramshowingtrisomy 21 in patient 590.

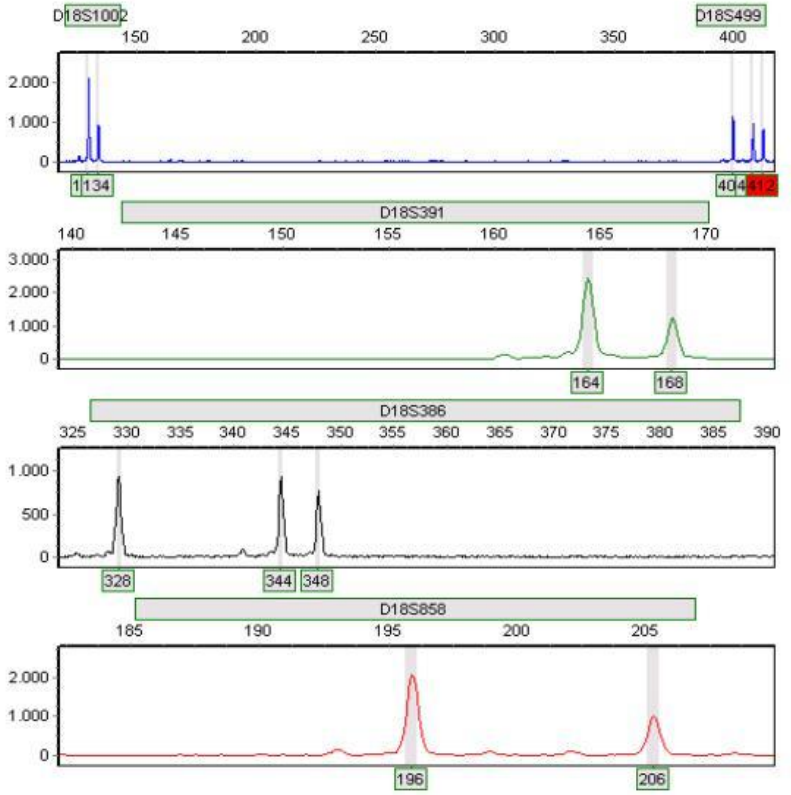


Figura 6 QF-PCR eletropherogramshowingtrisomy 18 in patient 592.

En conjunto, estos estudios moleculares efectuados en pacientes con HPE o sus microformas, explican las causas de alrededor de 20 % de los casos.

SÍNDROMES CON CRANIOSINOSTOSIS

SÍNDROME	GENES
• Apert	• FGFR2
• Crouzon	• FGFR2
• Muenke	• FGFR3, pro250arg
• Pfeiffer	• FGFR1, FGFR2
• Jackson-Weiss	• FGFR2
• Crouzonconacanthosisnigricans	• FGFR2
• Saethre-Chotzen	• TWIST
• Beare Stevenson	• FGFR2
• Displasia craniofrontonasal	• EFNB1

Enfermedad (gene)	Manifestaciones Clinicas
Síndrome de Muenke (FGFR3)	Sinostosis coronal unilateral o bilateral , radiograficamente falanges medias de manos en dedal, otras anomalías; autosómica dominante.
Síndrome de Beare Stevenson (FGFR2)	Cráneo en trébol o cruzonoide, cutis gyrata, palmas y plantas arrugadas, apéndices cutaneo/mucosos, coto umbilical prominente; mutación autosómica dominante.

Caso 4372

American Journal of Medical Genetics Part A 146A:658–660 (2008)

Clinical Report

Second Case of Beare–Stevenson Syndrome With an *FGFR2* Ser372Cys Mutation

Renata Fonseca,[1] Marcelo A. Costa-Lima,[1,2] Viviana Cosentino,[3] and Iêda M. Orioli[1]*

[1]ECLAMC (Estudo Colaborativo Latino Americano de Malformações Congênitas) at Departamento de Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[2]Departamento de Biologia Celular e Genética, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
[3]ECLAMC at Centro de Educación Médica e Investigaciones Clínicas (CEMIC), Buenos Aires, Argentina

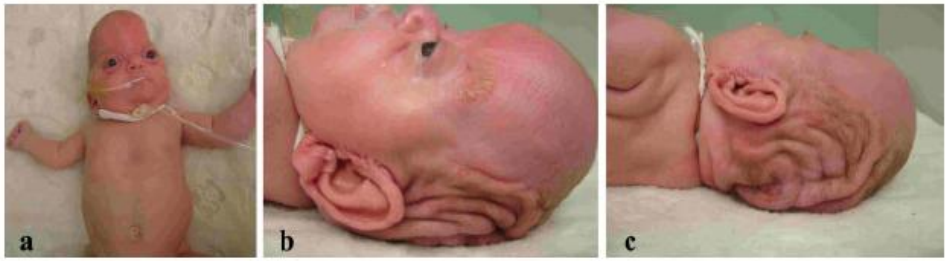


Fig. 1. The patient with Beare–Stevenson syndrome: (**a**) striking cutis gyrata of the posterior scalp, redundant skin on the nape of the neck, and abnormal ears; (**b**) patient at 3 months. Note cloverleaf skull, hypertelorism, prominent eyes, downslanting palpebral fissures, mildface hypoplasia, low nasal bridge, anteverted nares, small mouth, and slight prominence of the umbilical stump; (**c**) lateral view showing ocular proptosis, ptosis of the eyelids, posteriorly angulated ears with preauricular creases, and cutis gyrata. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

c.1115 C>G

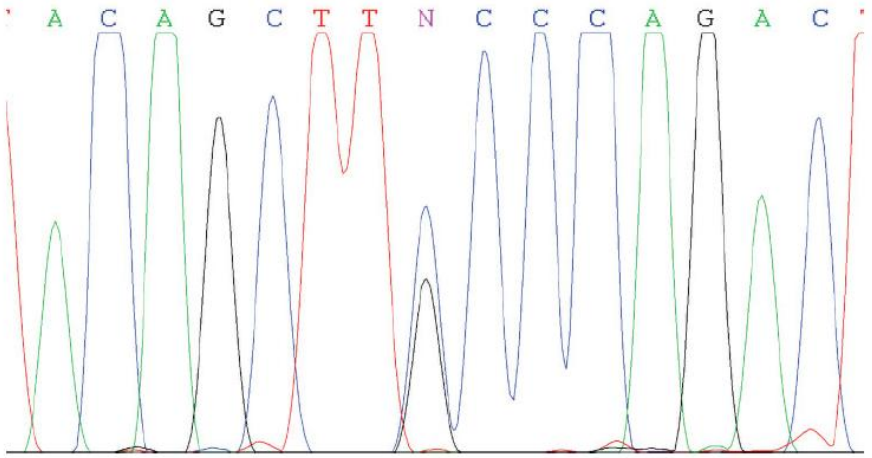


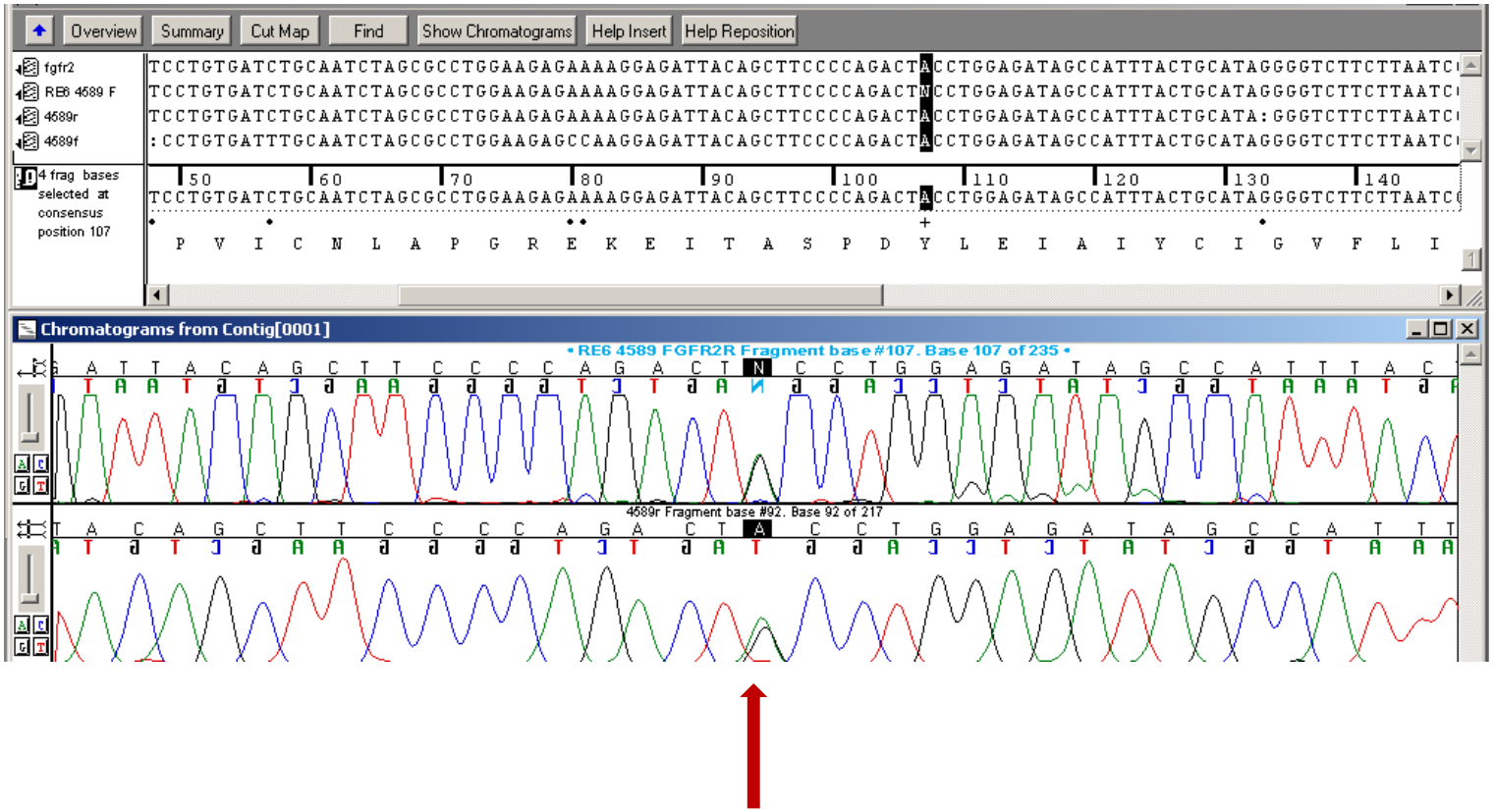
Fig. 2. Chromatogram showing the presence of a 1115C→G transversion in the patient. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

MOLECULAR MEDICINE REPORTS 1: 753-755, 2008

Caso 4589

Beare-Stevenson cutis gyrata syndrome: A new case of a c.1124C→G (Y375C) mutation in the *FGFR2* gene

RENATA FRAGELLI FONSECA[1], MARCELO AGUIAR COSTA-LIMA[1,2], ELIANA TERNES PEREIRA[3], EDUARDO ENRIQUE CASTILLA[4] and IÊDA MARIA ORIOLI[1]



Agradecimientos