Developmental Genetics and Birth Defects

Cleft Lip and/or Palate

BeSHG Interuniversity Course in Genetics
09 February 2024
UCL, Brussels

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outline

lip and palate embryological development

characteristics – classification – prevalence

genetic aspects - etiology

(management, genetic testing strategy and genetic counselling are beyond the scope of this course; they will be covered in the MaNaMa course in 04/2024)

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LIP AND PALATE DEVELOPMENT

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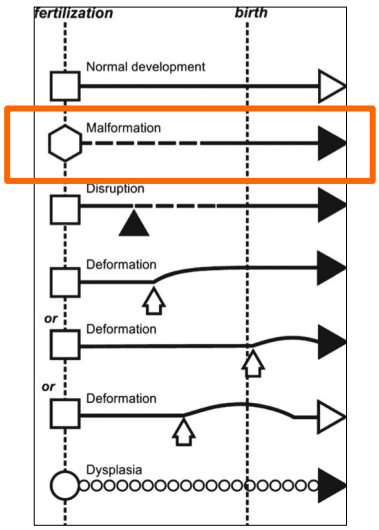
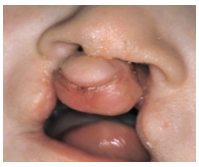
birth defects : mechanisms





Raoul C. Hennekam, AJMG, 2013

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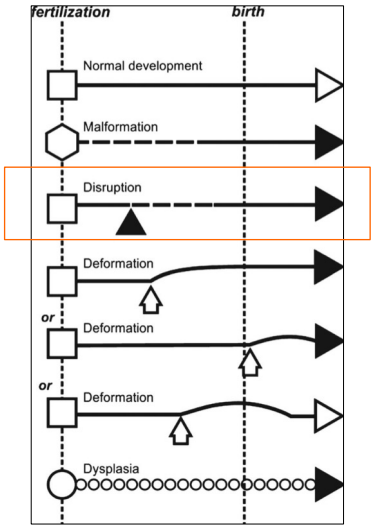
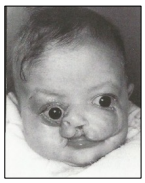
birth defects : mechanisms



secondary to amniotic bands (rare)



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

Raoul C. Hennekam, AJMG, 2013

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lip and palate embryological development

derive from the cranial neuronal crest cells

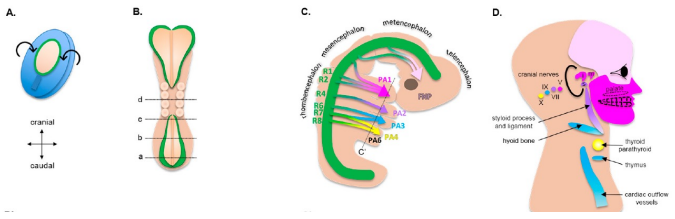
proliferation

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

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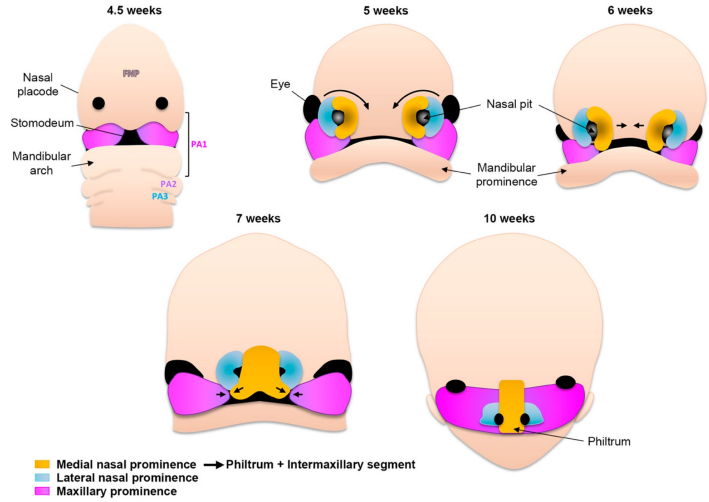
lip and palate embryological development



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

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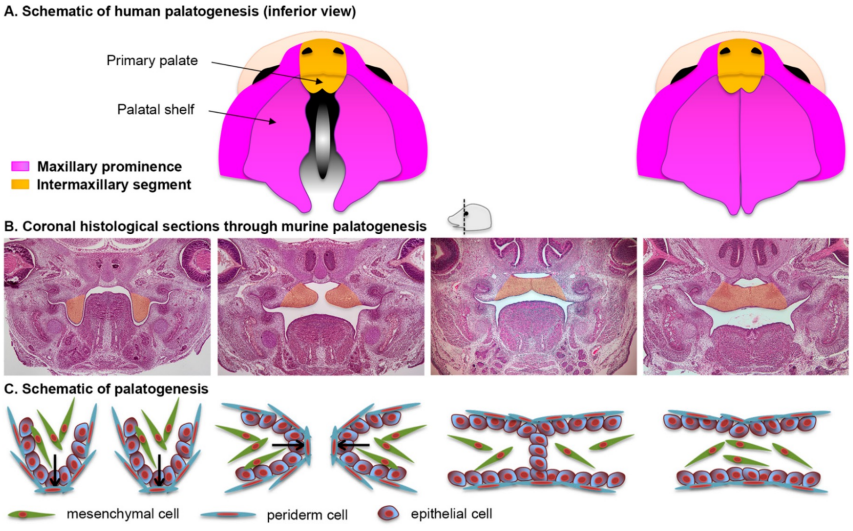
lip and palate embryological development



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

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pathways implicated in lip and palate formation

transcription factors

ligands

receptors

adhesion molecules

WNT

FGF

BMP

TGFβ

SHH

PDGF

NOTCH

proliferation, migration, differentiation, apoptosis, fusion, ….

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9

pathways implicated in lip and palate formation

WNT

FGF

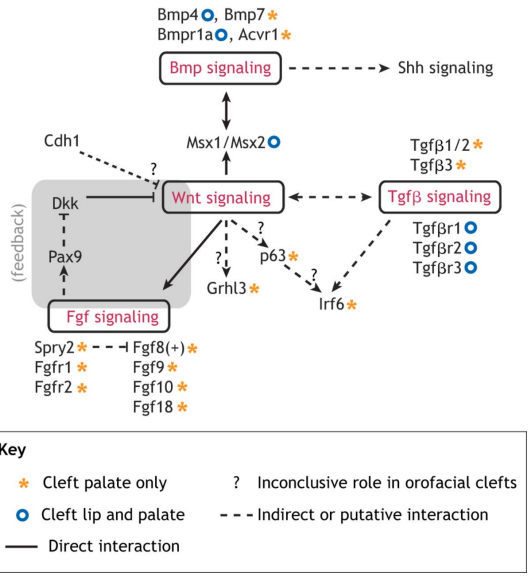
BMP

TGFβ

SHH

PDGF

NOTCH



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

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CHARACTERISTICS – CLASSIFICATION PREVALENCE

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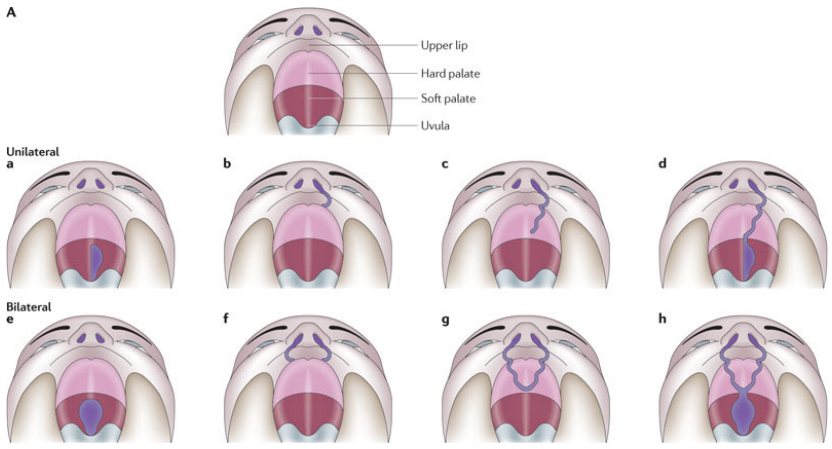
cleft types



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

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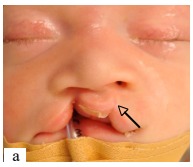
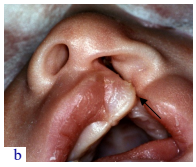
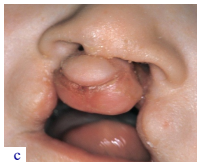
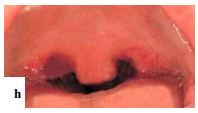
cleft types

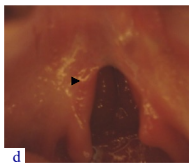

a
incomplete bilateral cleft lip

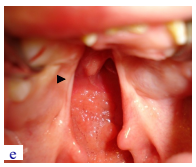

b
complete unilateral cleft lip

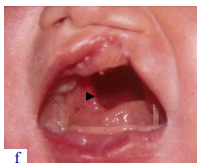

c
bilateral cleft lip

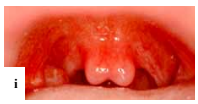

g
absent uvula

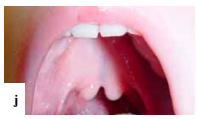

h
broad uvula


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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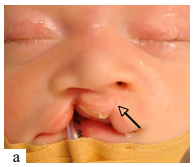
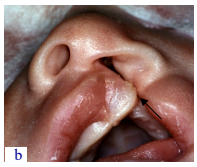
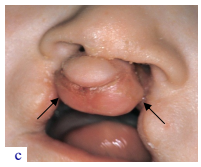
cleft classification

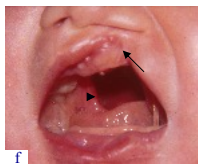

a


b

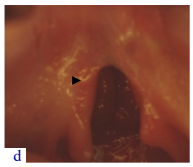

c

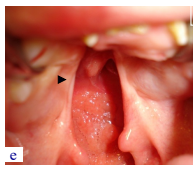
group 1: cleft lip with or without the palate (CL/P)

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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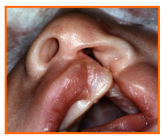
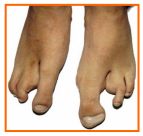
cleft characteristics

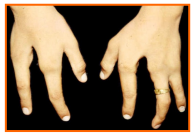
isolated : cleft is the only feature

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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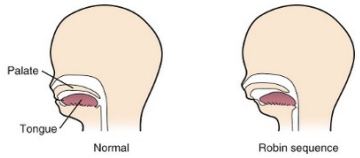
Pierre Robin sequence/syndrome



microretrognathia

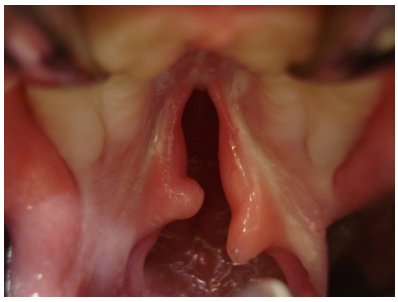
glossoptosis

cleft palate

respiratory obstruction

sequence

syndrome



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

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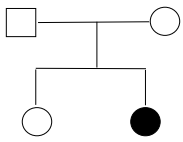
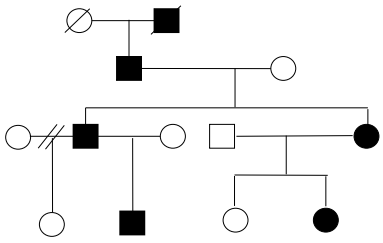
cleft characteristics

80% sporadic



20% familial



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GENETIC ASPECTS

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cleft etiology

is highly heterogenous

isolated

syndromic

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cleft etiology

is highly heterogenous

isolated

syndromic **> 300 syndromes**
> 75% known etiology
- cytogenetic abnormalities : chromosomal, CNVs
- monogenic (> 150 genes)
- teratogens

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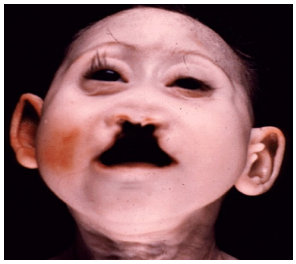
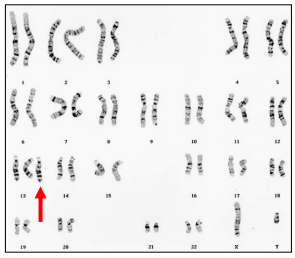
chromosomal abnormalities : Patau syndrome (trisomy 13)

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

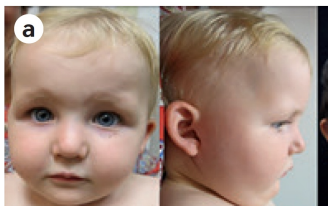
22q11.2 deletion syndrome

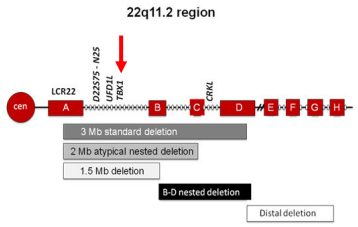
(prevalence 1:2000-4000 live births)

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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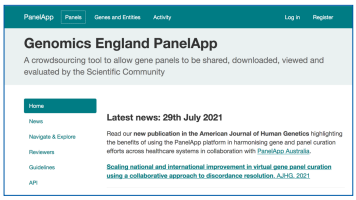
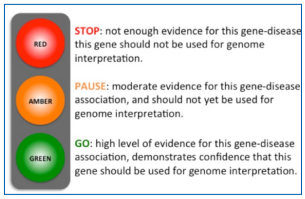
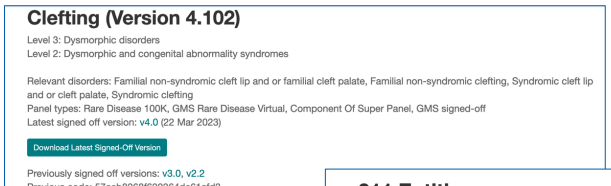
monogenic







311 Entities
301 reviewed, 156 green

Expert – curated list of genes for :
- syndromic cleft lip and/or cleft palate
- familial non-syndromic cleft lip and/or cleft palate

https://panelapp.genomicsengland.co.uk/

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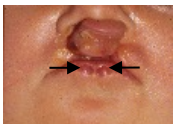
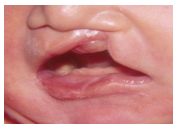
monogenic

van der Woude syndrome

most common cleft syndrome (2%)

prevalence: 1/ 35 000 (3 patients/year in Belgium)

autosomal dominant

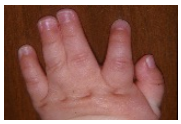
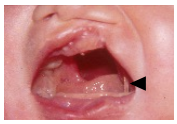
high penetrance and variable expressivity

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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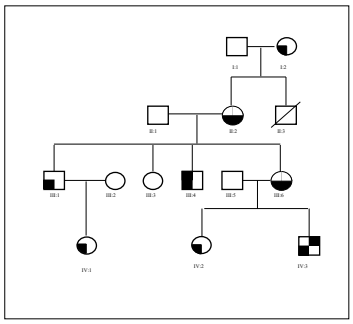
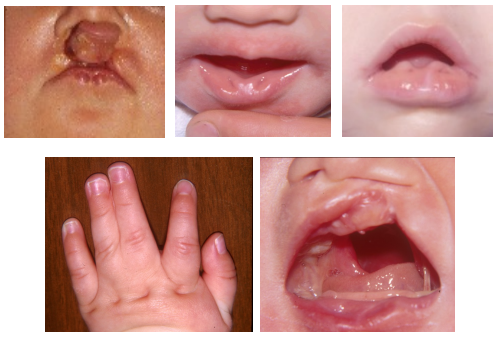
VWS / PPS syndromes – variable expressivity





ONLINE MUTATION REPORT

Six families with van der Woude and/or popliteal pterygium syndrome: all with a mutation in the *IRF6* gene

M Ghassibé, N Revencu, B Bayet, Y Gillerot, R Vanwijck, C Verellen-Dumoulin, and M Vikkula

J Med Genet 2004;**41**:e15 (http://www.jmedgenet.com/cgi/content/full/41/2/e15). doi: 10.1136/jmg.2003.009274

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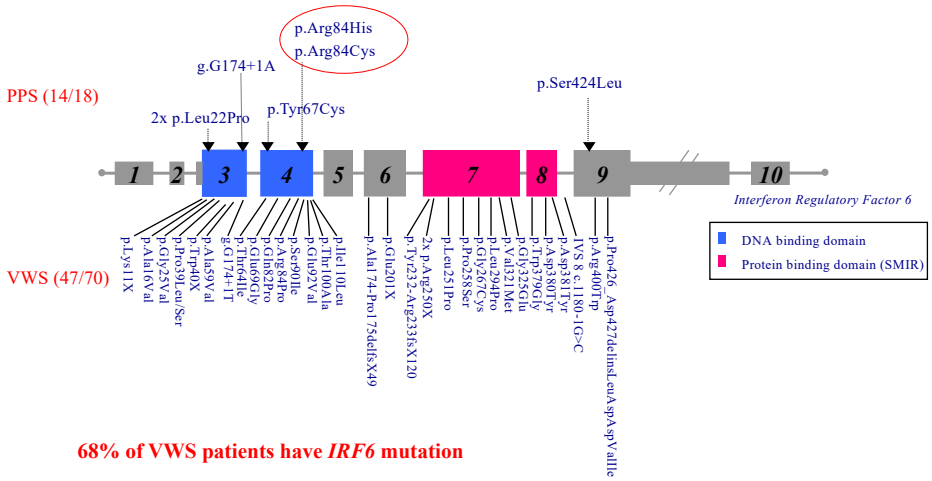
distribution of *IRF6* mutations



68% of VWS patients have *IRF6* mutation

Ghassibe et al., *J Med Genet* 2004
Desmyter, Ghassibe et al., *Molecular Syndromology 2010*

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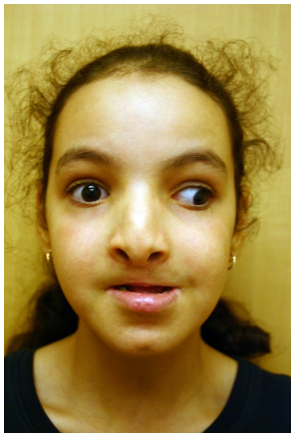
more complexe patients

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



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

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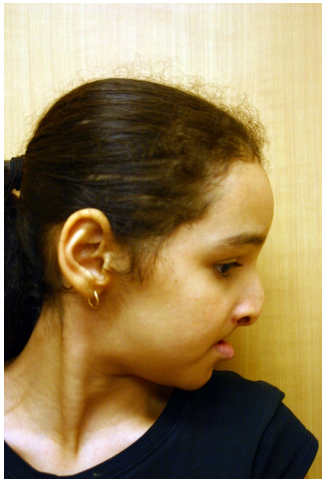
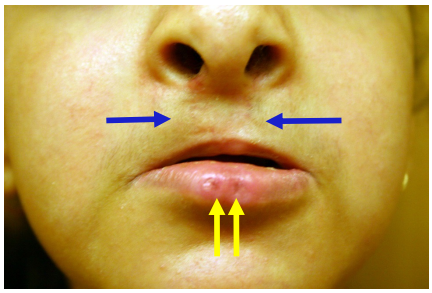
more complexe patients





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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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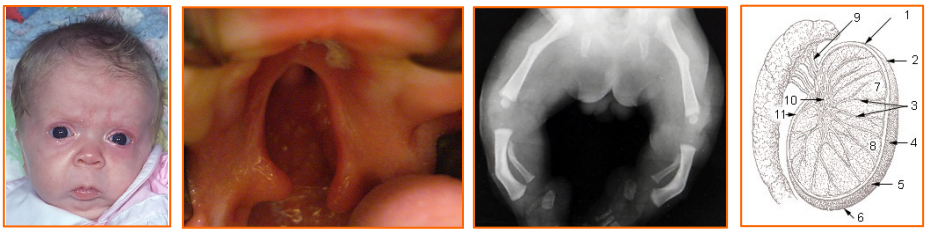
SOX9 and campomelic dysplasia



heterozygous loss-of-function mutations in the *SOX9* gene

Gorlin collection

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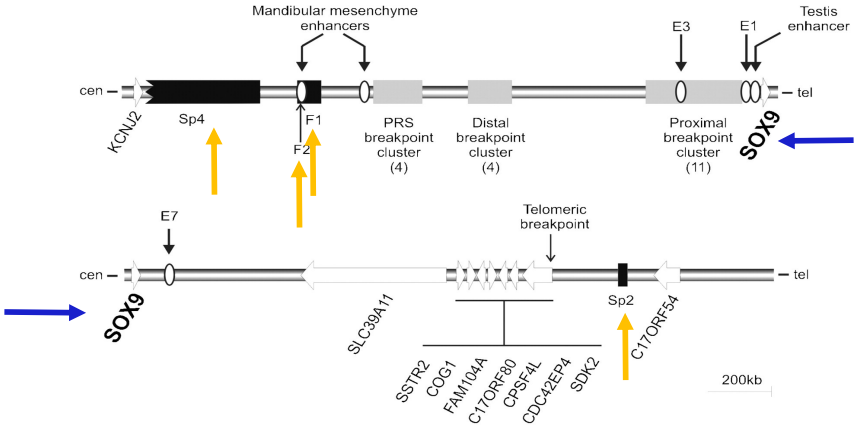
deletions in **regulatory regions**

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

deletions upstream and downstream *SOX9* 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

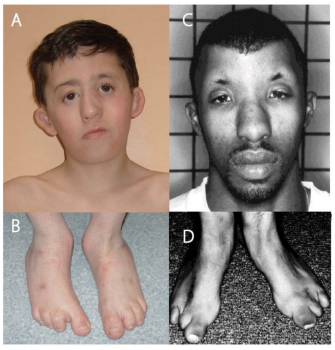
cupped ears

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

different approaches have been used to identify genetic risk factors :

linkage, GWAS, sequencing of candidate genes, WES

many loci identified

current theory : 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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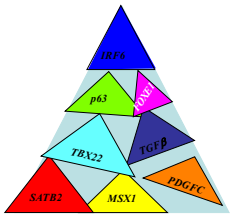
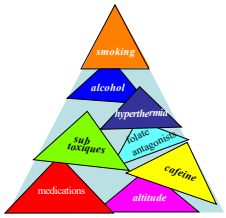
isolated cleft lip / palate : multifactorial origin

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

maternal illnesses

- diabetes mellitus before pregnancy
- obesity (body mass index ≥ 30)
- infections
- hyperthermia

medication

- antiepileptic drugs : topiramate or valproic acid
- steroid treatment

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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

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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linkage studies in isolated cleft

- linkage studies are based on co-segregation of genetic loci with disease
- performed in large, multiplex families (two or more affected members)
- linkage analysis is a powerful approach for mapping individual genes for traits following clear Mendelian patterns within multiplex families, but it is less effective in mapping genes for complex traits
- succes is limited by the genetic complexity of isolated cleft
- meta-analysis combining six studies identified 6 loci : 1q32, 2p13, 3q27–28, 9q21, 14q21–24, and 16q24

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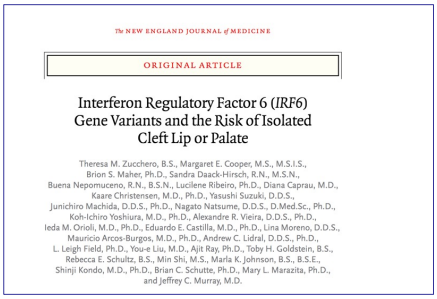
common variants in and near *IRF6* are associated with isolated cleft



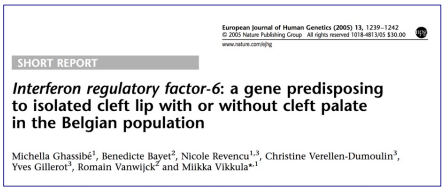
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Interferon Regulatory Factor 6 (*IRF6*) Gene Variants and the Risk of Isolated Cleft Lip or Palate

Theresa M. Zucchero, B.S., Margaret E. Cooper, M.S., M.S.I.S., Brion S. Maher, Ph.D., Sandra Daack-Hirsch, R.N., M.S.N., Buena Nepomuceno, R.N., B.S.N., Lucilene Ribeiro, Ph.D., Diana Caprau, M.D., Kaare Christensen, M.D., Ph.D., Yasushi Suzuki, D.D.S., Junichiro Machida, D.D.S., Ph.D., Nagato Natsume, D.D.S., D.Med.Sc., Ph.D., Koh-Ichiro Yoshiura, M.D., Ph.D., Alexandre R. Vieira, D.D.S., Ph.D., Ieda M. Orioli, M.D., Ph.D., Eduardo E. Castilla, M.D., Ph.D., Lina Moreno, D.D.S., Mauricio Arcos-Burgos, M.D., Ph.D., Andrew C. Lidral, D.D.S., Ph.D., L. Leigh Field, Ph.D., You-e Liu, M.D., Ajit Ray, Ph.D., Toby H. Goldstein, B.S., Rebecca E. Schultz, B.S., Min Shi, M.S., Marla K. Johnson, B.S., B.S.E., Shinji Kondo, M.D., Ph.D., Brian C. Schutte, Ph.D., Mary L. Marazita, Ph.D., and Jeffrey C. Murray, M.D.

1q32 locus



European Journal of Human Genetics (2005) 13, 1239–1242

www.nature.com/ejhg

SHORT REPORT

***Interferon regulatory factor-6*: a gene predisposing to isolated cleft lip with or without cleft palate in the Belgian population**

Michella Ghassibé[1], Benedicte Bayet[2], Nicole Revencu[1,3], Christine Verellen-Dumoulin[3], Yves Gillerot[3], Romain Vanwijck[2] and Miikka Vikkula*[,1]

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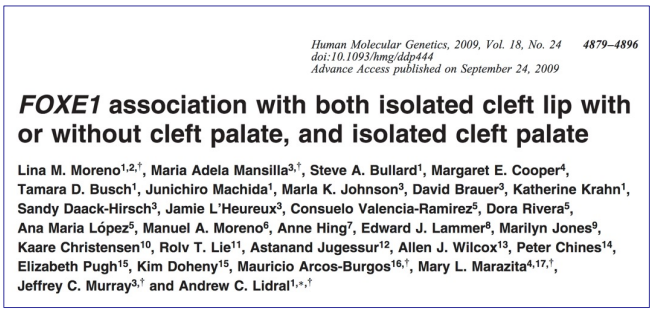
variants within *FOXE1* are associated with isolated cleft

- mutations in *FOXE1* - congenital hypothyroidism, spiky hair, and cleft palate, AR
- fine-mapping of the 9q21 region



Human Molecular Genetics, 2009, Vol. 18, No. 24 ***4879–4896***
doi:10.1093/hmg/ddp444
Advance Access published on September 24, 2009

FOXE1 association with both isolated cleft lip with or without cleft palate, and isolated cleft palate

Lina M. Moreno[1,2,†], Maria Adela Mansilla[3,†], Steve A. Bullard[1], Margaret E. Cooper[4], Tamara D. Busch[1], Junichiro Machida[1], Marla K. Johnson[3], David Brauer[3], Katherine Krahn[1], Sandy Daack-Hirsch[3], Jamie L'Heureux[3], Consuelo Valencia-Ramirez[5], Dora Rivera[5], Ana Maria López[5], Manuel A. Moreno[6], Anne Hing[7], Edward J. Lammer[8], Marilyn Jones[9], Kaare Christensen[10], Rolv T. Lie[11], Astanand Jugessur[12], Allen J. Wilcox[13], Peter Chines[14], Elizabeth Pugh[15], Kim Doheny[15], Mauricio Arcos-Burgos[16,†], Mary L. Marazita[4,17,†], Jeffrey C. Murray[3,†] and Andrew C. Lidral[1,*,†]

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genome wide association studies in isolated cleft

- GWASs : test for differences in frequencies of common markers in samples of affected and unaffected individuals from a population
- GWAS accounts for about 30% of the heritability
- > 40 genes and loci associated with isolated CL/P
- the SNPs identified through GWASs might themselves be functional, but many are in linkage disequilibrium with causal variants
- GWASs do not identify rare and de novo variants

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rare causal variants identified by candidate gene approach

- selection of the candidate genes to study requires previous knowledge on the molecular pathways involved in the disease mechanism
- several genes have been found to be associated with isolated cleft
  - the first one *IRF6*
  - *TBX22, FGFR1, …*

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rare causal variants identified by candidate gene approach

***IRF6* mutation screening in nonsyndromic orofacial clefting: analysis of 1521 families**

Elizabeth J. Leslie[1], Daniel C. Koboldt[2], Chul Joo Kang[2], Lian Ma[3], Jacqueline T. Hecht[4], George L. Wehby[5], Kaare Christensen[6], Andrew E. Czeizel[7], Frederic W.-B. Deleyiannis[8], Robert S. Fulton[2], Richard K. Wilson[2], Terri H. Beaty[9], Brian C. Schutte[10], Jeffrey C. Murray[11], and Mary L. Marazita[1]

Clin Genet. 2016 Jul;90(1):28-34.

- lip pits are absent in 15% VWS cases, resulting in a phenotype mimicking non-syndromic cleft
- 0.3% of individuals with isolated cleft should have *IRF6* mutation
  - 1,521 case-parent trio
  - seven presumably pathogenic variants identified 0.46%

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isolated cleft lip / palate : some have monogenic origin

the line between syndromic and non-syndromic clefts is 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]

J Med Genet. 2018 Jul;55(7):449-458

Received: 2 May 2018 | Revised: 15 August 2018 | Accepted: 17 August 2018
DOI: 10.1002/ajmg.a.40630
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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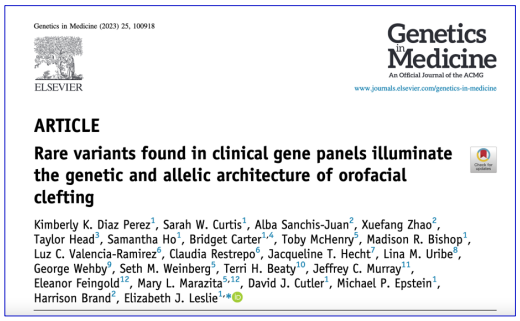
isolated cleft lip / palate : some have monogenic origin



Genetics in Medicine (2023) 25, 100918

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Genetics in Medicine
An Official Journal of the ACMG
www.journals.elsevier.com/genetics-in-medicine

ARTICLE

Rare variants found in clinical gene panels illuminate the genetic and allelic architecture of orofacial clefting

Kimberly K. Diaz Perez[1], Sarah W. Curtis[1], Alba Sanchis-Juan[2], Xuefang Zhao[2], Taylor Head[3], Samantha Ho[1], Bridget Carter[1,4], Toby McHenry[2], Madison R. Bishop[1], Luz C. Valencia-Ramirez[6], Claudia Restrepo[6], Jacqueline T. Hecht[7], Lina M. Uribe[8], George Wehby[9], Seth M. Weinberg[5], Terri H. Beaty[10], Jeffrey C. Murray[11], Eleanor Feingold[12], Mary L. Marazita[5,12], David J. Cutler[1], Michael P. Epstein[1], Harrison Brand[2], Elizabeth J. Leslie[1,*]

Cohort
841 index cases (621 "simplex")
most trios (child and parents)
- CLP: 660
- CP : 74
- CL: 107

heterogenous cohort with incomplete phenotypic data
no other major malformations
no intellectual deficiency

Control sample : 294 child-parent trios

418 genes analysed

76 likely pathogenic variants identified in 39 genes
9.04% of cases and 1.02% of controls

likely pathogenic variants identified in :

- CLP : 9.09% (60/660)
- CP : 17.6% (13/74)
- CL: 2.80% (3/107)

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conclusions

clefts are common birth defects

complex disorder with heterogeneous etiology : monogenic, CNV, chromosomal, teratogens, multifactorial

sporadic versus familial

isolated versus syndromic

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

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