Developmental Genetics and Birth Defects

Cleft Lip and/or Palate

Permanent Education Course
in Human Genetics
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outline

- lip and palate embryological development
- characteristics – classification – prevalence
- etiology
- genetic counselling

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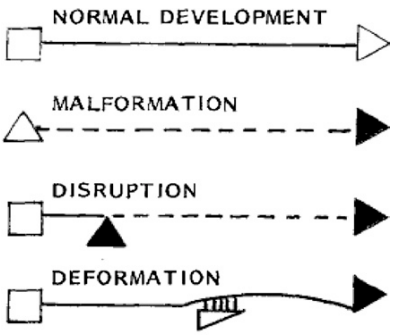
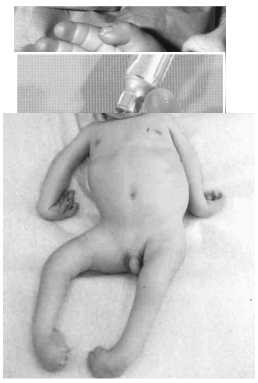
LIP AND PALATE DEVELOPMENT

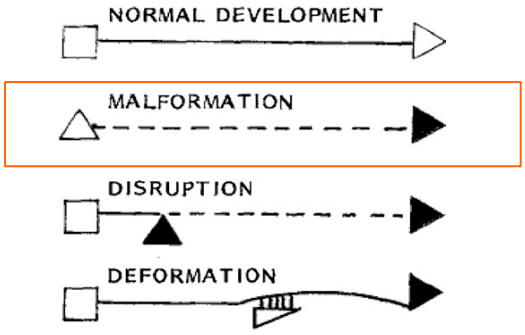
birth defects – mechanisms





Emery and Rimoins's Principles and practice of medical genetics, 6th edition

birth defects – mechanisms



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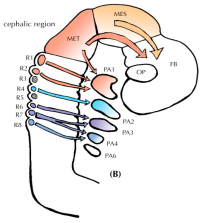
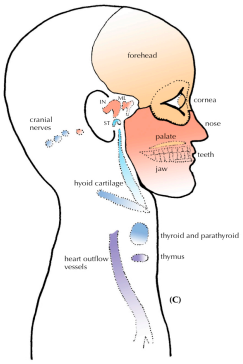
lip and palate embryological development

- proliferation
- migration
- differentiation
- apoptosis
- fusion





http://commons.wikimedia.org/wiki/File:Cranial_Neural_Crest_Cells_-_migration.jpg

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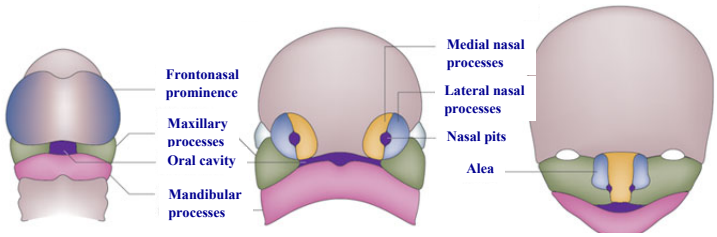
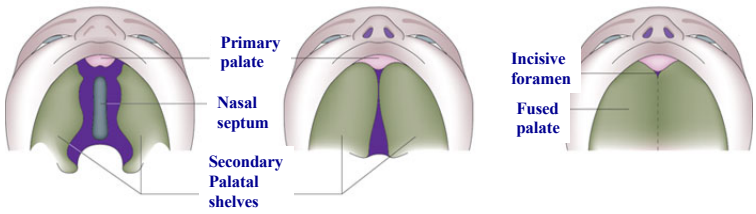
lip and palate embryological development



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

7

lip and palate embryological development



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

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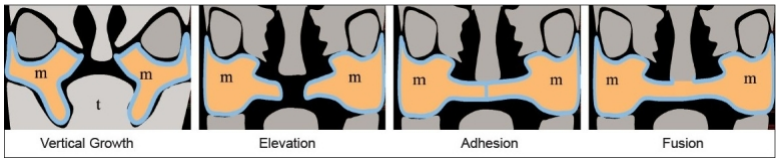
lip and palate embryological development



Indian J Plast Surg 2009 Oct; 42(Suppl): S35–S50.

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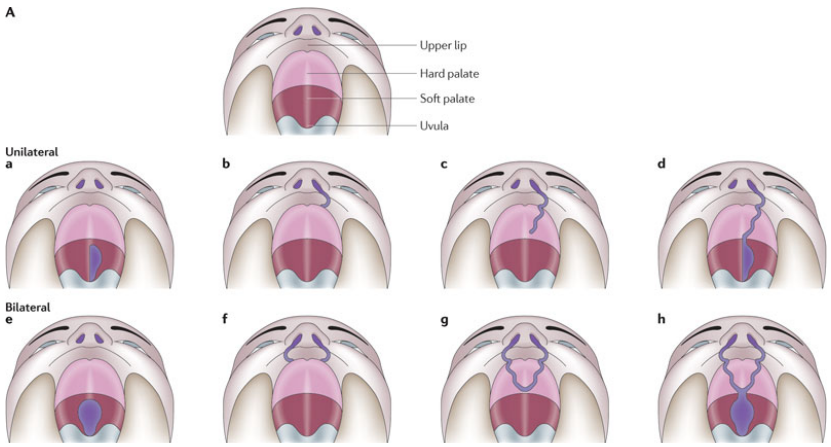
CHARACTERISTICS – CLASSIFICATION CLINICAL APPROACH

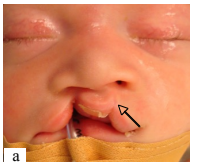
10

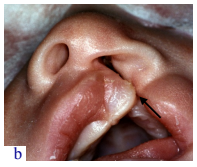
cleft types

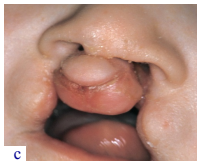


Nat Rev Genet. 2011 Mar; 12(3): 167–178.

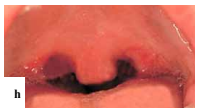
cleft types

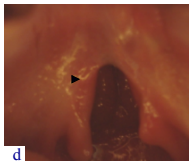

a
incomplete bilateral cleft lip

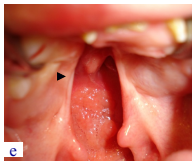

b
complete unilateral cleft lip

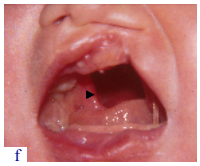

c
bilateral cleft lip

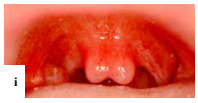

g
absent uvula


h
broad uvula


d
cleft of the soft palate


e
complete cleft palate

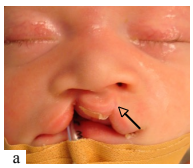

f
cleft lip and palate


i
bifid uvula


j
bifid uvula and submucosal cleft

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

cleft classification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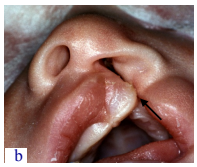
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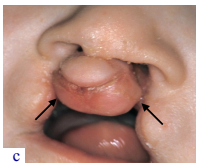
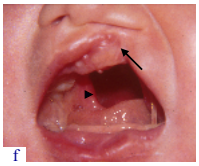
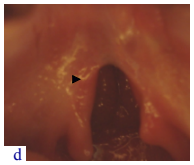

c

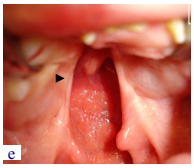
group 1: cleft lip with or without the palate (CL/P)

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

- prevalence : 1/700 (frequent consultation in medical genetics)
- most common craniofacial malformation
- 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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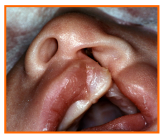
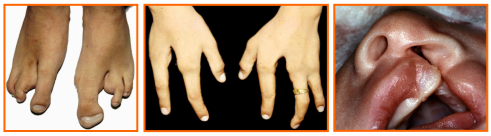
cleft characteristics

- isolated – cleft is the only feature
  - 85% CL
  - 70% CLP
  - 50% CP



- syndromic - additional physical/cognitive abnormalities
  - 15% CL
  - 30% CLP
  - 50% CP

  > 300 syndromes



- Pierre Robin

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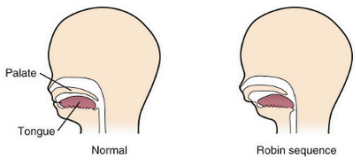
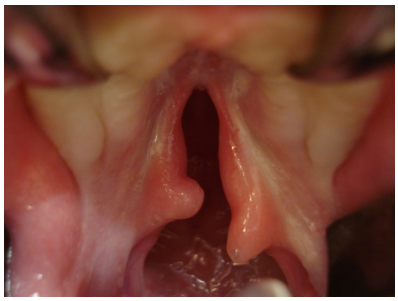
Pierre Robin sequence/syndrome



- 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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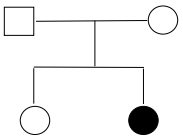
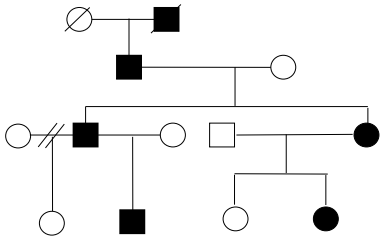
cleft characteristics

- 80% sporadic



- 20% familial



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cleft characteristics

- sporadic and isolated = 1 individual **NO** other anomalies
- familial and isolated = several individuals **NO** other anomalies
- sporadic and syndromic = 1 individuals **AND** other anomalies
- familial and syndromic = several individuals **AND** other anomalies

→ **implications for the management and the genetic counseling**

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clinical approach

- prenatal diagnosis : prospective study on 36.000 pregnancies (W. Maarse, 2011)
  - 88 % of cleft lip +/- cleft palate
  - 0% cleft palate
- type of cleft : CL; CLP; CP; PR
- associated anomalies (cardiac, renal, cerebral, …) : isolated / syndromic
- growth
- development
- detailed clinical examination, minor signs !
- three generation family history tree : sporadic/familial
- etiology
- 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 $

require multiple interventions from birth until the end of puberty

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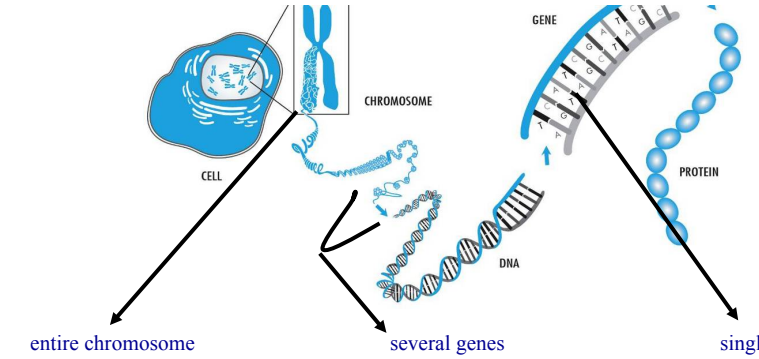
ETIOLOGY

syndromic cleft etiology

- sporadic and isolated
- familial and isolated
- sporadic and syndromic
- familial and syndromic

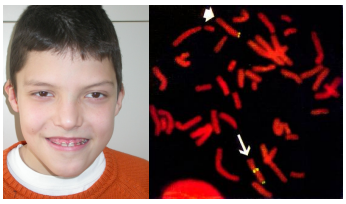
- > 300 syndromes
- > 75% known etiology
- 147 genes on Genomics England PanelApp

syndromic cleft - etiology

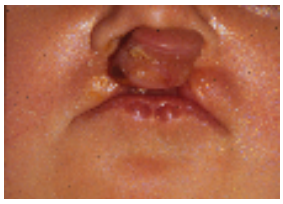


entire chromosome | several genes | single gene

Patau syndrome (trisomy 13)



22q11.2 microdeletion



van der Woude syndrome

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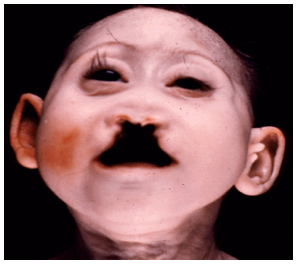
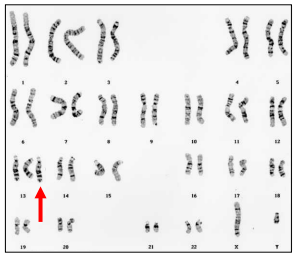
chromosomal abnormalities : Patau syndrome (trisomy 13)

- *in utero* death > 95%
- intrauterine growth retardation
- holoprosencephaly 70%
- **cleft**
- congenital cardiac anomaly
- facial dysmorphism
- ocular anomalies
- postaxial polydactyly
- severe psychomotor retardation
- ……





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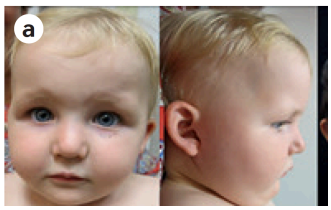
copy number variants

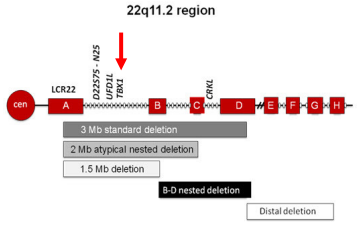
22q11.2 deletion syndrome

(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
- ……

- deletion of 3Mb flanked by LCR
- FISH, MLPA or molecular carotyping





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

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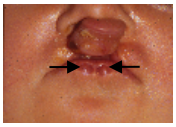
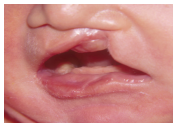
monogenic

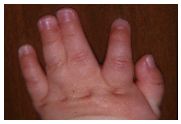
van der Woude syndrome

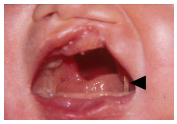




- most common cleft syndrome (2%)
- prevalence: 1/ 35 000 (3 patients/year in Belgium)
- autosomal dominant
- high penetrance and variable expressivity
- pits on the lower lip (80%)
- cleft lip and/or palate (50%)
- hypodontia (25%)

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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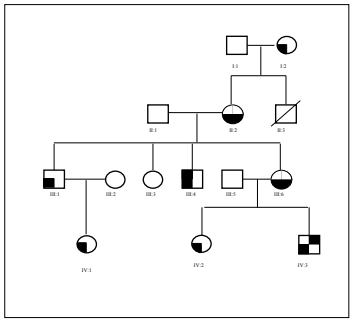
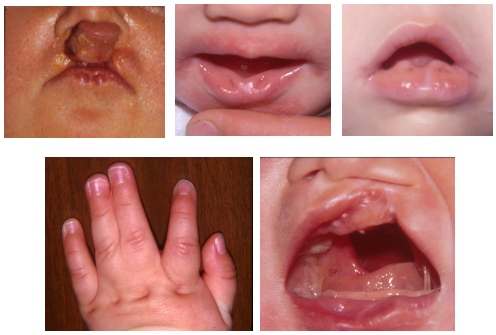
VWS / PPS syndromes – variable expressivity





CP
bifid uvula
CL/P
lip pits

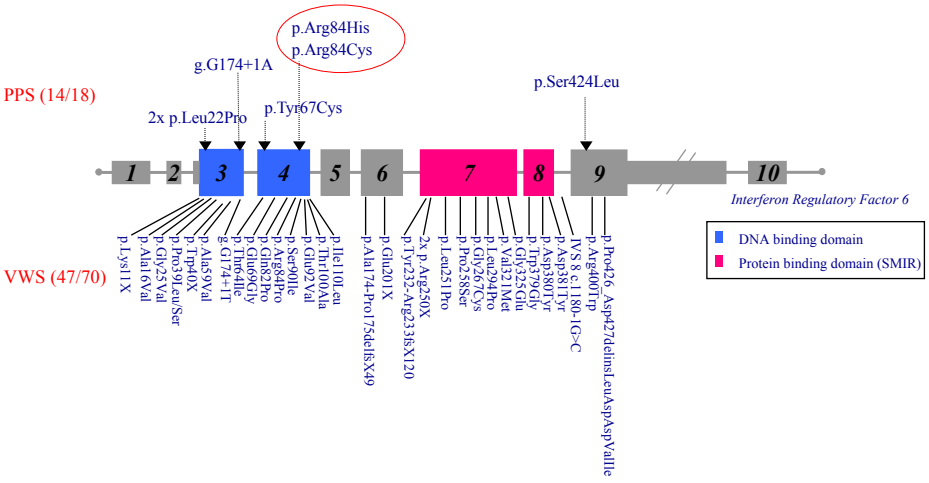
ONLINE MUTATION REPORT

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

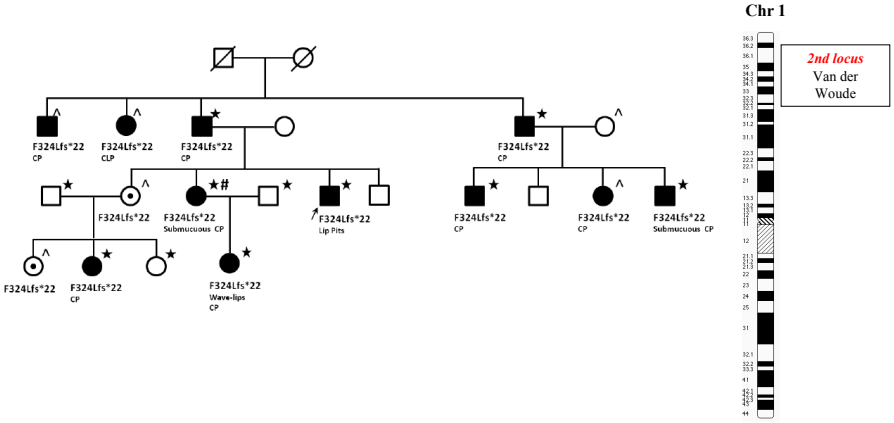
non-random distribution of *IRF6* mutations



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

68% of VWS patients have *IRF6* mutation

VWS 2nd locus



Koillien et al. EJHG, 2001

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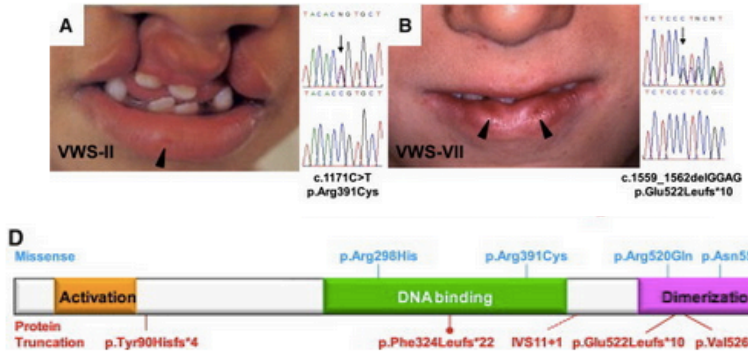
identification of mutations in *GRHL3* in patients with VWS without *IRF6* mutation



5% of patients with VWS have *GRHL3* mutation

Peyrard-Janvid et al. AJHG, 2014

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comparison of VWS phenotypes caused by mutations in *IRF6* and *GRHL3*

Has Phenotype?	CL/P	CP	Cleft Only	Lip Pits	Lip Pits Only	Dental Anomalies	Limb Defects	Pterygia
***GRHL3* (n = 27)**								
yes	3	19	12	14	5	2	2	0
no	24	8	15	13	22	25	25	27
%	11	70	44	52	19	7	7	0
***IRF6* (n = 632)**								
yes	267	159	141	445	158	70	45	10
no	365	473	491	187	474	562	587	622
%	46	27	24	76	27	12	8	2
p value	0.001	2.0×10^{-6}	0.02	0.05	0.65	0.76	1	1

Peyrard-Janvid et al. AJHG, 2014

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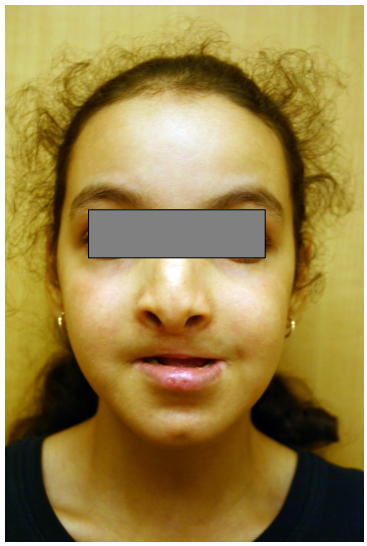
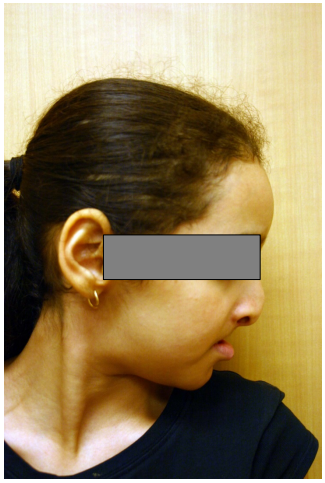
copy number variants

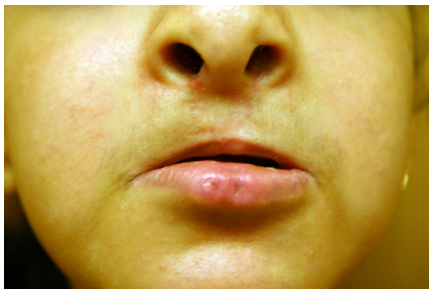
- 14-year-old girl
- consanguineous parents
- **bilateral cleft lip and palate**
- developmental delay
- no language
- strabismus



32

copy number variants





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copy number variants

- *IRF6* sequencing : normal
- CMA : 5Mb **interstitial deletion 1q32.2-q32.3** covering 38 genes, including *IRF6*
  → some patients with smaller deletion reported with : clet lip/palate, pits lower lip, growth retardation, +/- ID

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

MOLECULAR CYTOGENETICS

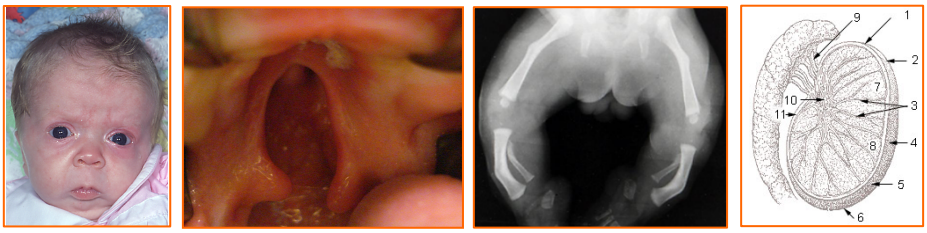
CASE REPORT Open Access

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]

34 34

SOX9 and campomelic dysplasia



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

Gorlin collection

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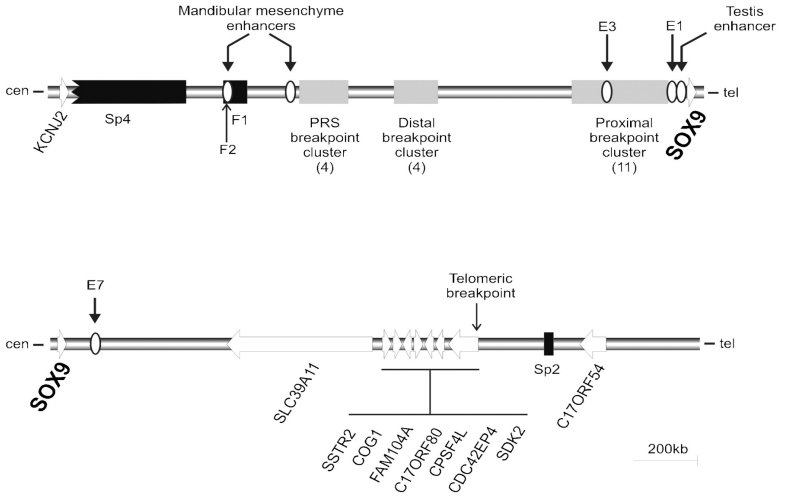
deletions in the *SOX9* **regulatory regions**

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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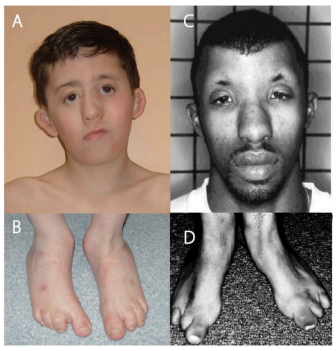
teratogens

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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isolated cleft etiology

- **sporadic and isolated**
- **familial and isolated**
- sporadic and syndromic
- familial and syndromic

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isolated cleft lip / palate - etiology

- complex disorder with complex etiology
- most are sporadic (no family history)
- strong genetic component
  - relative risk to a first-degree relative (sibling, offspring)
    - CL/P x 32
    - CP x 56
- but 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 gene, WES
- could represent « mixed models »
  - polygenic - combined effects of many independent genes
  - monogenic - rare variants in single major genes (Mendelian)

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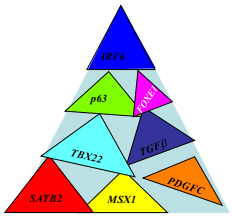
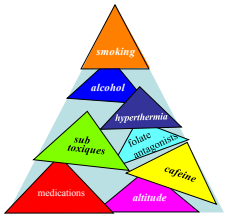
isolated cleft lip / palate - etiology

- most have a **multifactorial origin** : interaction of multiple genetic and environmental risk factors

genetic predisposition



environmental factors



MZ twins : 40-60% (genetic and non-genetic components)

DZ twins 5%

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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)
- difficult as the condition is genetically heterogeneous
- 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
- 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]

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,*,†]

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 only for about 30% of the heritability
- 40 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

→ the interest of targeted sequencing of loci identified by GWAS

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rare causal variants identified by candidate gene screening

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

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

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]

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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GENETIC COUNSELLING

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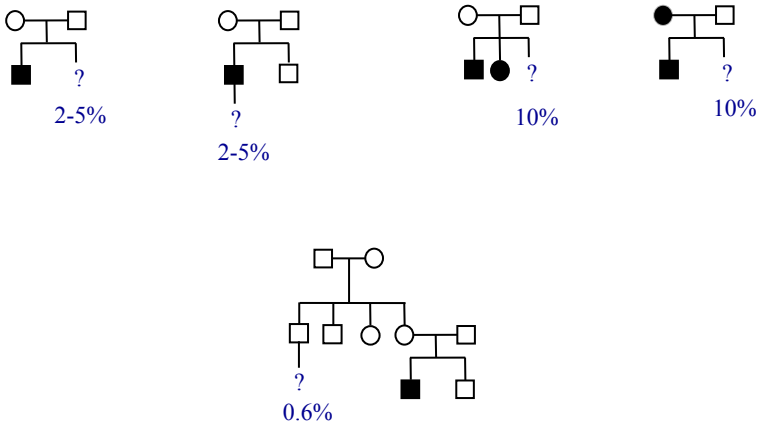
recurrence risk for isolated cleft



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recurrence risk for syndromic cleft

- precise estimation possible if the diagnosis is made and the mutation identified
- majority – autosomal dominant inheritance
  - incomplete penetrance
  - variable expressivity
  - « de novo » mutation, germline mosaicism
- some – autosomal recessive inheritance
- some – X-linked inheritance

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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
- interest of genomic sequencing studies

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