Developmental Genetics and Birth Defects

Cleft Lip and/or Palate

BeSHG Interuniversity Course in Genetics, Day 5
11 February 2022
UCL, Brussels

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Brussels, Belgium

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outline

lip and palate embryological development

characteristics – classification – prevalence

etiology

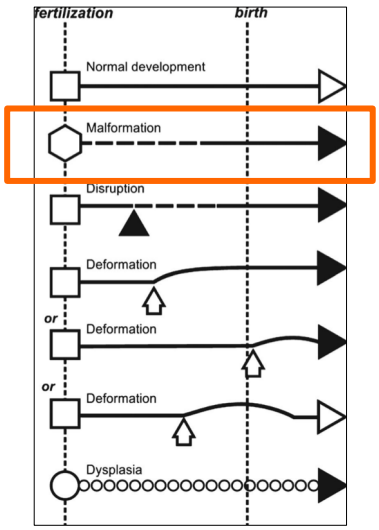
clinical approach

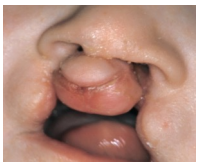
genetic counselling

2

LIP AND PALATE DEVELOPMENT

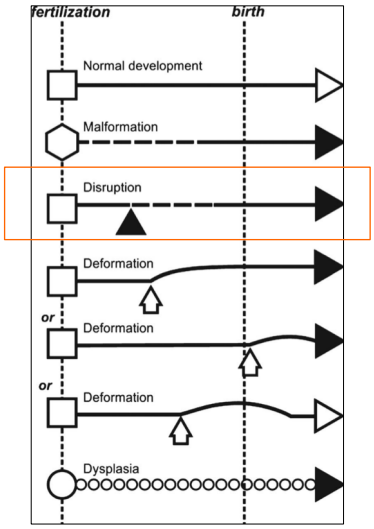
birth defects – mechanisms



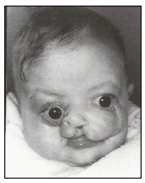


Raoul C. Hennekam, AJMG, 2013

birth defects – mechanisms



secondary to amniotic bands (rare)



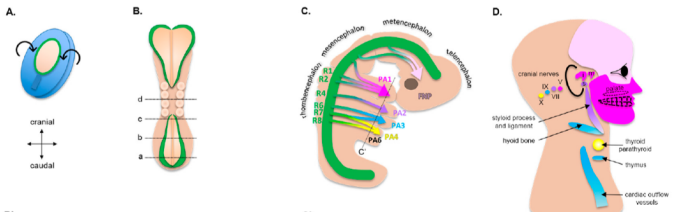
Human Malformations and Related Anomalies. 2nd Ed. Oxford Univ Press. 2006.

Raoul C. Hennekam, AJMG, 2013

lip and palate embryological development

derive from the cranial neuronal crest cells

- proliferation
- migration
- differentiation
- apoptosis
- fusion

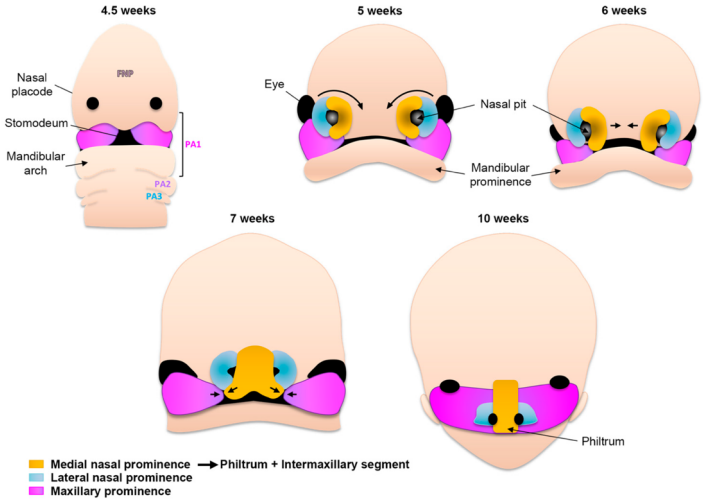


16 days **22days** **32 days**

morphogenesis of pharyngeal arches and their derivatives

Biology **2022**, *11*(2), 153; https://doi.org/10.3390/biology11020153

lip and palate embryological development



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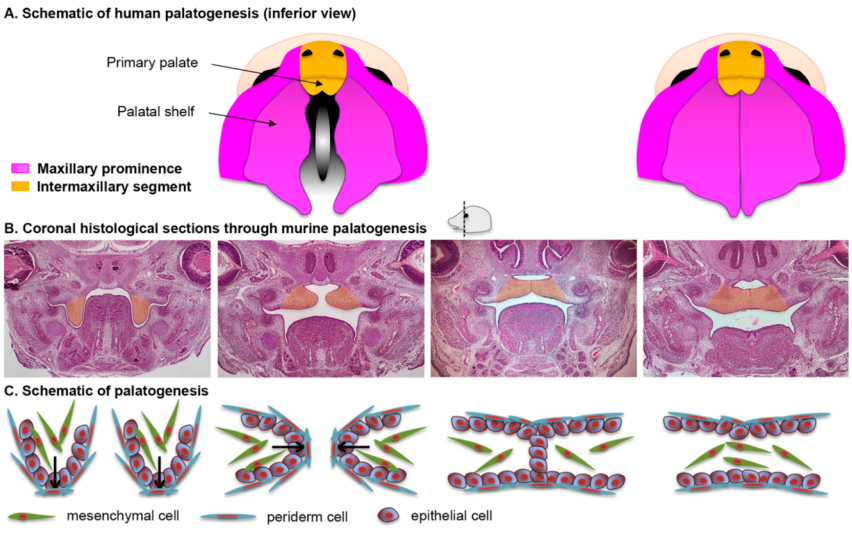
Biology **2022**, *11*(2), 153; https://doi.org/10.3390/biology11020153

lip and palate embryological development



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Biology **2022**, *11*(2), 153; https://doi.org/10.3390/biology11020153

pathways implicated in lip and palate formation

transcription factors

ligands

receptors

adhesion molecules

WNT

FGF

BMP

TGFβ

SHH

PDGF

NOTCH

pathways implicated in lip and palate formation

WNT

FGF

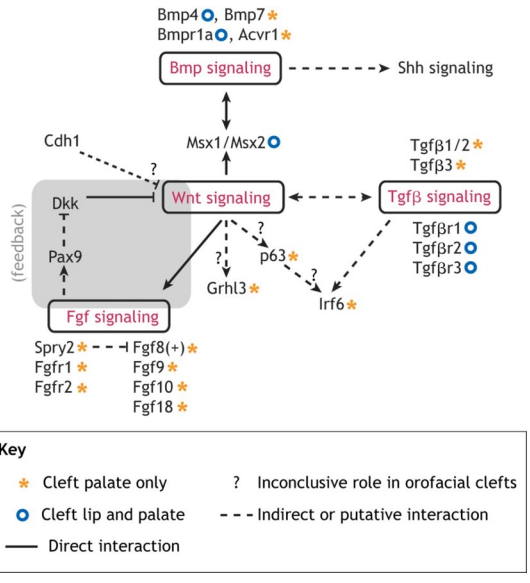
BMP

TGFβ

SHH

PDGF

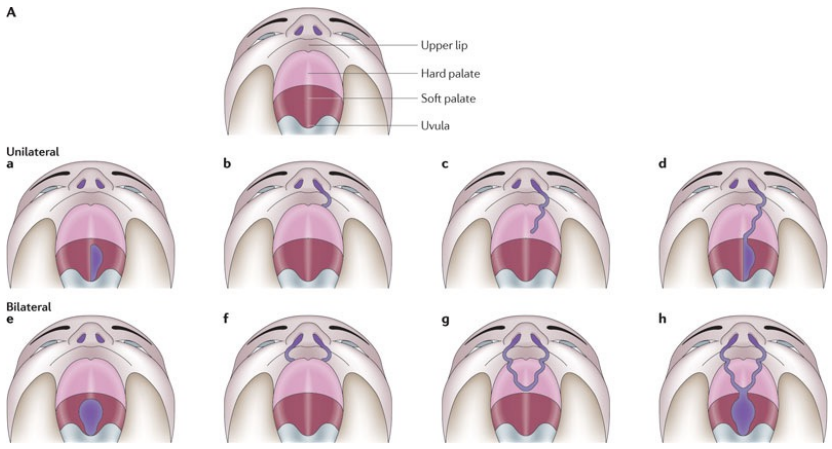
NOTCH



Kurt Reynolds et al. Dis. Model. Mech. 2019;12:dmm037051

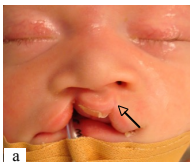
CHARACTERISTICS – CLASSIFICATION CLINICAL APPROACH

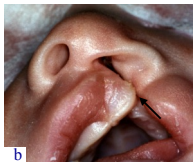
cleft types

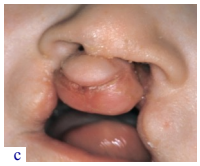


Nat Rev Genet. 2011 Mar; 12(3): 167–178.

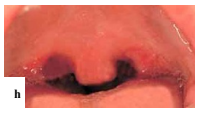
cleft types

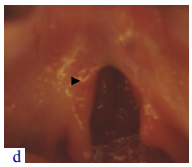

a
incomplete bilateral cleft lip

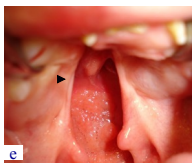

b
complete unilateral cleft lip

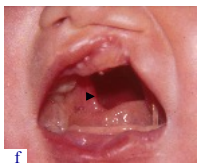

c
bilateral cleft lip

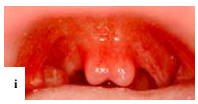

g
absent uvula

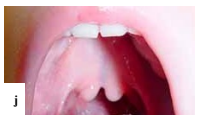

h
broad uvula


d
cleft of the soft palate


e
complete cleft palate


f
cleft lip and palate


i
bifid uvula


j
bifid uvula and submucosal cleft

Photos courtesy of Dr Bénédicte Bayet, Centre Labiopalatin, Cliniques universitaires Saint-Luc, Brussels, Belgium

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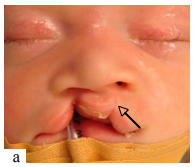
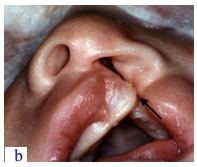
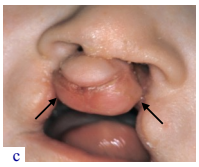
cleft classification

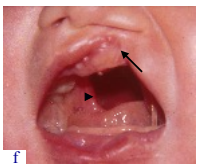

a


b

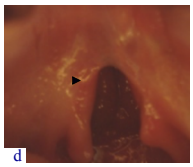

c

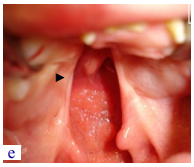
group 1: cleft lip with or without the palate (CL/P)

epidemiological and embryological studies


f

group 2: cleft palate only (CPO)


d


e

Photos courtesy of Dr Bénédicte Bayet, Centre Labiopalatin, Cliniques universitaires Saint-Luc, Brussels, Belgium

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cleft characteristics

most common craniofacial malformation

prevalence : 1/700 (frequent consultation in medical genetics)

220,000 babies per year

cleft lip +/- cleft palate : 1/1000

- 1/500 Asians
- 1/1000 Caucasians
- 1/2500 Africans

cleft palate : 1/2000

cleft lip : 2M/1F

cleft palate : 1M/2F

unilateral cleft lip : 2 left/1 right

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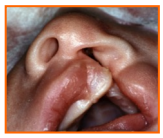
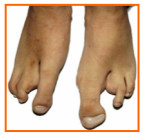
cleft characteristics

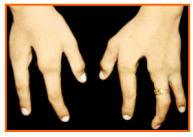
isolated – cleft is the only feature

- 85% CL
- 70% CLP
- 50% CP



syndromic : additional physical abnormalities
major anomaly
at least 3 minor anomalies
and/or intellectual disability





- 15% CL
- 30% CLP
- 50% CP

> 300 syndromes

Pierre Robin

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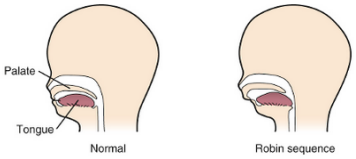
Pierre Robin sequence/syndrome



microretrognathia

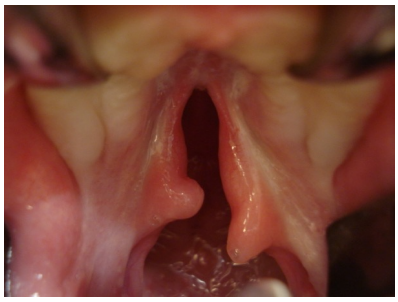
glossoptosis

cleft palate

respiratory obstruction

sequence

syndrome



Photos courtesy of Dr Bénédicte Bayet, Centre Labiopalatin, Cliniques universitaires Saint-Luc, Brussels, Belgium

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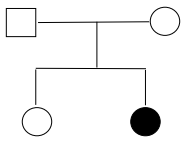
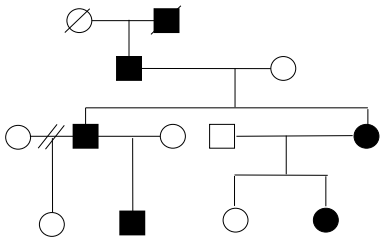
cleft characteristics

80% sporadic



20% familial



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ETIOLOGY

cleft etiology

is highly heterogenous

isolated

syndromic

cleft etiology

is highly heterogenous

isolated

syndromic **> 300 syndromes**
> 75% known etiology
- cytogenetic abnormalities : chromosomal, CNVs
- monogenic : 147 genes (Genomics England PanelApp)
- teratogens

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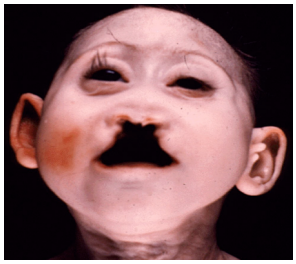
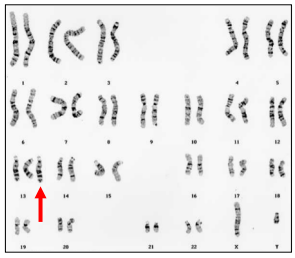
chromosomal abnormalities : Patau syndrome (trisomy 13)

severe condition
in utero death > 95%
intrauterine growth retardation
holoprosencephaly 70%
cleft lip and palate
cardiac malformation
facial dysmorphism
ocular anomalies
postaxial polydactyly
severe psychomotor retardation
…….





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copy number variants

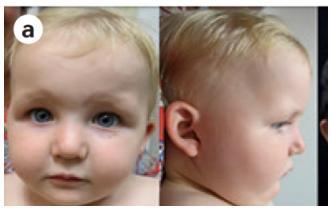
22q11.2 deletion syndrome

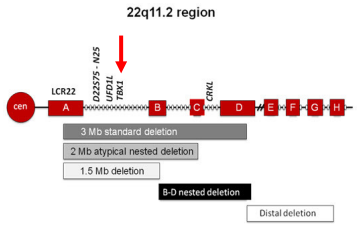
(prevalence 1:2000-4000 live births)

congenital heart defects (75%)
palatal anomalies 75%
(the most common cause of syndromic palatal anomalies)
- velopharyngeal insufficiency
- submucosal cleft palate
- cleft palate
- bifid uvula
- (CL/P)

facial dysmorphism
developmental delay
immune deficiency
neuropsychiatric disorders
……

most frequently : deletion of 3Mb (85%)





https://www.ncbi.nlm.nih.gov/books/NBK1523/; Nat Rev Dis Primers. 2015 Nov 19;1:15071.

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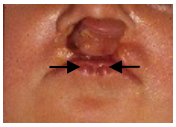
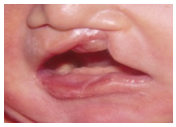
monogenic

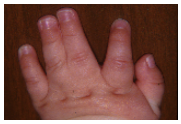
van der Woude syndrome

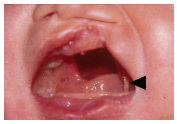




most common cleft syndrome (2%)
prevalence: 1/ 35 000 (3 patients/year in Belgium)
autosomal dominant
high penetrance and variable expressivity
pits on the lower lip (80%)
cleft lip and/or palate (50%)
hypodontia (25%)

popliteal pterygium syndrome





prevalence: 1/ 300 000
autosomal dominant
van der Woude signes +
- buccal synechiae
- popliteal webs
- syndactyly-polydactyly
- genital anomalies
- nail anomalies

mutations in Interferon Regulatory Factor 6 (*IRF6*)

Kondo et al, Nat Genet, 2002

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more complexe patients

14-year-old girl
consanguineous parents
bilateral cleft lip and palate
lip pits
developmental delay
no language
strabismus

NO IMAGE AVAILABLE

phenotype suggestive of van der Woude syndrome + intellectual disability
→ 2 different conditions?

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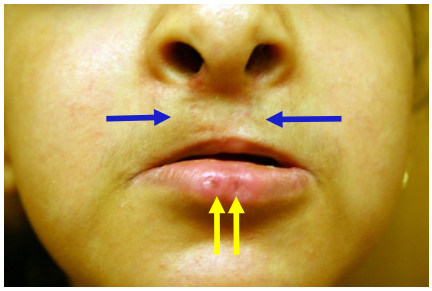
more complexe patients

NO IMAGE AVAILABLE



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more complexe patients

IRF6 sequencing : normal

molecular karyotyping : 5Mb **interstitial deletion 1q32.2-q32.3** covering 38 genes, including *IRF6*

→ **contiguous gene deletion disorder**

Tan *et al. Molecular Cytogenetics* 2013, 6:31
http://www.molecularcytogenetics.org/content/6/1/31

MOLECULAR CYTOGENETICS

CASE REPORT Open Access

De novo 2.3 Mb microdeletion of 1q32.2 involving the Van der Woude Syndrome locus

Ene-Choo Tan[1,2*], Eileen CP Lim[1] and Seng-Teik Lee[3]

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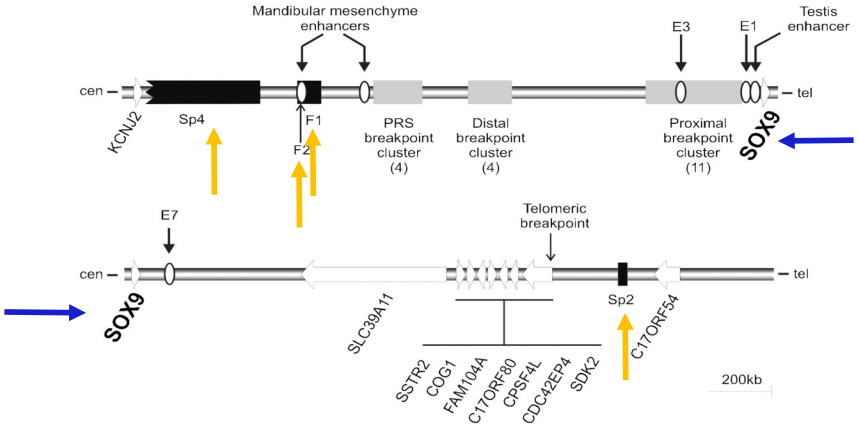
deletions in **regulatory regions**

heterozygous variants in *SOX9* are involved in (acampomelic) campomelic dysplasia

deletions upstream and downstream *SOX9* are associated with Pierre Robin sequence



Gordon C T et al. J Med Genet 2009;46:649-656

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teratogens

Miller syndrome

autosomal recessive

DHODH gene (2010 by WES)

pyrimidine biosynthesis

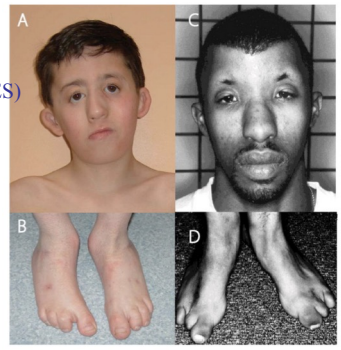
cupped ears

prominent nose

cleft lip and/or palate

micrognathia

absence of the 5th toes



acrofacial dysostosis

methotrexate embryopathy

anti-mitotic activity

cupped ears

hypertelorism

sparse eyebrows

prominent nose

cleft palate

micrognathia

absence of the 4th and 5th toes

Ng et al. Nat Genet, 2010

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cleft etiology

is highly heterogenous

isolated

syndromic

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isolated cleft lip / palate - etiology

the etiology of most of the isolated clefts is unknown

most are sporadic (no family history)

strong genetic component : increased risk in relatives

relative risk to a first-degree relative (sibling, offspring)

CL/P x 32

CP x 56

some pedigrees show clear Mendelian inheritance

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isolated cleft lip / palate - etiology

many approaches have been used to identify genetic risk factors :

linkage, GWAS, sequencing of candidate genes, WES

many loci identified

could represent « mixed models »

multifactorial origin in most

monozygotic twins : 25 to 60%

dizygotic twins : 3 to 6%

monogenic causes in some

rare variants in single major genes : *IRF6*, *GRHL3*, *TP63*, *FGF8*, *FGFR1*, *TBX22*, *PVRL1*…

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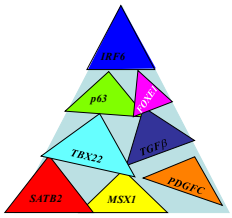
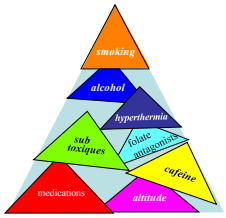
isolated cleft lip / palate : multifactorial origin

combined effects of many independent genes + environnemental factors

genetic predisposition



environmental factors



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isolated cleft lip / palate : environnemental factors

little is known about the environnemental factors

many environmental risk factors have been investigated, but relatively few associations have been clearly established and the contribution to cleft is modest

one example : maternal smoking (active or passive), has been extensively studied

it has consistently been associated with increased risk of CLP

the risk seems to be stronger for CL/P than for CP, and increases with the number of cigarettes per day in CL/P

4% of all orofacial clefts and 12% of bilateral CL/P could be attributed to periconceptional maternal smoking

genetic susceptibility in the context of maternal smoke exposure :

polymorphisms in *TGFA, TGFB3, BMP4, MSX1*,… genes have been associated with increased risk of cleft

M. Garland et al. Birth Defects Res, 2020

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isolated cleft lip / palate : some have monogenic origin

the line between syndromic and non-syndromic orofacial appears to be blurred

WES : genes involved in **syndromes** can also be involved in **isolated** cleft lip/palate

Whole exome sequencing identifies mutations in 10% of patients with familial non-syndromic cleft lip and/or palate in genes mutated in well-known syndromes

Mirta Basha,[1] Bénédicte Demeer,[1,2,3] Nicole Revencu,[1,4] Raphael Helaers,[1] Stephanie Theys,[5] Sami Bou Saba,[6] Odile Boute,[7] Bernard Devauchelle,[8] Geneviève Francois,[9] Bénédicte Bayet,[10] Miikka Vikkula[1]

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WILEY American Journal of medical genetics A

RESEARCH ARTICLE

Unmasking familial CPX by WES and identification of novel clinical signs

Bénédicte Demeer[1,2,3] | Nicole Revencu[1,4] | Raphael Helaers[1] | Bernard Devauchelle[3,5] | Geneviève Francois[6] | Bénédicte Bayet[7] | Miikka Vikkula[1]

- 106 individuals from 63 families
- mutations identified in 7 families
  - *TBX1*
  - *TBX22* (2 families)
  - *LRP6*
  - *GRHL3* (2 families)
  - *TP63*

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CLINICAL APPROACH

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clinical approach

when is the diagnosis made ?

most of the cleft lip (w/o palate) are diagnosed prenatally

prospective study on 36.000 pregnancies (W. Maarse, 2011)

88 % of cleft lip +/- cleft palate

0% cleft palate

cleft palate are usually diagnosed postnatally, in the first days of life

bifid uvula and submucosal cleft palate might not be diagnosed until later in life

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clinical approach

family tree on 3 generations : cleft, other malformations, miscarriages, osteoarthritis, severe myopia, missing teeth, etc…

associated anomalies (cardiac, renal, cerebral, …) : isolated / syndromic

growth

development

detailed clinical examination, minor signs (preauricular pits, tags, lip pits, ...)

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clinical approach

questions :

- is the cleft sporadic or familial ?
- is the cleft isolated or syndromic ?
  - not always obvious at the time of diagnosis, even postnatally
    - additional symptoms : can be mild or can develop later in life
    - importance of the follow-up : propose to see the patient again
  - *Rittler M et al. 2011 :* 7 to 9% of the clefts that are initially thought to be isolated cases are found to have associated abnormalities

→ genetic testing

→ genetic counselling

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multidisciplinary management

team

- pediatrician
- plastic surgeon
- otolaryngologist
- speech therapist
- pediatric dentist and orthodontist
- geneticist
- psychologist
- social worker
- …..

CLP/CP lifetime cost treatment : 200.000 $

patients need multidisciplinary follow-up until the end of puberty

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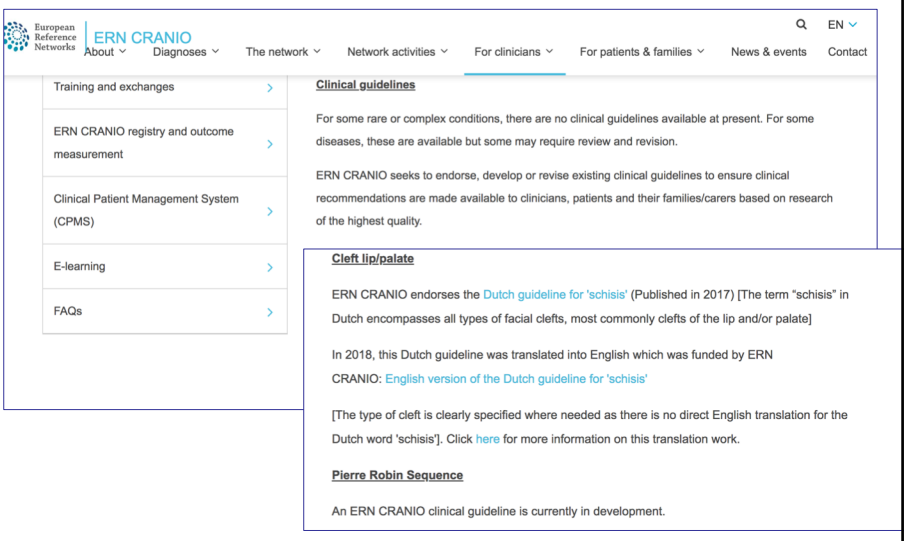
when and what genetic testing should be performed?

there are no guidelines on the genetic strategy in patients with cleft

https://ern-cranio.eu/for-clinicians/standards-of-care/

when and what genetic testing should be performed?

clinical management recommandations



https://ern-cranio.eu/for-clinicians/standards-of-care/

when and what genetic testing should be performed?

patients with cleft should be referred to genetic consultation, if possible before the first surgery

fast referral is indicated in case of associated anomalies (other malformation, growth problem/developmental delay, etc.)

if a genetic testing is performed, a clinical geneticist should be involved and the lab specialist must be well informed about the **phenotype** and **the family history**

→ **of paramount importance for the interpretation of the genetic data!**

when and what genetic testing should be performed?

ERN CRANIO endorses the Dutch guideline for cleft (published in 2017)

RECOMMENDATIONS

if the cleft is associated with additional anomalies, a positive family history or a suspicion of a specific syndrome a genetic testing is indicated

which technique?

- molecular karyotyping
- targeted gene testing (rare), e.g. *IRF6*
- gene panel
- virtual panel WES/WGS
- (… or all in one)

https://ern-cranio.eu/for-clinicians/standards-of-care/

when and what genetic testing should be performed?

ERN CRANIO endorses the Dutch guideline for cleft (published in 2017)

RECOMMENDATIONS

in patients with isolated cleft palate, it is advised to perform a molecular karyotyping first

if normal, +/- consider gene panel/WES (or all in one)

in patients with isolated CL or CLP : discuss the option of a genetic testing with the parents

keep in mind that :

the distinction between syndromic and non-syndromic is sometimes difficult, especially in young children

a non-syndromic cleft can be caused by genes involved in syndromic forms

the study of the yield of this approach is needed!

https://ern-cranio.eu/for-clinicians/standards-of-care/

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GENETIC COUNSELLING

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risk of recurrence

risk depends on :

- the underlying cause
- sporadic/familial
- the type of cleft
- sex of index

recurrence risk for isolated cleft lip w/o cleft palate

Relationship to index case	Recurrence risk (%)
sibling unilateral CL	2-3%
sibling unilateral CL/P	4%
sibling bilateral CL/P	5-6%
two affected siblings	10%
affected sibling and parent	10%*
affected parent	4%

*could represent dominant risk

some of the patients with isolated cleft have mutation in a single gene

recurrence risk for isolated cleft palate

Relationship to index case	Recurrence risk (%)
sibling	2-3%
parent	4%

some of the patients with isolated cleft have mutation in a single gene

recurrence risk for syndromic cleft

precise estimation possible if a diagnosis is known

majority – autosomal dominant inheritance

- incomplete penetrance
- variable expressivity
- « de novo » variants, germline mosaicism

some – X-linked (*TBX22*, *MID1*, etc.) or autosomal recessive (*NECTIN1*, *EPG5*, *etc.)*

conclusions

clefts are common birth defects

complex disorder with heterogeneous etiology : monogenic, polygenic, CNV, chromosomal, environment, teratogens

sporadic versus familial

isolated versus syndromic

cleft palate requires multidisciplinary management from birth to adulthood

major impact on the patient, family and public health

etiology known for the majority of syndromic cleft and for a minority of the isolated cleft

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