Genetics of congenital heart diseases
Old ideas and new concepts
Or Old concepts and new ideas

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IcarP Cardiology, Institut Hospitalo-Universitaire IMAGINE

Centre de Référence Maladies Rares
Malformations Cardiaques Congénitales Complexes-M3C
Centre de Référence Maladies Rares
Maladies Cardiaques Héréditaires- CARDIOGEN

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CRMR Malformations Cardiaques Congénitales Complexes
CRMR Maladies Cardiaques Héréditaires
Centre de Compétences du CRMR Hypertension pulmonaires
INSERM Embryology & Genetics of Congenital Malformations

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Malformations Cardiaques Congénitales Complexes

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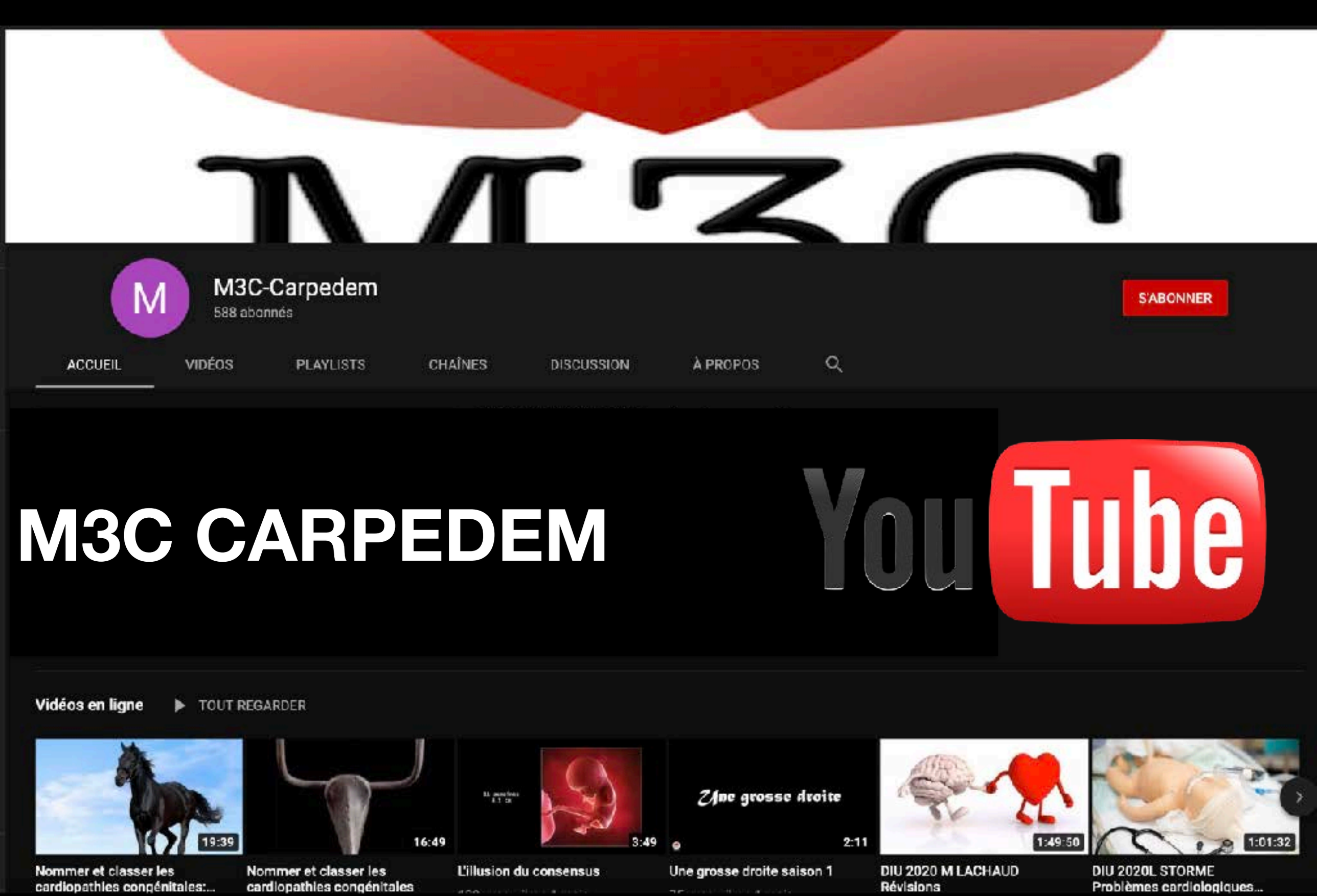
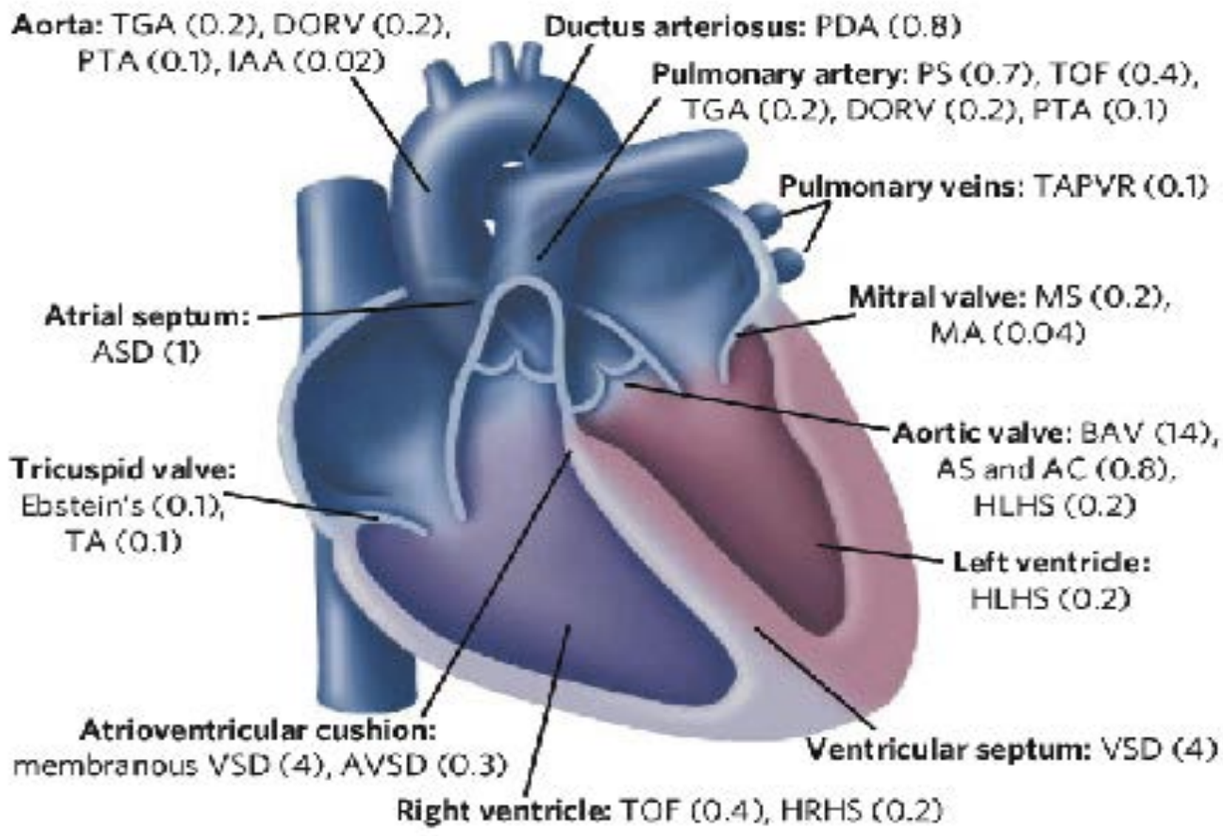


Congenital heart diseases

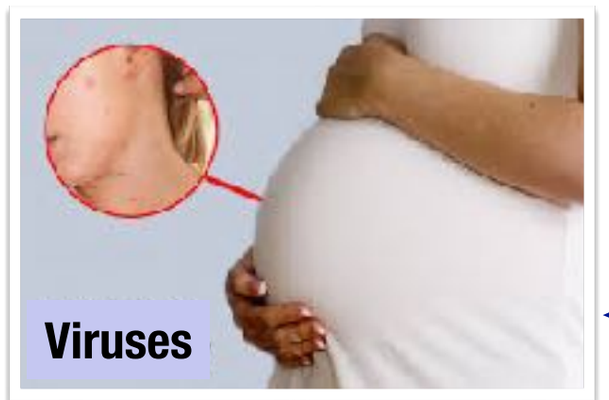
- Incidence: 8/1000 live-births
- 28% : associated anomalies
  > 600 entries in OMIM
- Genetic counseling is a challenge as survival is now the rule

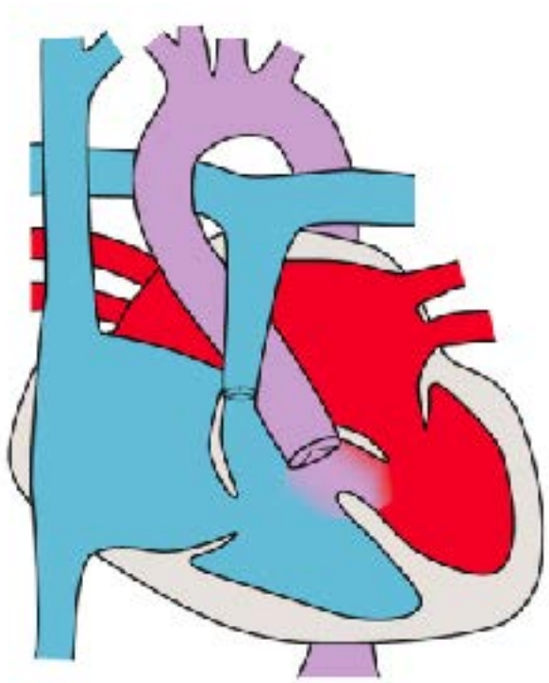


The developmental genetics of congenital heart disease

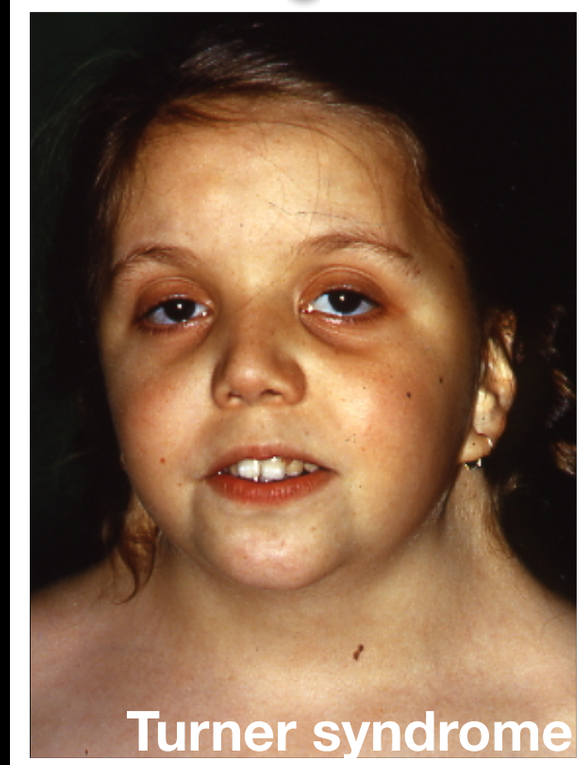
Benoit G. Bruneau

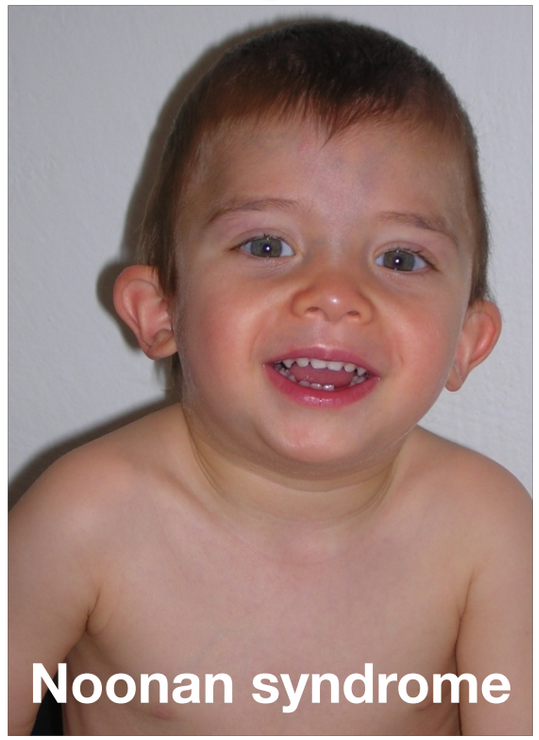
Well-known risk factors for congenital heart diseases

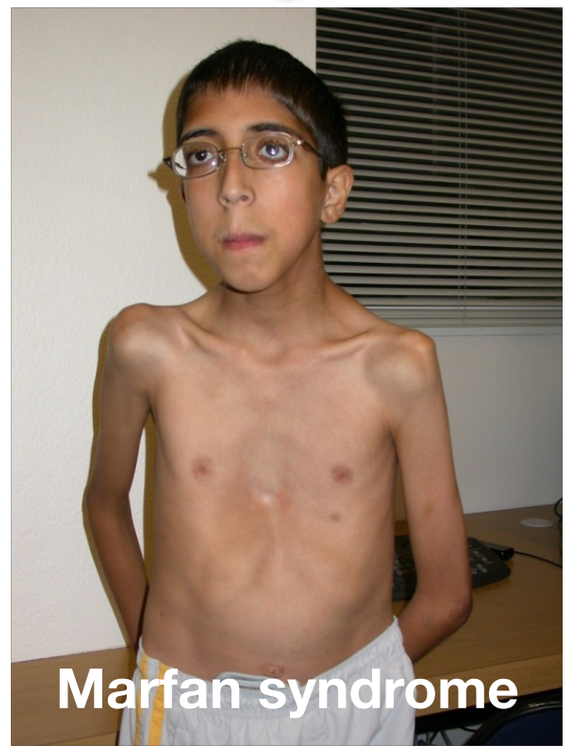


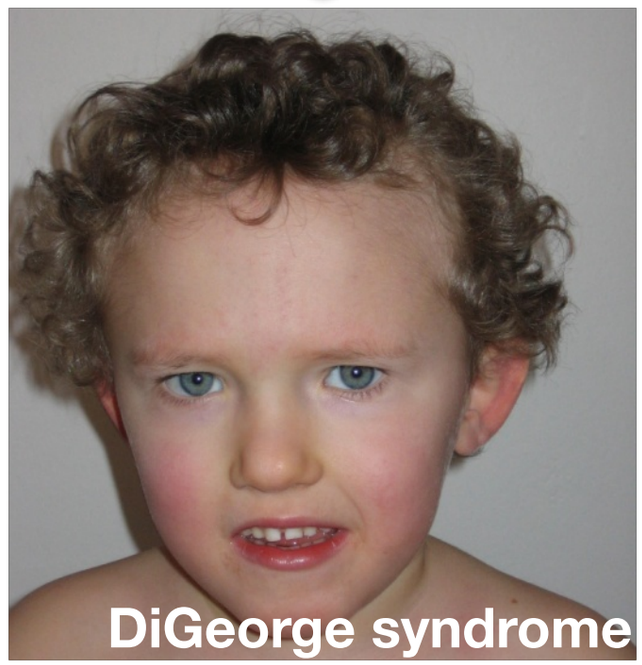


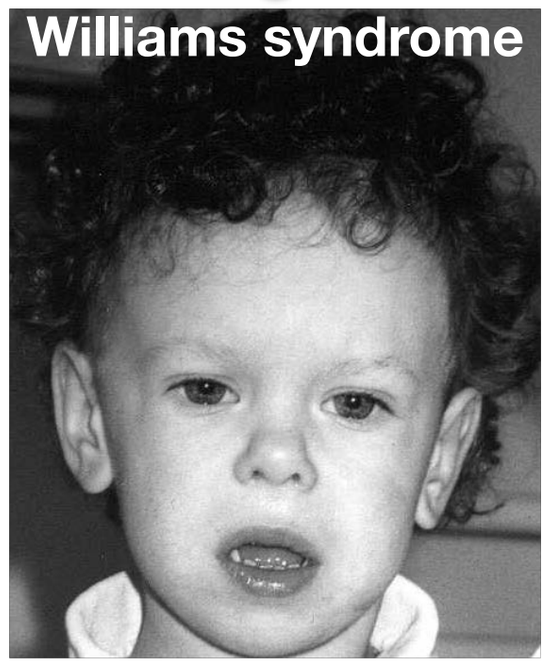
Old textbooks and clinical genetics

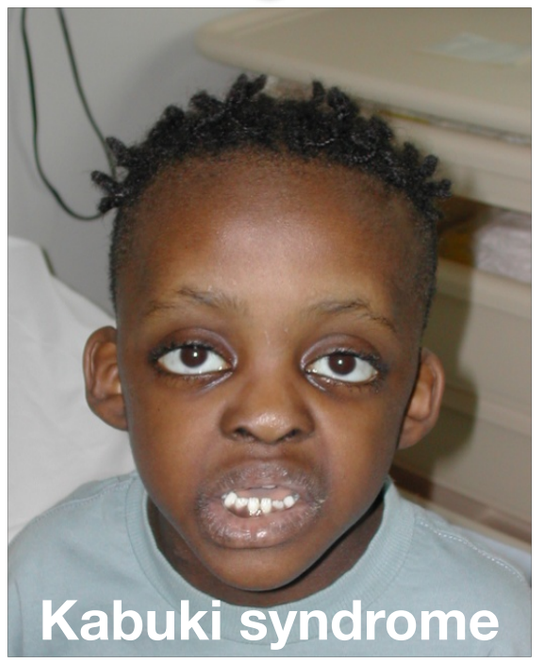


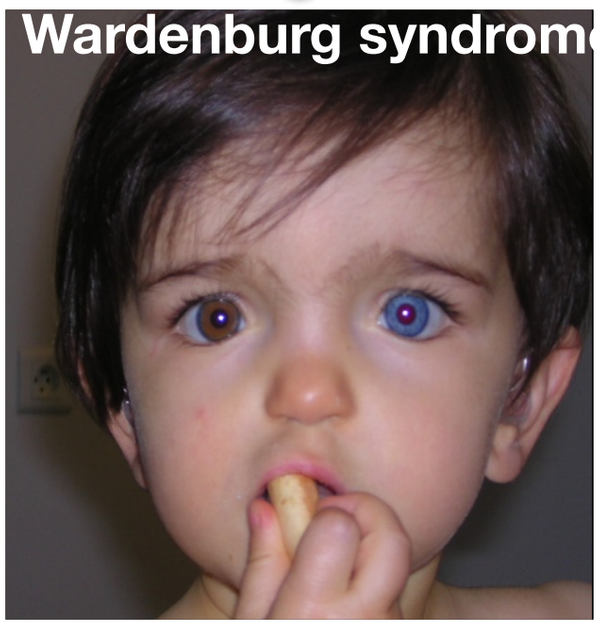




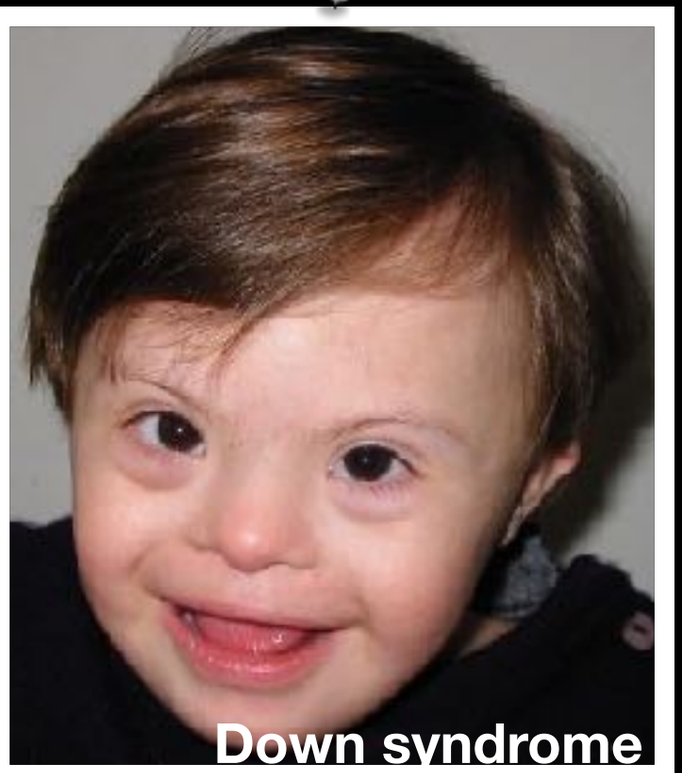


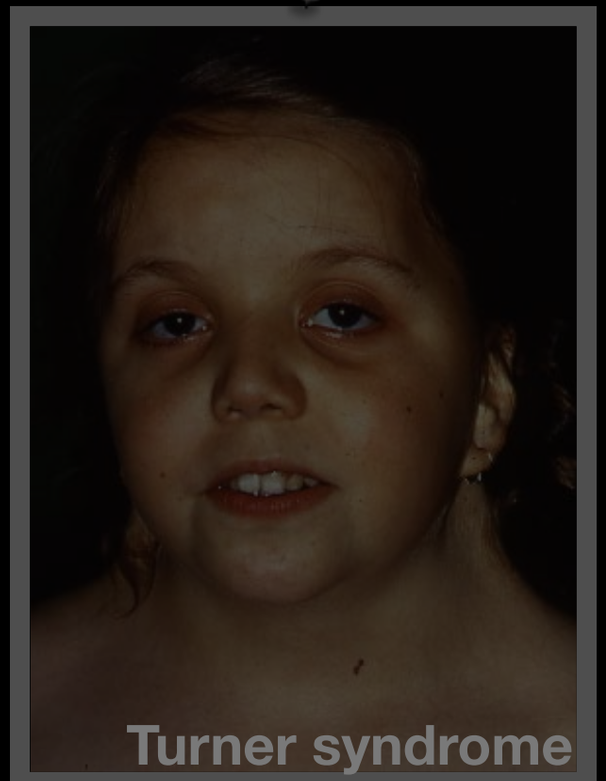


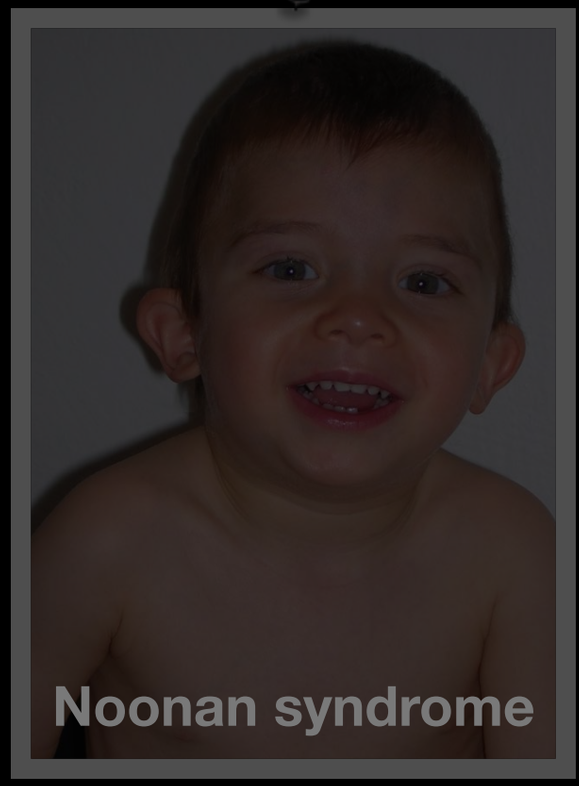


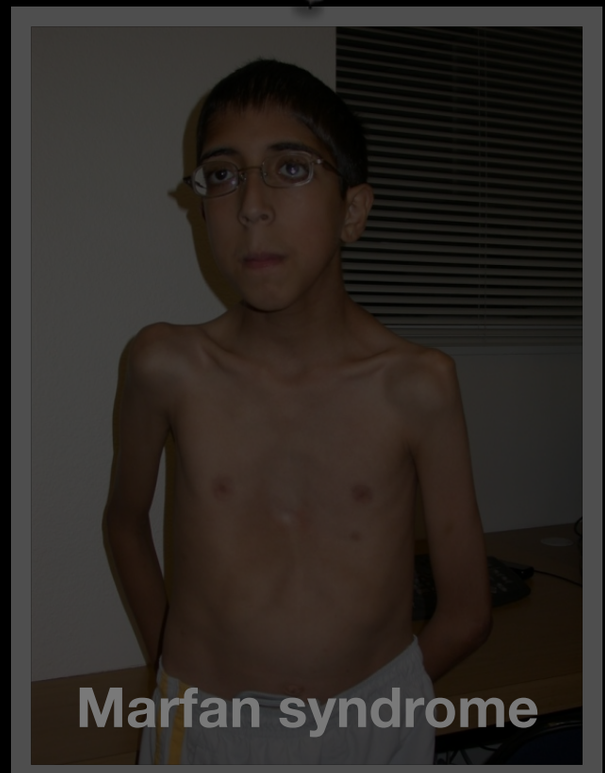


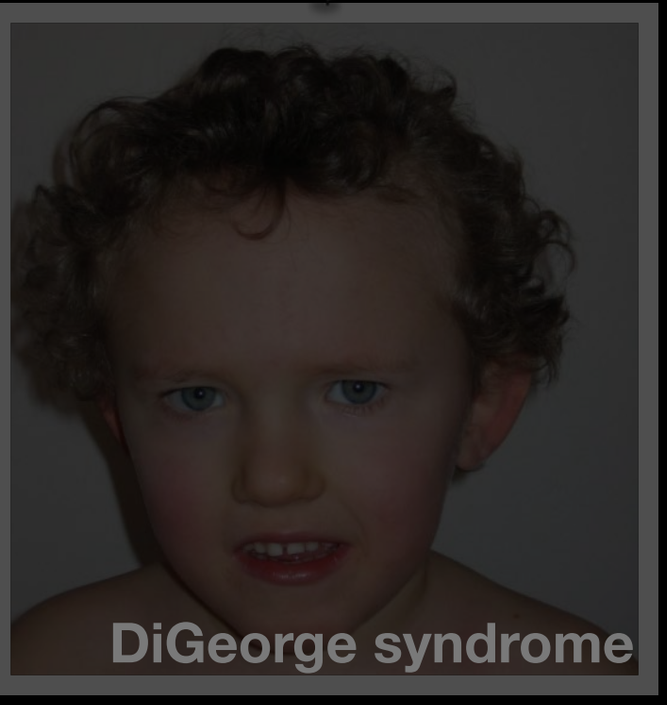
Old textbooks and clinical genetics

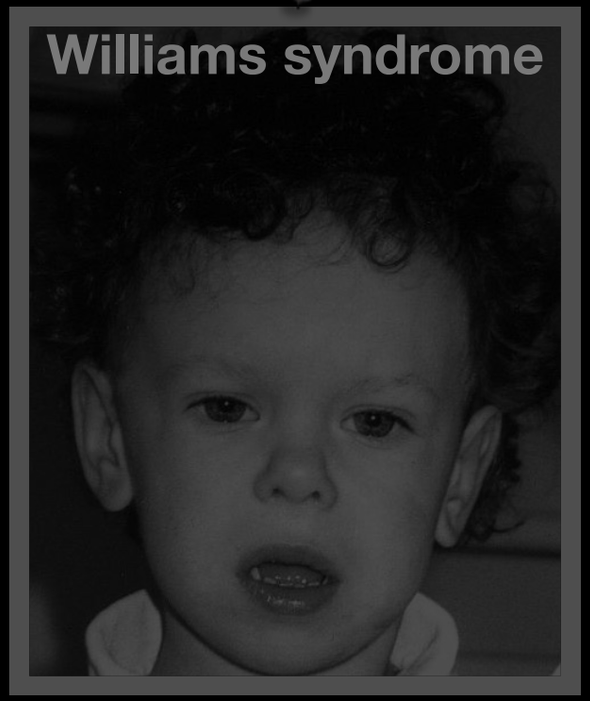


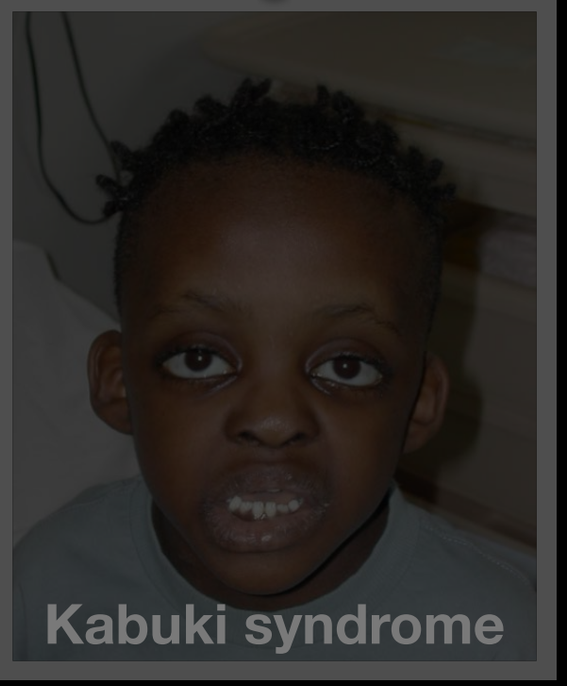


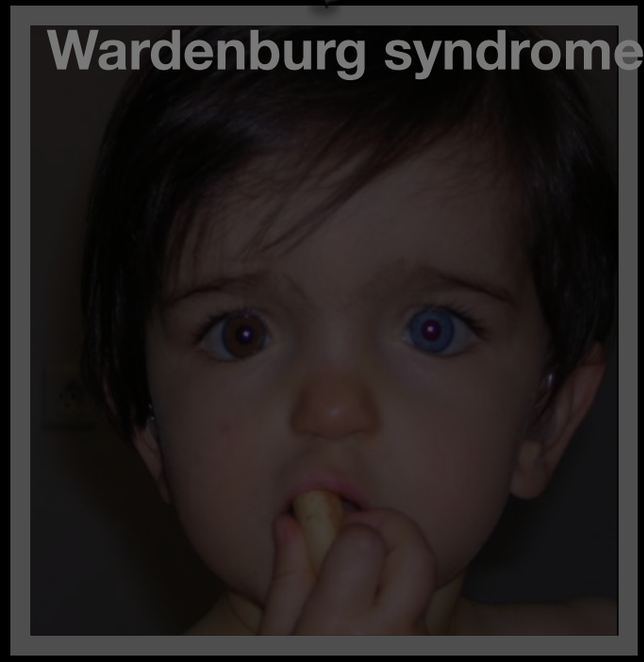


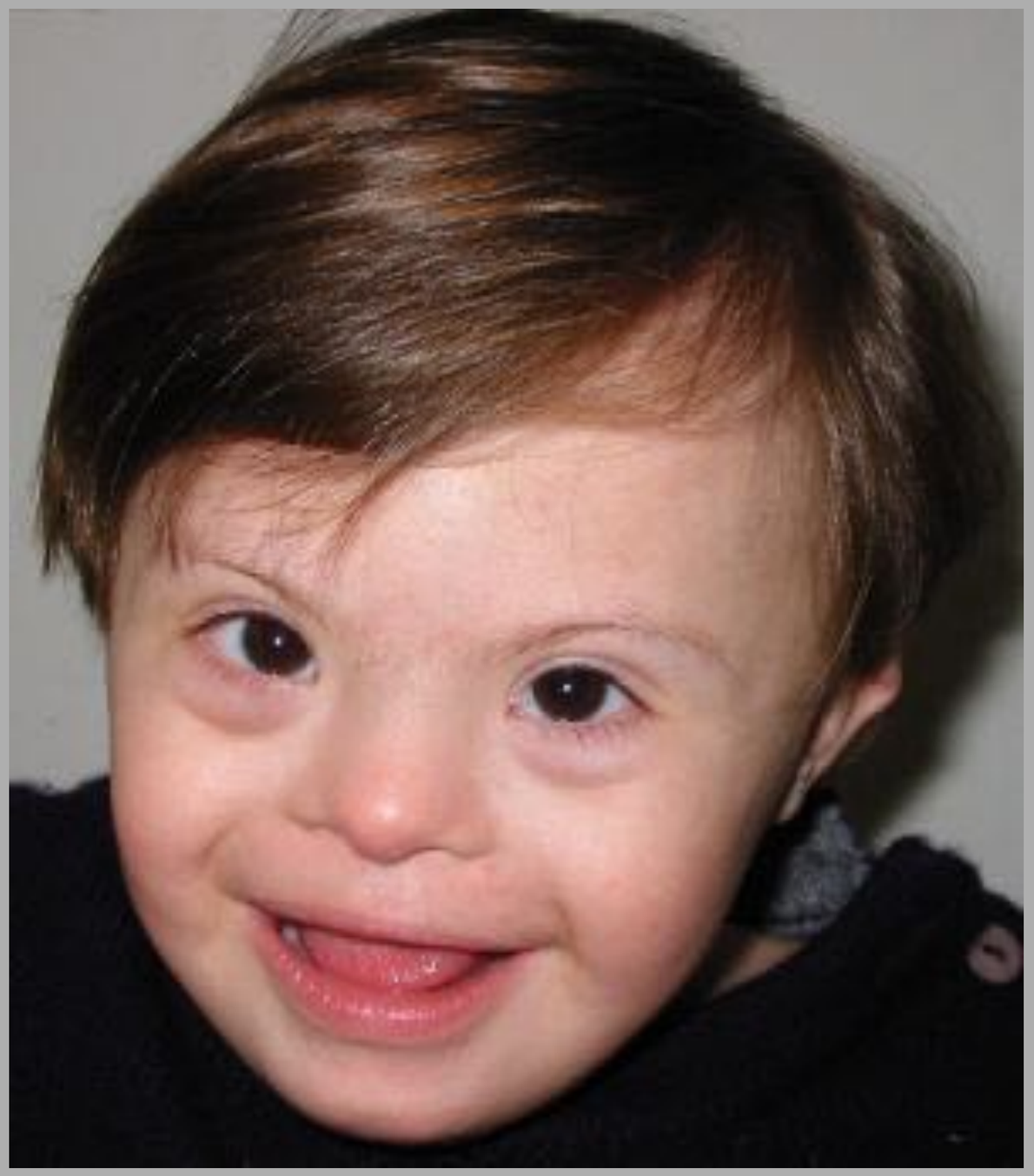


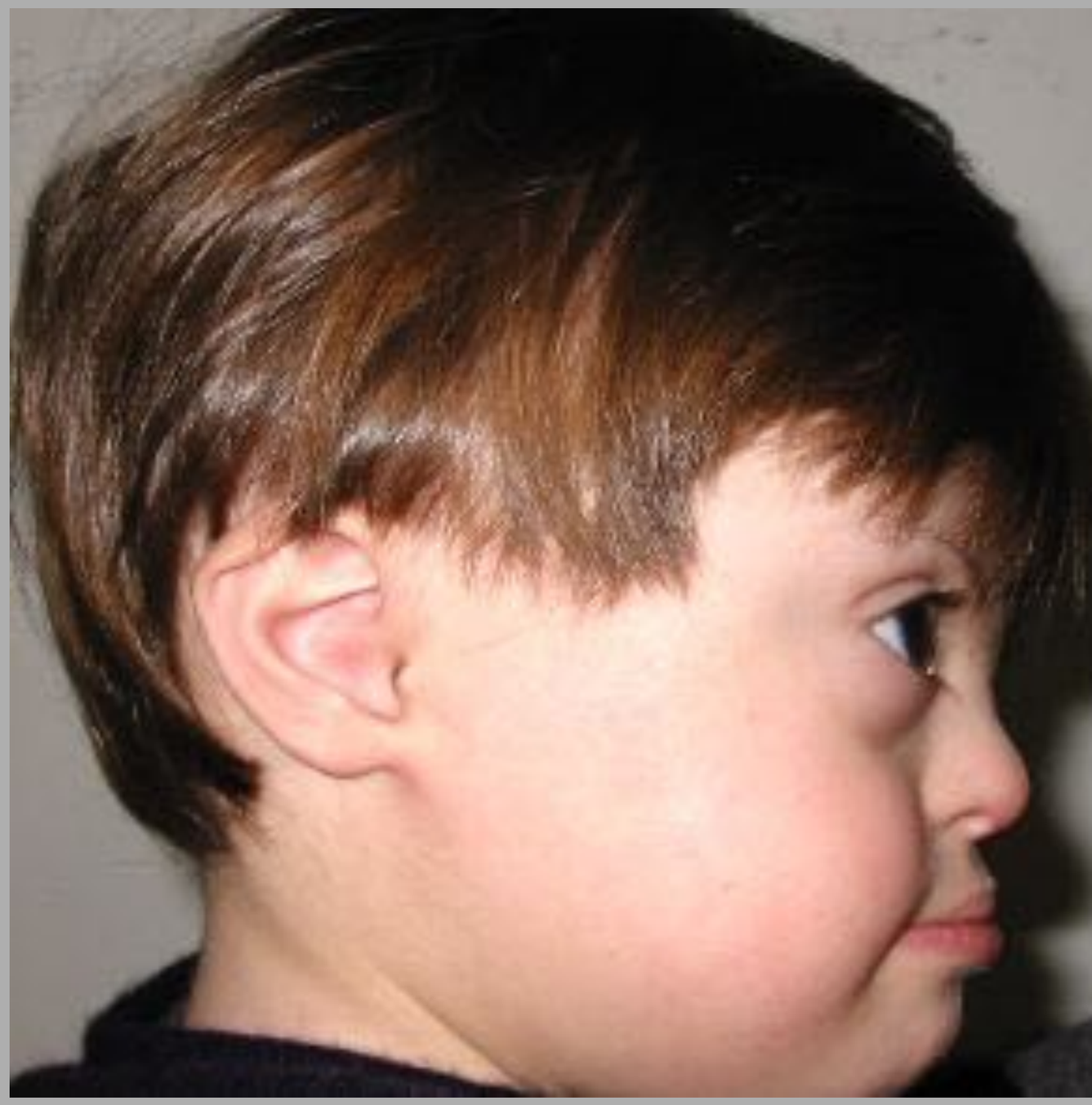




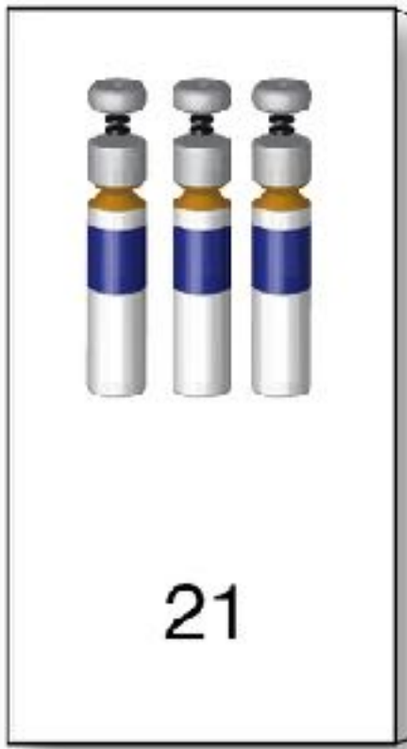








Trisomy 21 - Down syndrome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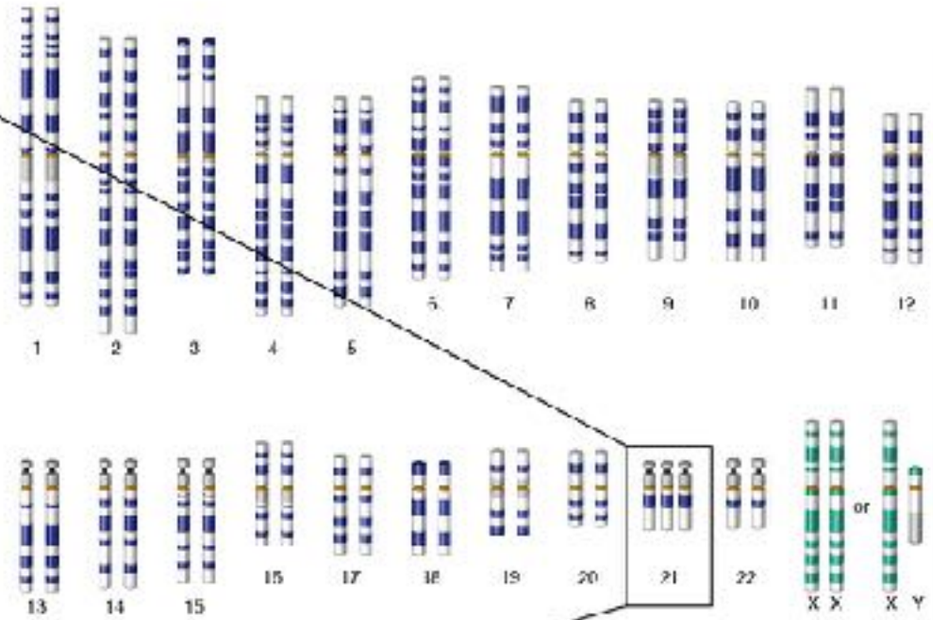
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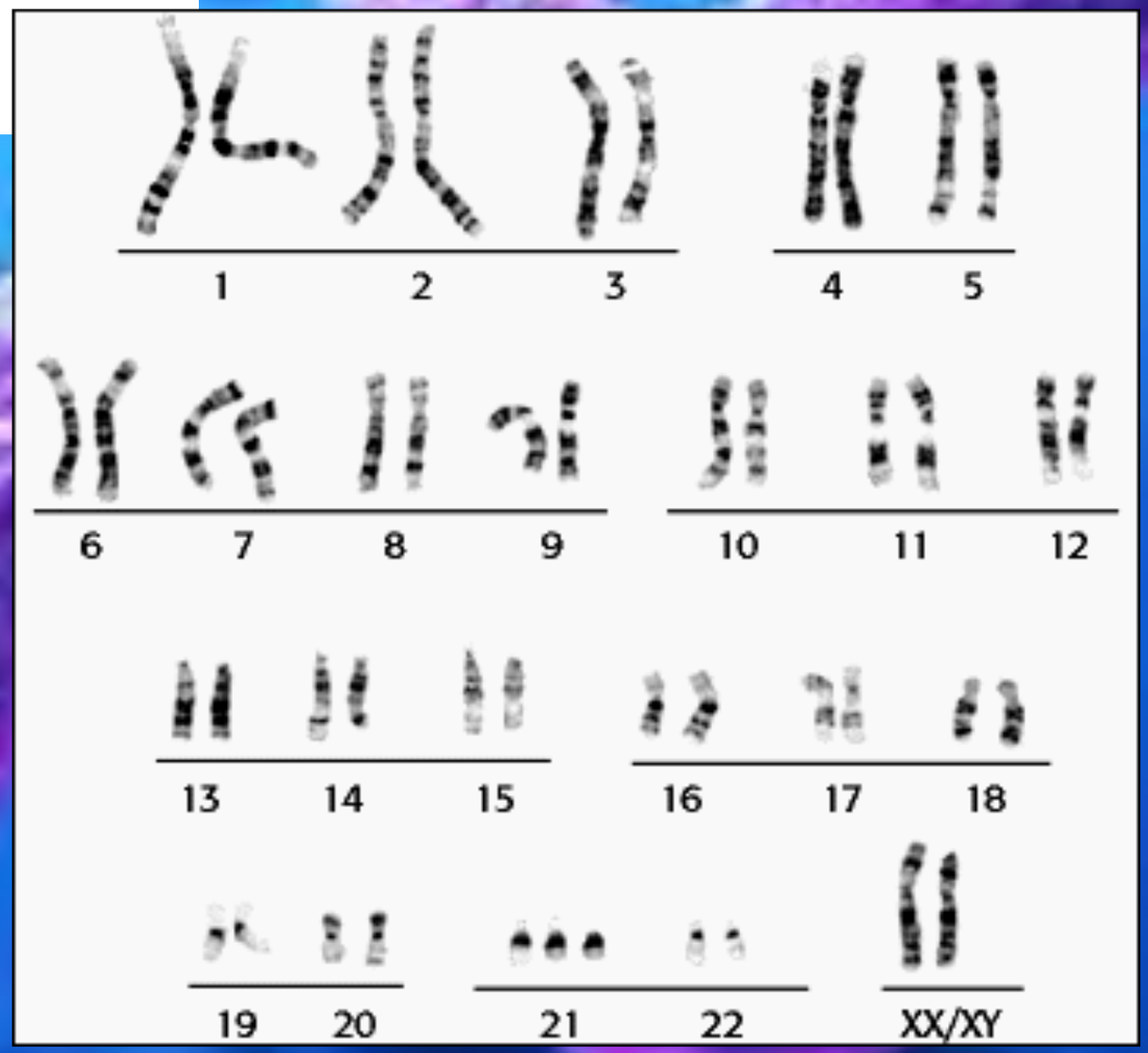
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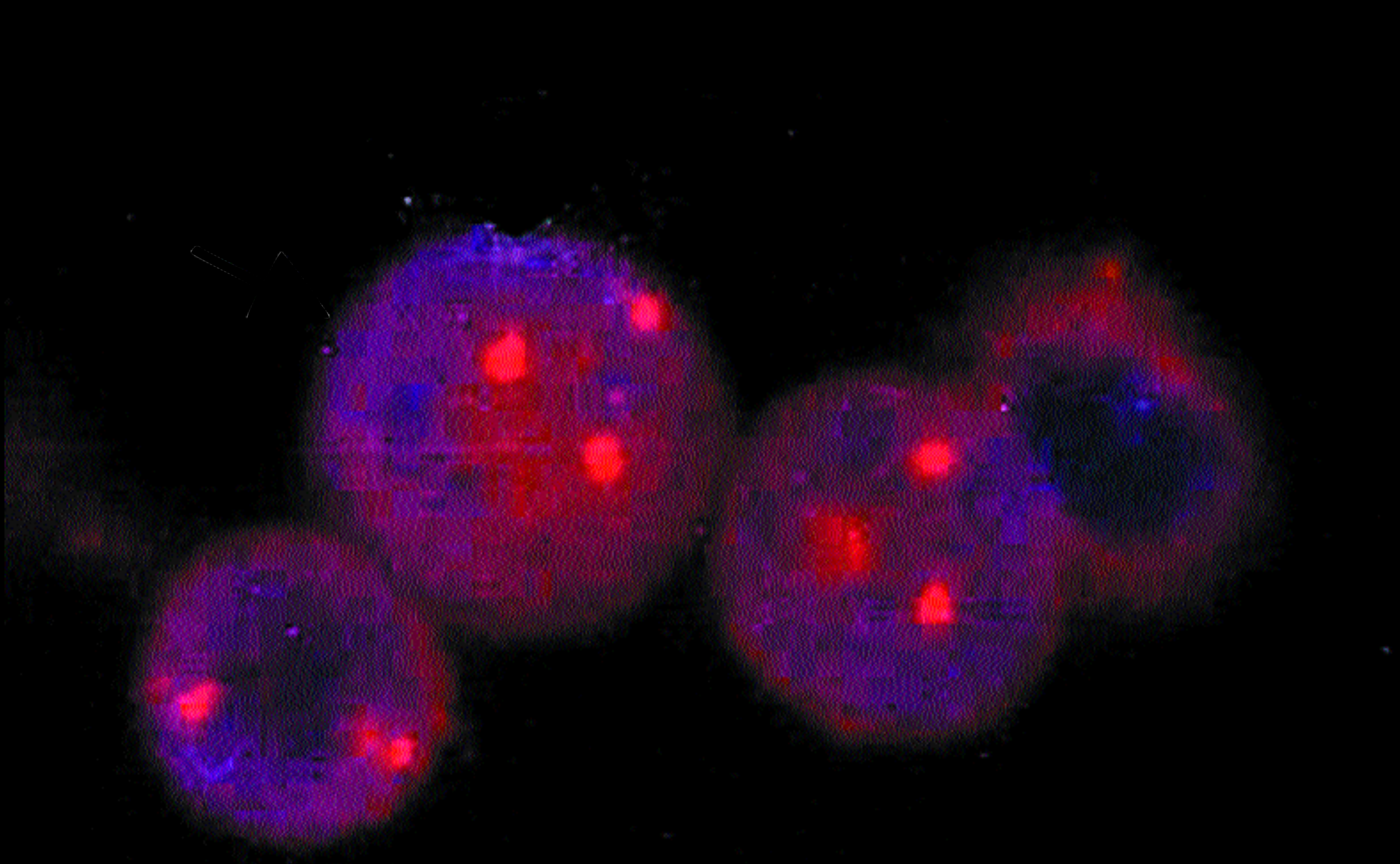
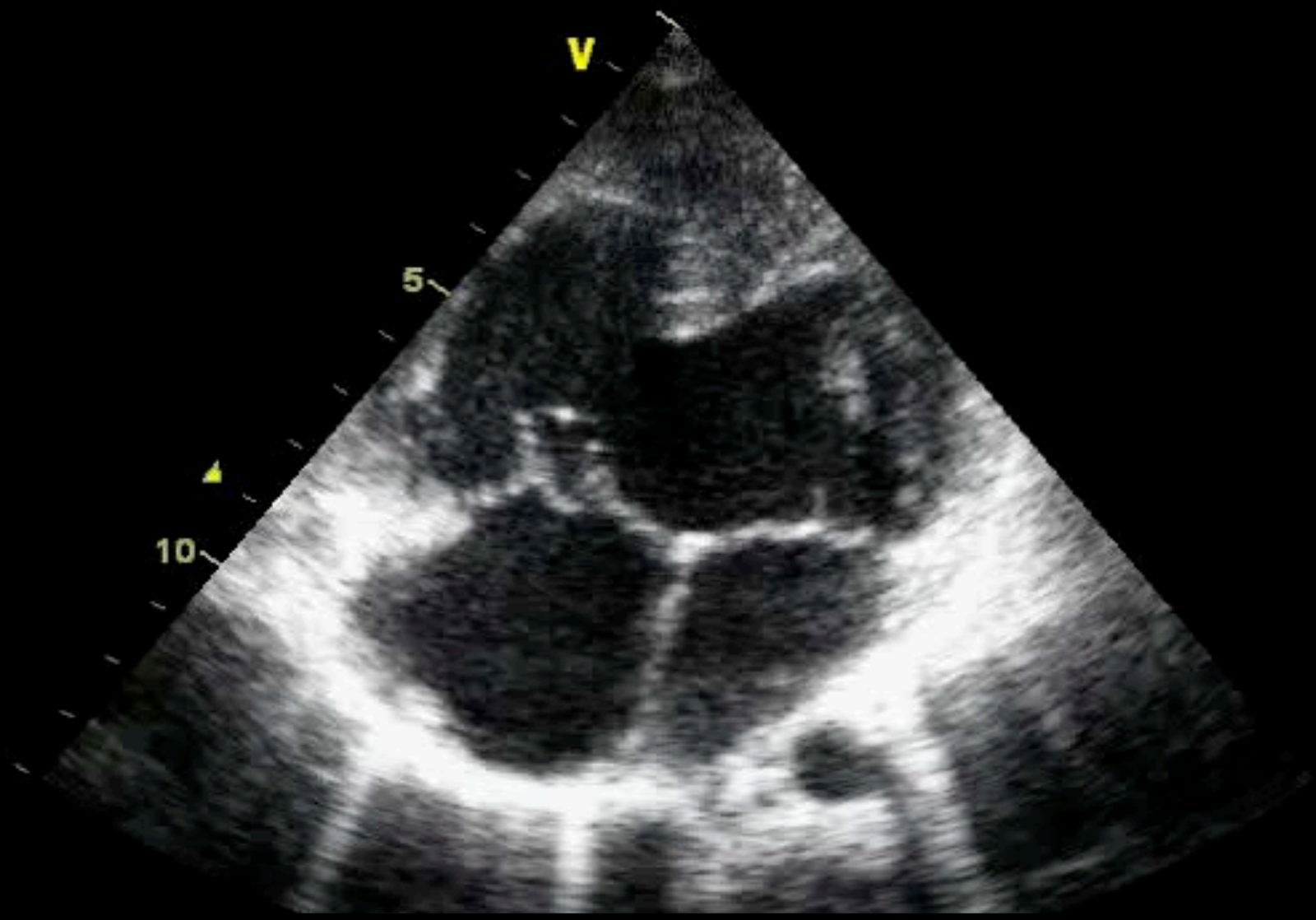
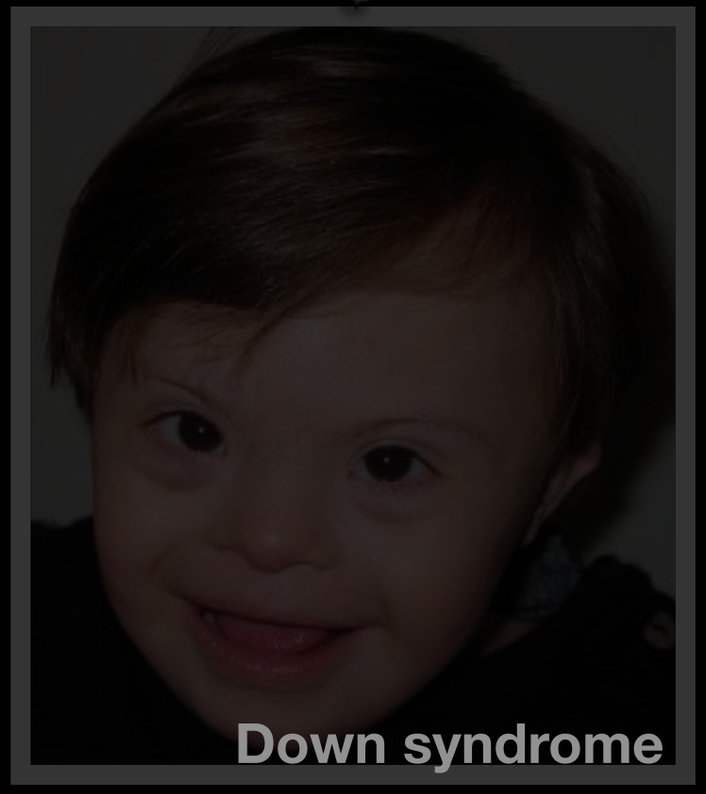


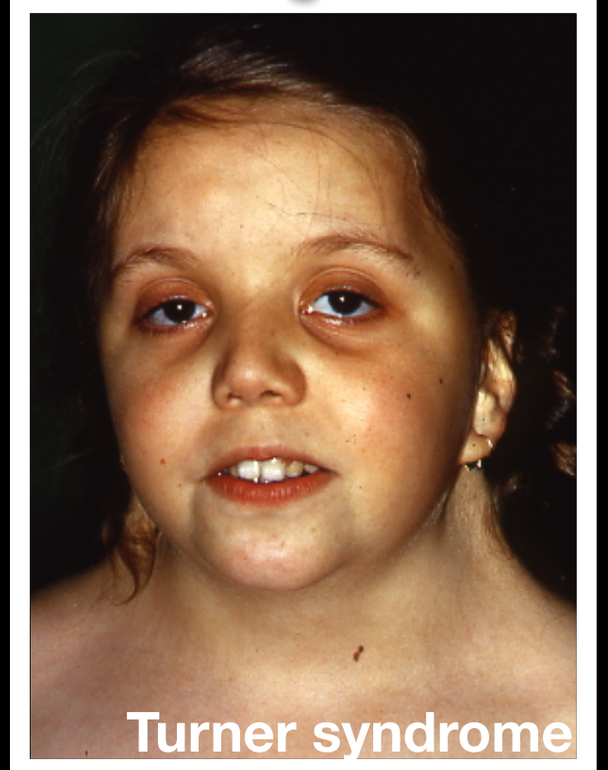
Trisomy 21 - Down syndrome

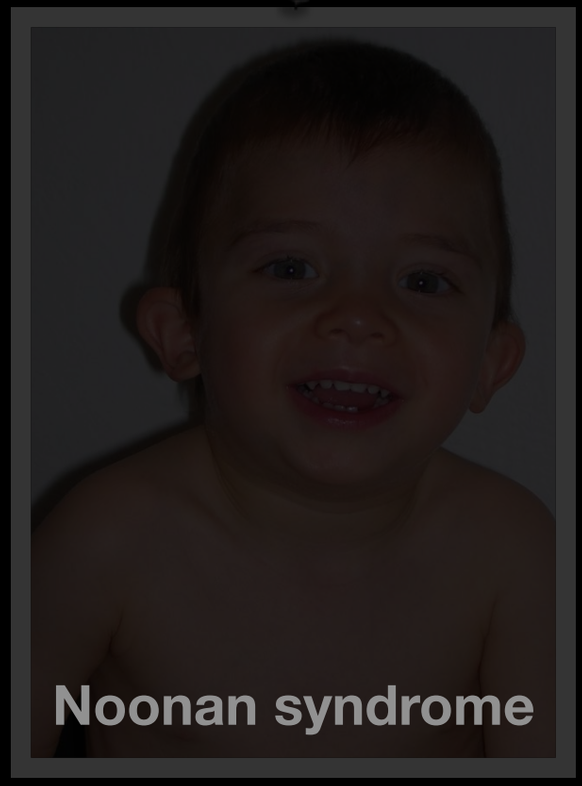
Inlet VSD

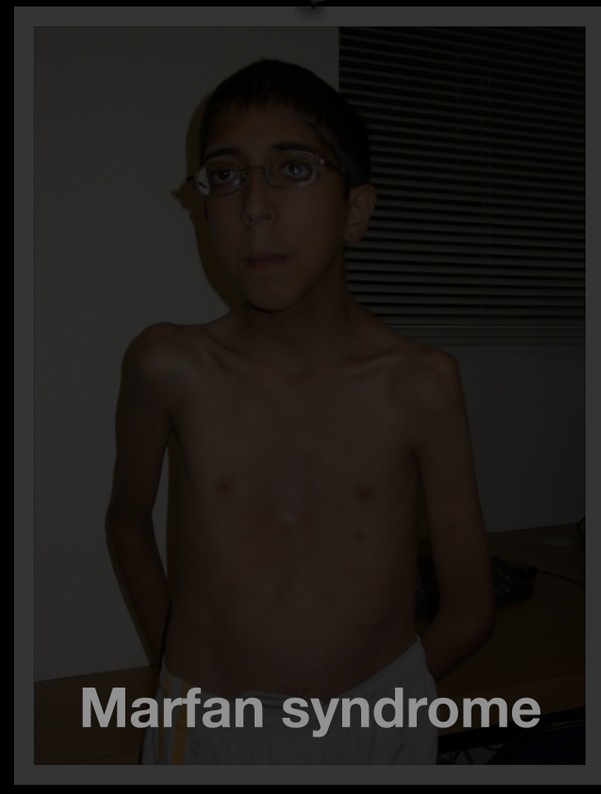


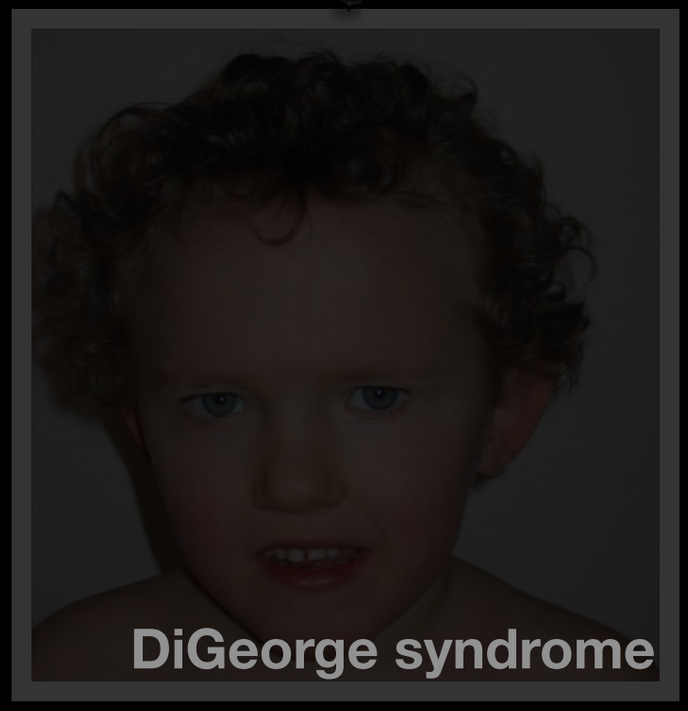
Old textbooks and clinical genetics

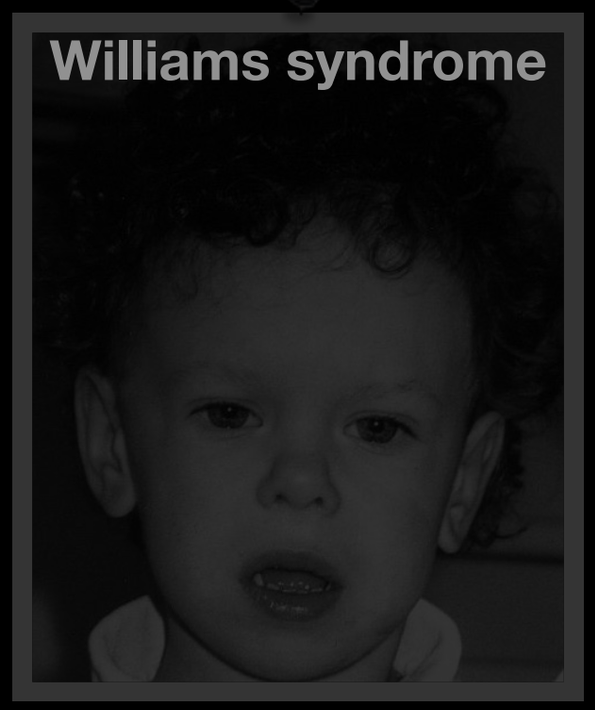


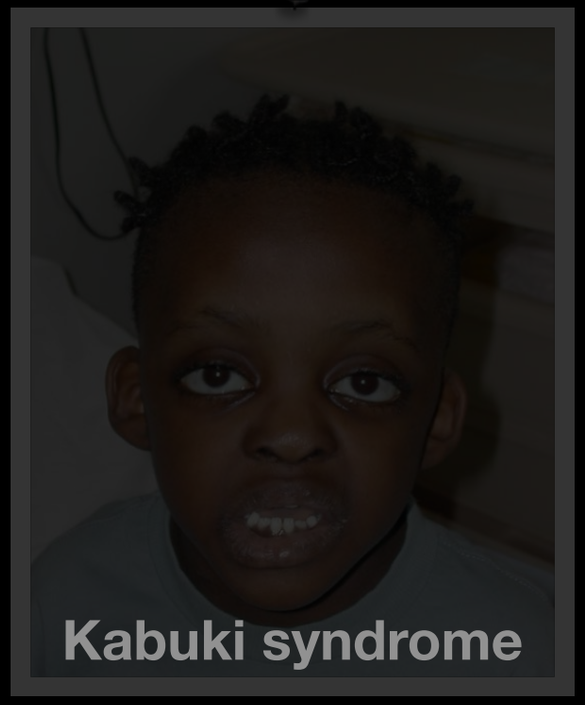


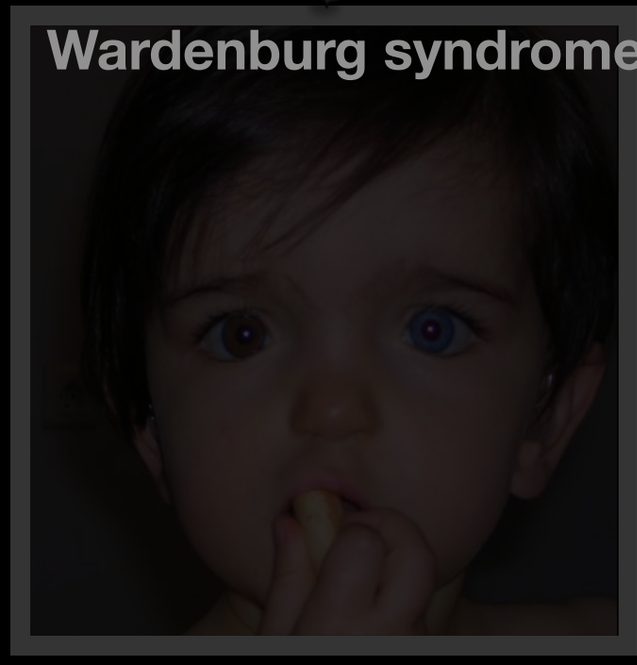


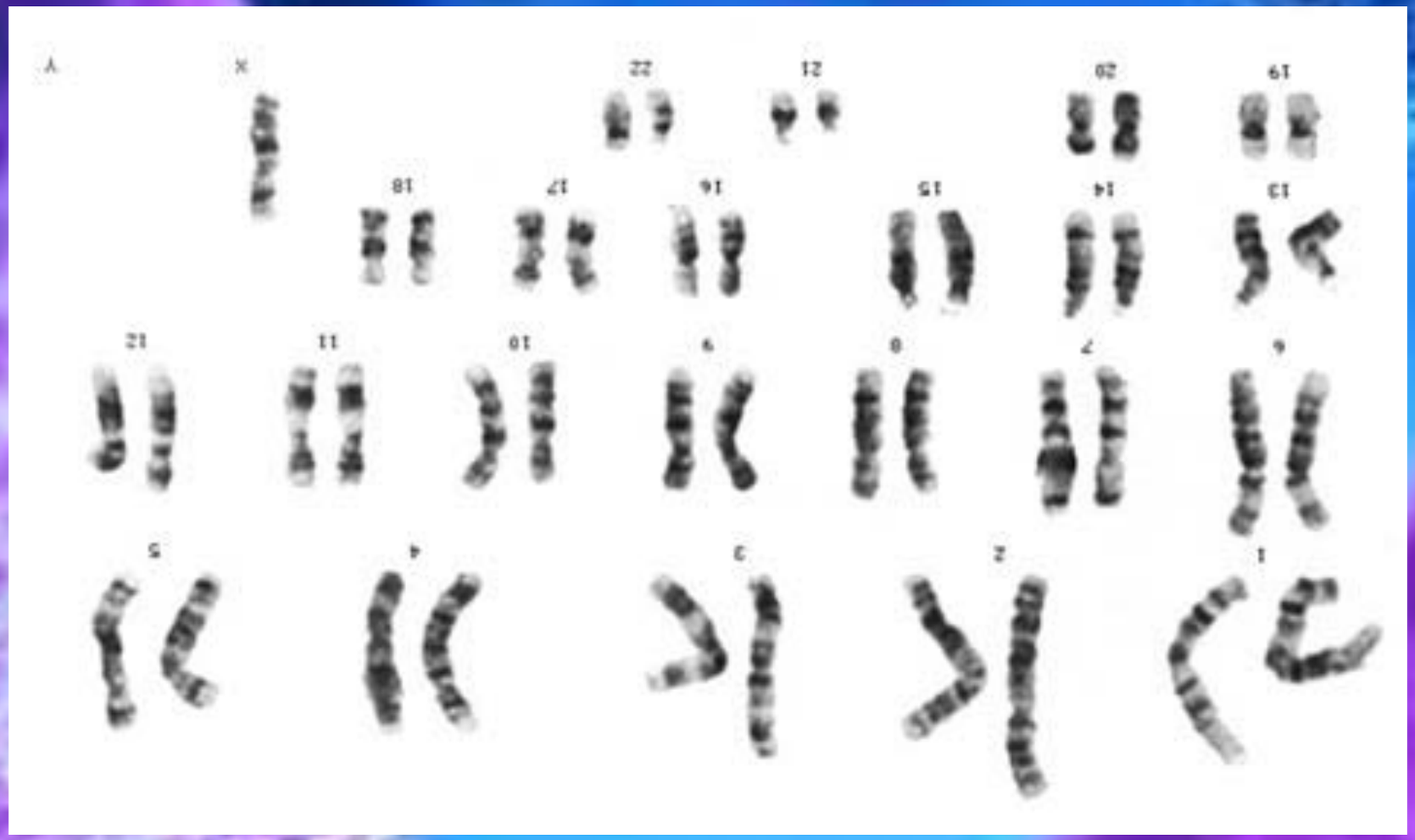








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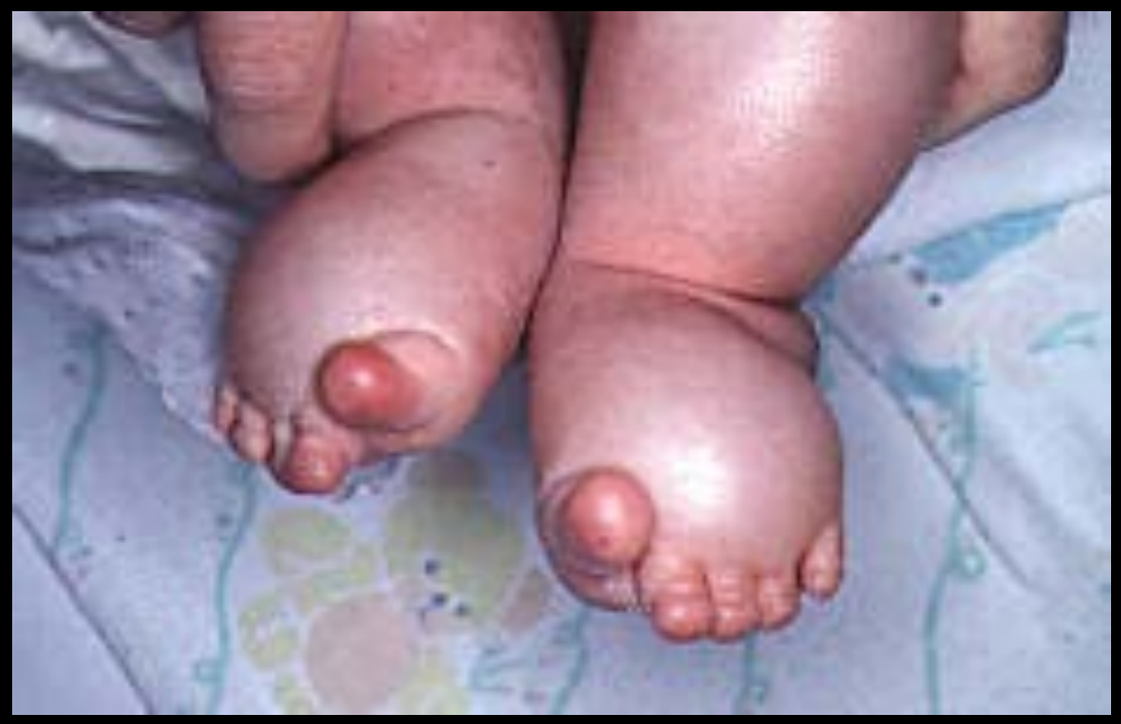
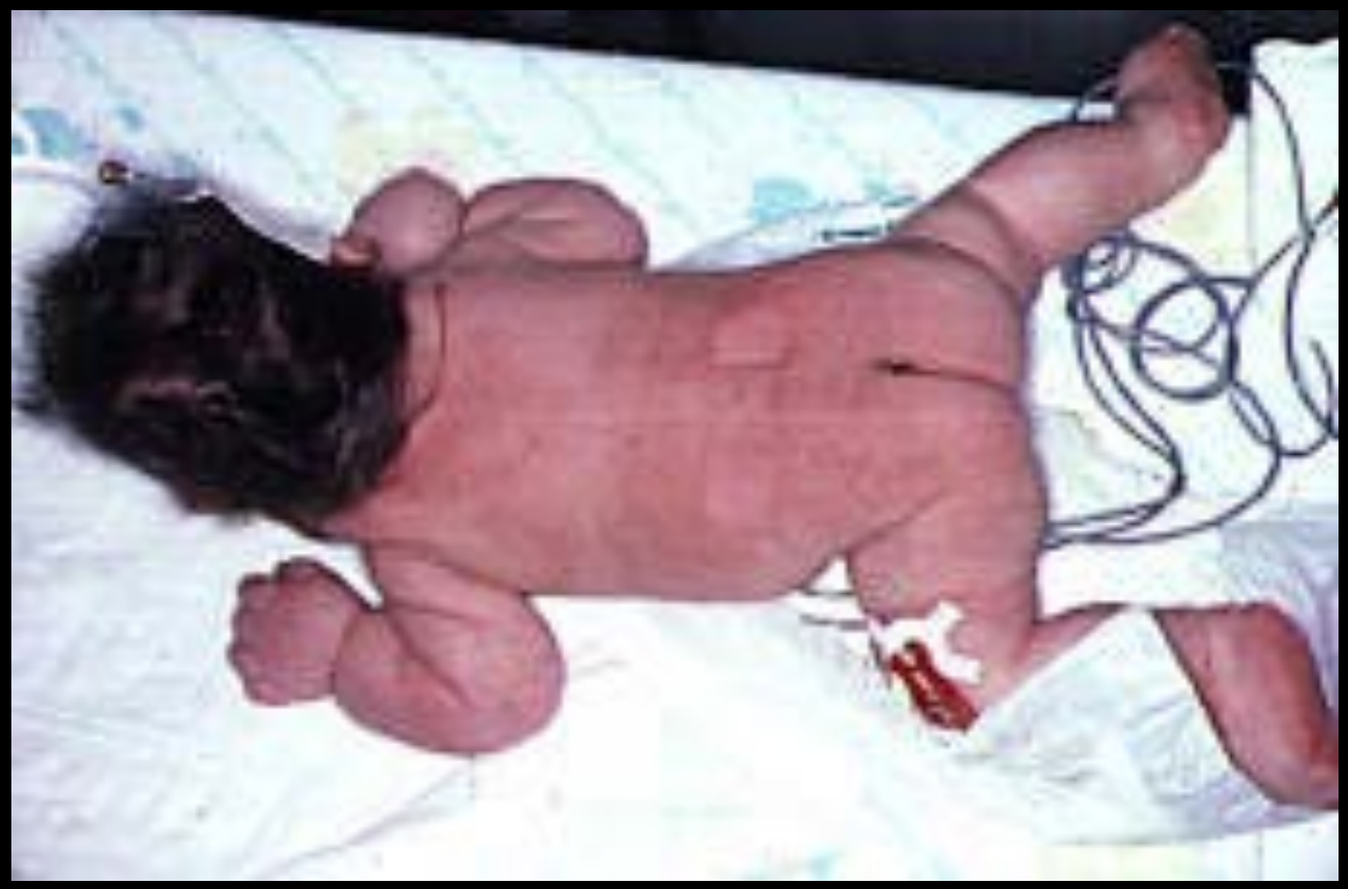
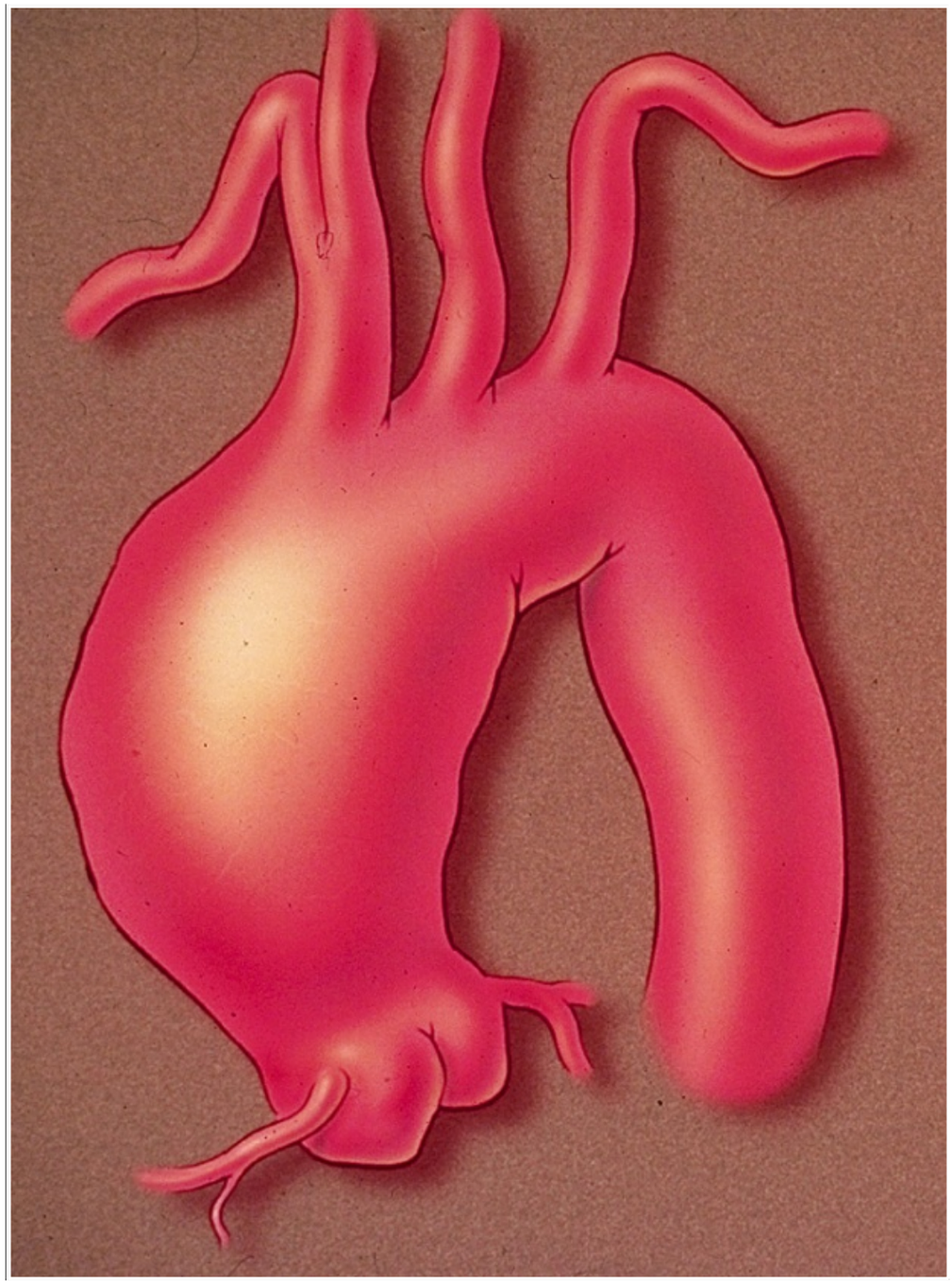
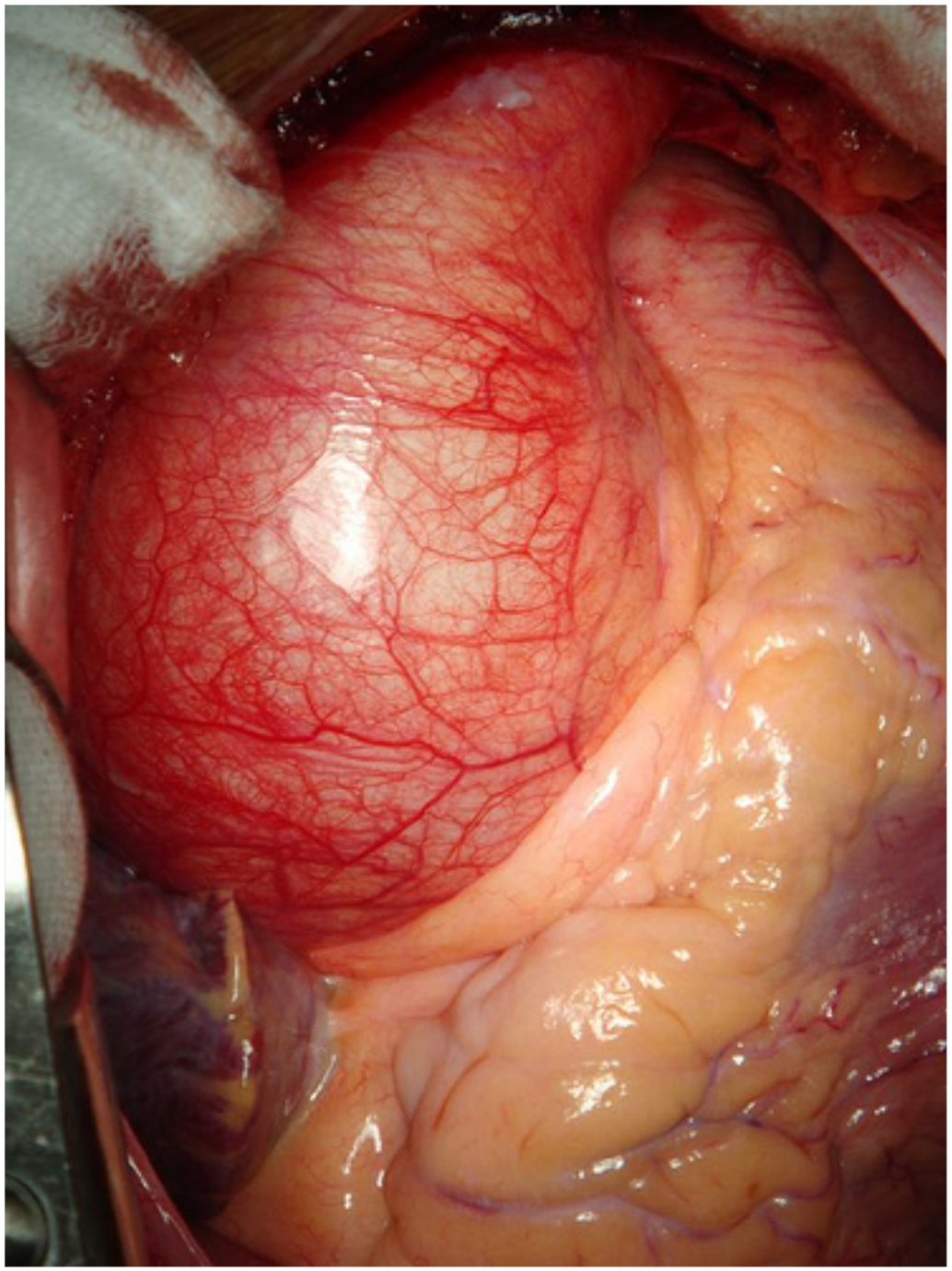


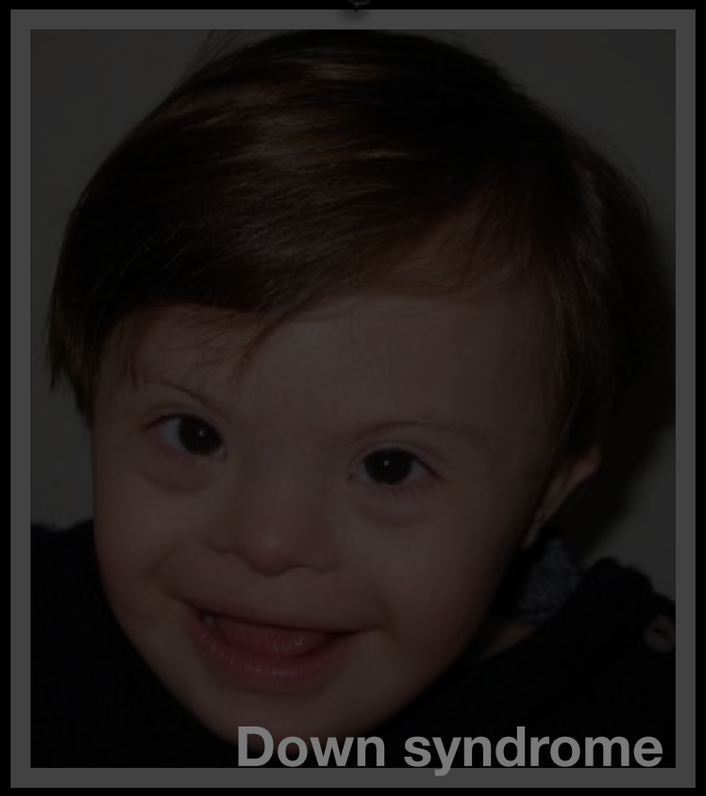


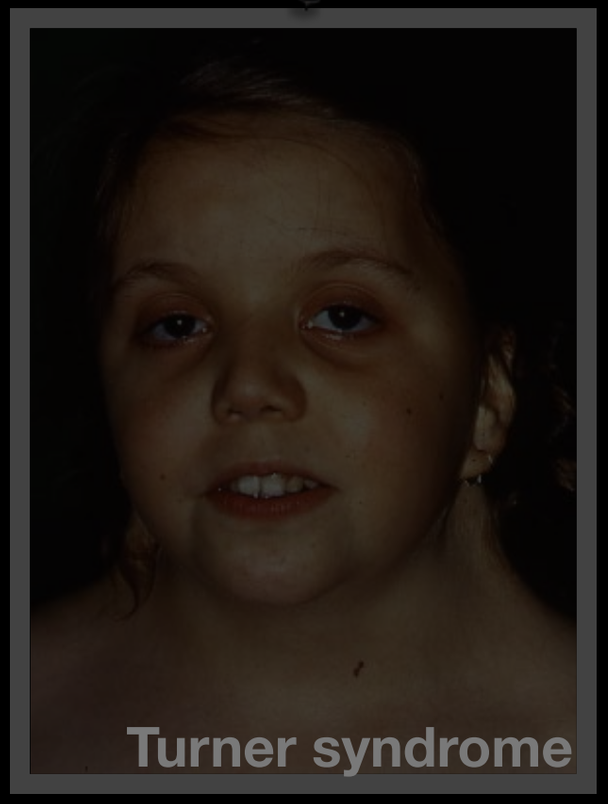
Bonnevie Ulrich syndrome

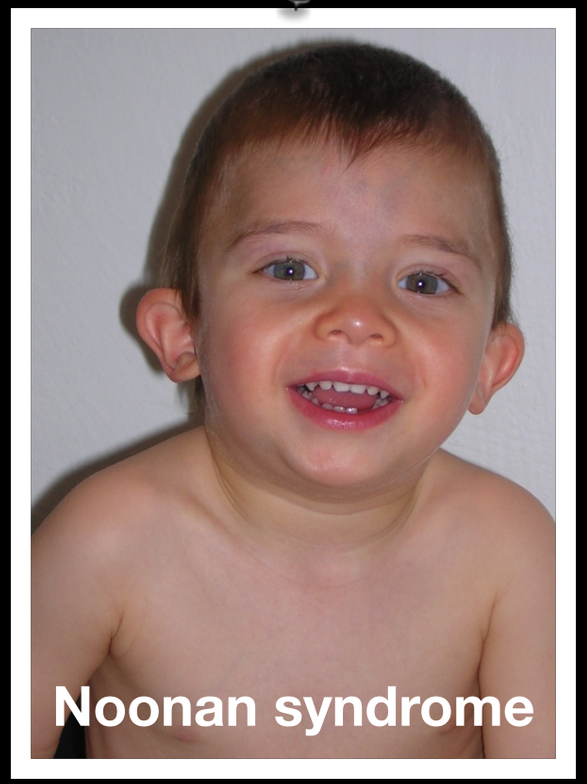


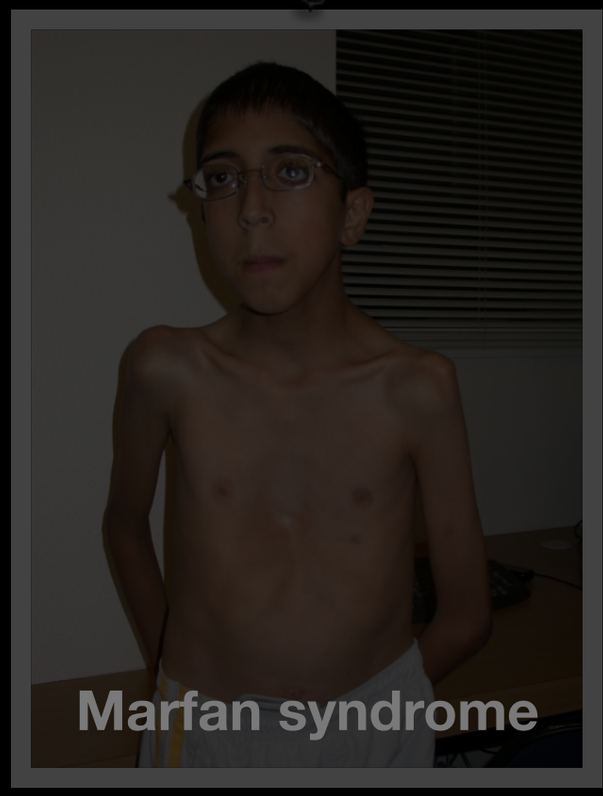


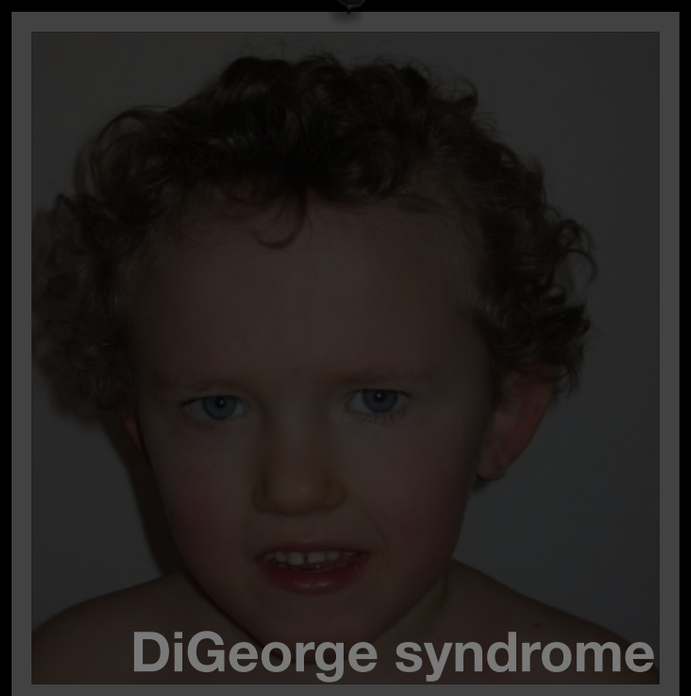
Old textbooks and clinical genetics

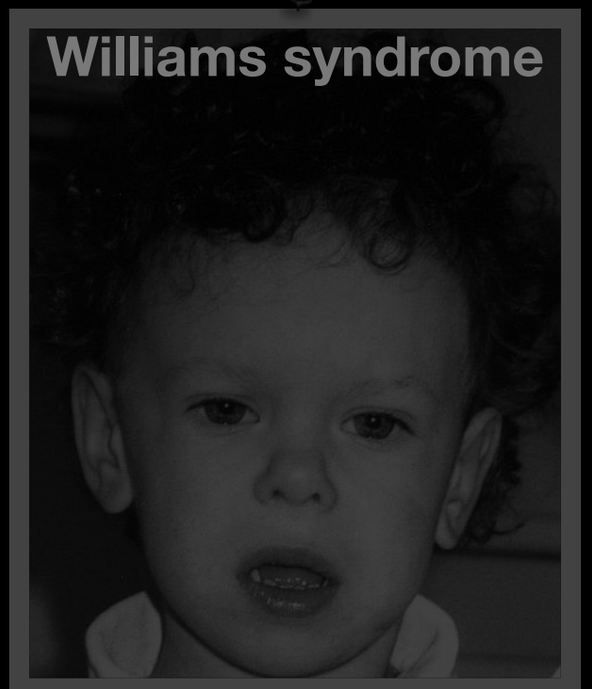


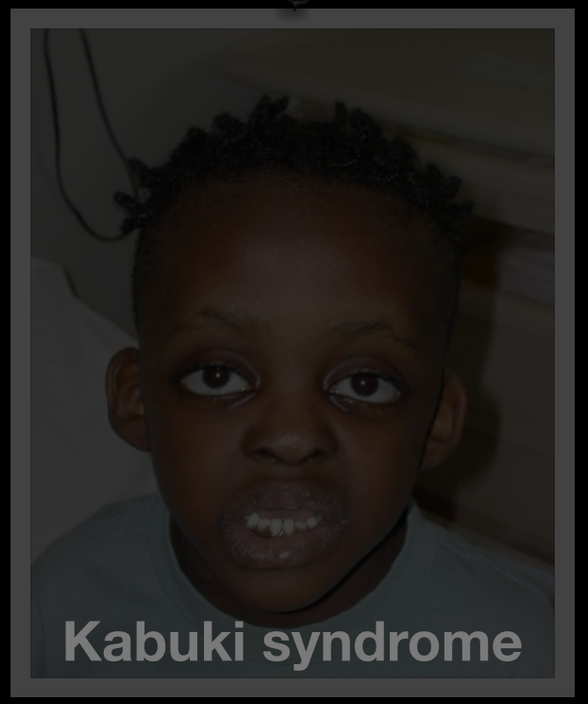


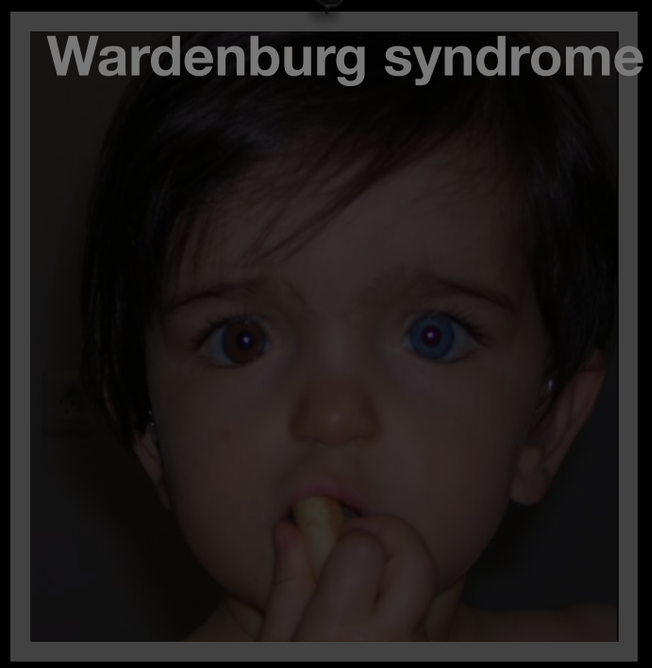










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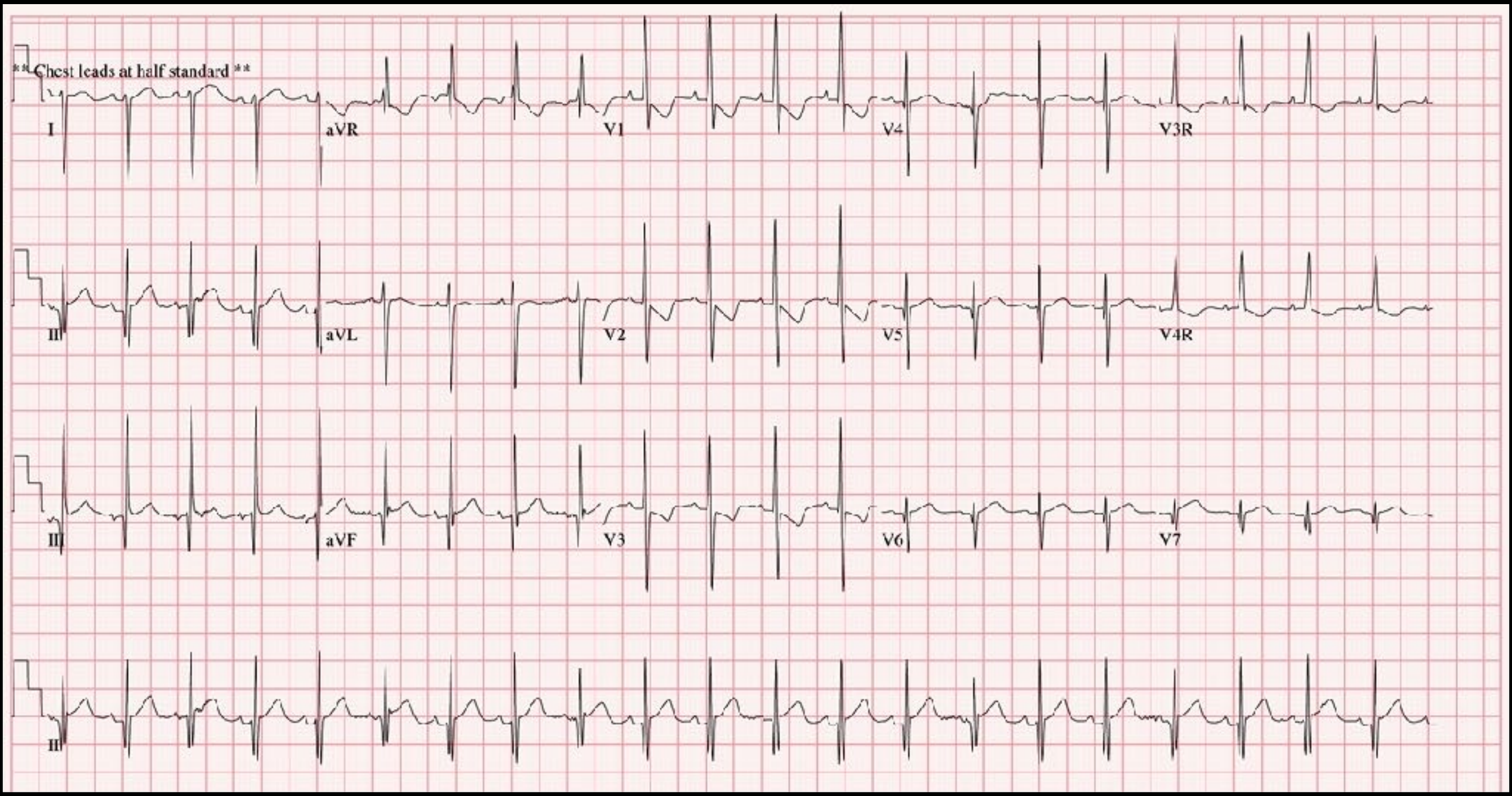
** Chest leads at half standard **
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V4R
III
aVF
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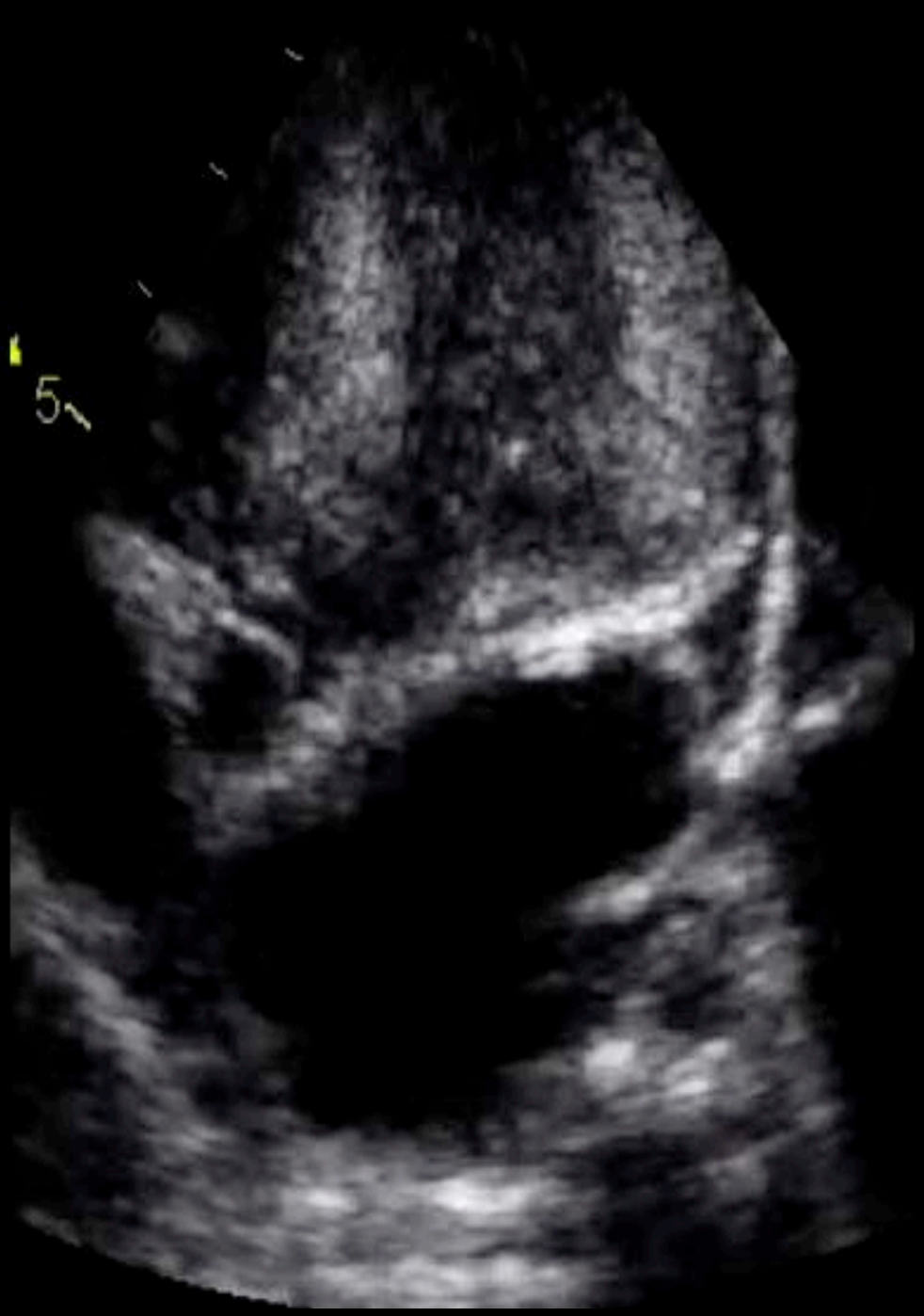
V6
V7
II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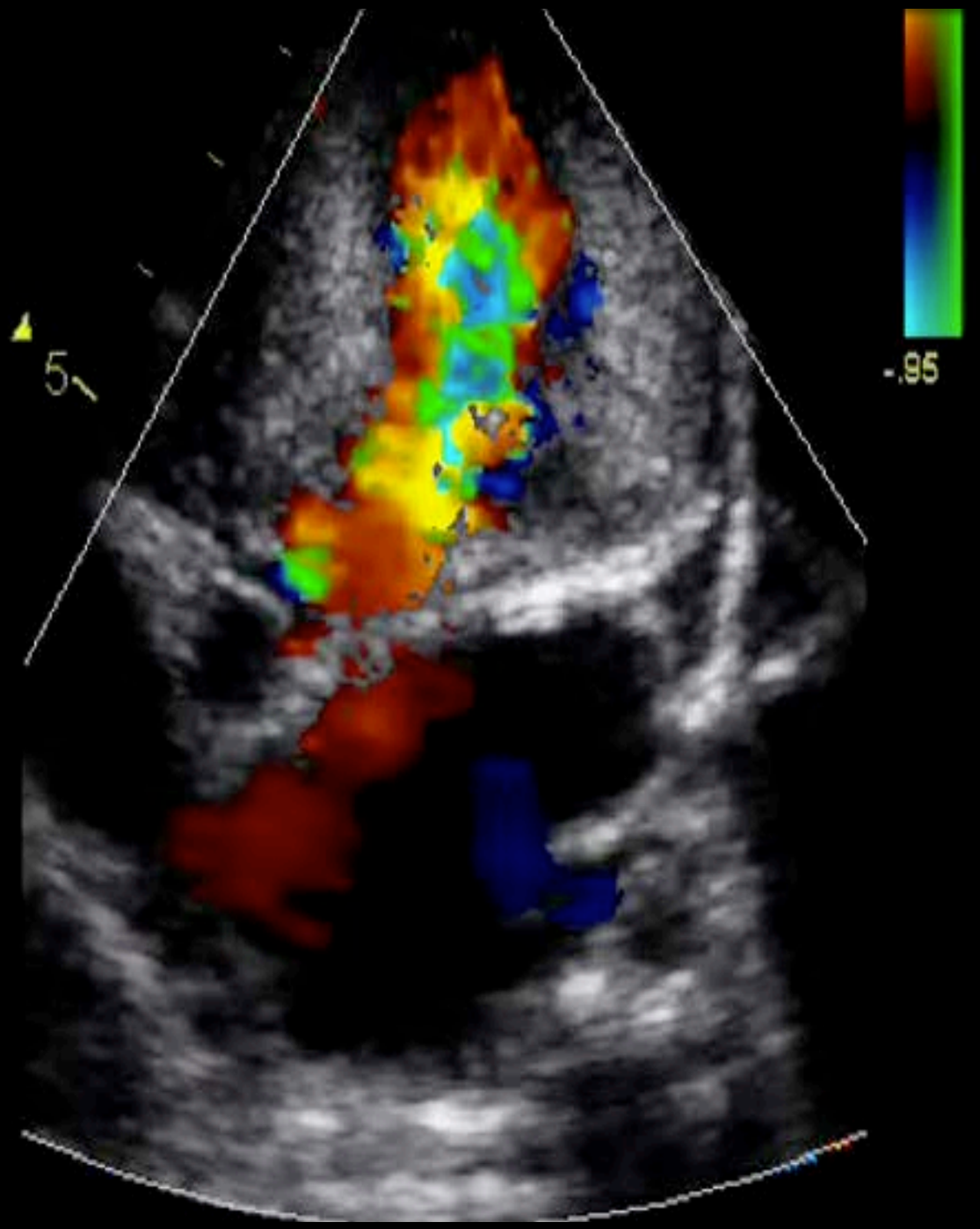
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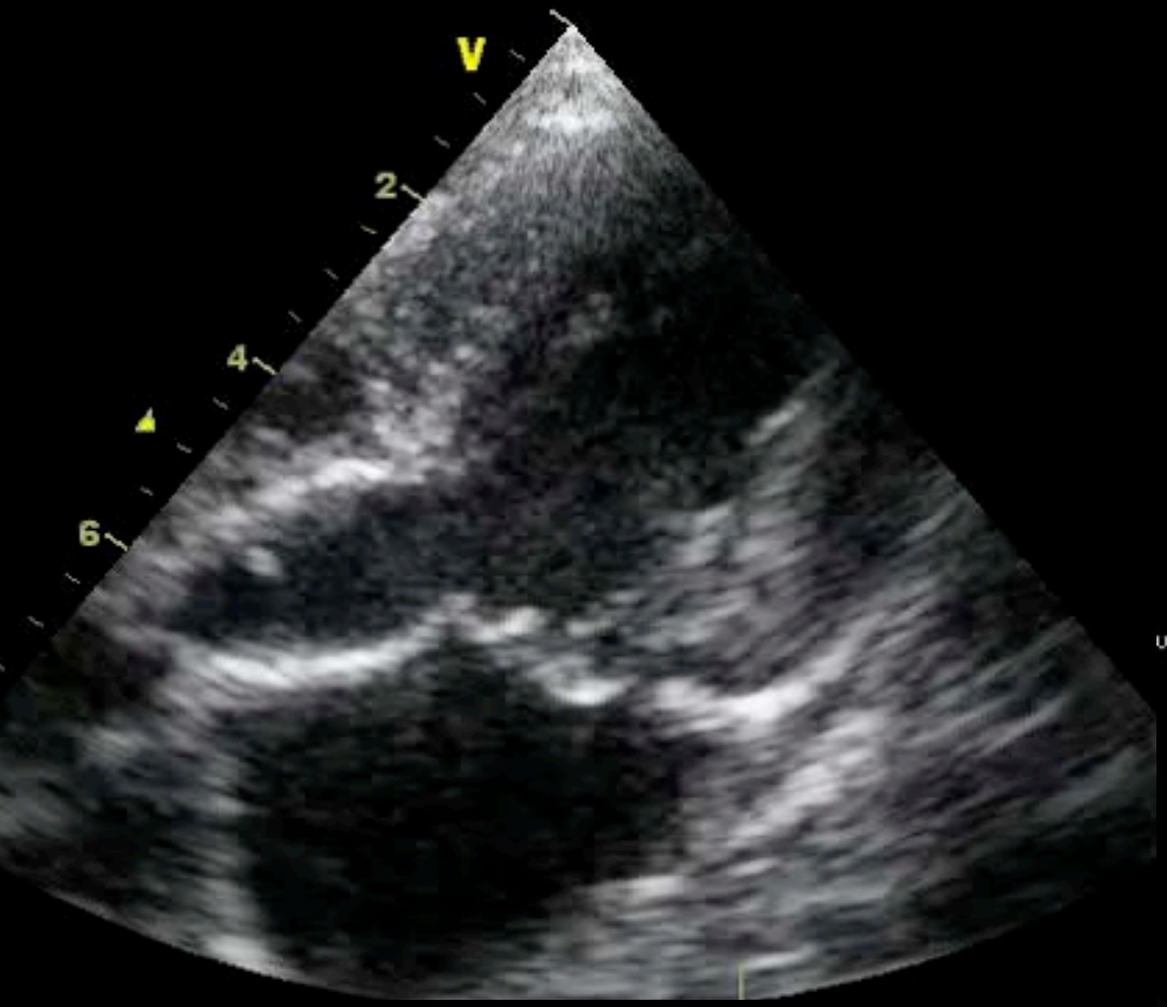
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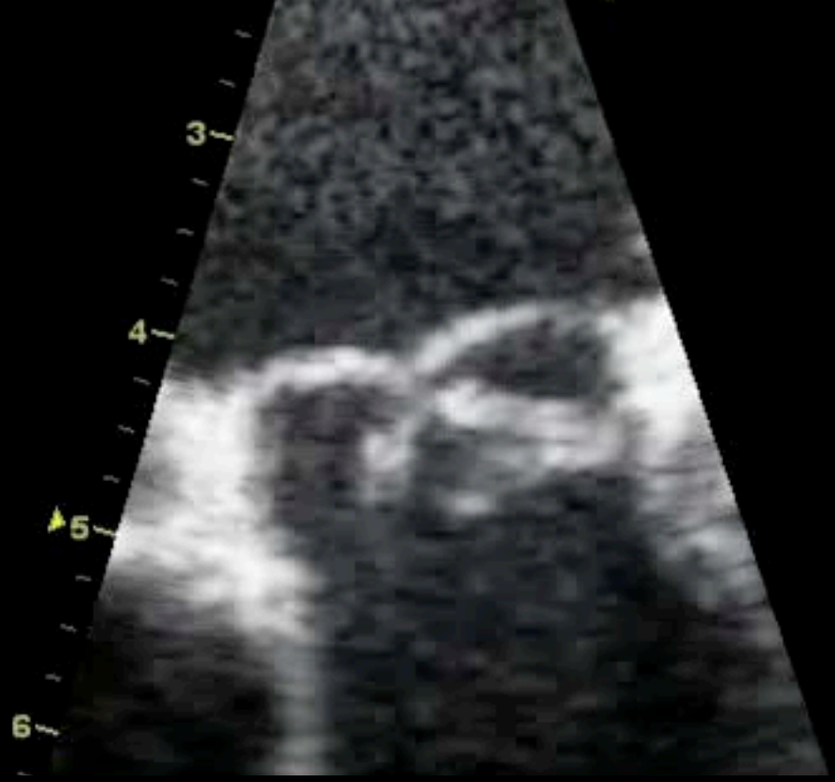
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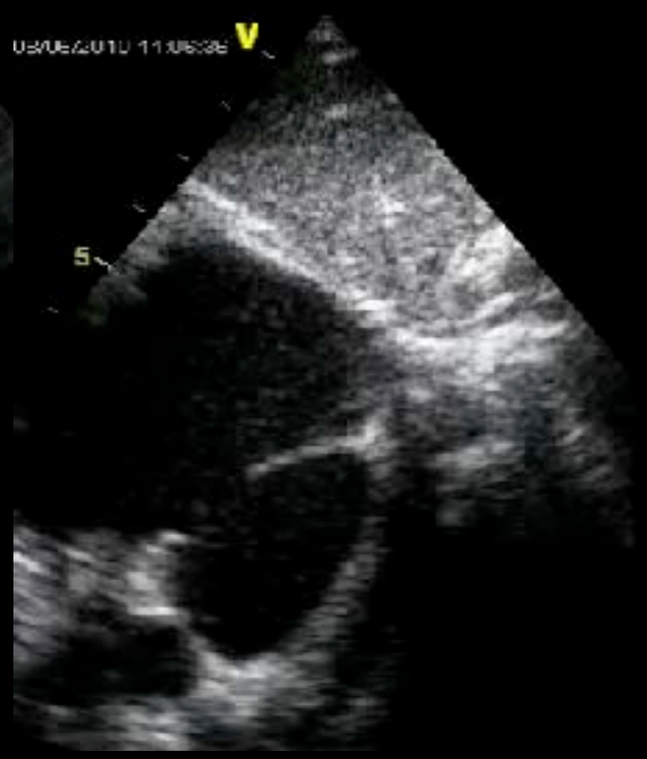
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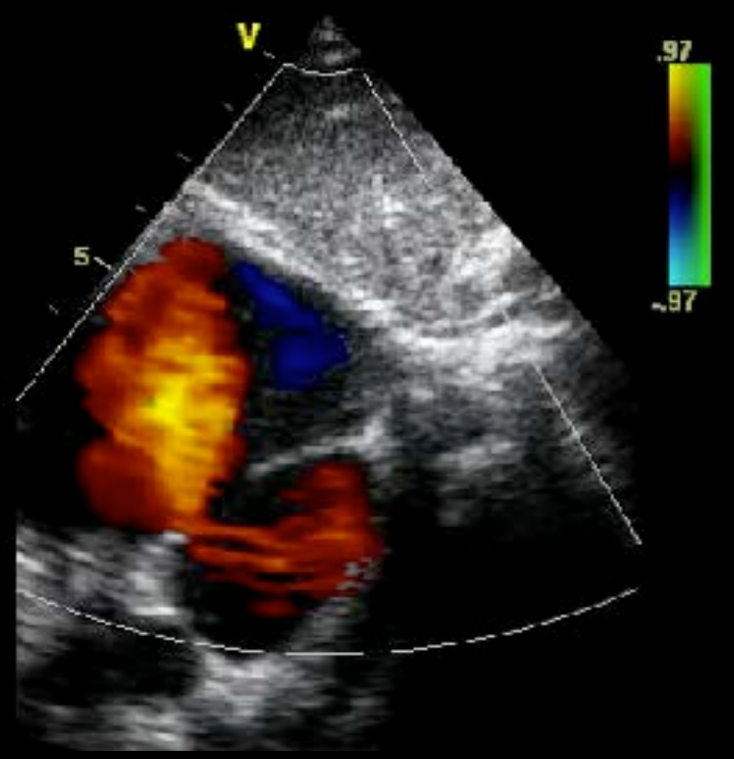
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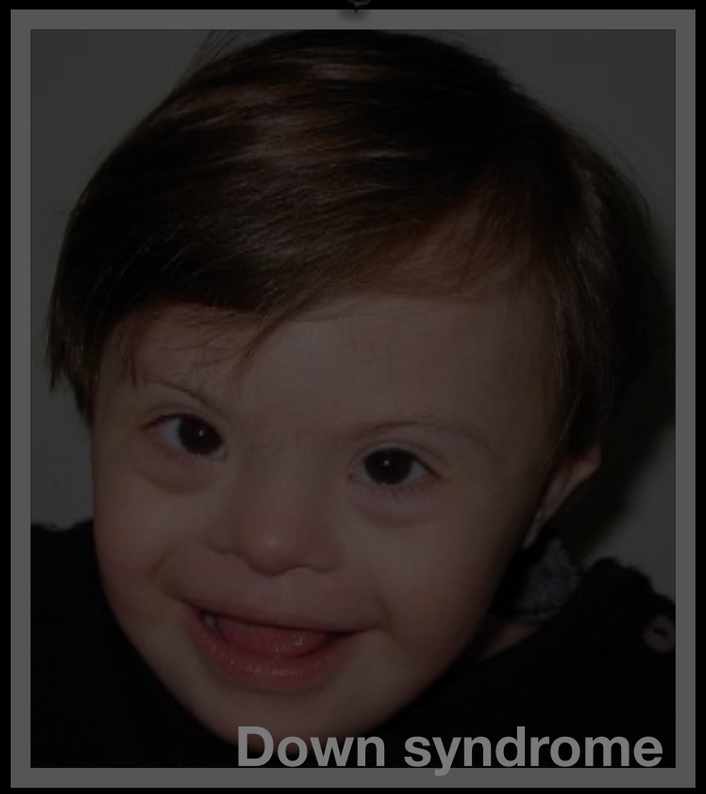
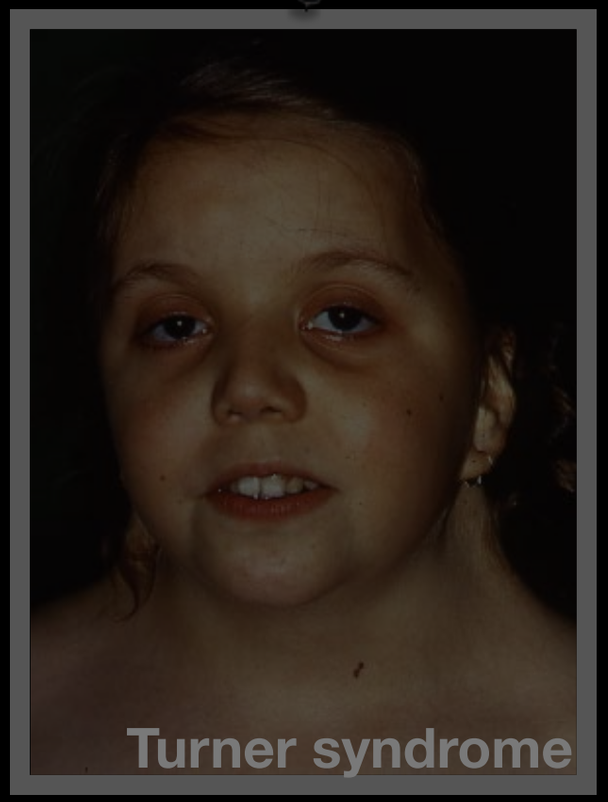
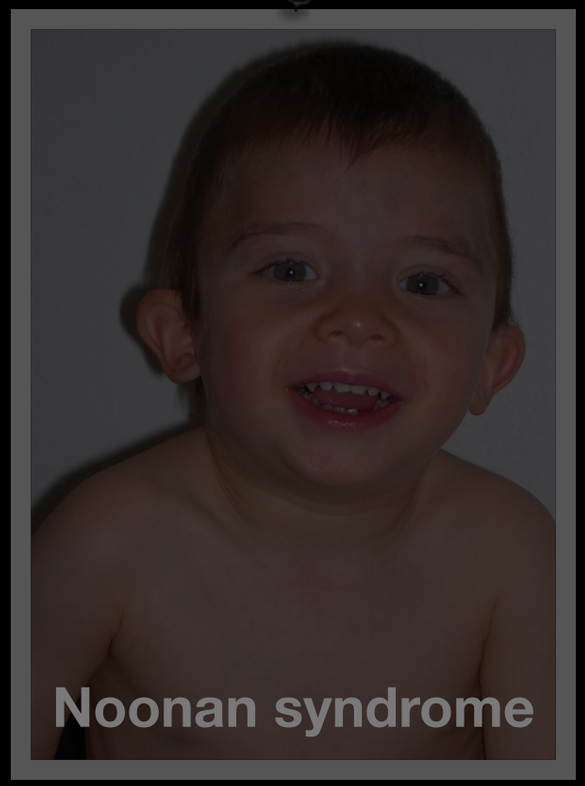
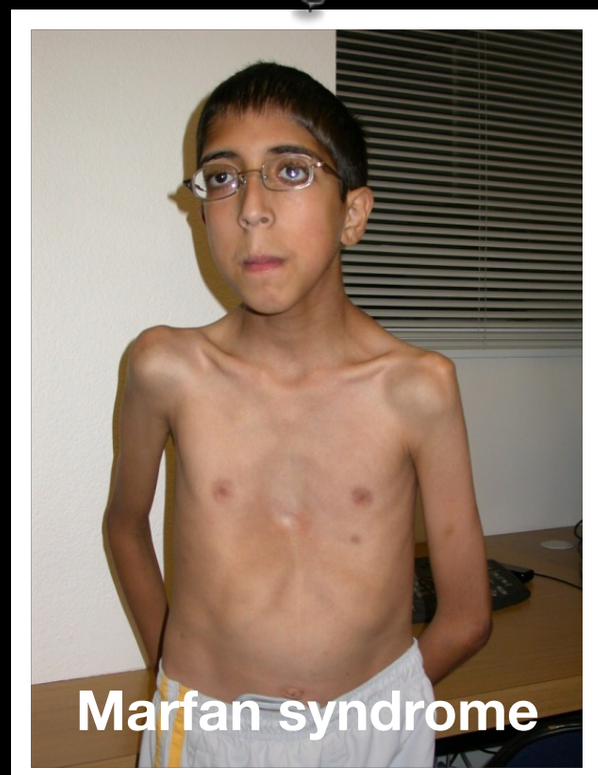
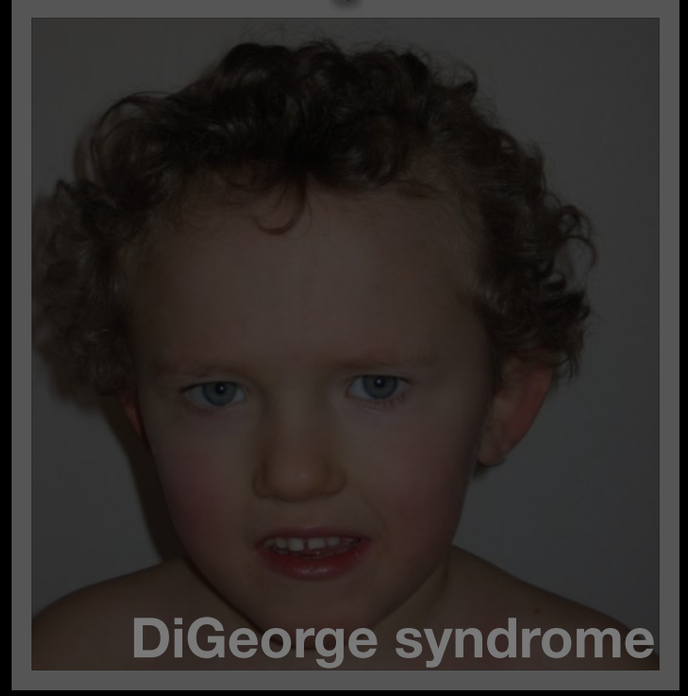
Old textbooks and clinical genetics

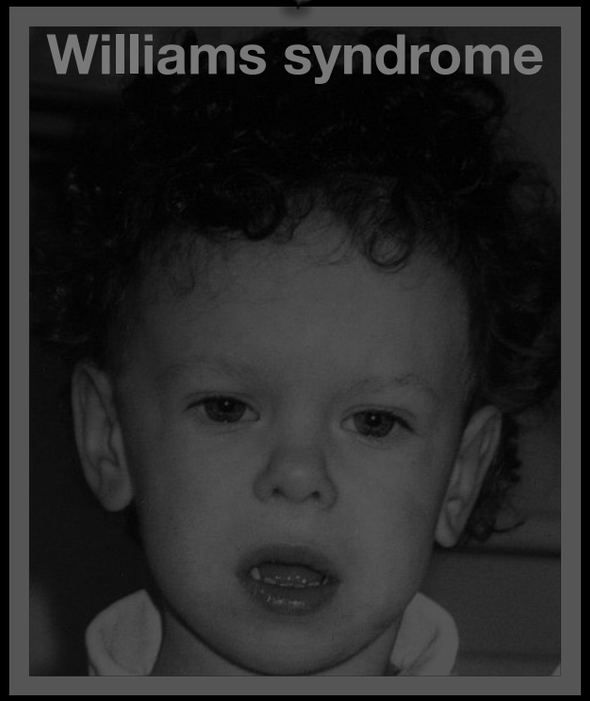


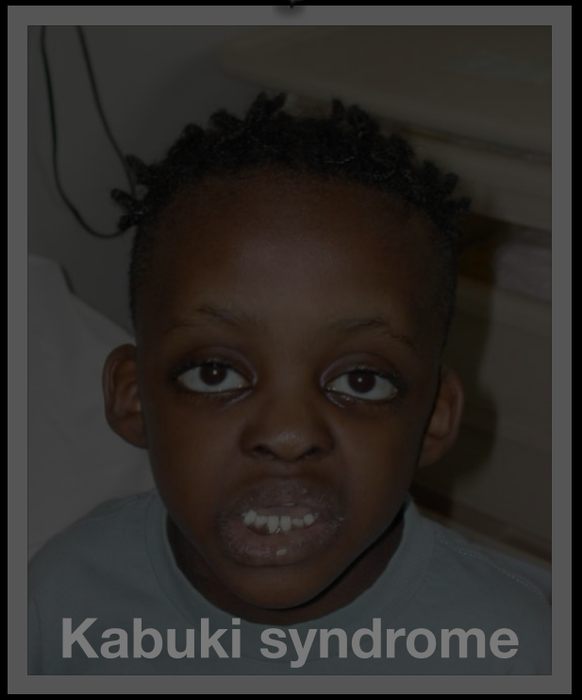


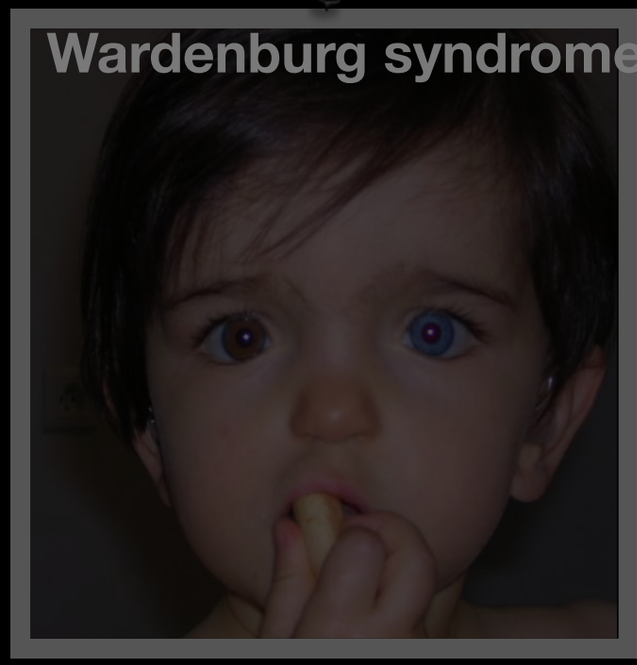


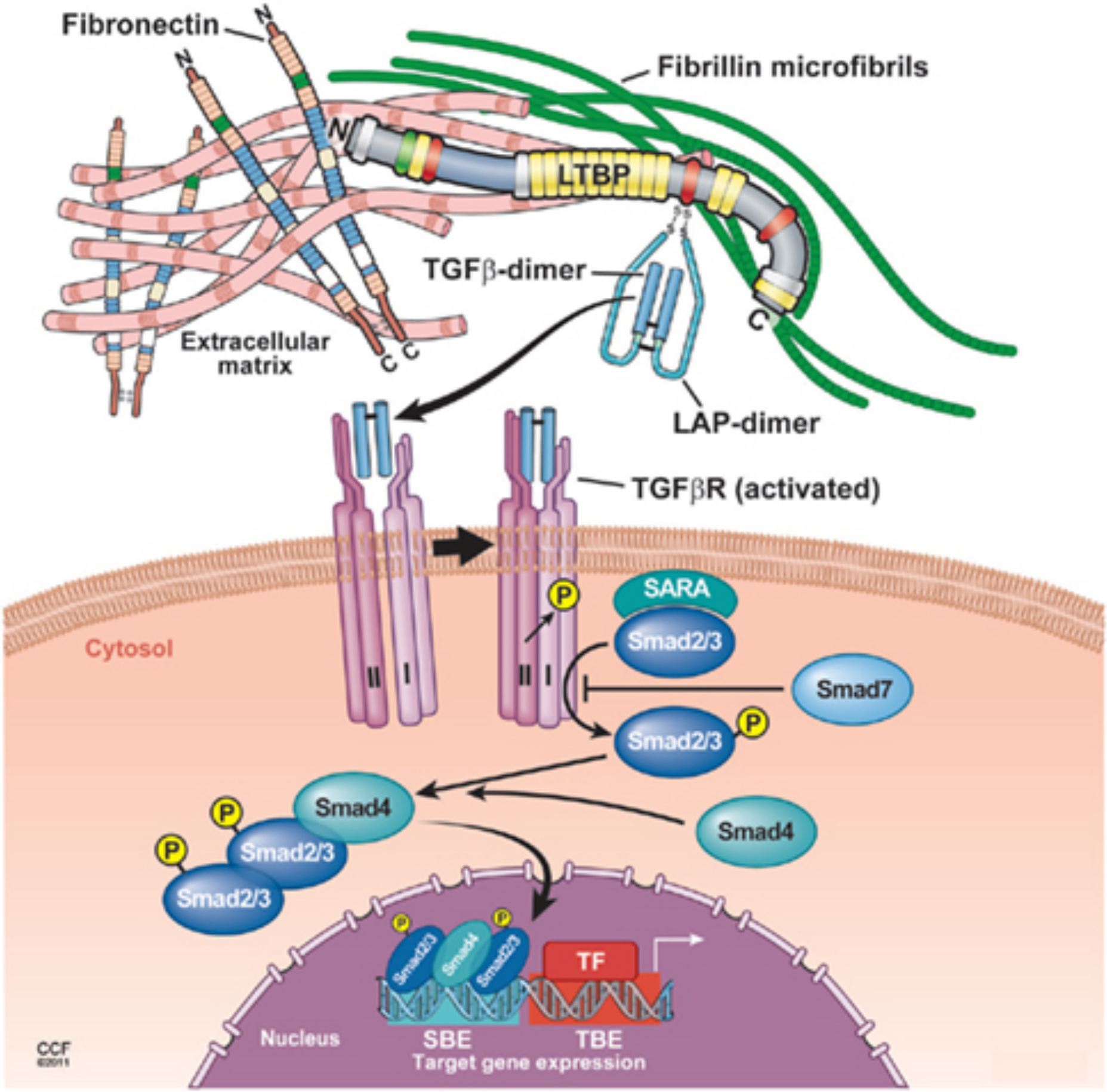


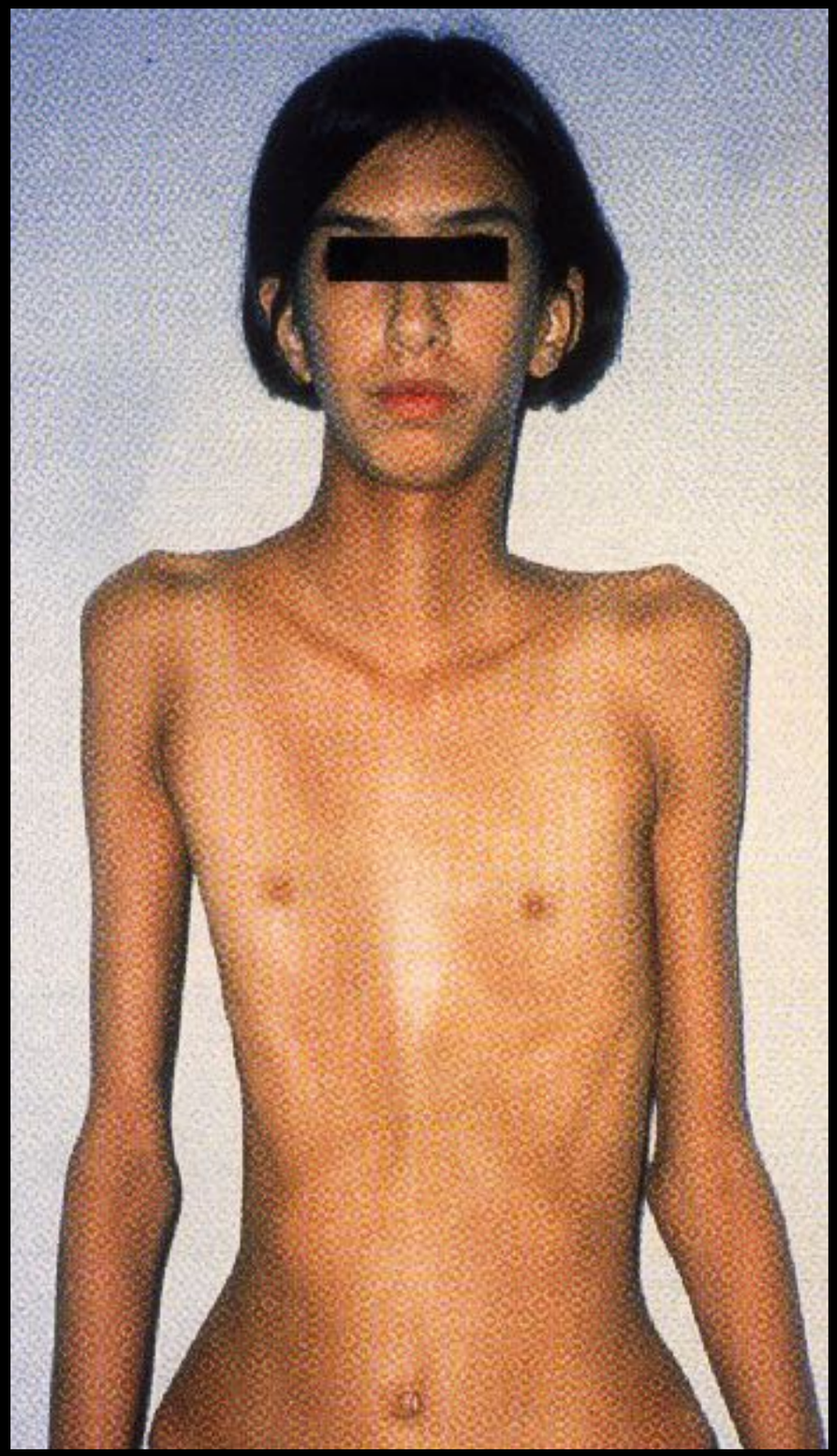


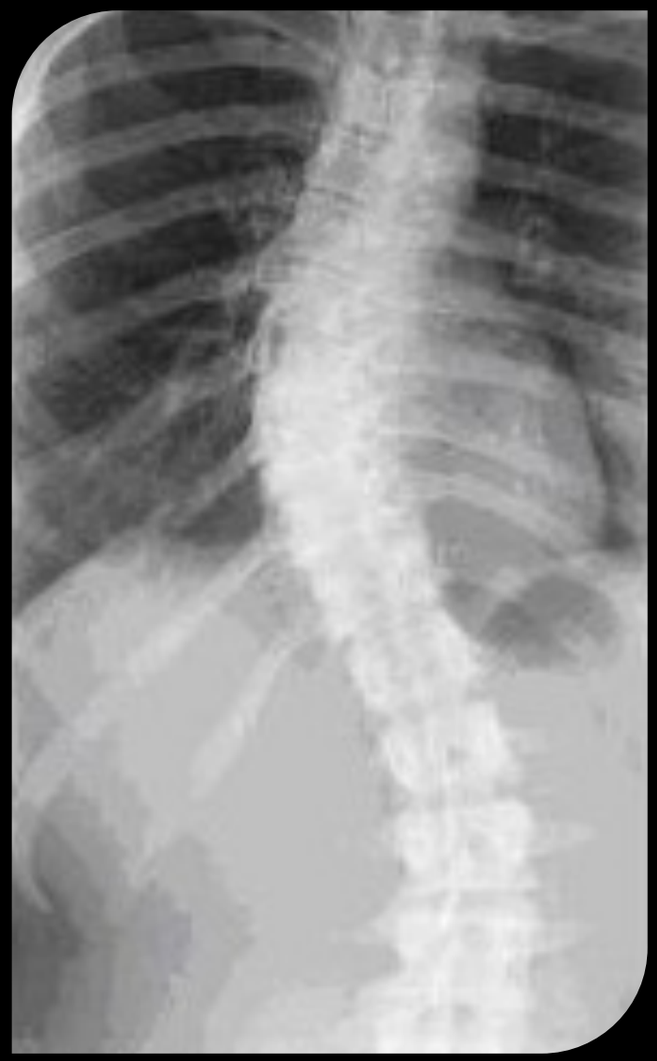


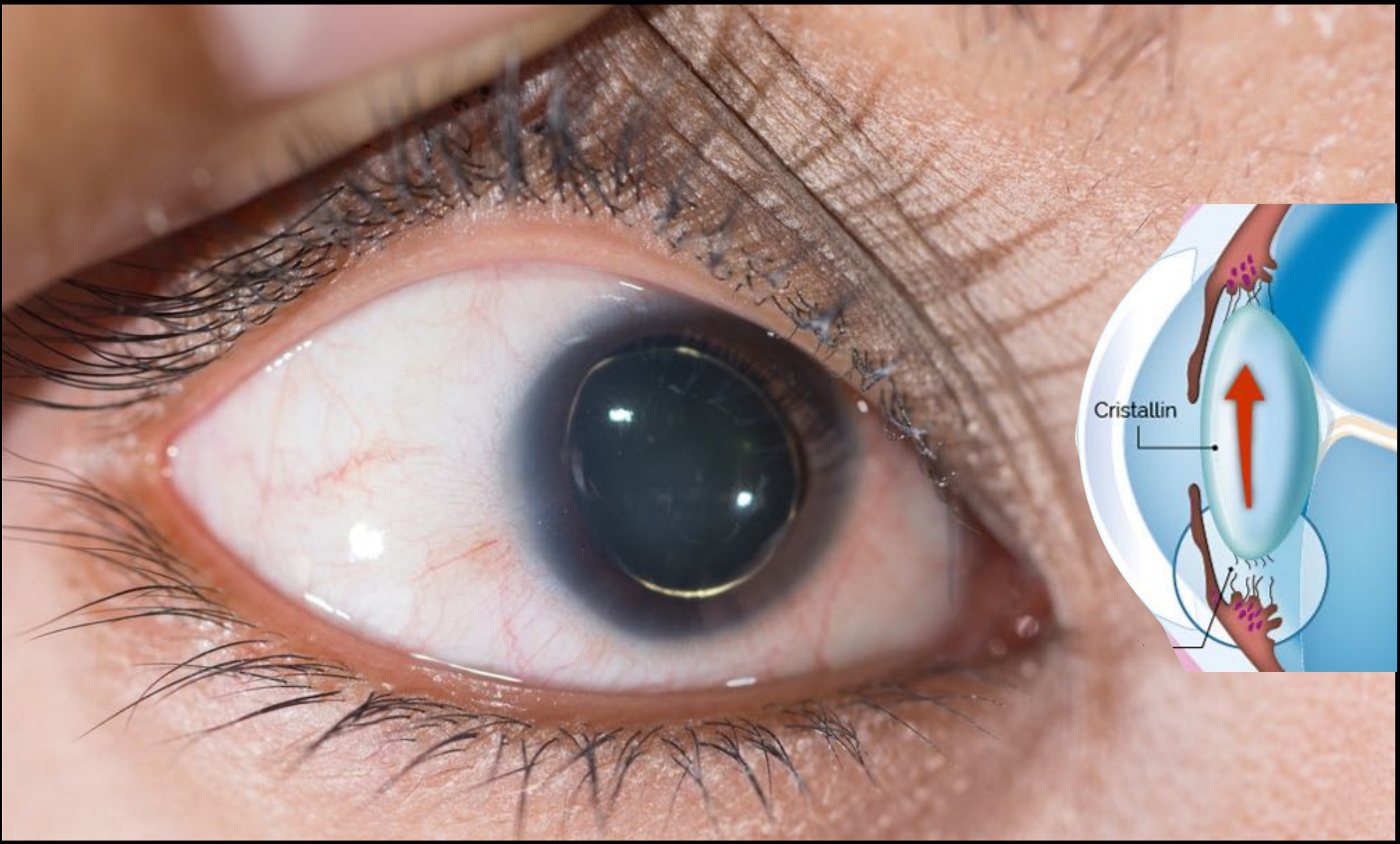


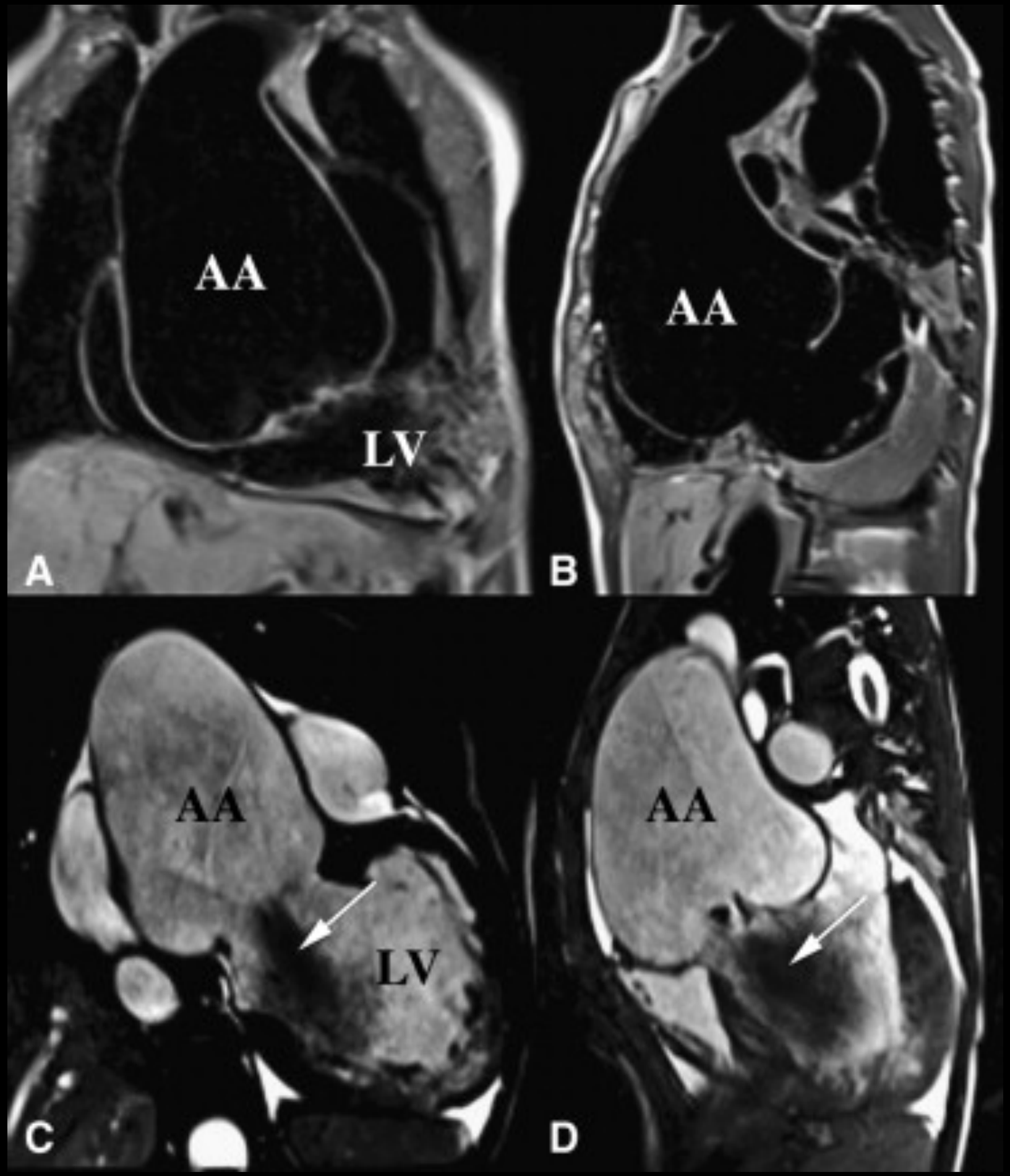



Fibronectin
Fibrillin microfibrils
N
LTBP
TGFβ-dimer
C
Extracellular matrix
C
C
LAP-dimer
TGFβR (activated)
Cytosol
SARA
Smad2/3
Smad7
P
Smad2/3
Smad4
P
Smad2/3
P
Smad2/3
Smad4
TF
Nucleus
SBE
TBE
Target gene expression
CCF
©2011



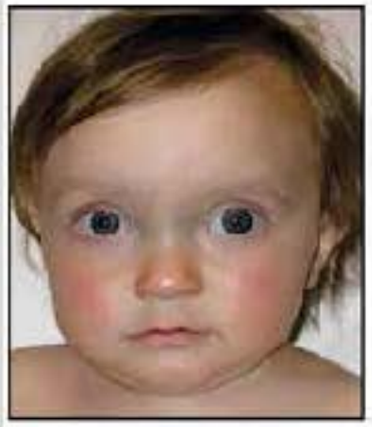


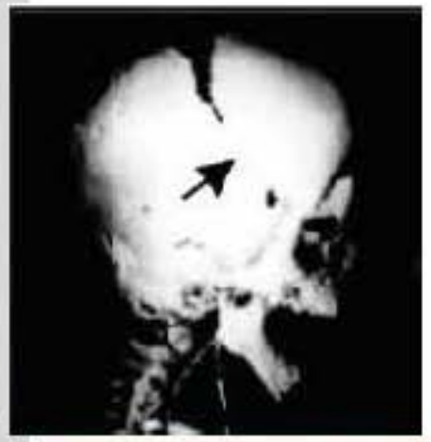

Cristallin

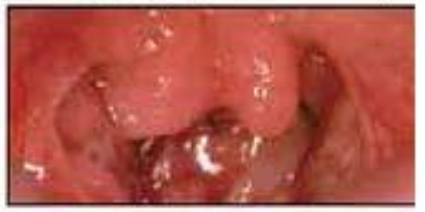

AA
LV
A
AA
B
AA
LV
C
AA
D

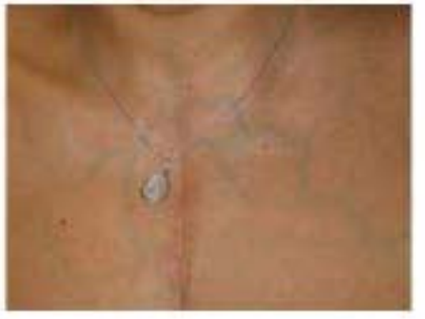
Syndrome de Loeys Dietz

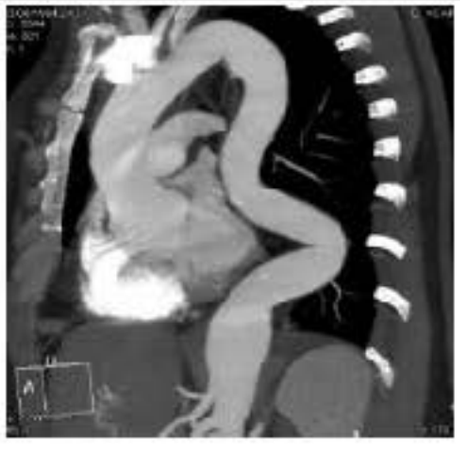
Système	Signes
Craniofacial	- Hypertélorisme - Luette bifide - Fente palatine - Craniosténose
Squelettique	- Pied bot - Malformation cervicale et/ou instabilité
Cutané	- Peau translucide - Cicatrices dystrophiques
Vasculaire	- Anévrysmes, Tortuosité Aortique - Tortuosité des vaisseaux du cou

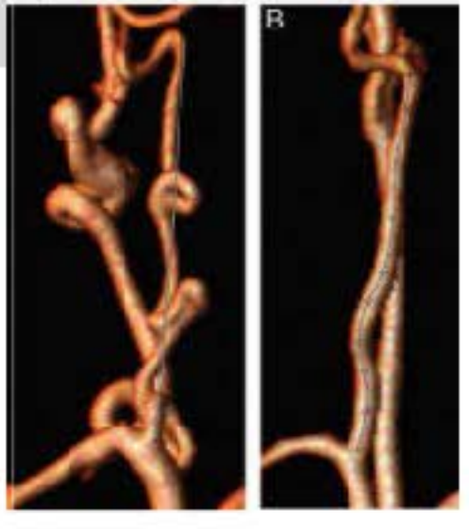






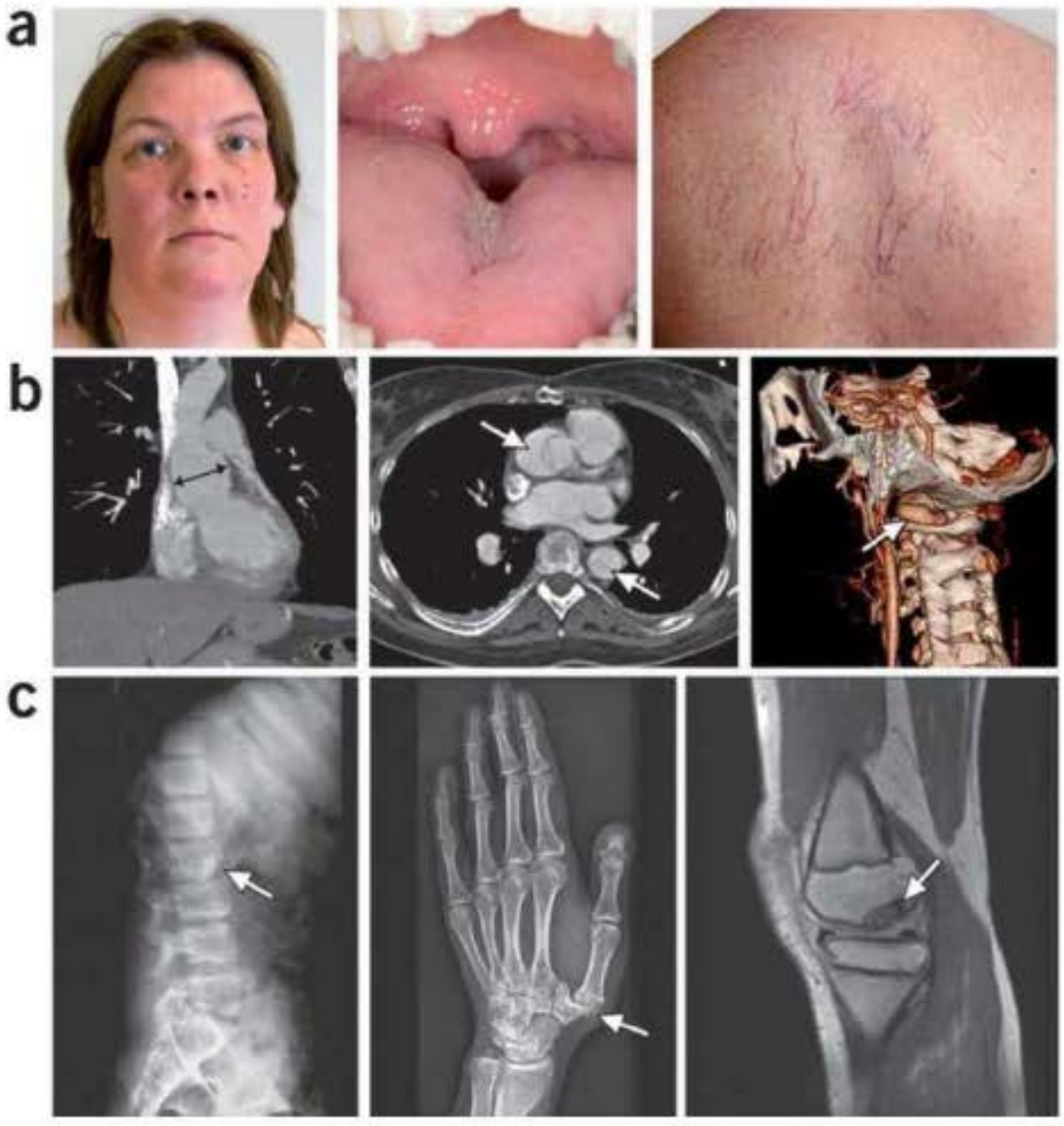






NEJM 2006;355:8

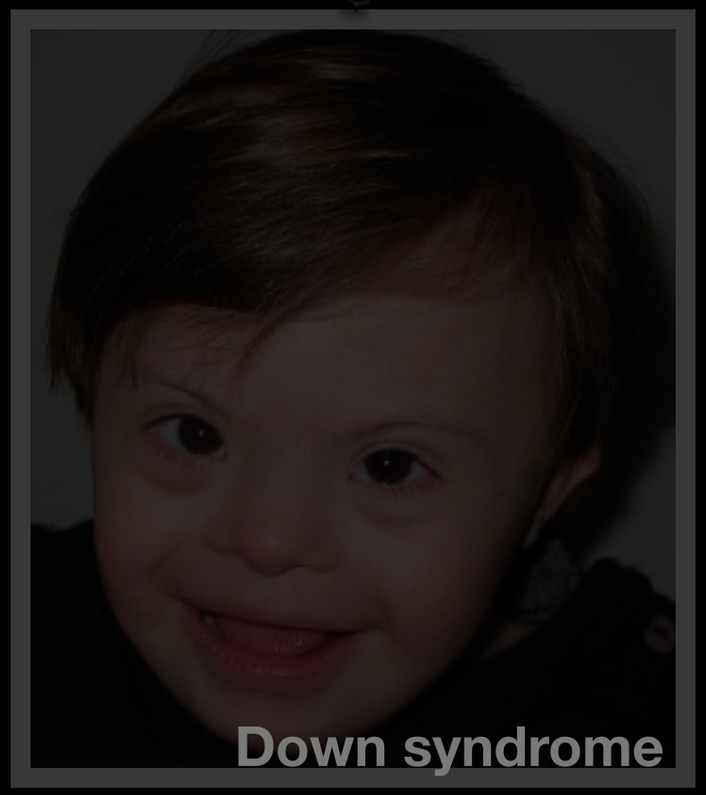
Mutation SMAD3

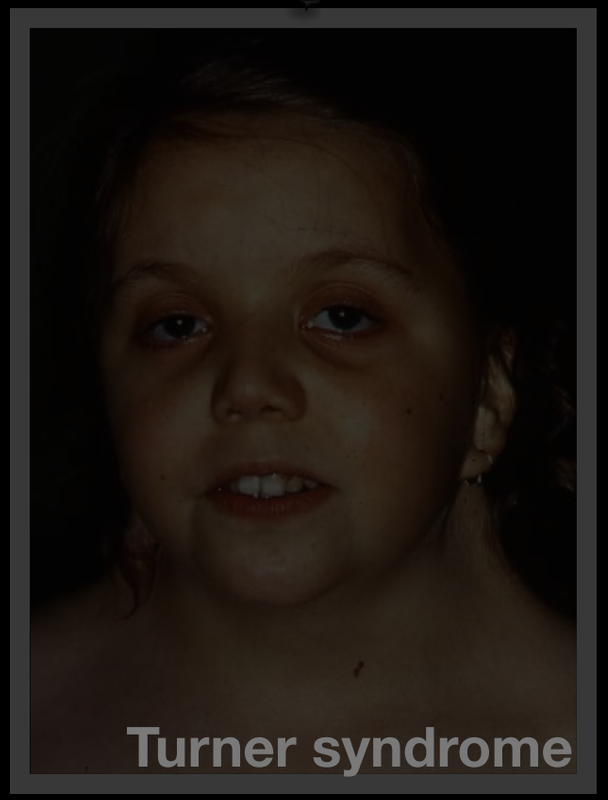


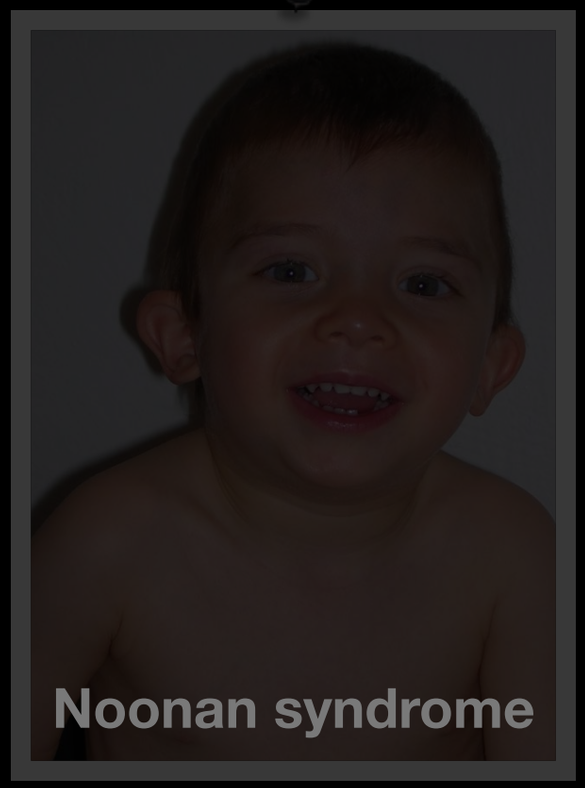
- hypertélorisme
- luette bifide et/ou fente palatine
- anévrysmes aortiques
- ostéoarthrite précoce

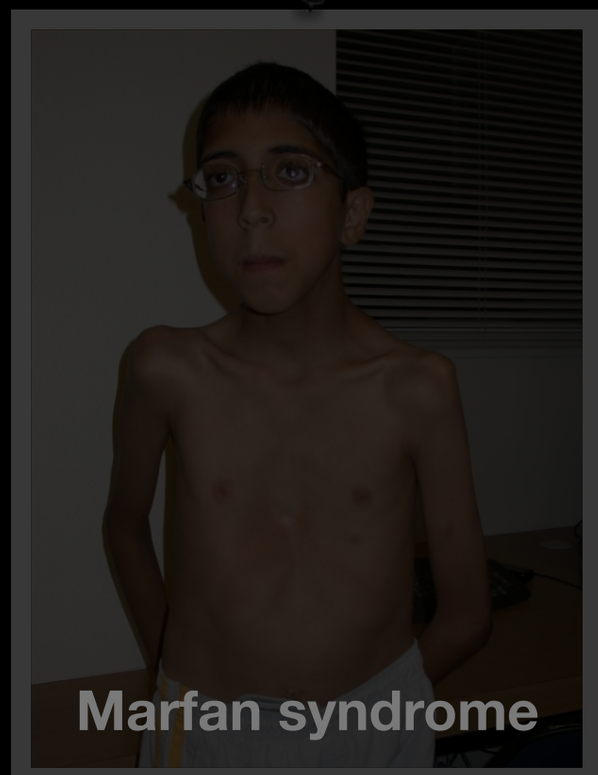
Van De Laar, Nat Genet. 2011

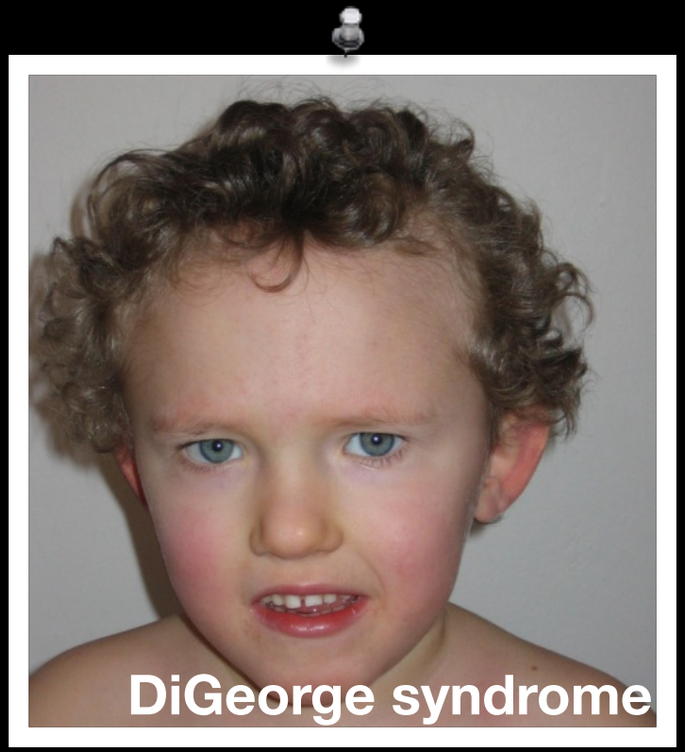
Old textbooks and clinical genetics

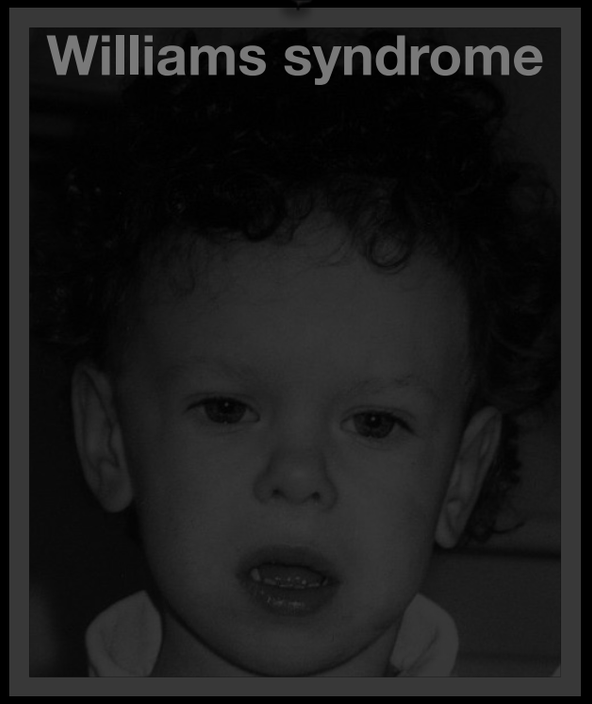

Down syndrome

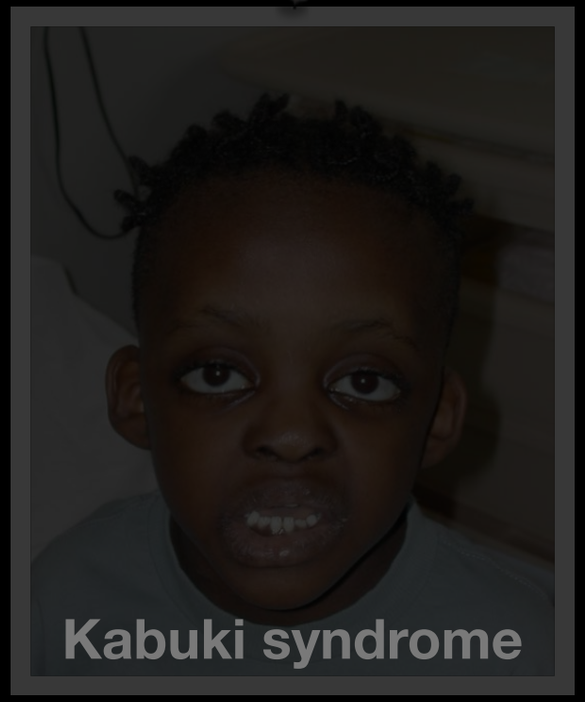

Turner syndrome

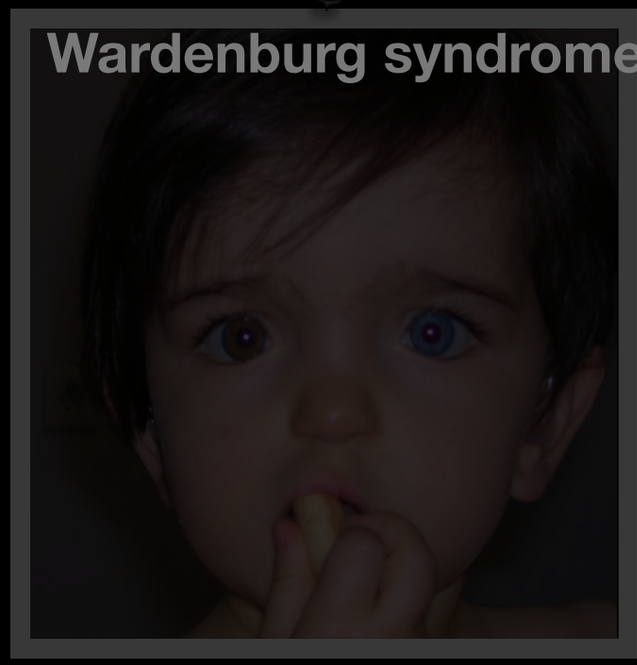

Noonan syndrome

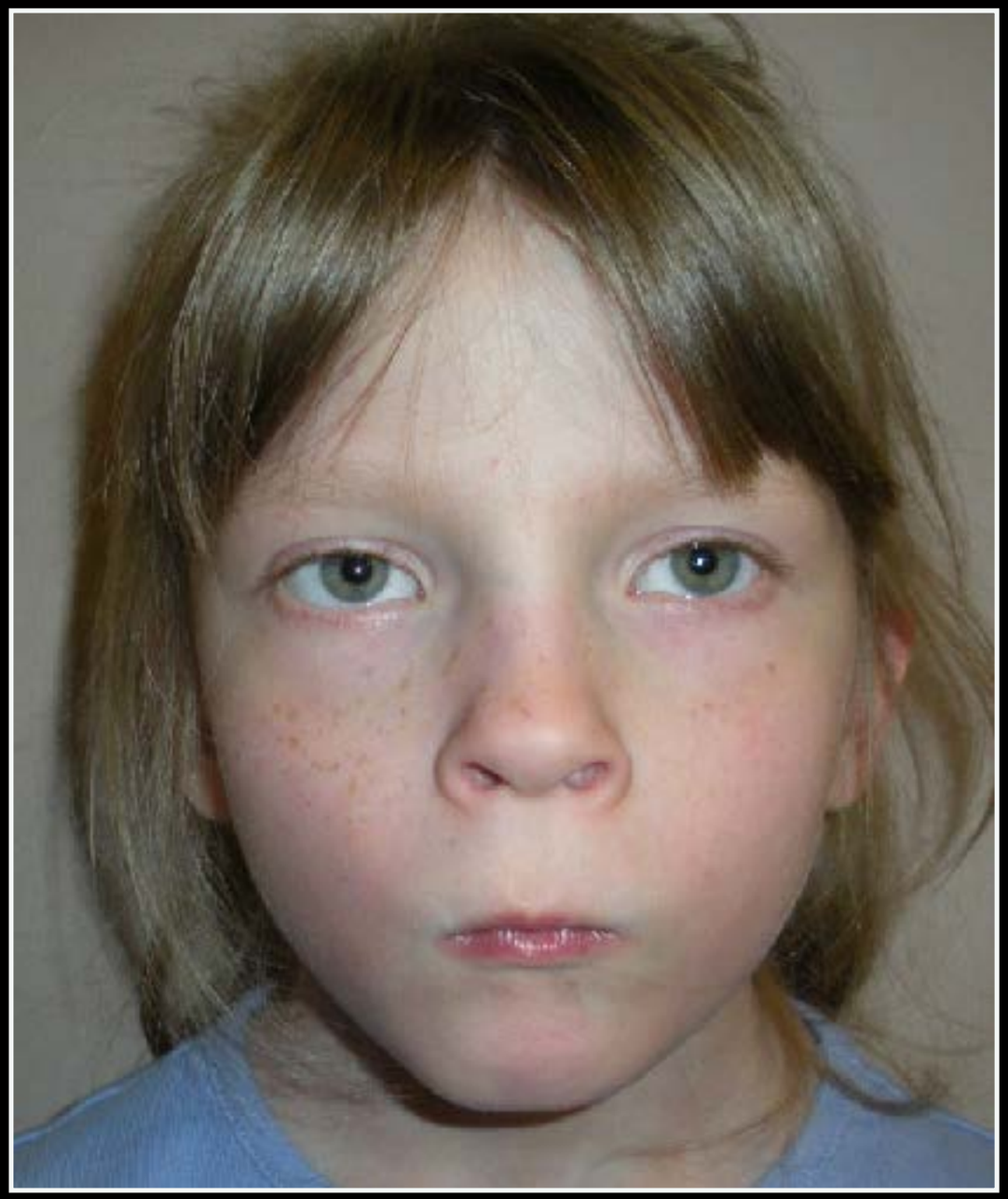

Marfan syndrome

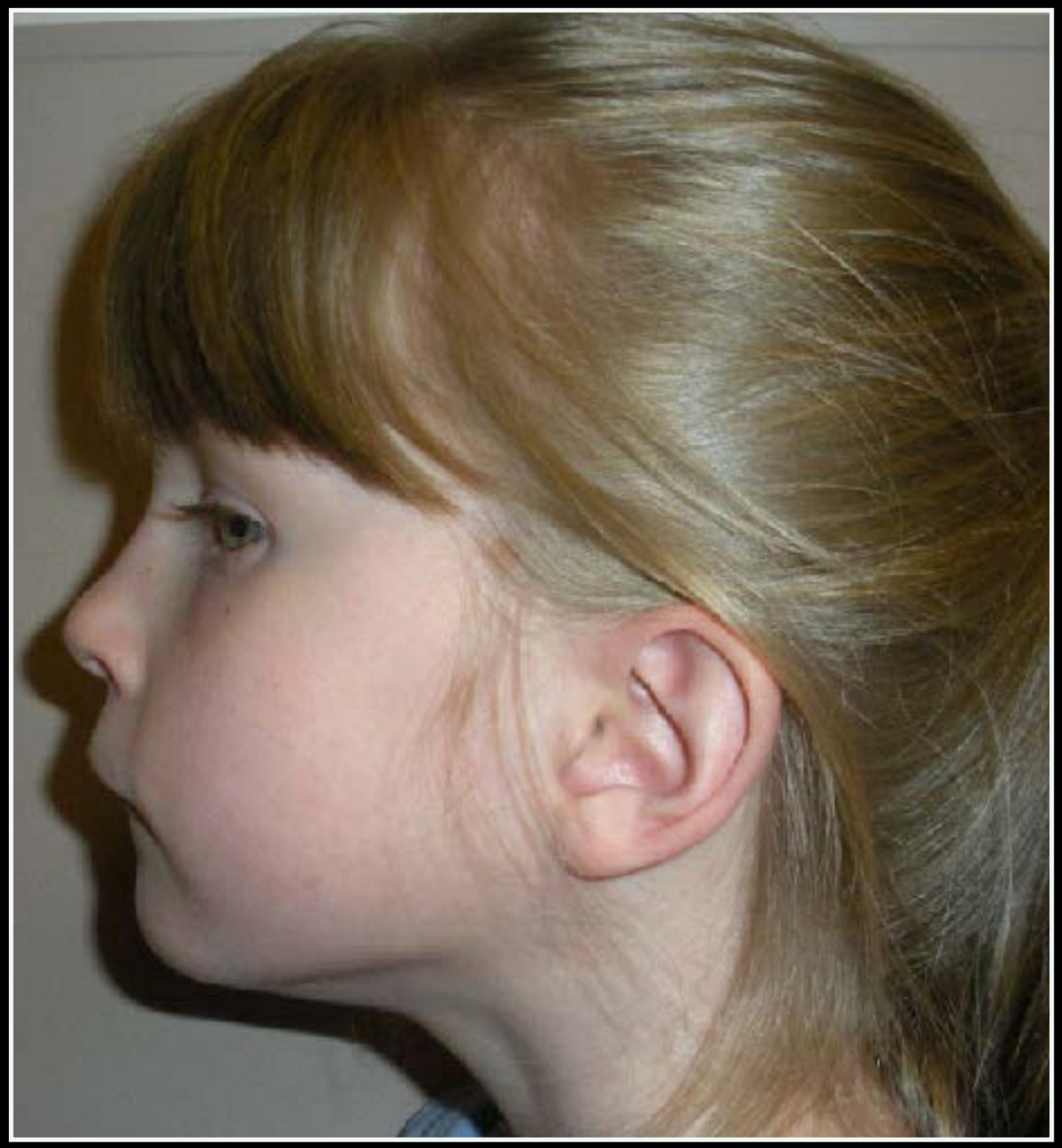

DiGeorge syndrome


Williams syndrome

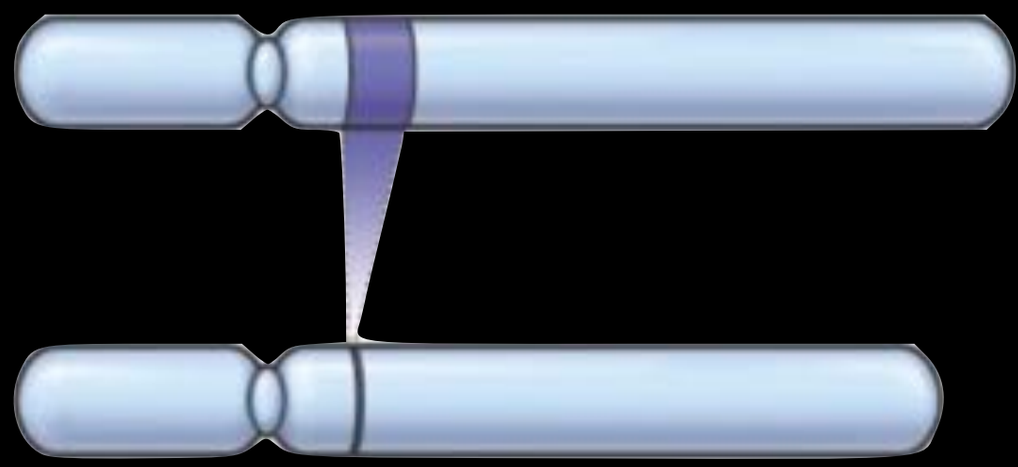

Kabuki syndrome


Wardenburg syndrome

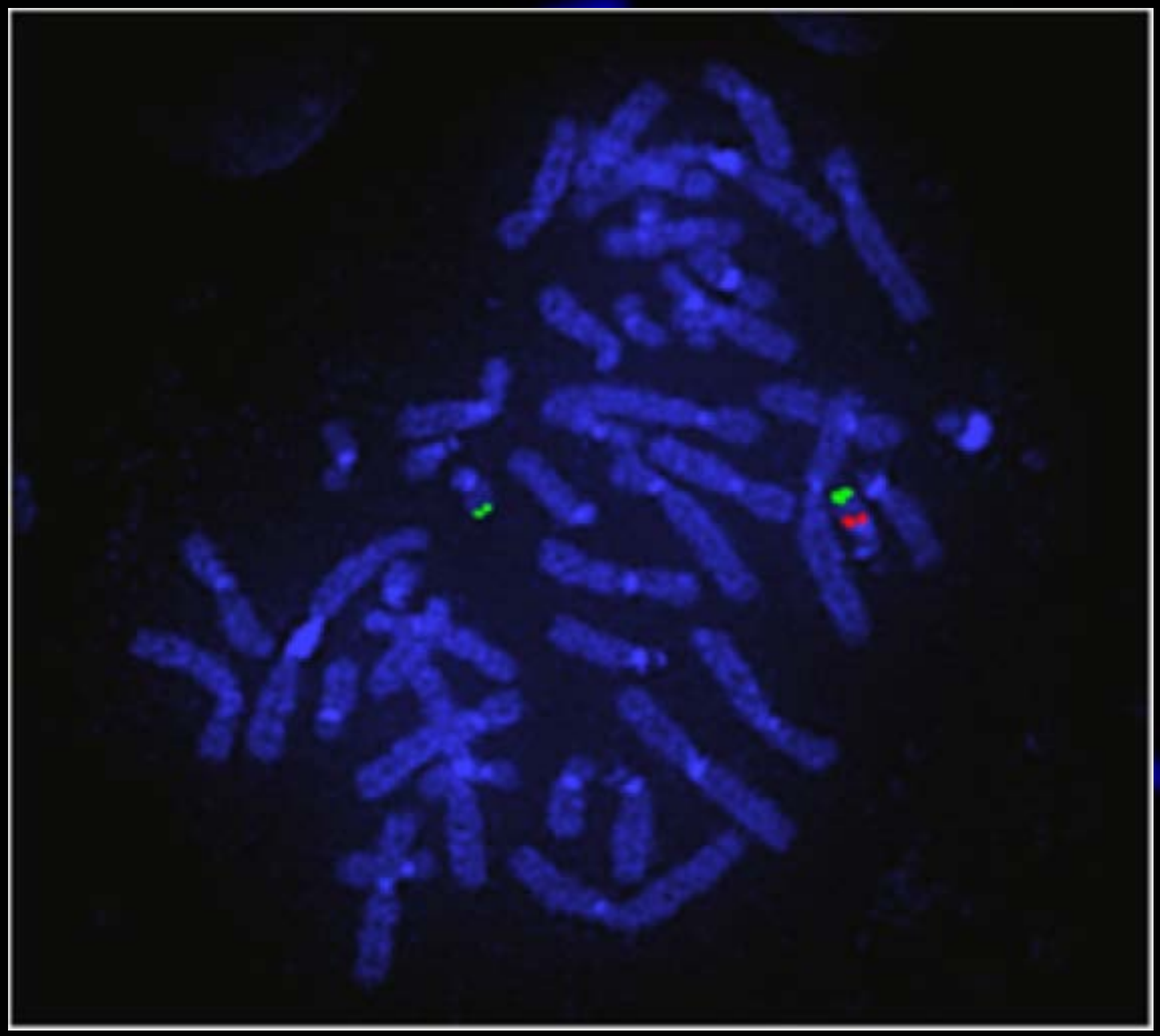




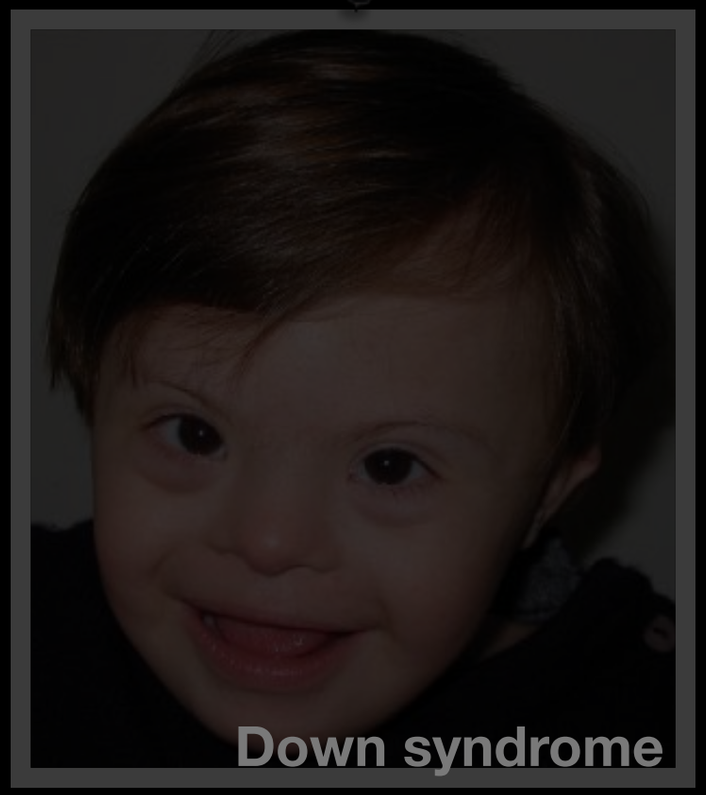
Normal chromosome 22

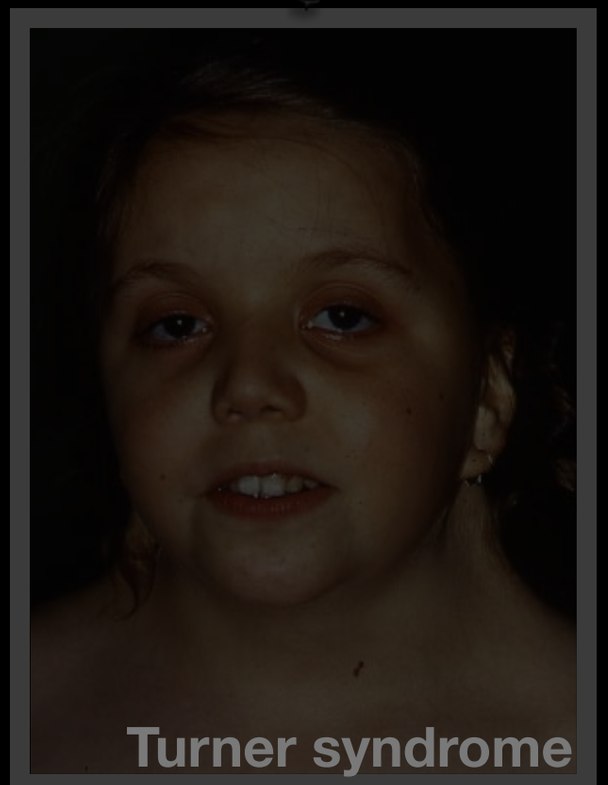


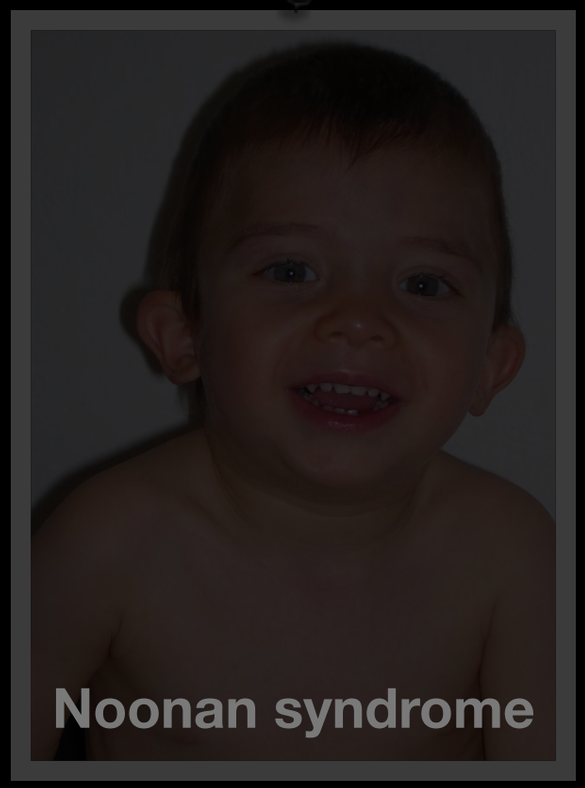
Chromosome 22q11 deletion

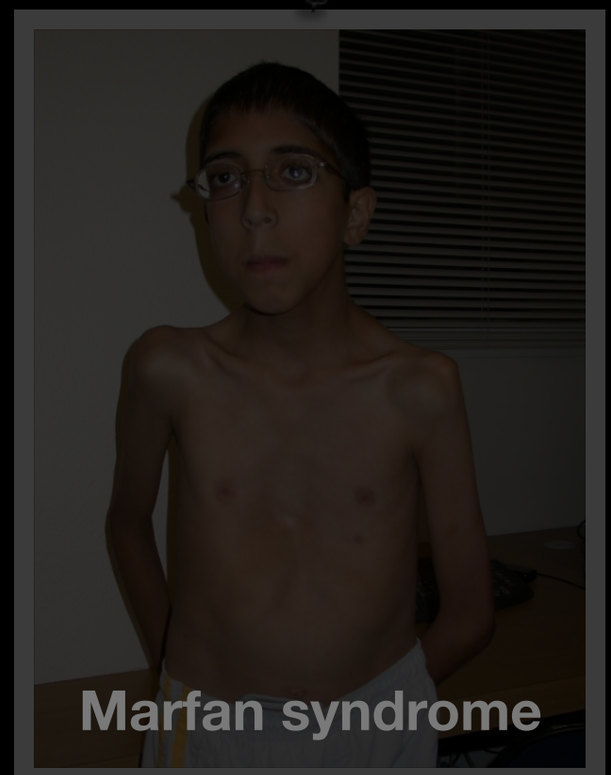


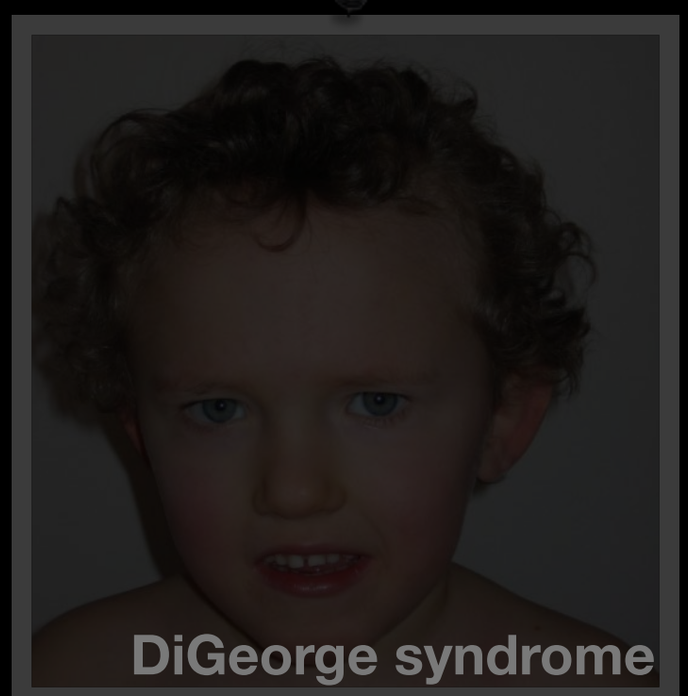
Old textbooks and clinical genetics

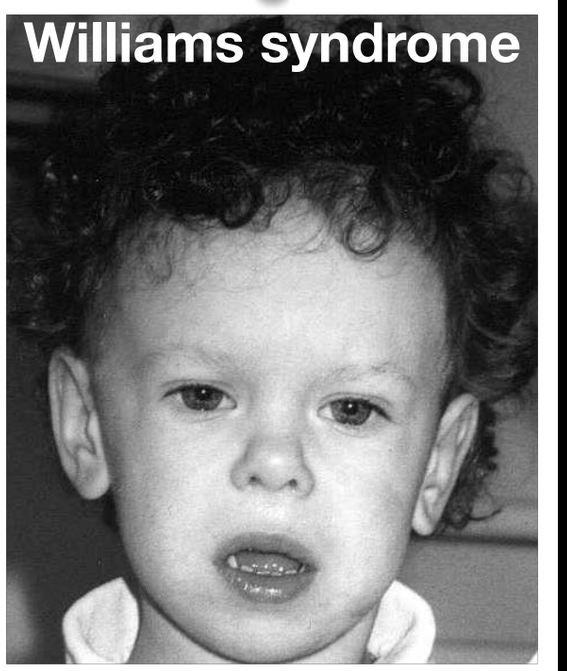


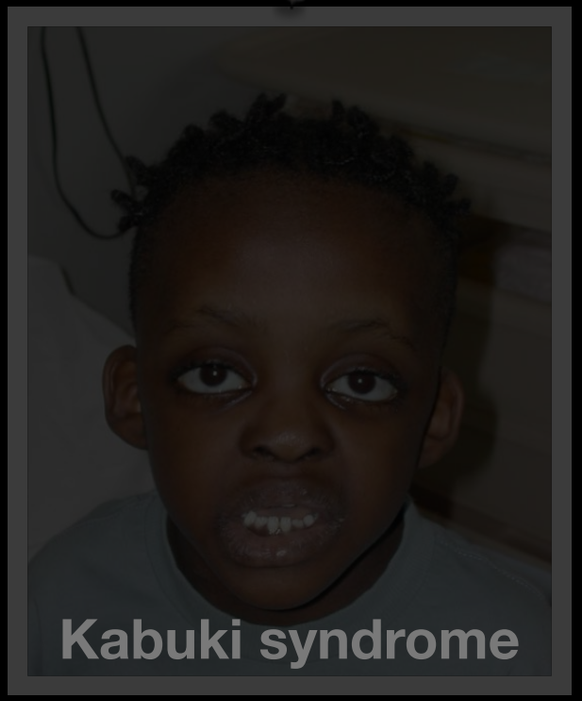


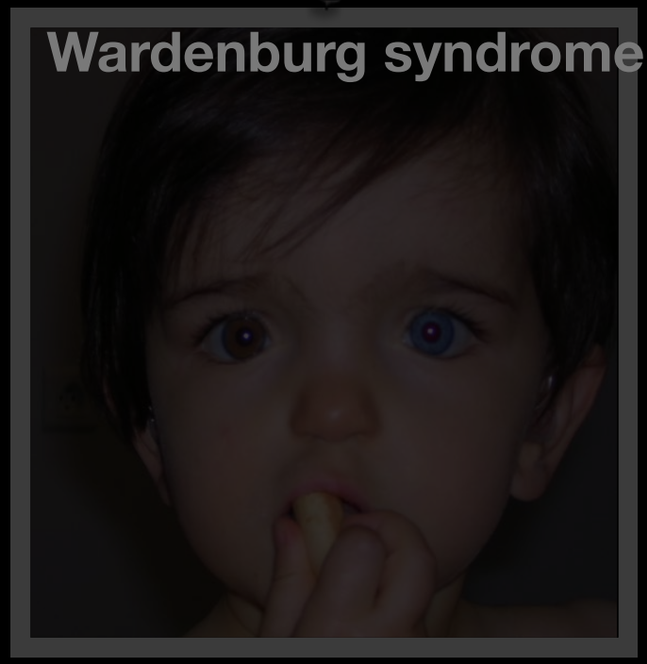


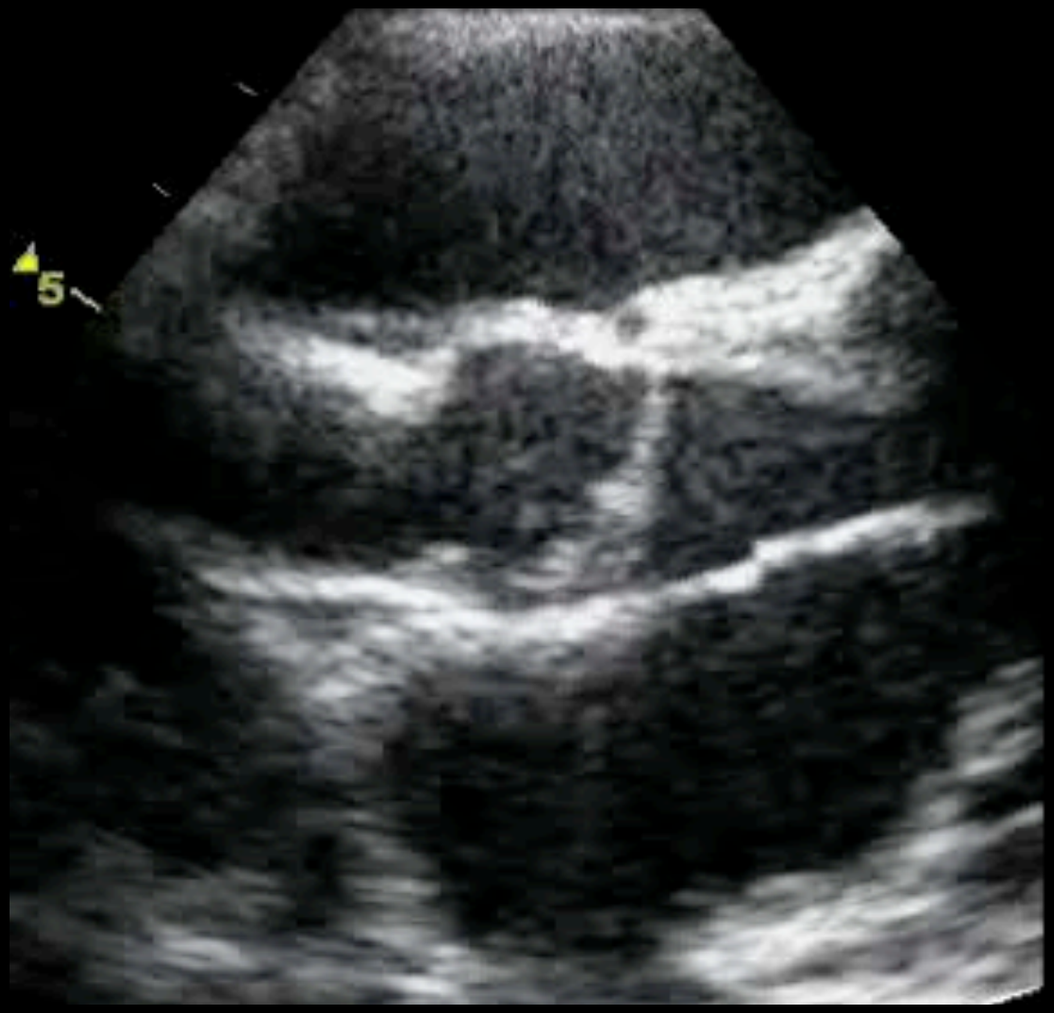








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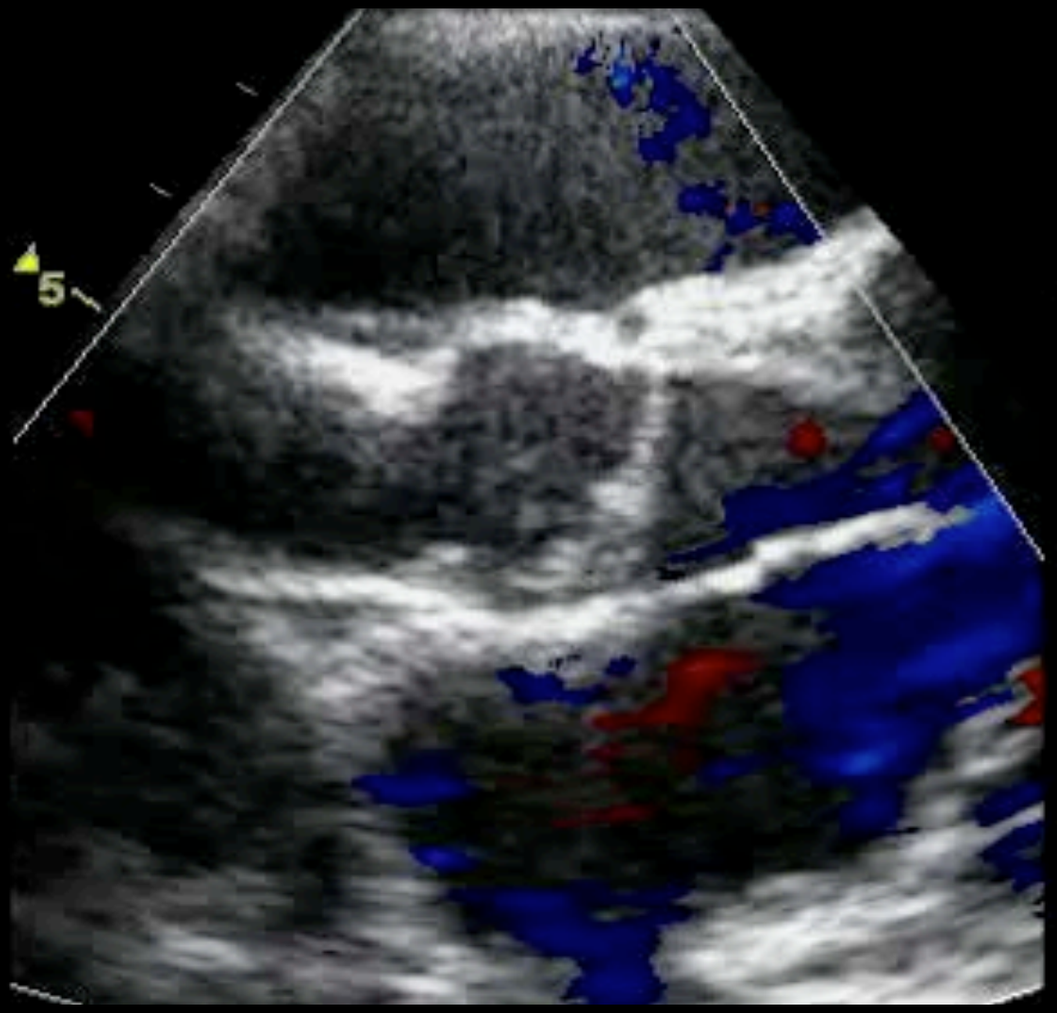
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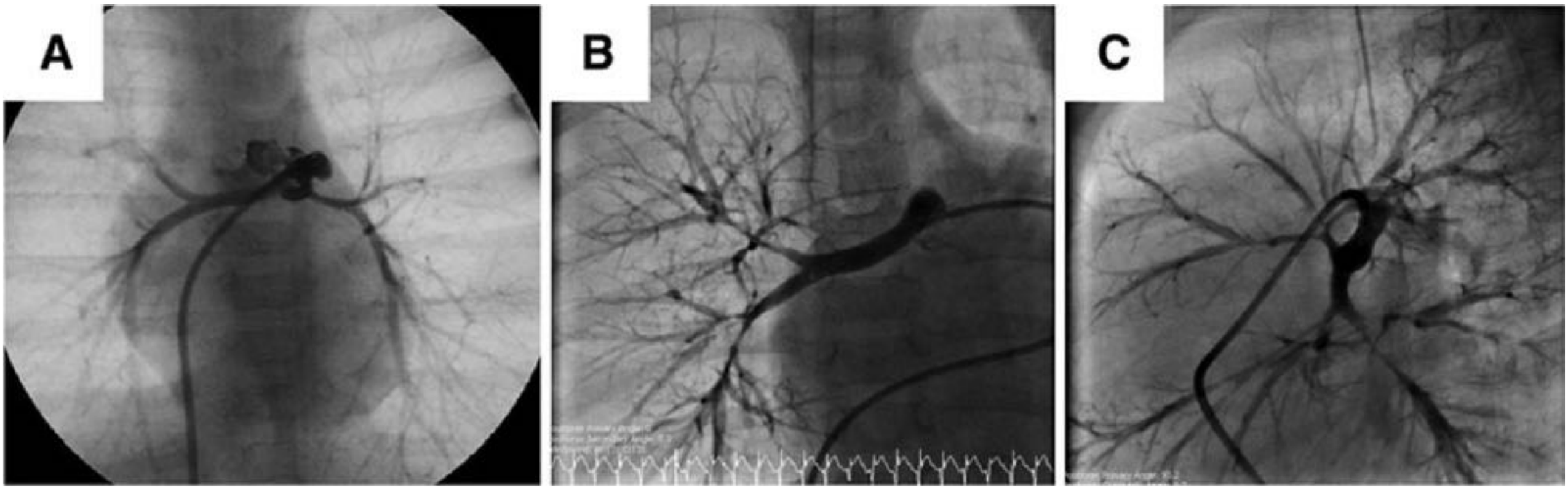
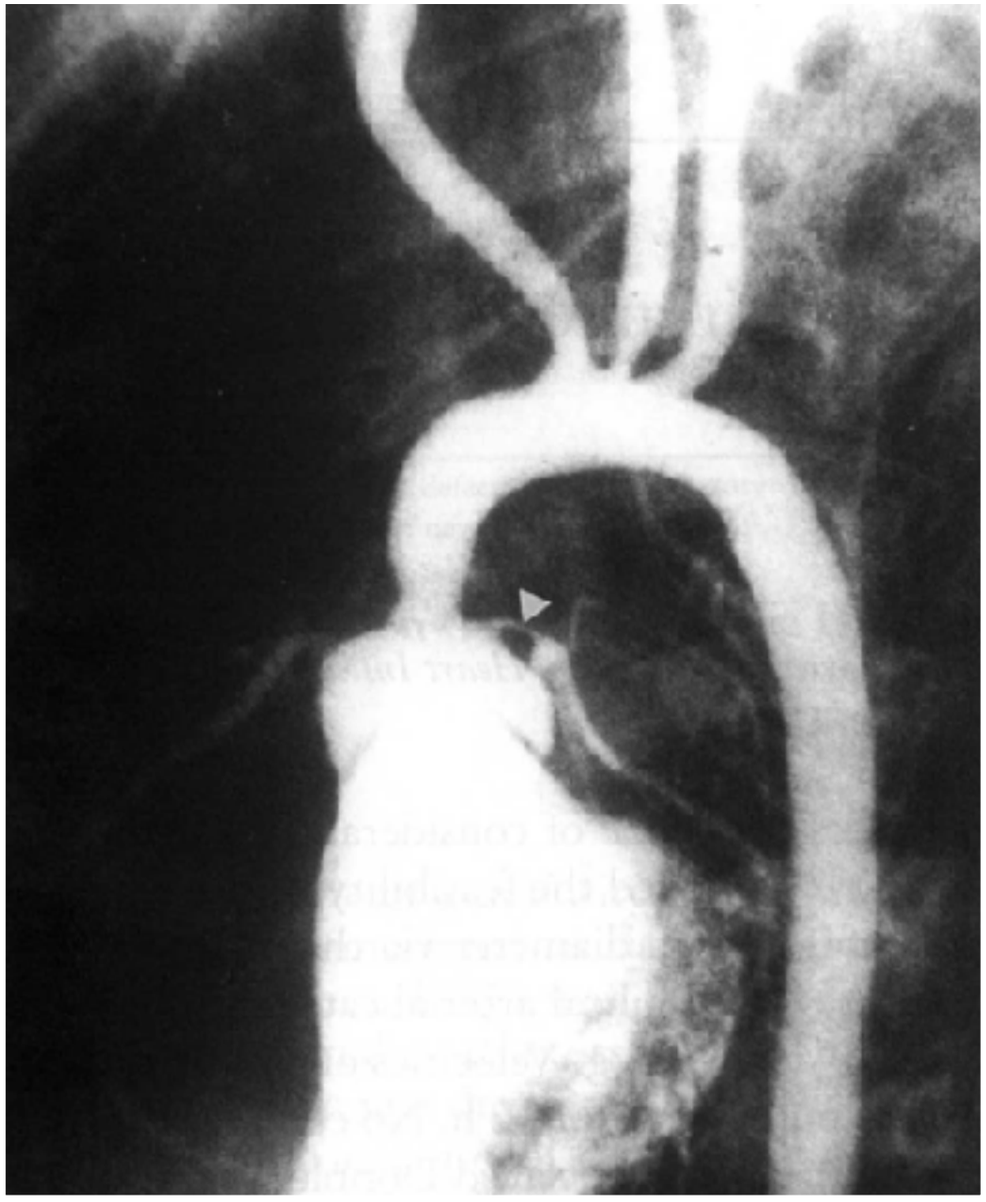
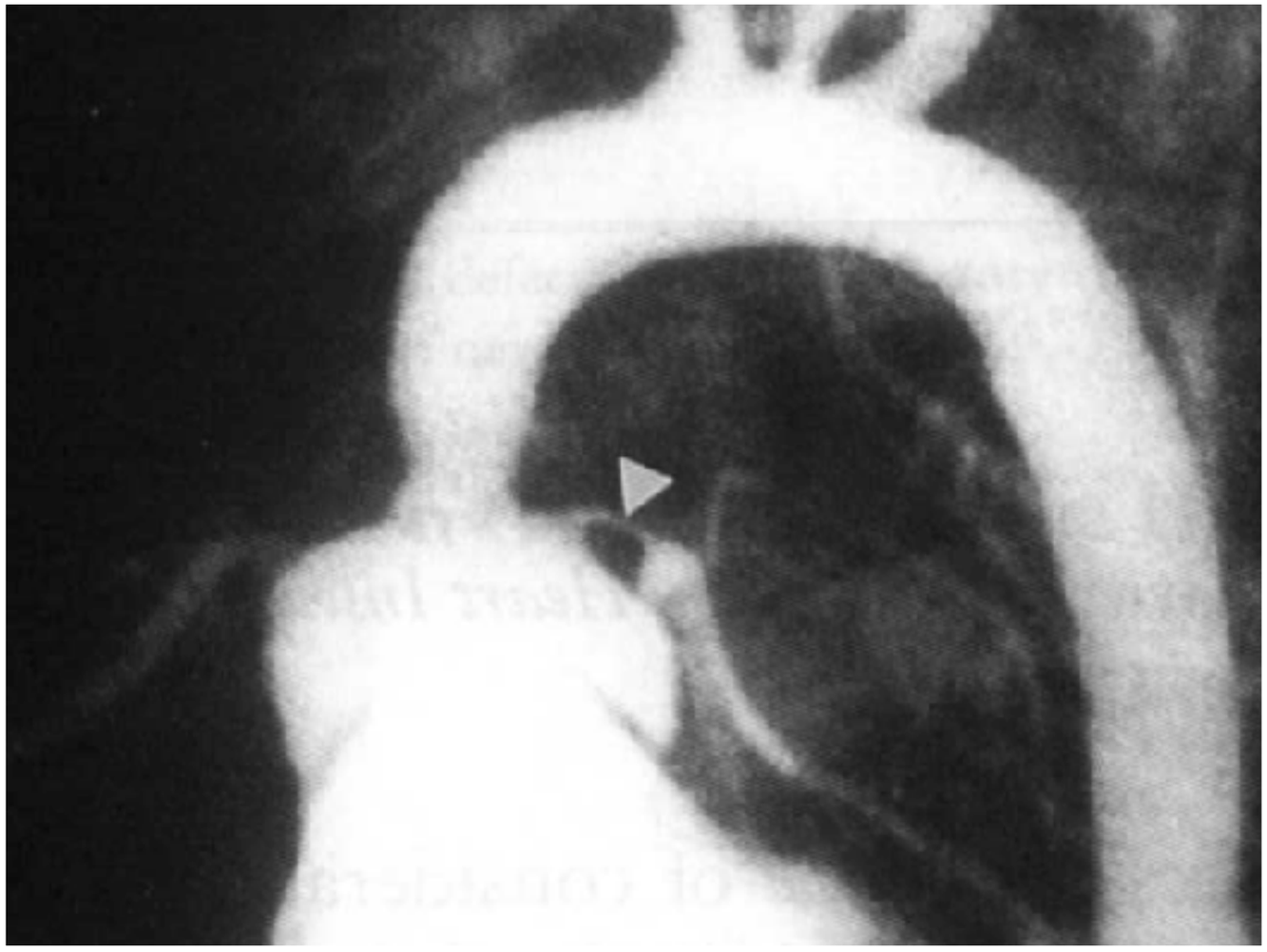
Peripheral pulmonary arterial stenosis



Collins R T Circulation. 2013;127:2125-2134

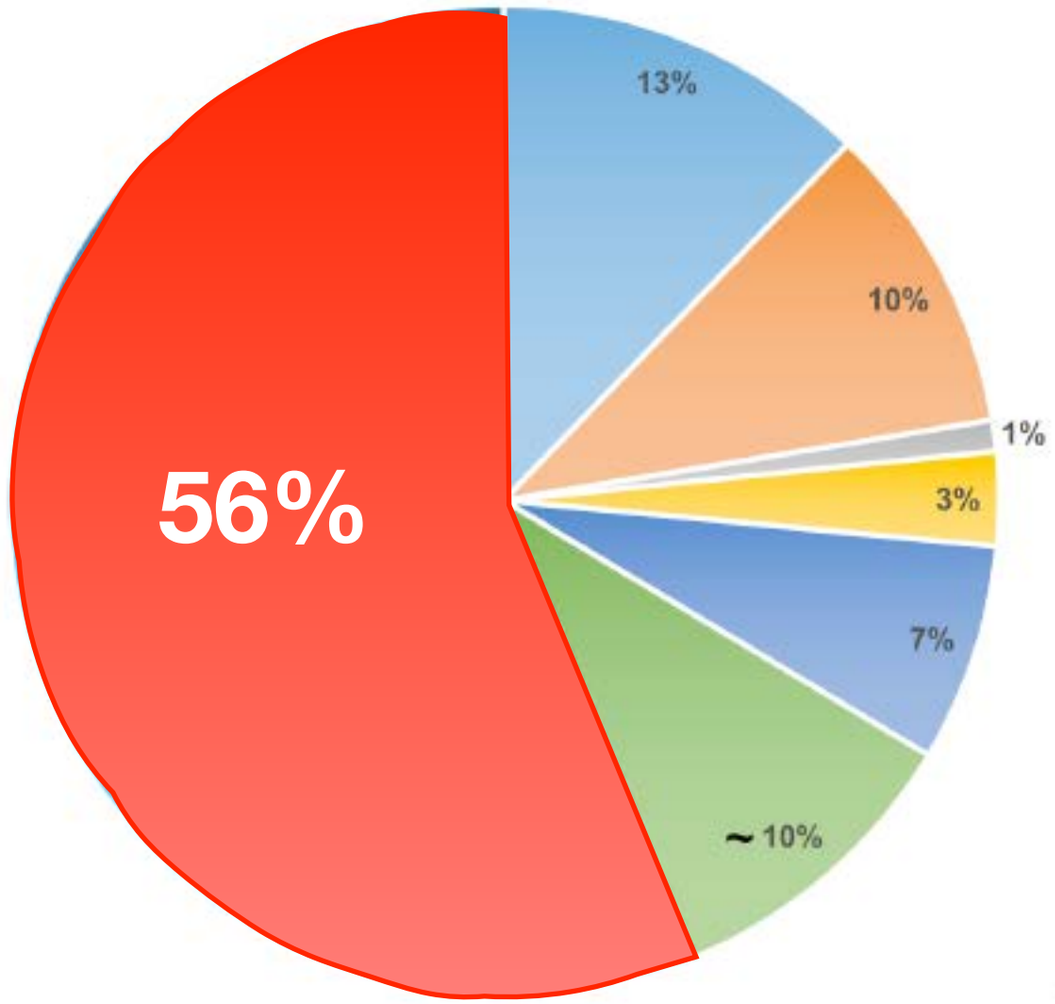
Coronary artery abnormalities in WS





Concepts in genetics of isolated CHD

Percentages of known and unknown genetic causes of CHD



Aneuploidy

CNV

Known gene inherited

De novo chromatin CNV

Other de novo SNV

Environmental

Unknown

Zaidi S & Brueckner M. Circ Res 2017;120:923-40

Genome content variations in CHD

- Genomic (Copy Number Variation) : 5-15 %

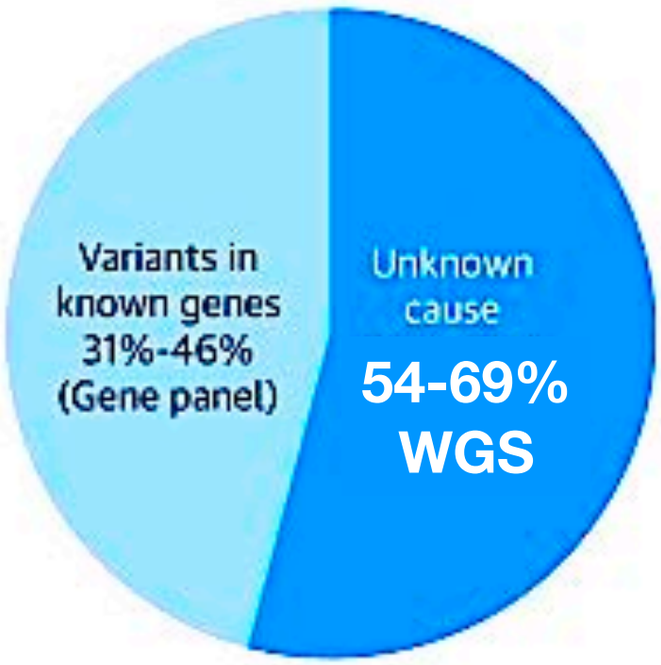
- CNVs : 5% in isolated tetralogy of Fallot (*Greenway et al. Nat Genet 2009*)
- CNVs : 15 % in isolated CHD (*Soemedi R et al. Am J Hum Genet 2012*)
- CNVs : 25-35% in syndromic CHD (*Cooper et al. Nat Genet 2011*)

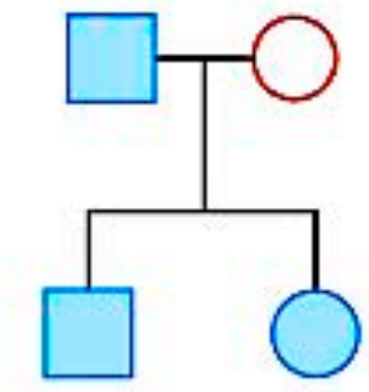
- Single gene mutation : 5 %

- Multifactorial model : 85% avec héritabilité $h^2 = 35$

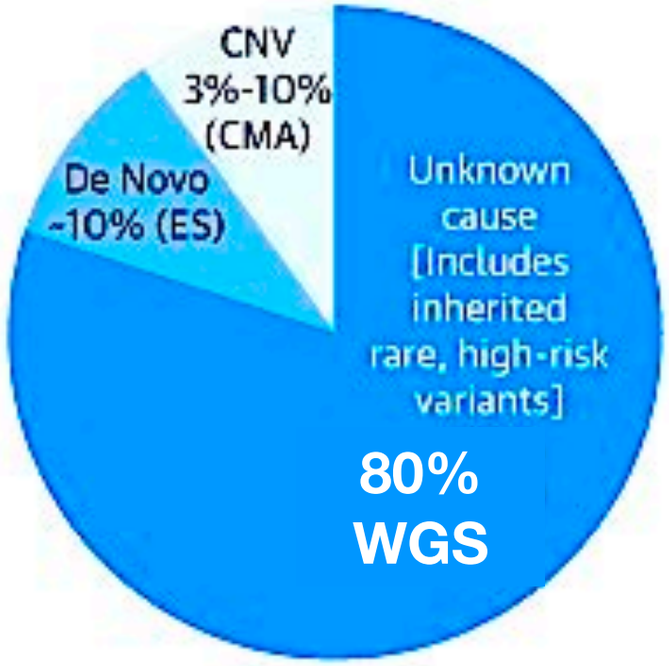
Percentages of known and unknown causes of the different forms of presenting **non-syndromic patients**

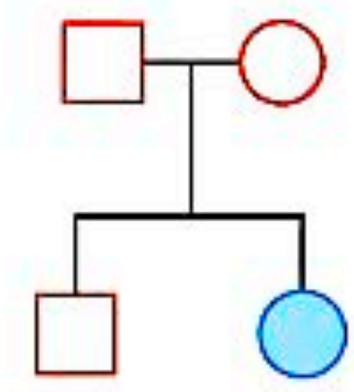
Familial CHD



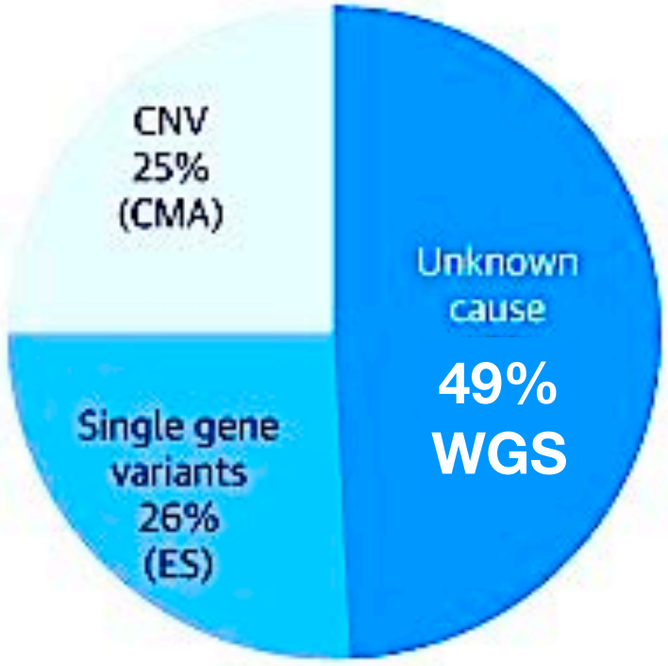


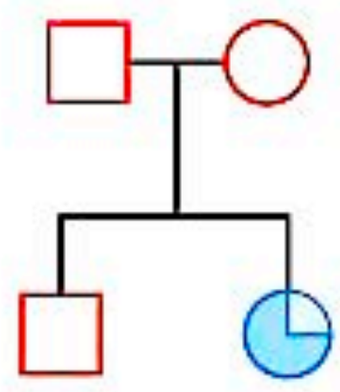
Sporadic CHD





CHD + ECA





Blue GM et al. J Am Coll Cardiol 2017;69:859-70

Half a century and the same old story !

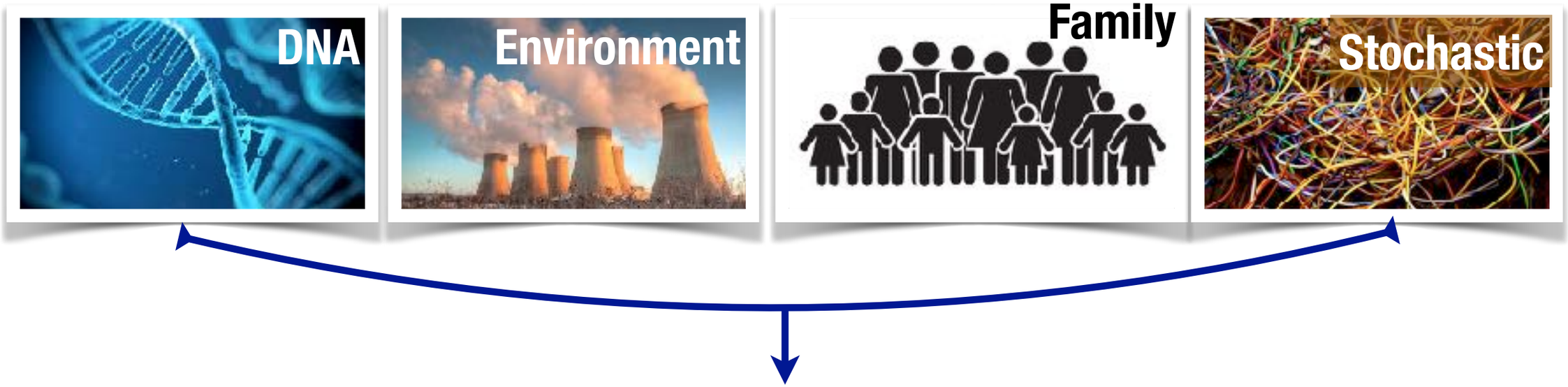
Multifactorial Inheritance Hypothesis for the Etiology of Congenital Heart Diseases

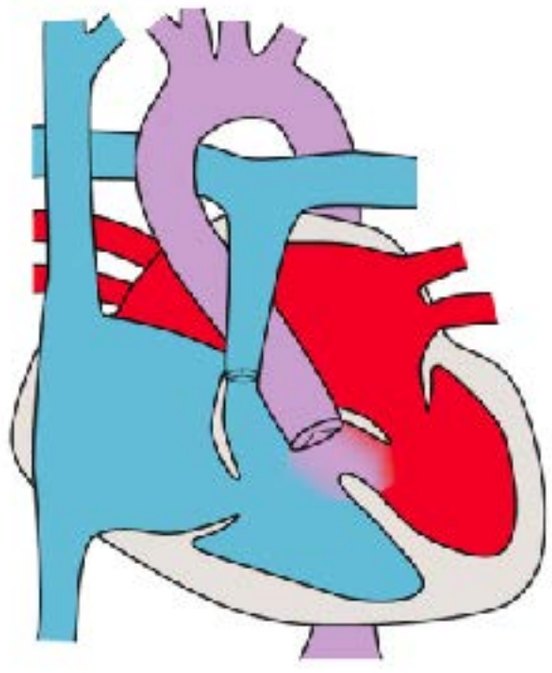
The Genetic-Environmental Interaction

By JAMES J. NORA, M.D.

Circulation 1968

The multifactorial hypothesis





The 4 hypotheses relevant for the genetic basis of congenital heart diseases

- There is no genetic basis for CHD
- ~~Gross chromosomal aberrations are responsible for the majority of CHD~~
- Single gene mutations are the main cause for CHD
- Congenital heart disease are a heterogeneous category of developmental anomalies, representing in most cases the multifactorial inheritance of threshold characters, the expression of which is the product of genetic-environment interaction

Nora JJ. Circulation 1968

Recurrence of CHDs in families

All types of defects

	Relative risk
Twin same sex	9.25
Twin unlike sex	3.33
First degree relative	3.45
Seconde degree relative	1.39
Third degree relative	1.18

High recurrence rate
but not as expected for mendelian inheritance

Oyen N et al. Circulation 2009

Congenital heart defect is more common in twins than in singletons, and the increased occurrence is not restricted to monochorionic twins

	Singletons	All twins	Monozygotic	Dizygotic	Unknown zygocity
CHD all types	0.87	1.41	1.14	1.15	2.07

Role of shared genes but also related to twining process

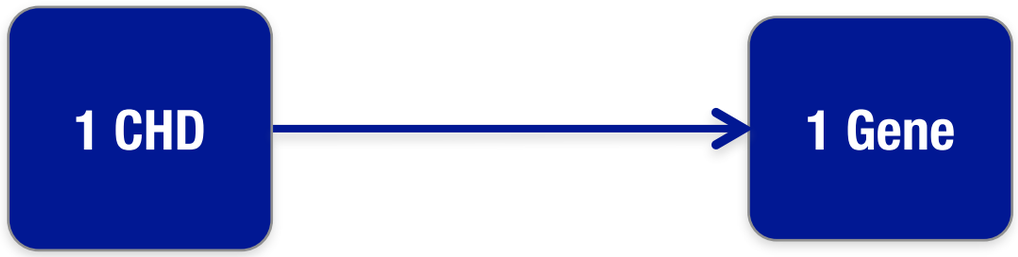
Herskind AM et al. Circulation 2013

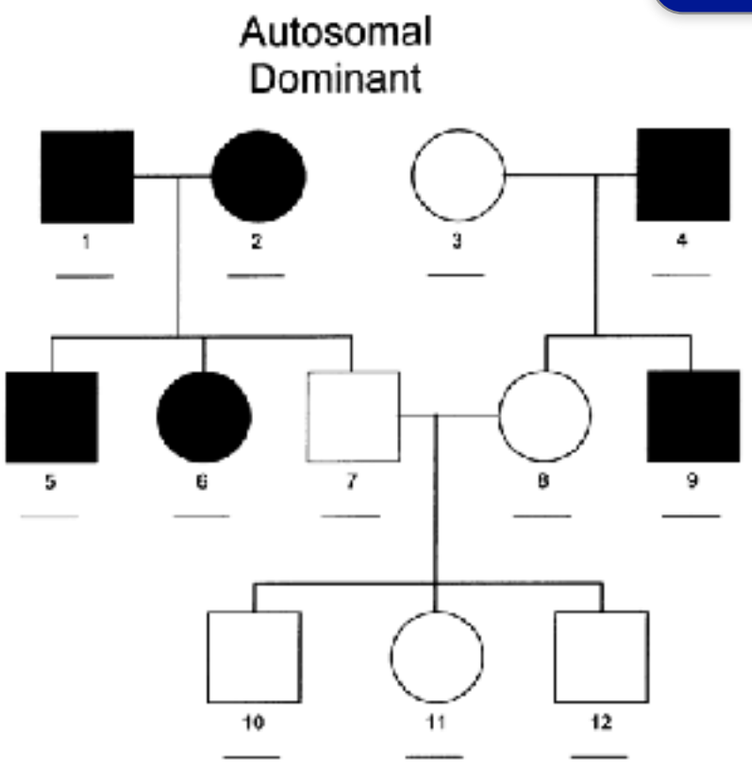
The 4 hypotheses relevant for the genetic basis of congenital heart diseases

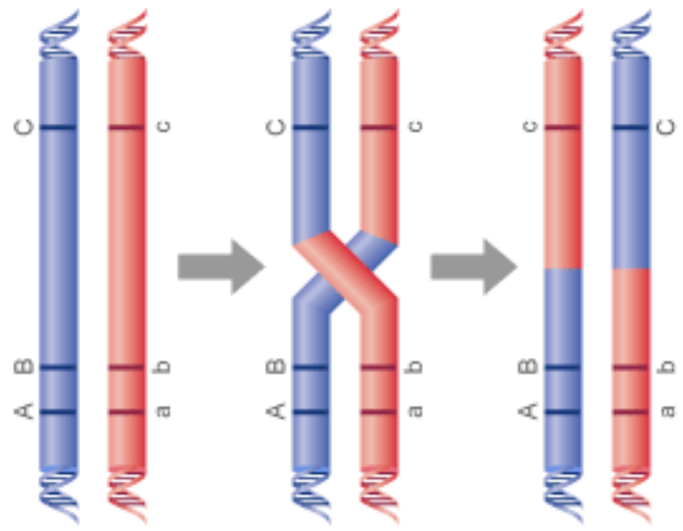
- ~~There is no genetic basis for CHD~~
- ~~Gross chromosomal aberrations are responsible for the majority of CHD~~
- Single gene mutations are the main cause for CHD
- Congenital heart disease are a heterogeneous category of developmental anomalies, representing in most cases the multifactorial inheritance of threshold characters, the expression of which is the product of genetic-environment interaction

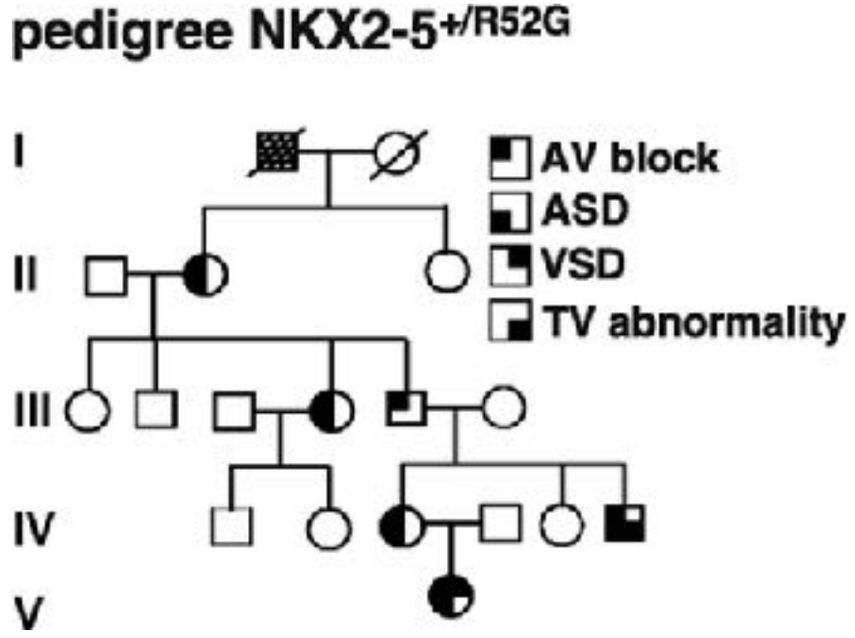
Nora JJ. Circulation 1968

The monogenic hypothesis









The positional cloning strategy

Recurrence of CHDs in families

Same defect *in affected members*

Heart defect phenotype in first degree relative	Relative risk
Heterotaxia	79.1
Conotruncal	11.7
AVSD	24.3
APVR	…
LVOTO	12.9
RVOTO	48.6
ASD	7.07
VSD	3.41
Overall same heart defect	8.15

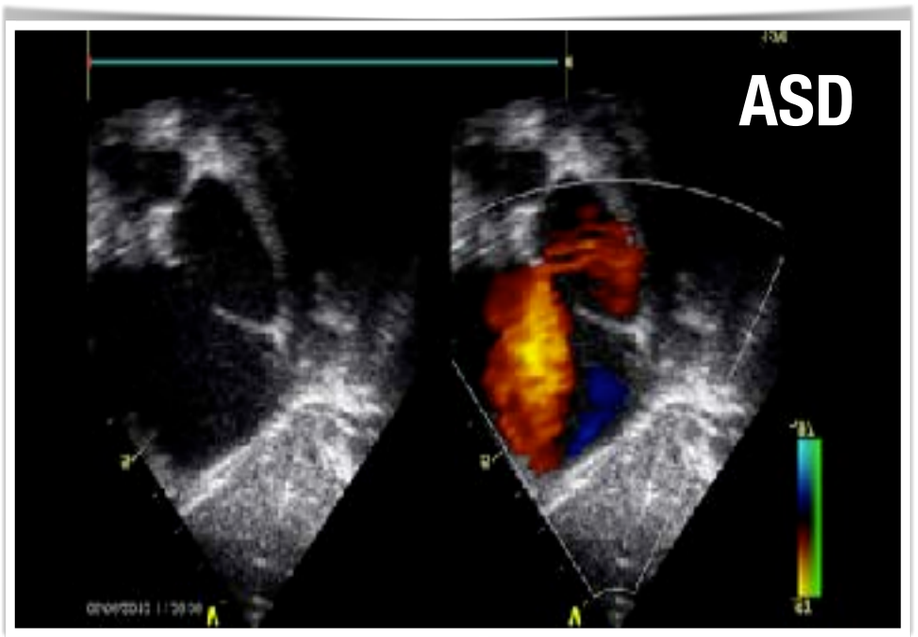
Recurrence of the same type can be due to inheritance of a single gene mutation

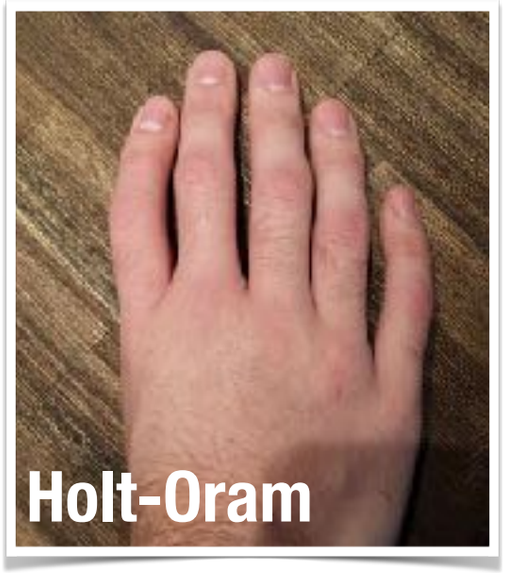
Oyen N et al. Circulation 2009

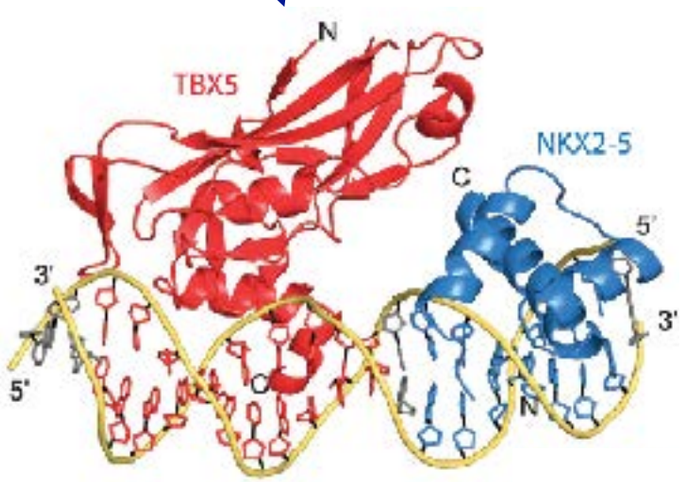
Genetic heterogeneity

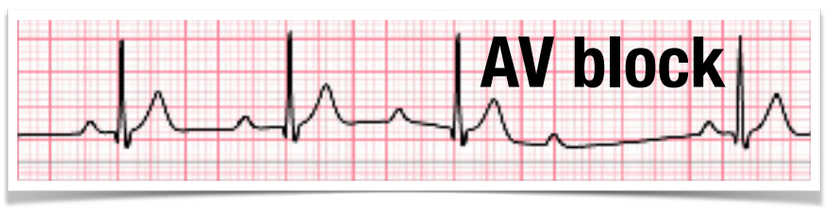
One cardiac phenotype-Different genotypes

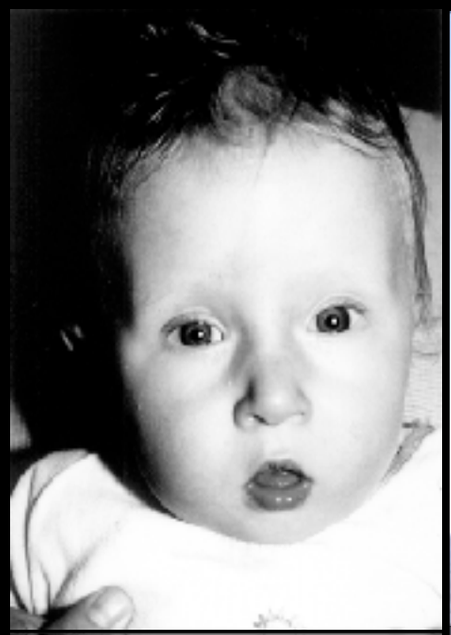
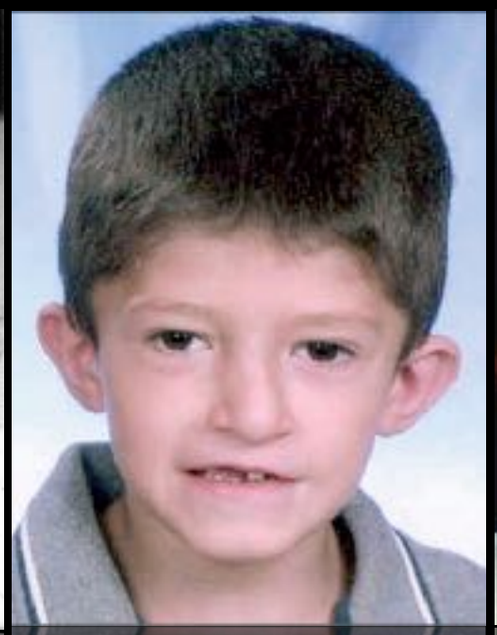
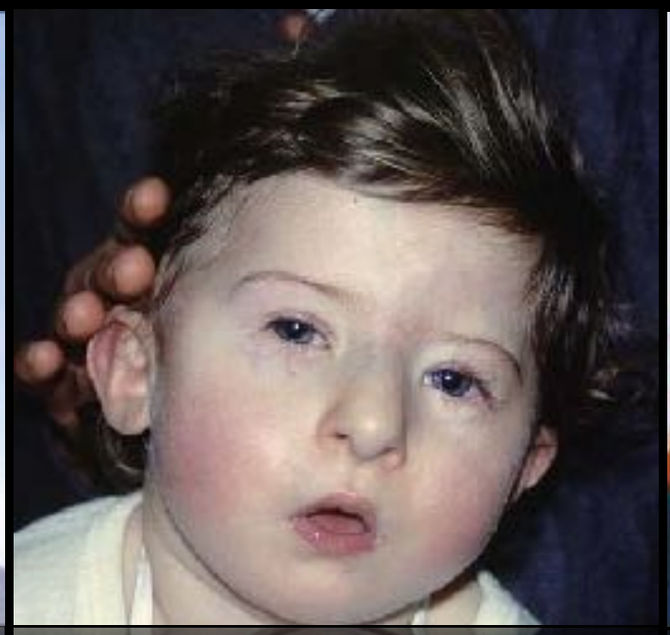
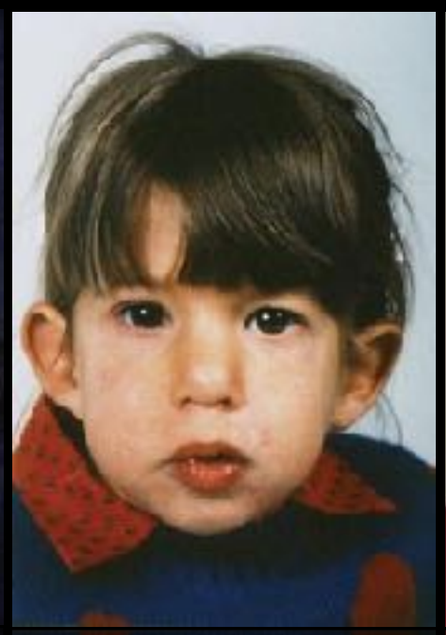
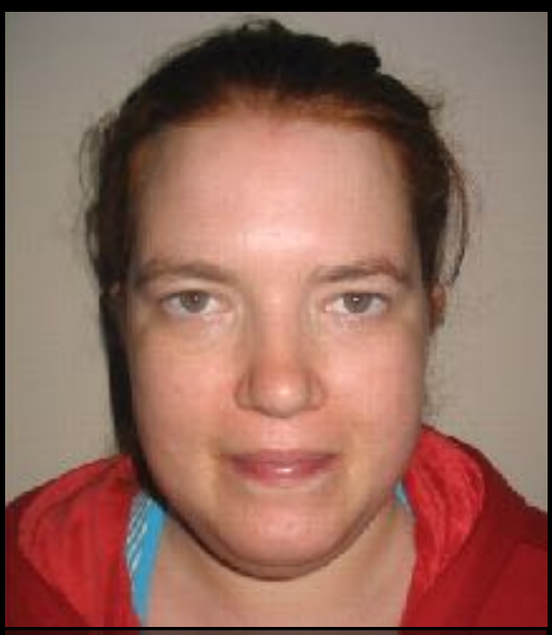
1 CHD → **X Genes**





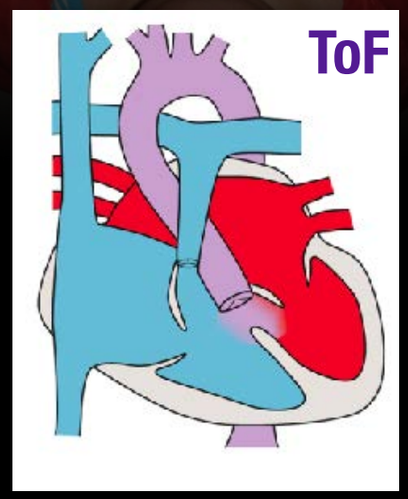


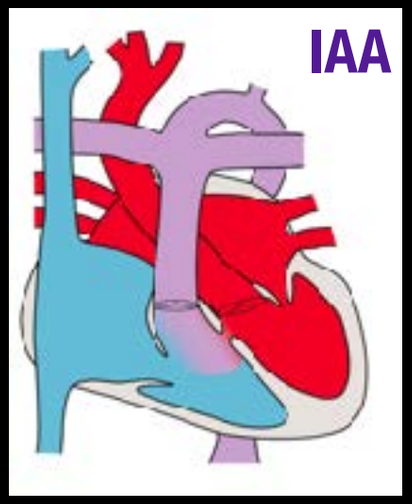


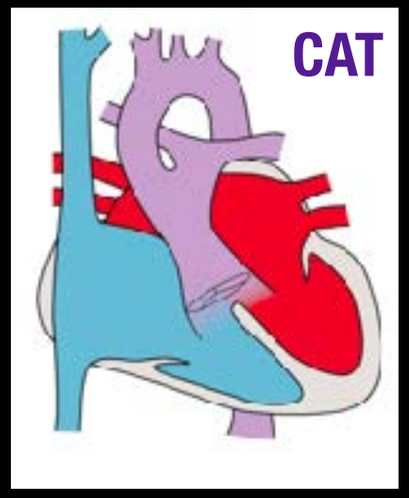






Phenotypic heterogeneity

One genotype-Different cardiac phenotypes



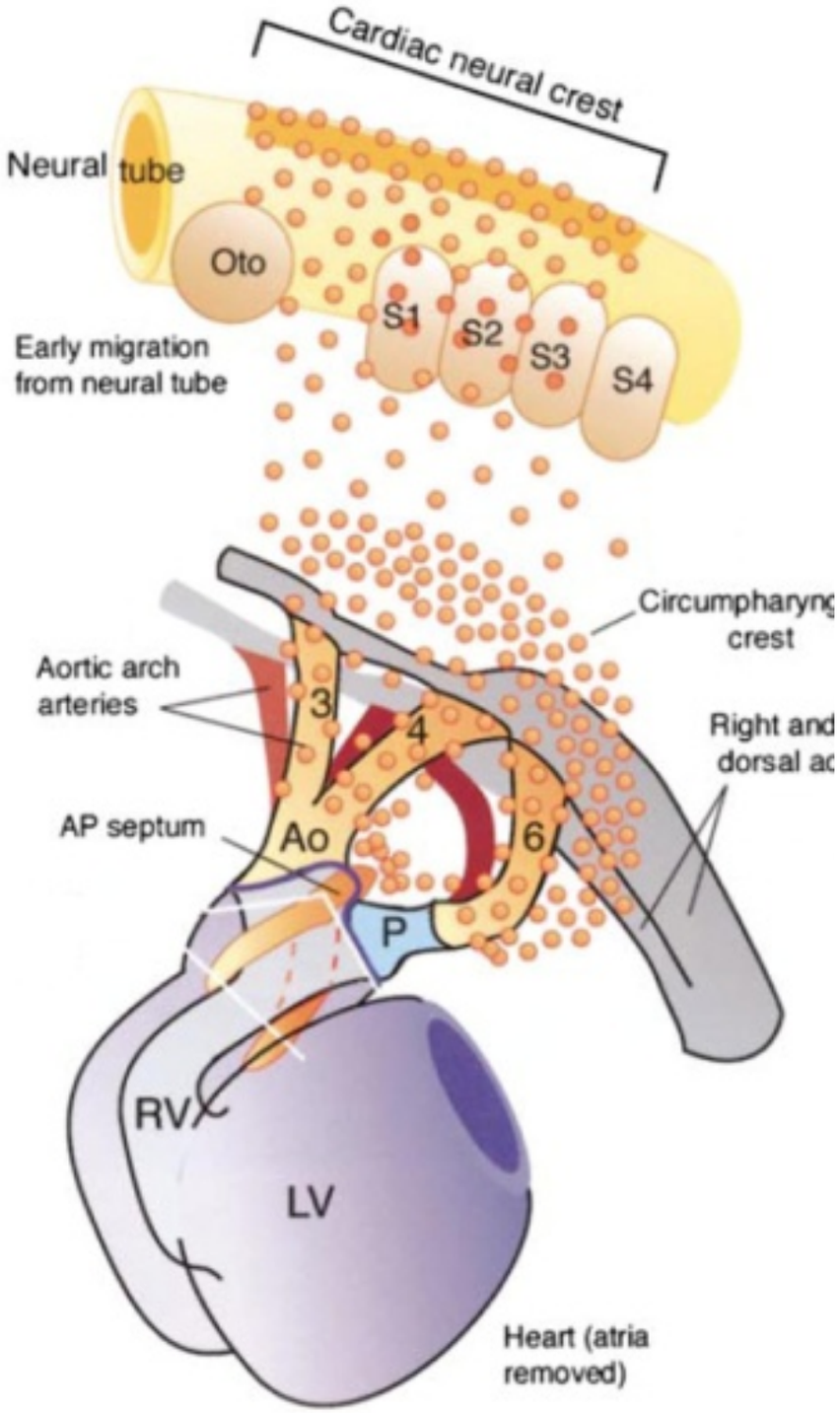


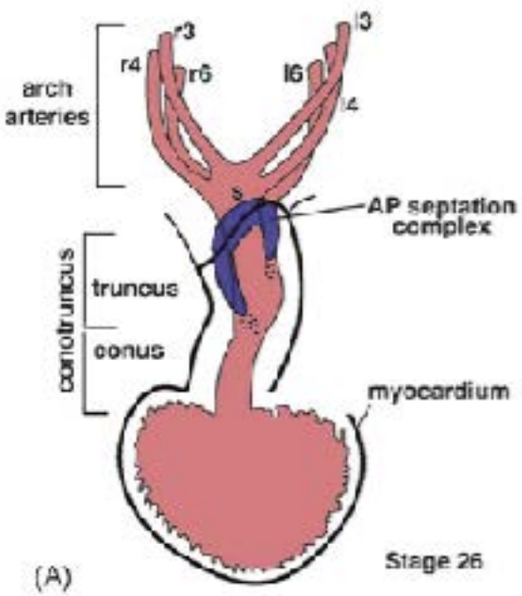


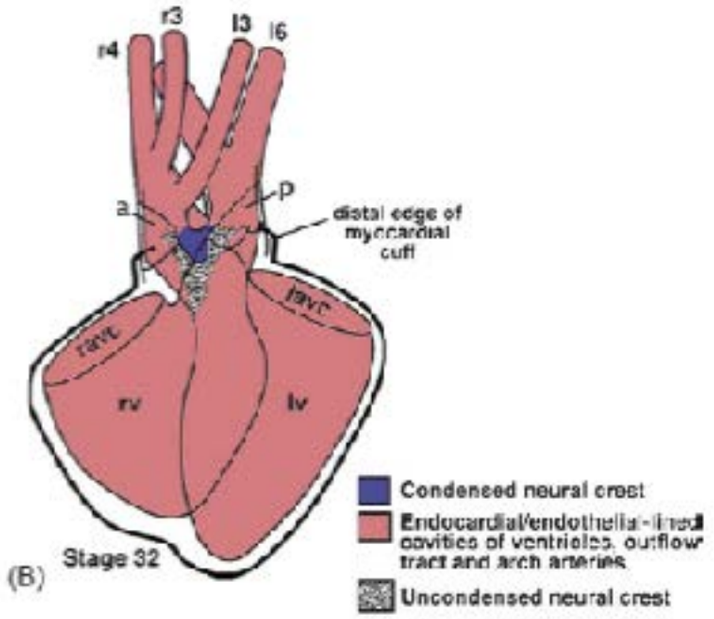
1 gene → X CHD

FISH 22q11 deletion

Migration of neural crest cells into the outflow tract

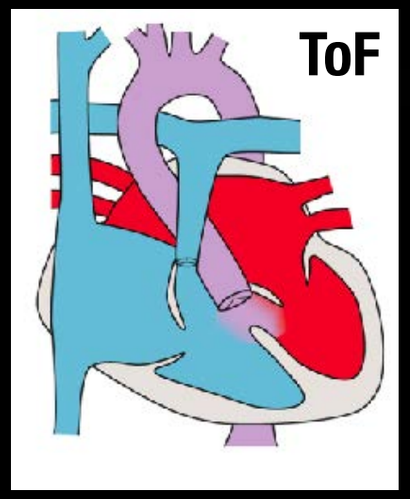




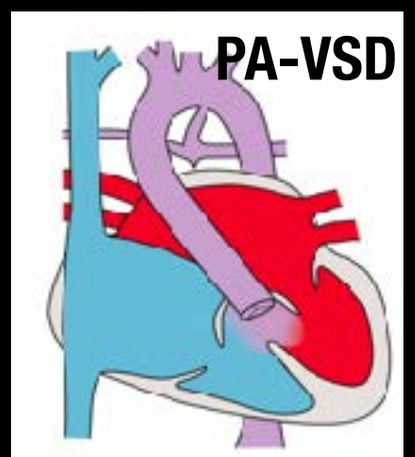


Hutson & Kirby 2007

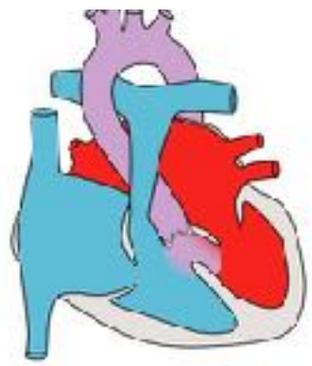
ToF

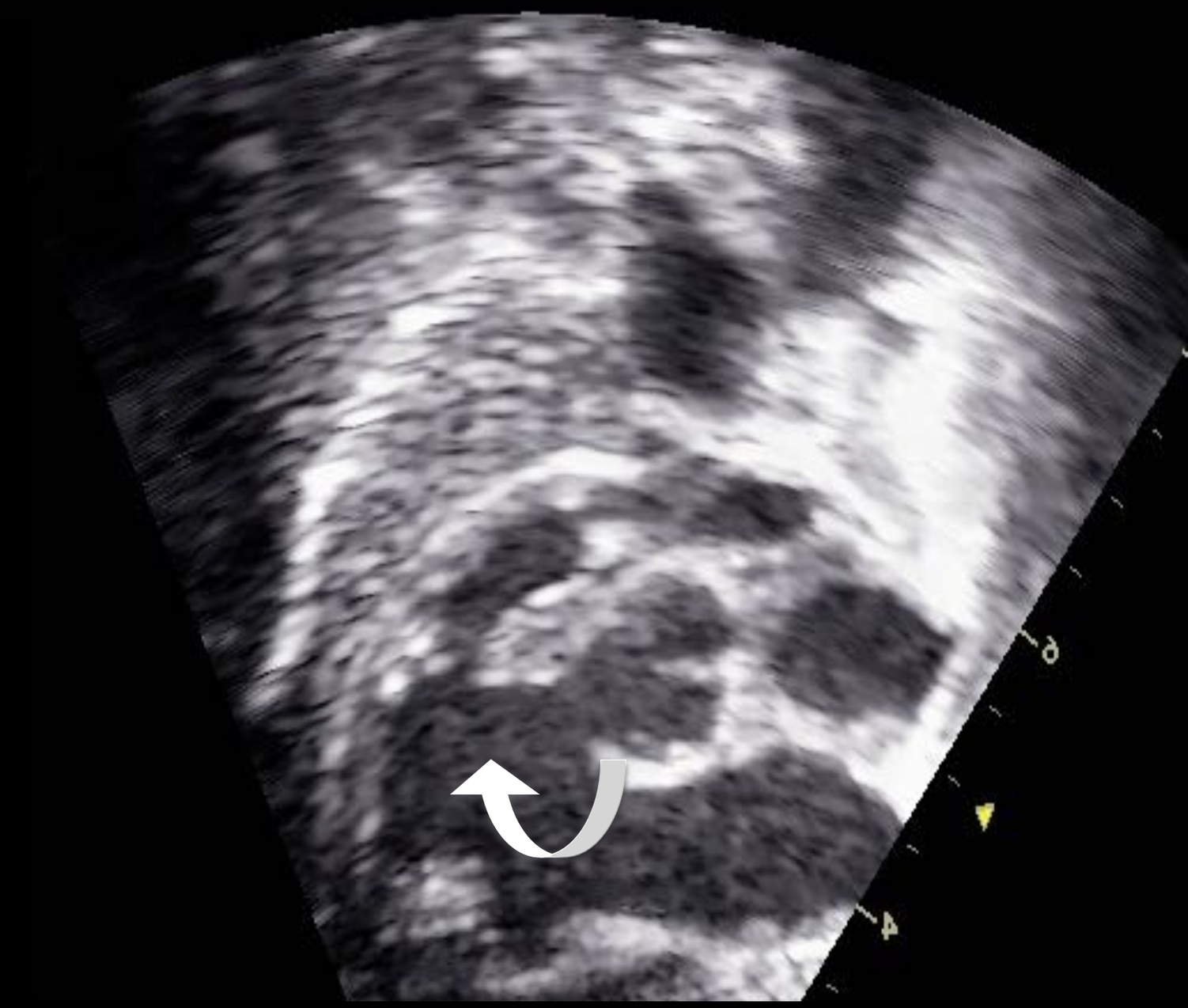


PA-VSD

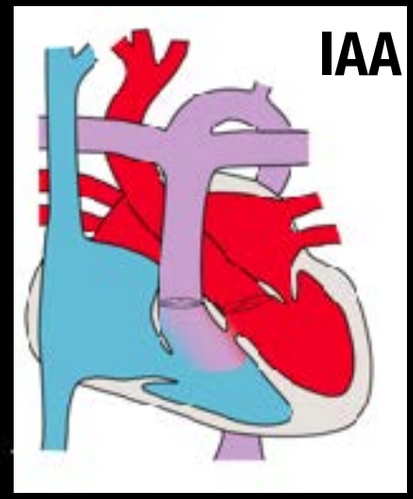


ToF-Abs PV

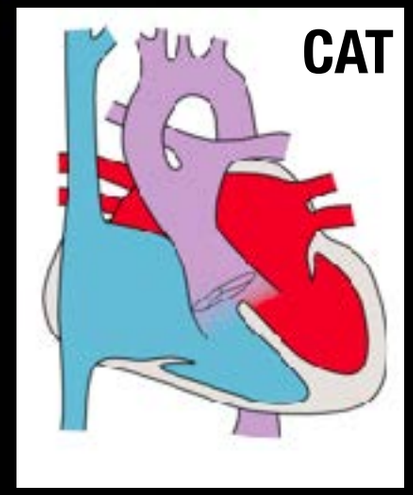




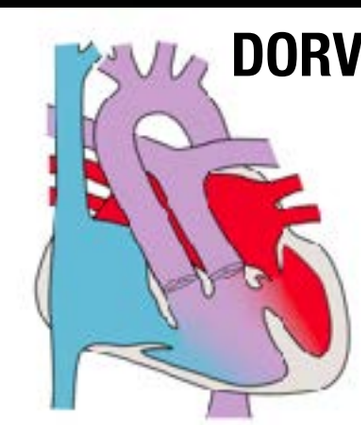
IAA



CAT



DORV



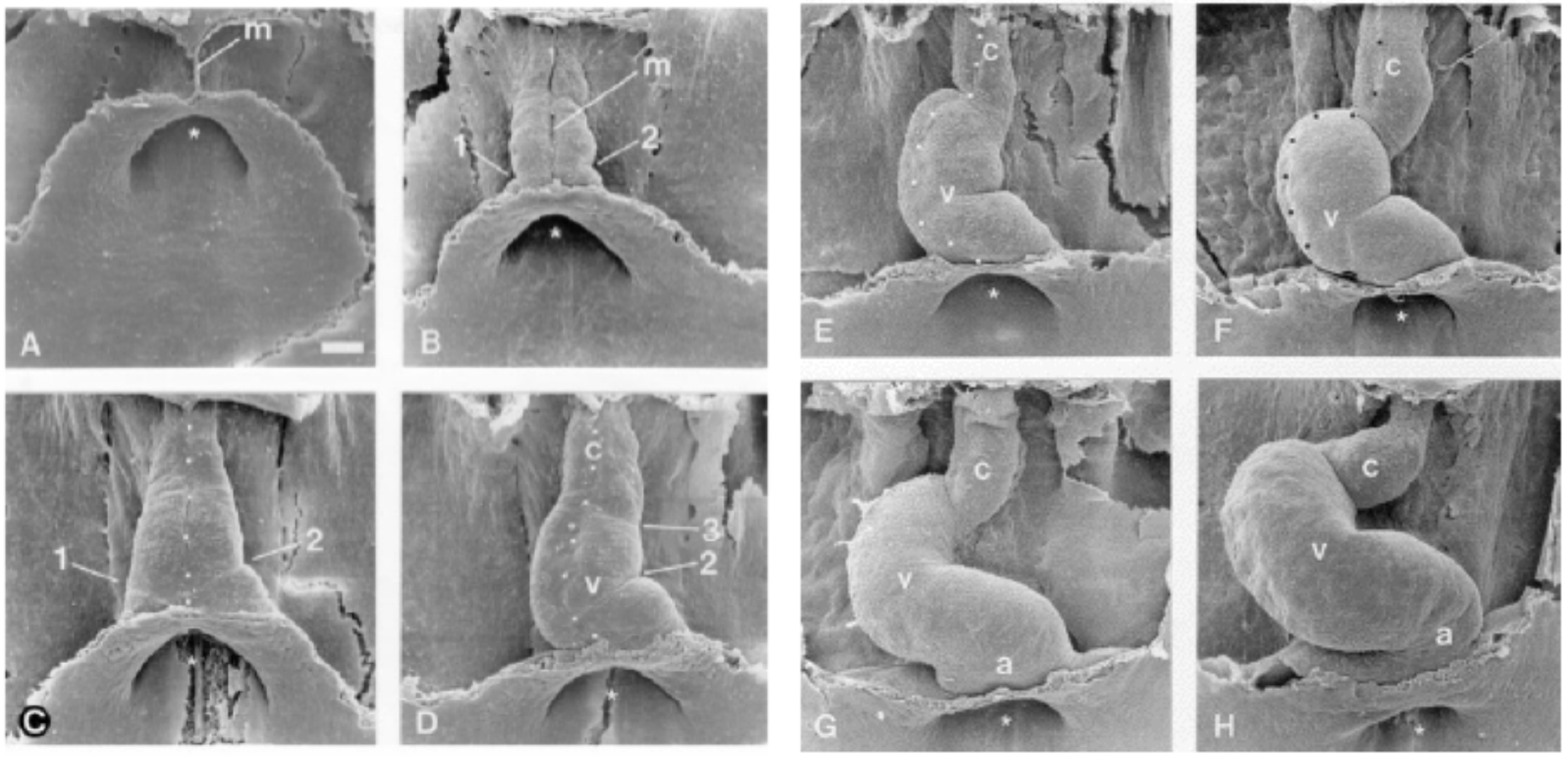
The mechanistic hypothesis

Neural crest cell migration defects

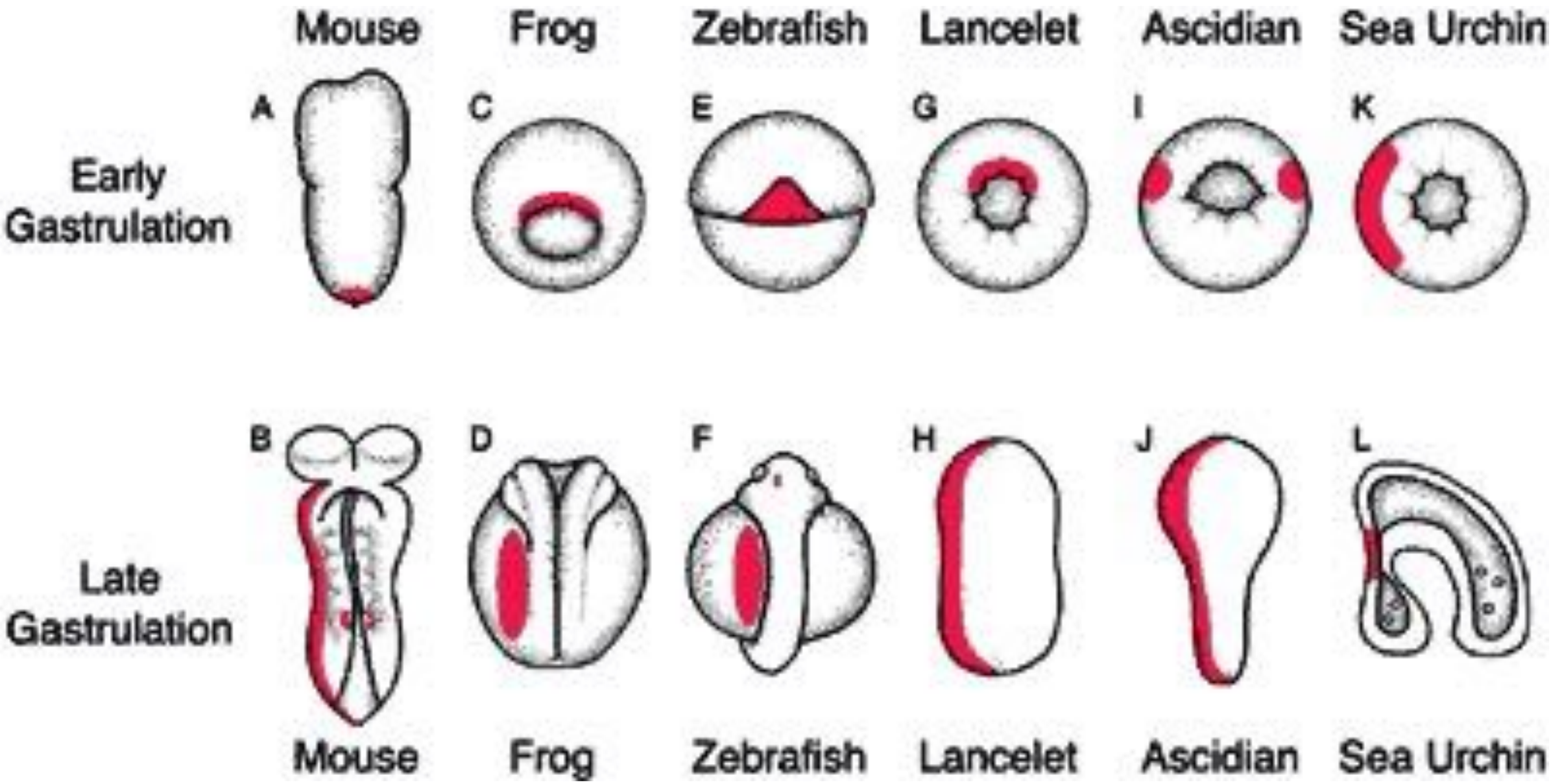
- Conotruncal malformations
  - Flow defects
  - Hypoplastic left heart
- Targeted developmental defects
  - TAPVR
- Extracellular matrix defects
  - Ventricular Septal Defects
- Endocardial cushions defects
  - Atrioventricular septal defects
- Looping anomalies-laterality defects
  - Heterotaxia

Clark EB. Am J Dis Child. 1986;140:41-4.

The mechanistic hypothesis



The example of laterality defects

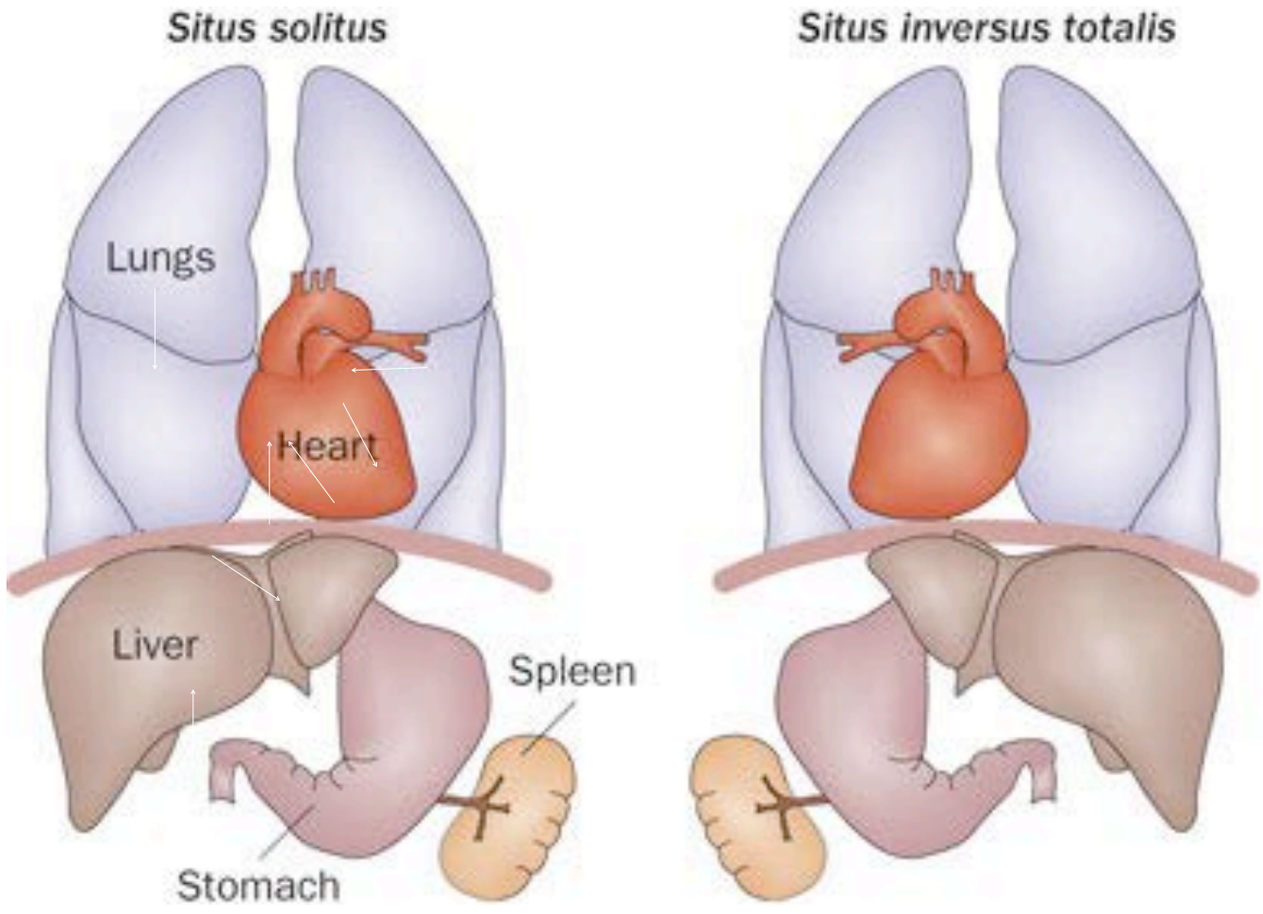


Expression of Nodal in different species is on the left side

What happens in the absence of left-right signaling ?

1.1/10,000 live births

3% of all Congenital Heart Diseases



Impairment of Left/Right signaling

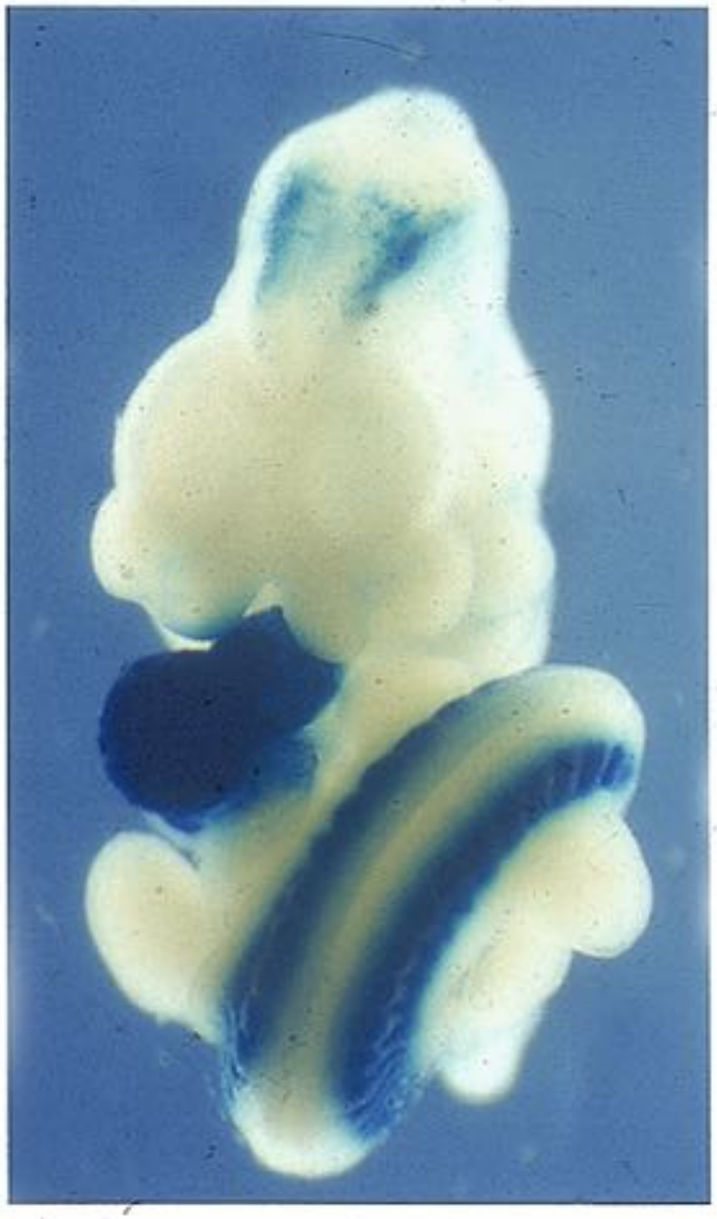
Formation of the node : *ZIC3, MMP21*

Ciliogenesis : *DNAH11, INVS*

Nodal signalling : *NODAL, LEFTY2, CFC1, ACVR2A*

Mouse mutant with absent left-right signaling

Situs solitus



Situs inversus

Mlc3f-2 X iv/iv

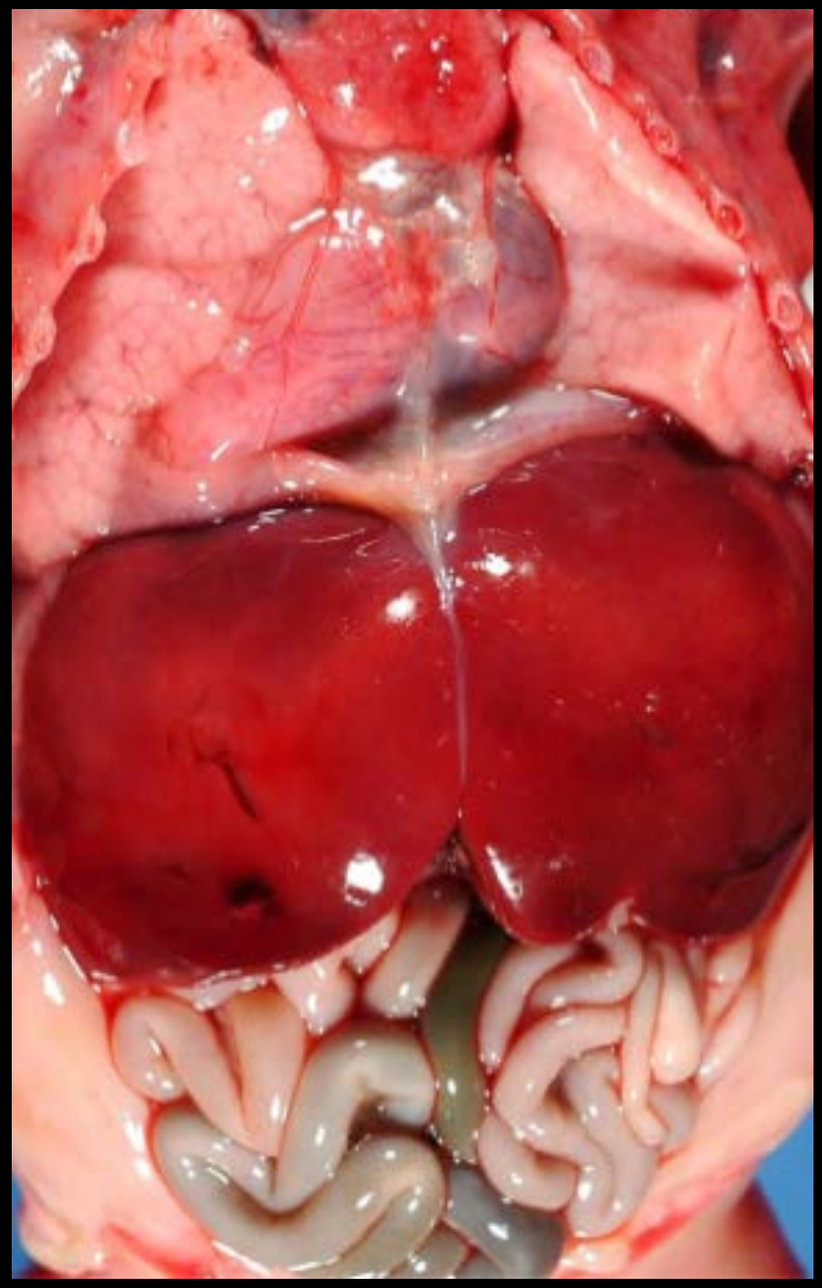
Lymnaea stagnalis

Absence of left-right signaling

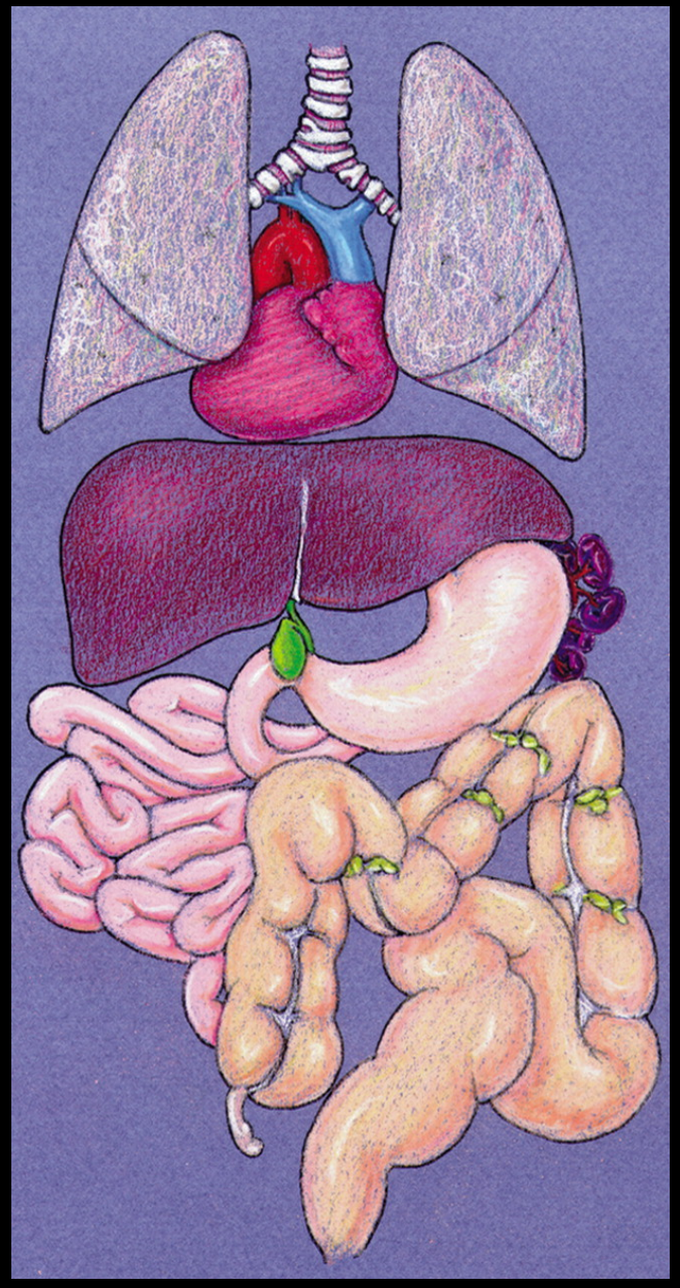
Inversion-mirror image, Isomerism-Heterotaxy

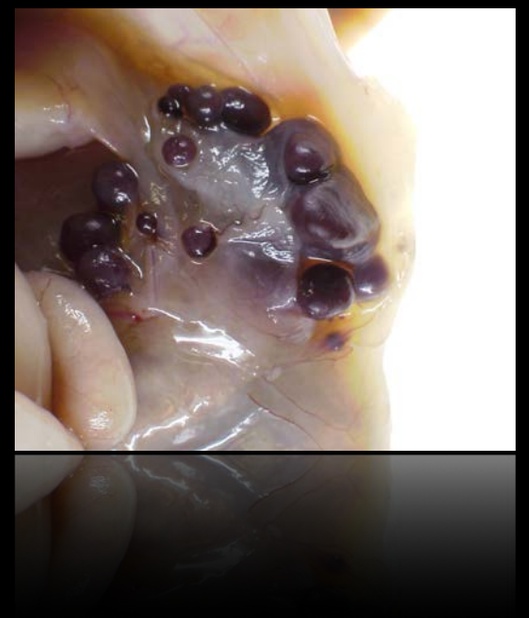
Isomerism is easy to understand for pair organs

Heterotaxy is abnormality of visceral asymmetry

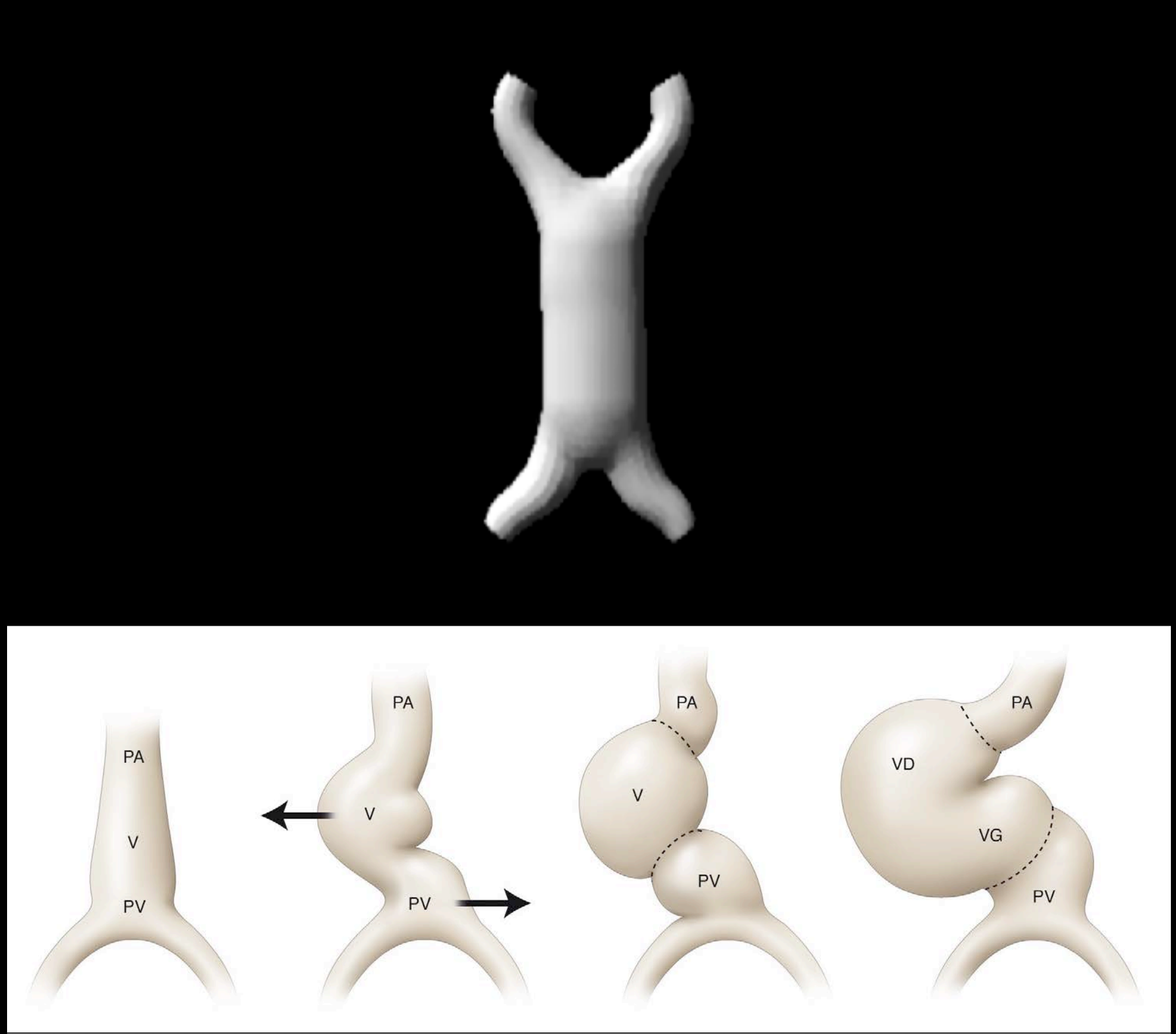


Right and left liver





Polysplenia

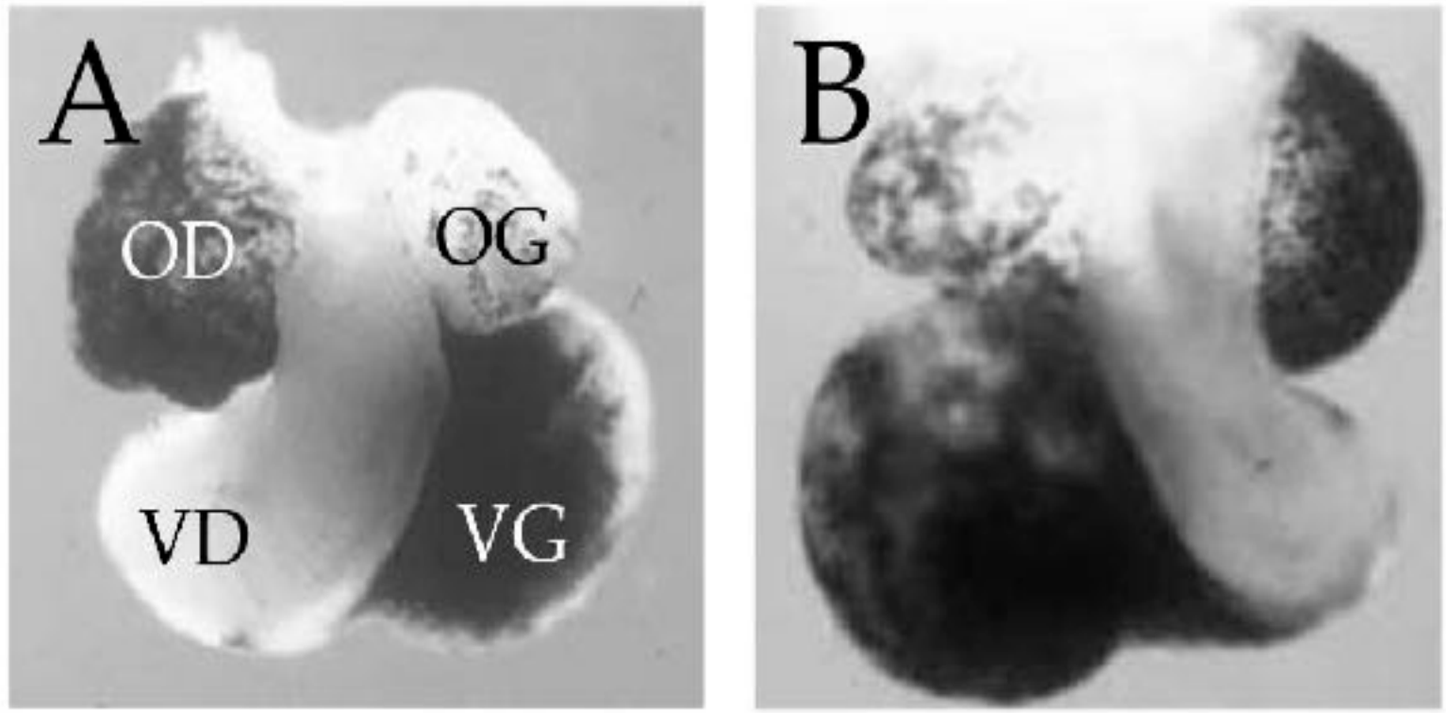


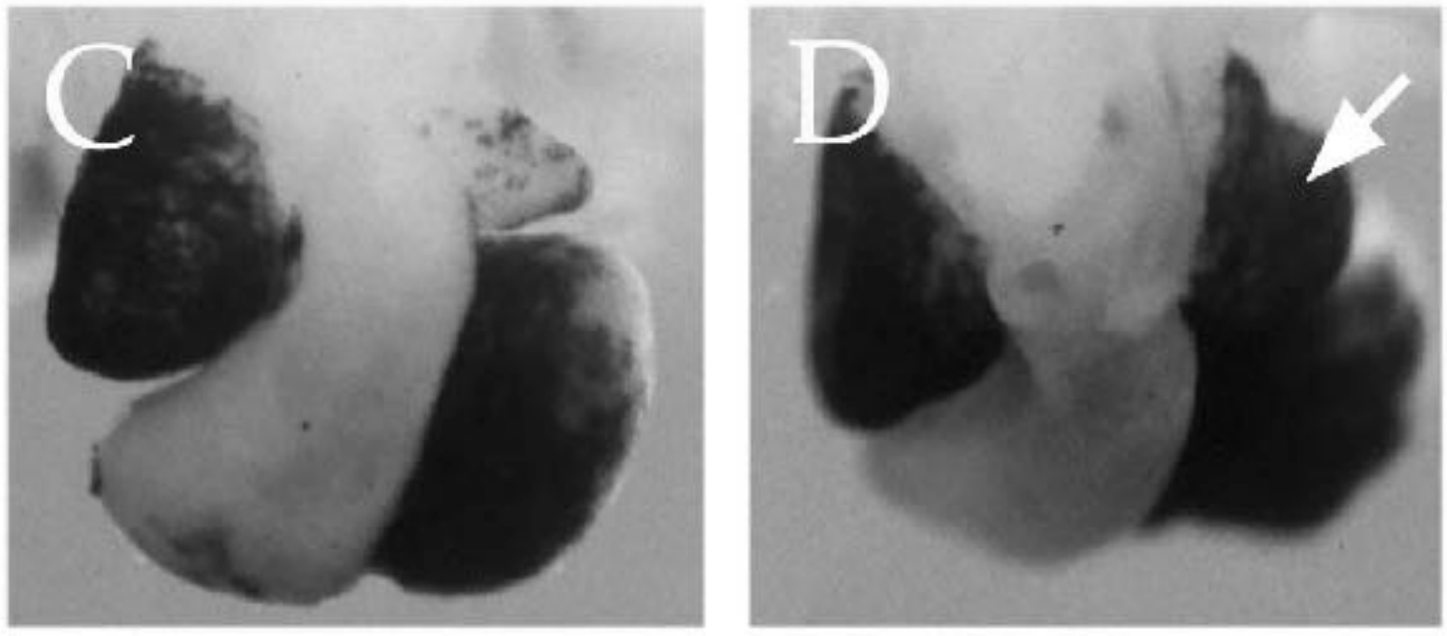
Right-sidedness and left-sidedness of cardiac structures are acquired during development, not present *de novo*

Transgenic mouse model for heterotaxy

Situs inversus

E10.5

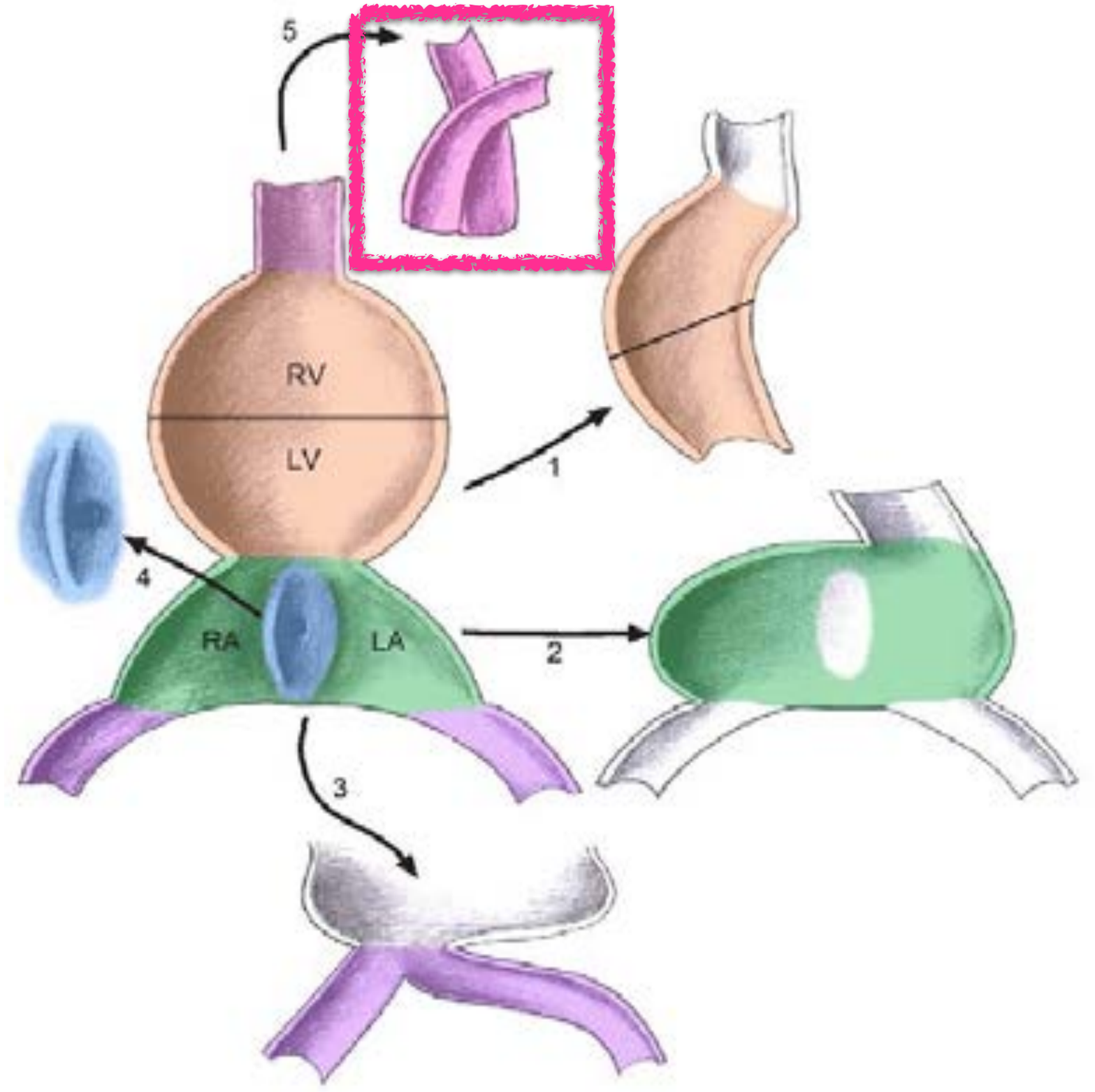




Situs solitus

Situs ambigus

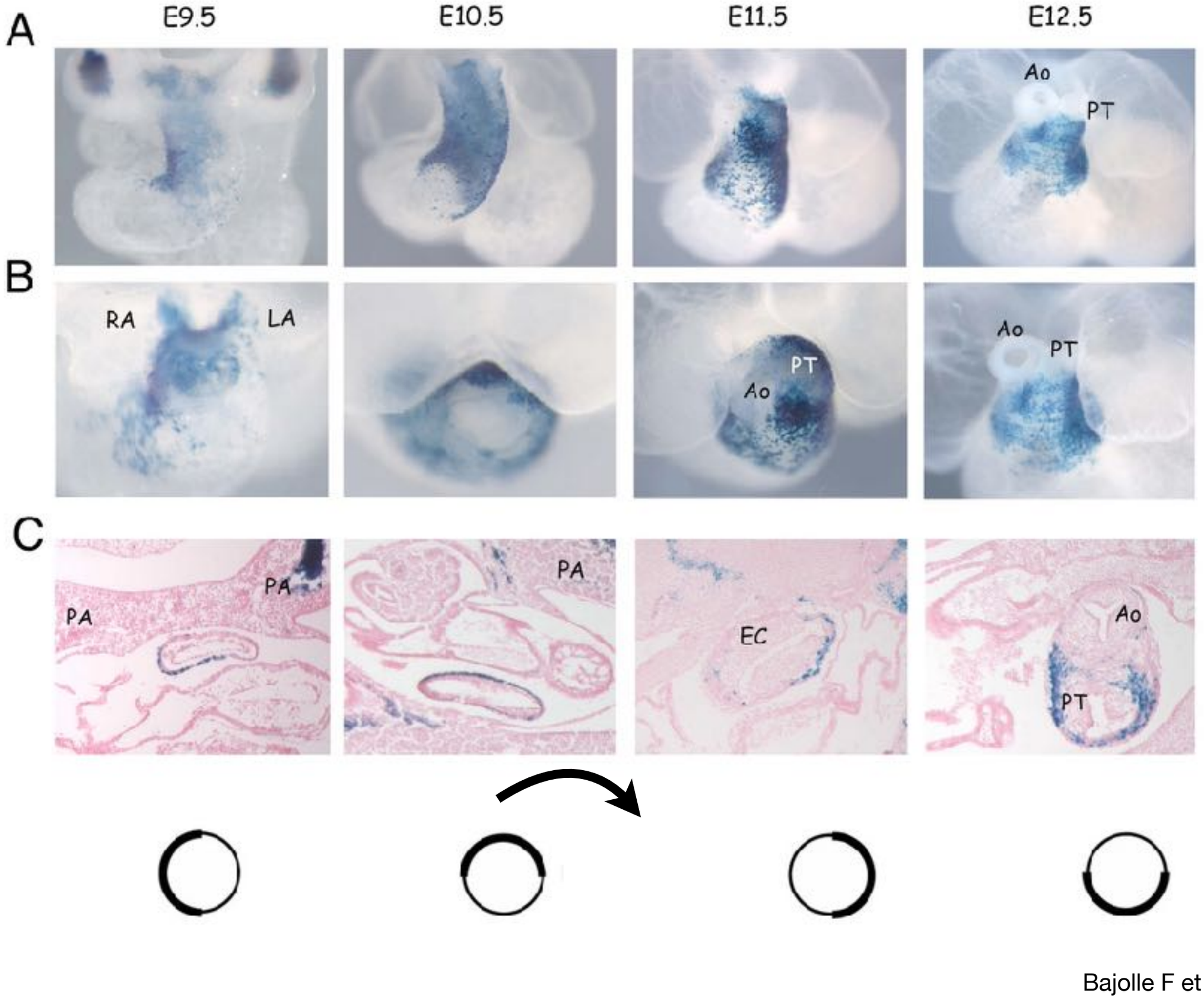
Mlc3f-2 X iv/iv



5 levels of asymmetry in the developing heart

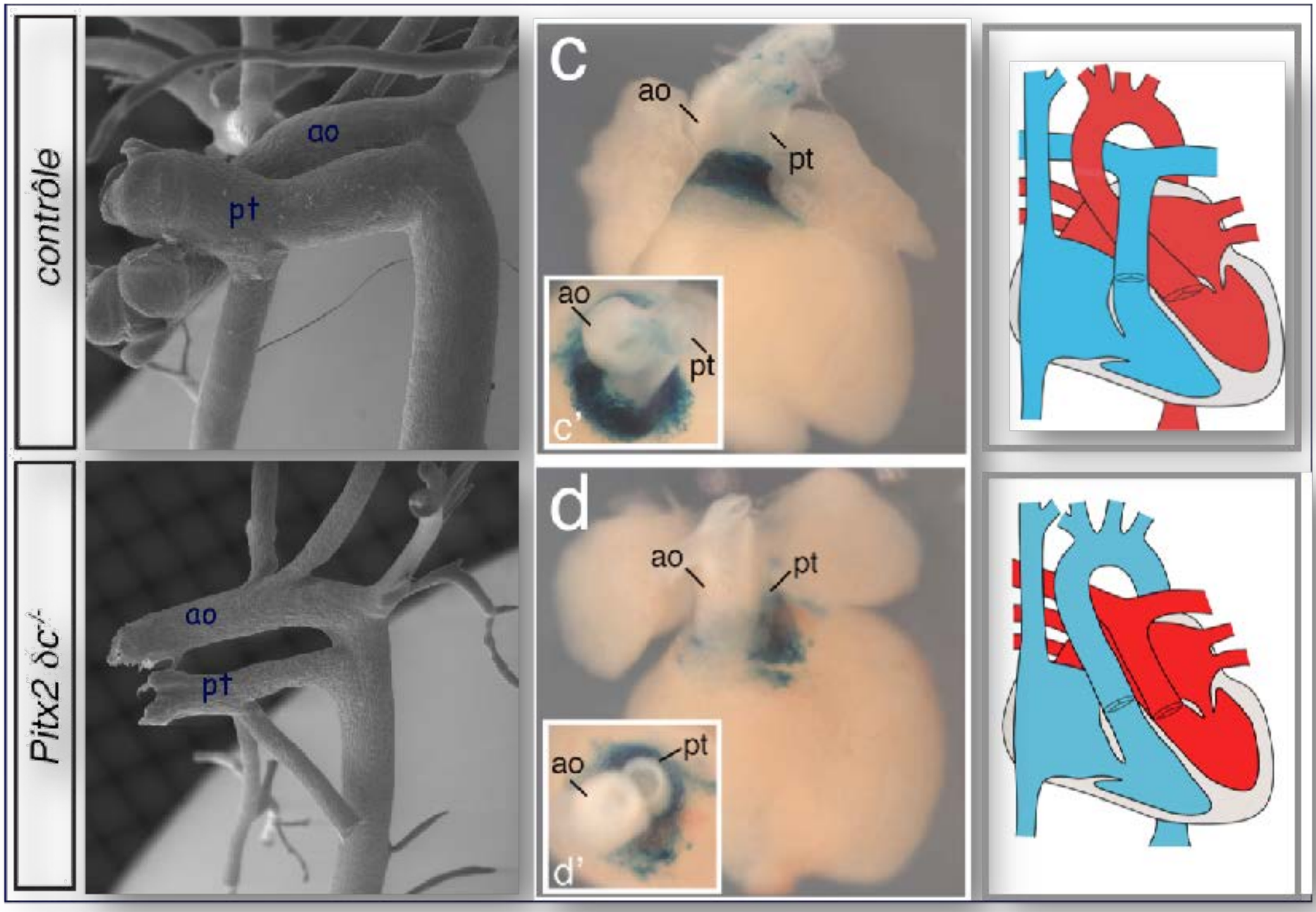
Nigel Brown

Rotation of the myocardium in cardio sensor mouse



Bajolle F et al. Circ Res

96-16 expression in Pitx2δc heart with TGA



Transposition of the great arteries with a rotation defect

Normal septation and normal neural crest cell migration

Defect of left-right signaling

Familial transposition of the great arteries caused by multiple mutations in laterality genes

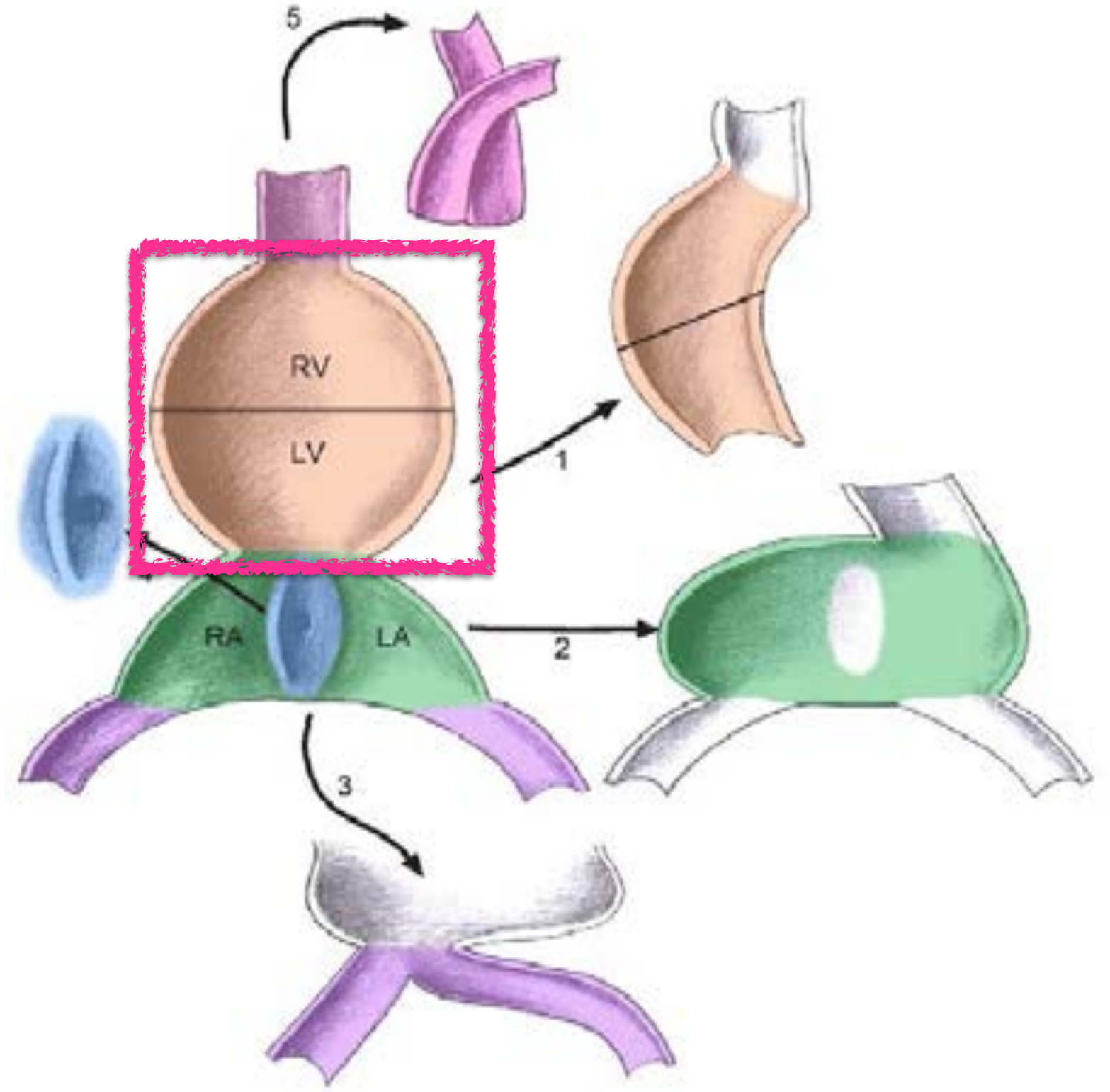
Alessandro De Luca,[1] Anna Sarkozy,[1,6] Federica Consoli,[1] Rosangela Ferese,[1] Valentina Guida,[1] Maria Lisa Dentici,[1] Rita Mingarelli,[1] Emanuele Bellacchio,[1] Giulia Tuo,[2] Giuseppe Limongelli,[3] Maria Cristina Digilio,[4] Bruno Marino,[5] Bruno Dallapiccola[1]

Heart 2010;**96**:673–677.

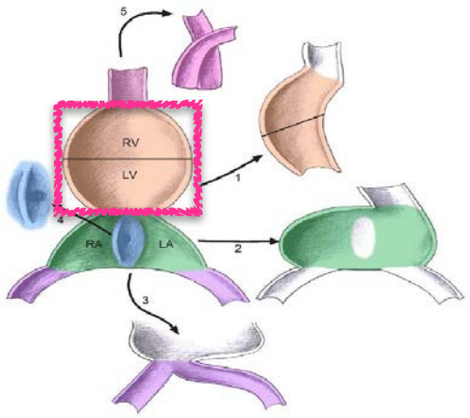
TGA is a laterality defect

It is not a conotruncal defect

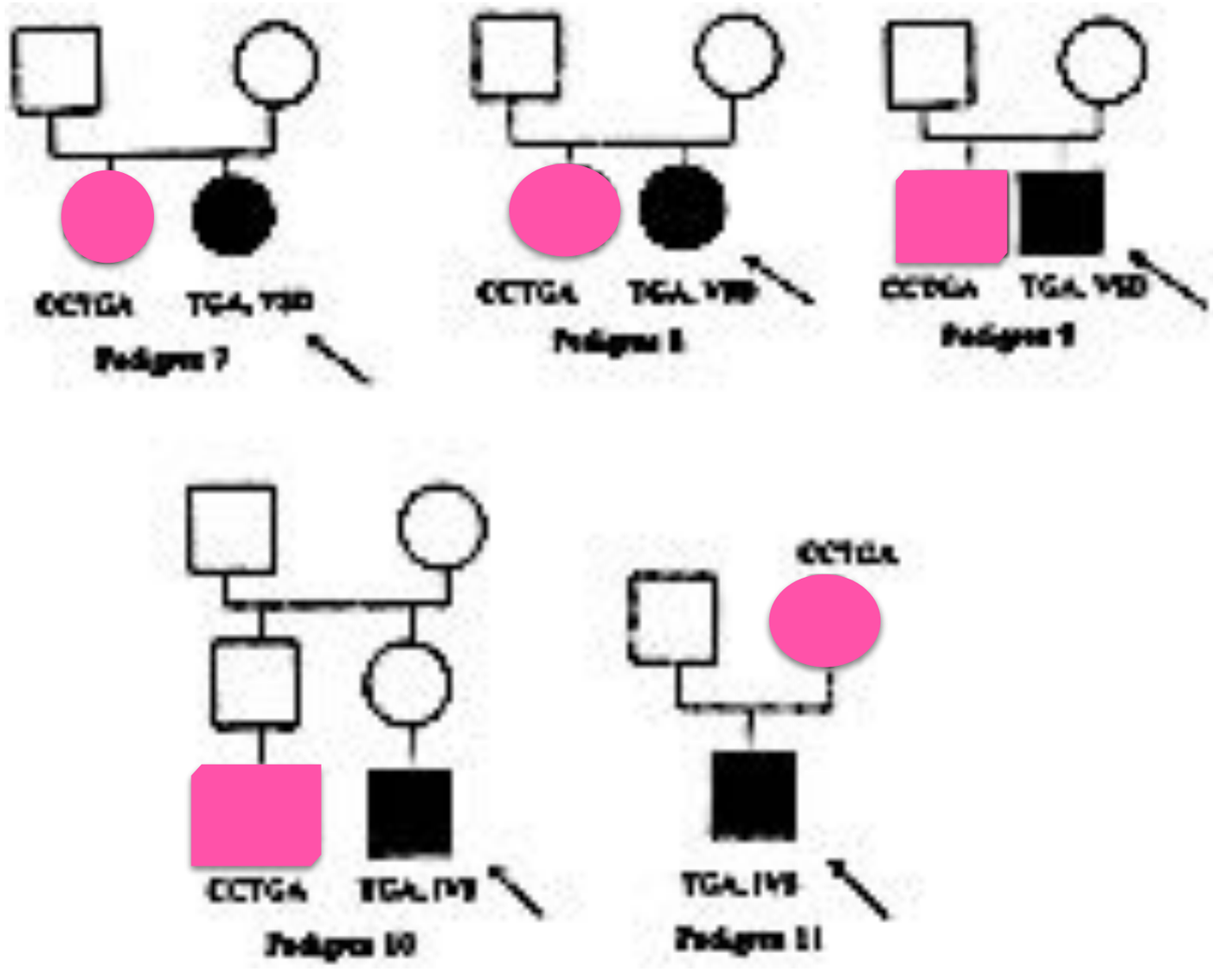
It is a laterality (rotation) restricted to a single segment of the developing heart



5 levels of asymmetry in the developing heart



Families TGA & CC-TGA



Digilio MC et al. Circulation. 2001;104:2809-2814.

Second heart field

-Abnormal contribution
-Abnormal proliferation

Neural crest cells

-Abnormal migration
-Abnormal proliferation

Myocardium

-Abnormal rotation
-Abnormal laterality

Endocardium

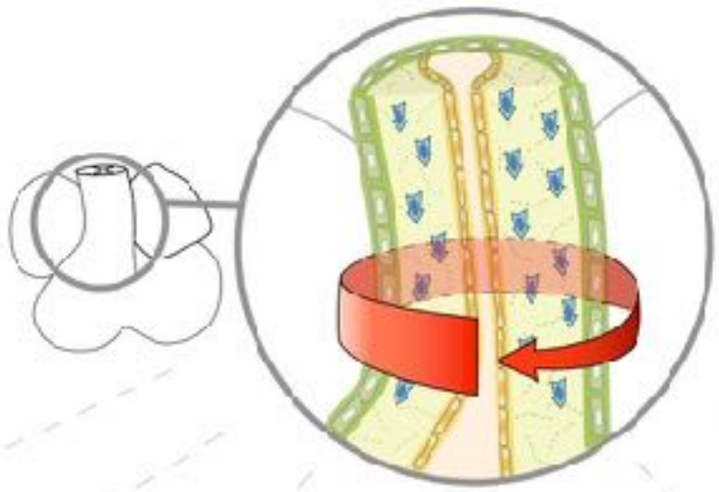
-Abnormal EM-transformation
-Abnormal proliferation

Elongation defect

Septation defect

Alignment defect

Cushion defect



Remodeling of
the outflow tract

Myccardium

Neural crest cells

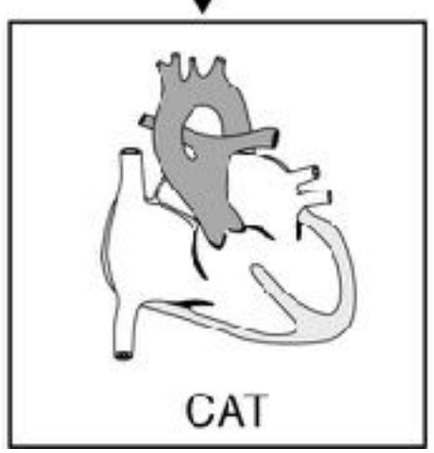
Endocardium

Endocardial
cushions

TOF

TOF&PA

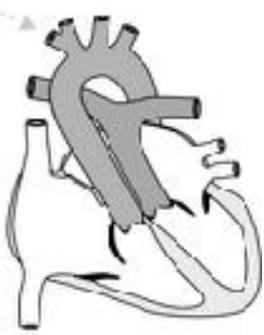
IAA



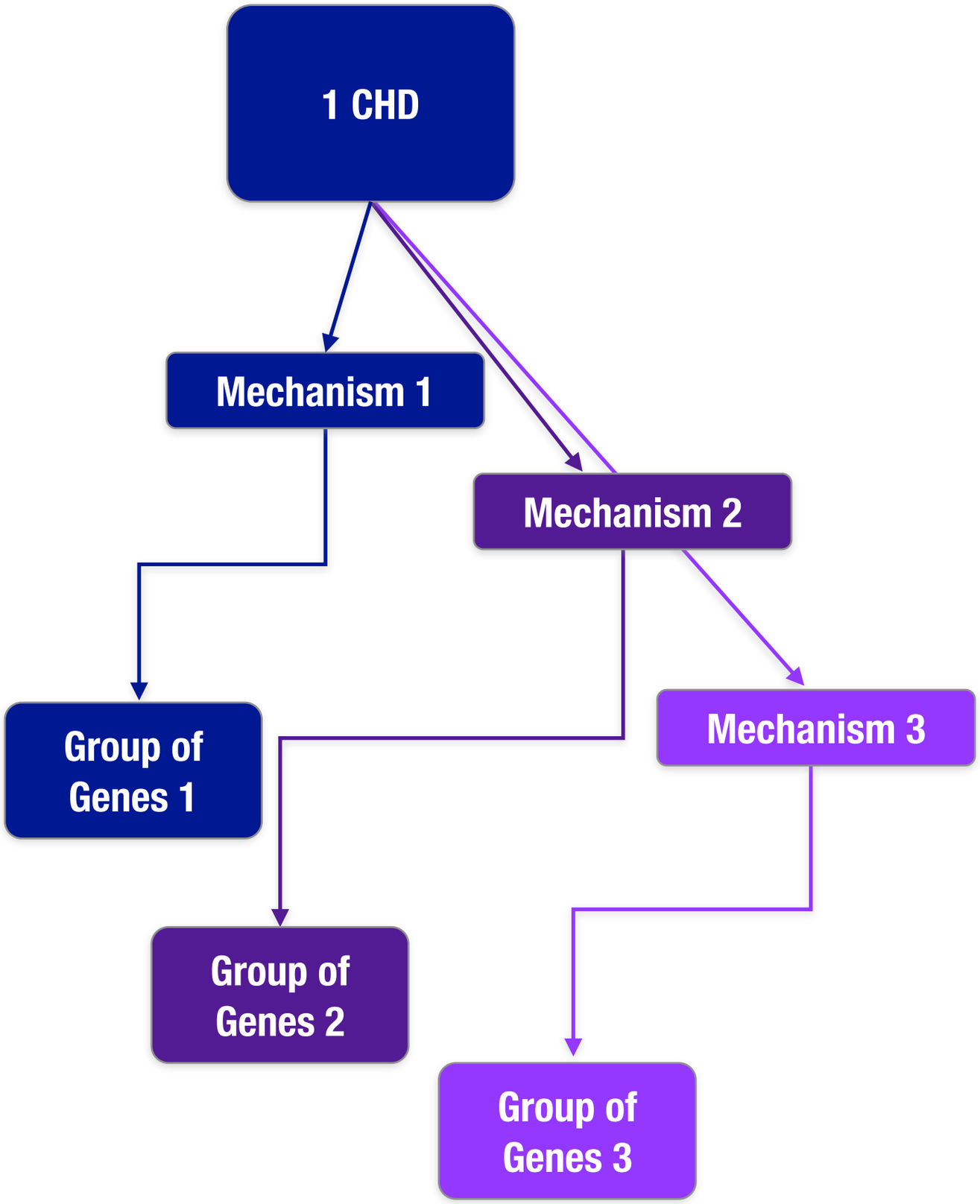
CAT

VSD

DORV



TGA



Coarctation of the aorta

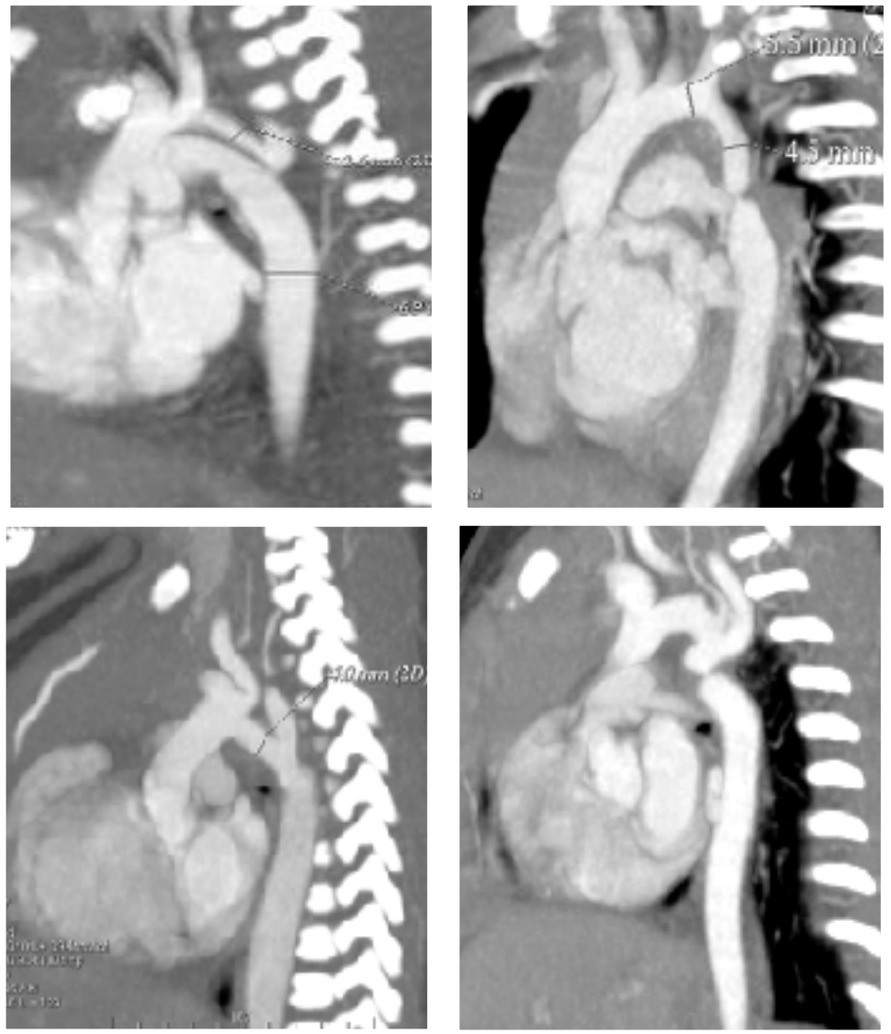
Disease of the aortic isthmus

Flow defect : spectrum of HLHS

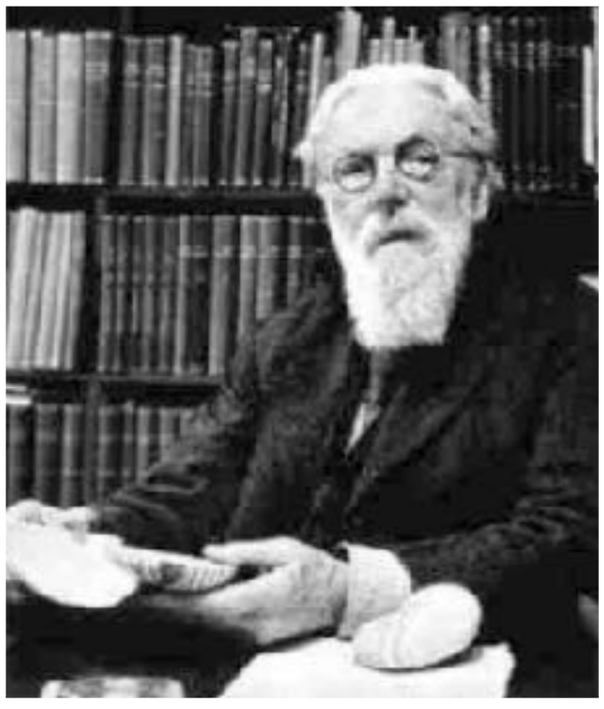
Conotruncal defect

Interrupted aortic arch

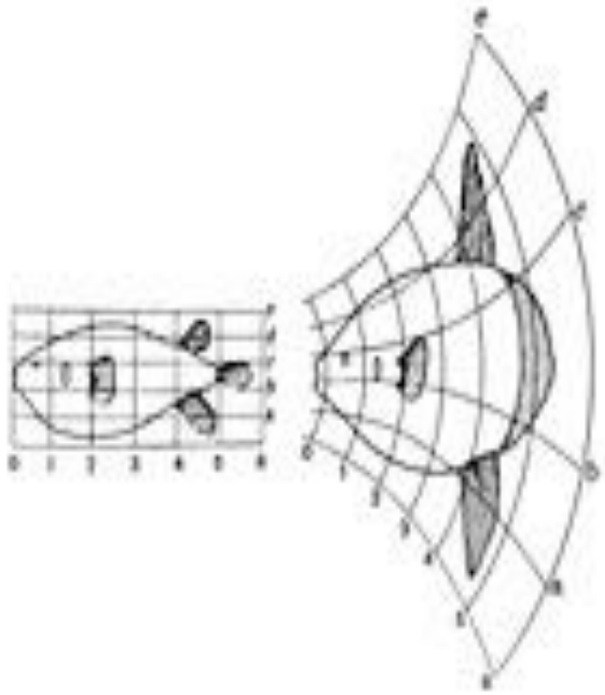
Laterality defect with persisting LSCV

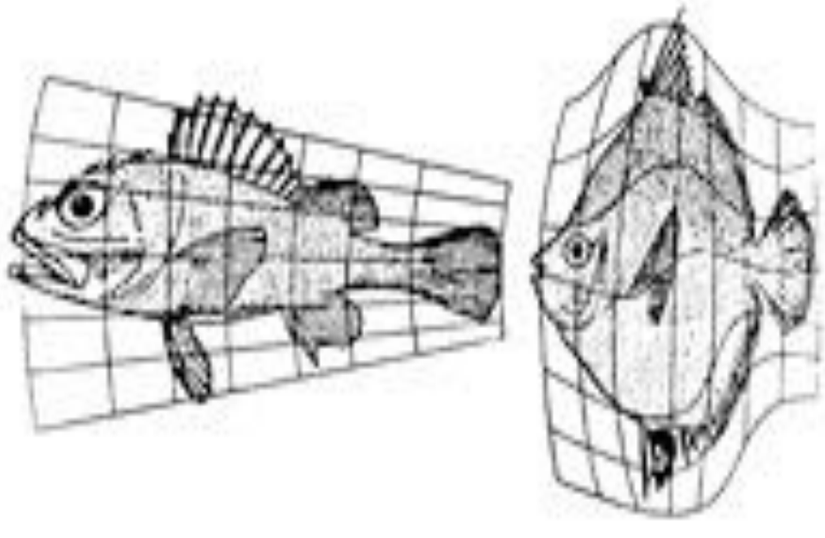


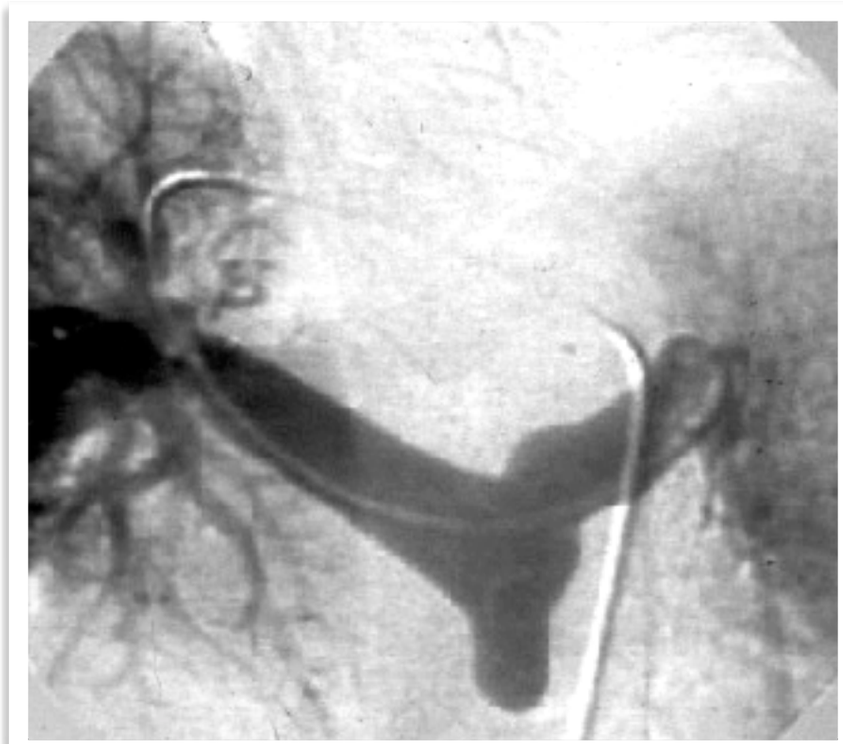
How to explain the variability inside a specific defect due to a single gene/CNV variant ?

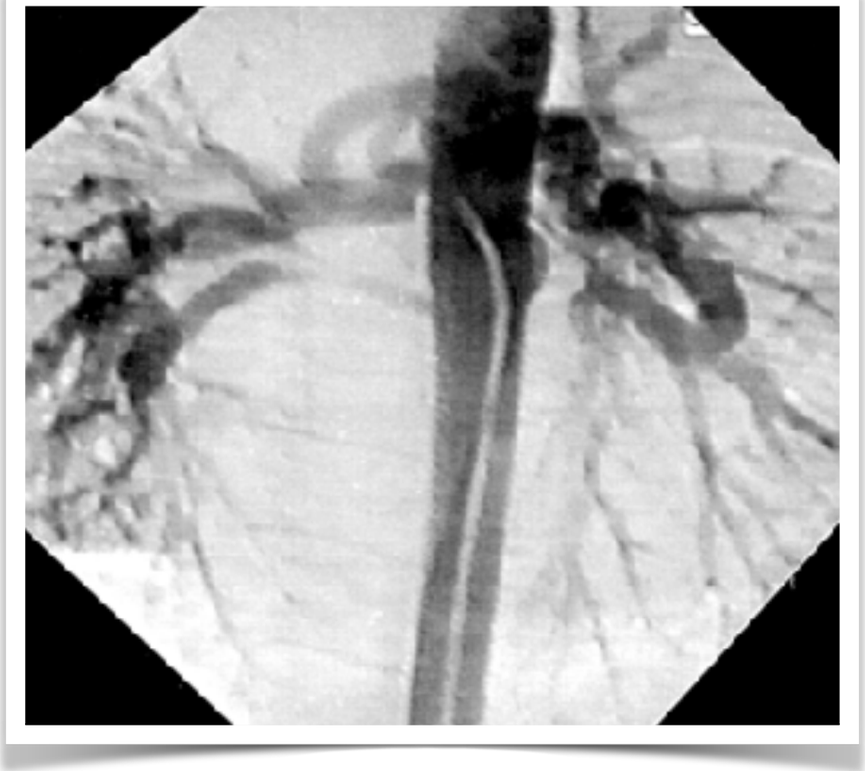


D’Arcy Thompson



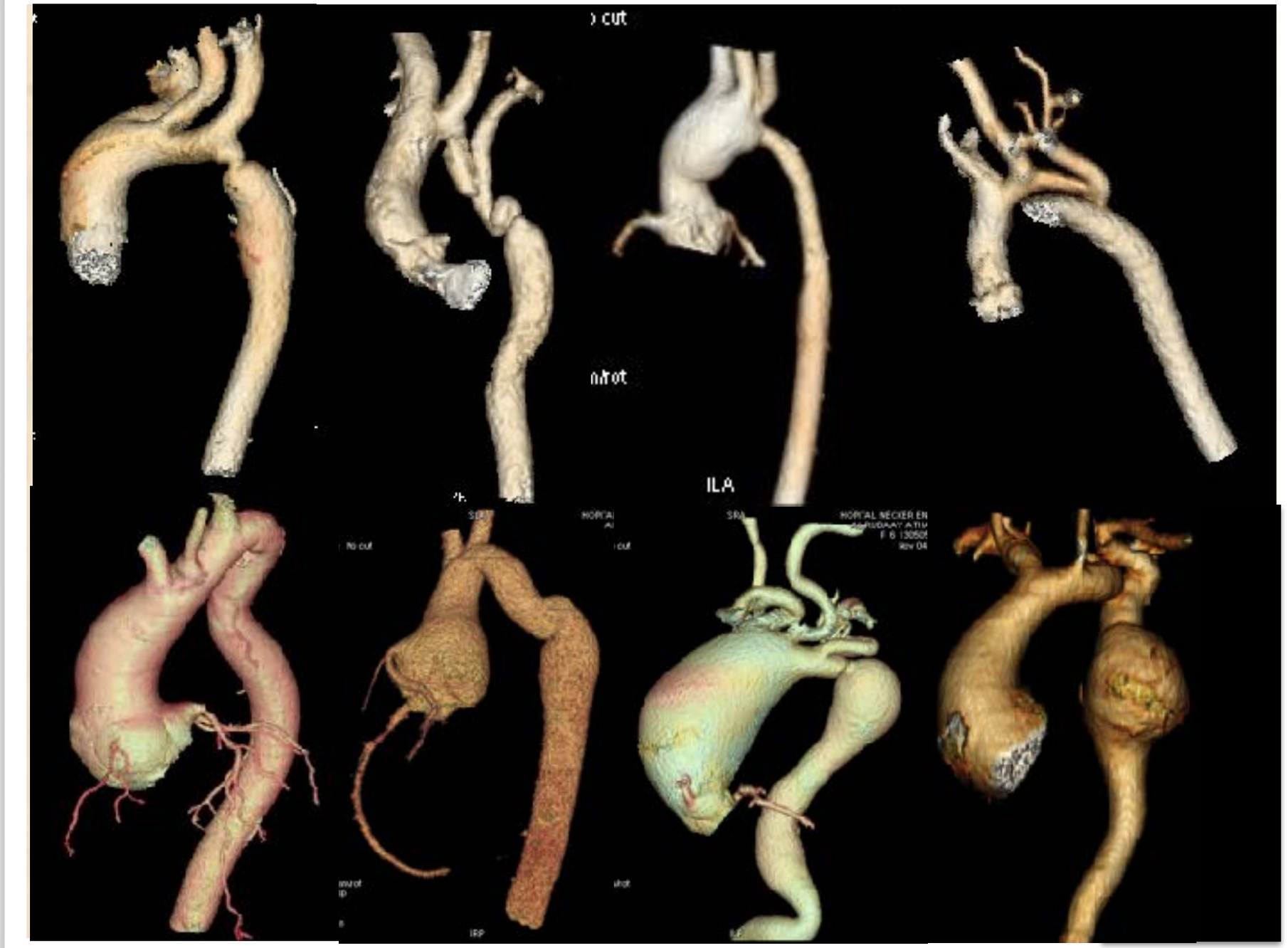






The gene dosage hypothesis

Bmp4 and morphological variation of beaks in Darwin’s Finches



Genotype–phenotype observations suggest that CHD are not because of a global change in genomic content, but rather from altered dose of specific genes.

Abzhanov A et al. Nature 2011

Genetic models of CHD

Familial CHD mutations
Different modes of inheritance
High penetrance
Variable clinical manifestations

Genetic heterogeneity
Interdependency of molecules
involved in heart development

Phenotypic heterogeneity
Genomic context-Gene dosage
Maternal-foetal environment
Foetal hemodynamics
Placenta function

An evolutionary perspective of CHD mutations predicts that reduced reproductive fitness and early mortality would cause substantial negative selection that eliminates CHD mutations from human populations.

Genetic models of CHD

Dominant or X-linked mutations do not contribute much to genetics of CHD : only 2.2% of affected patients have a first degree relative with CHD

Recessive models : higher risk in consanguineous families or in inbred populations

Somatic mutations during cardiac development

The polygenic hypothesis : Multiple variants, which individually contribute small risks that can be maintained throughout evolution, collectively cause CHD.

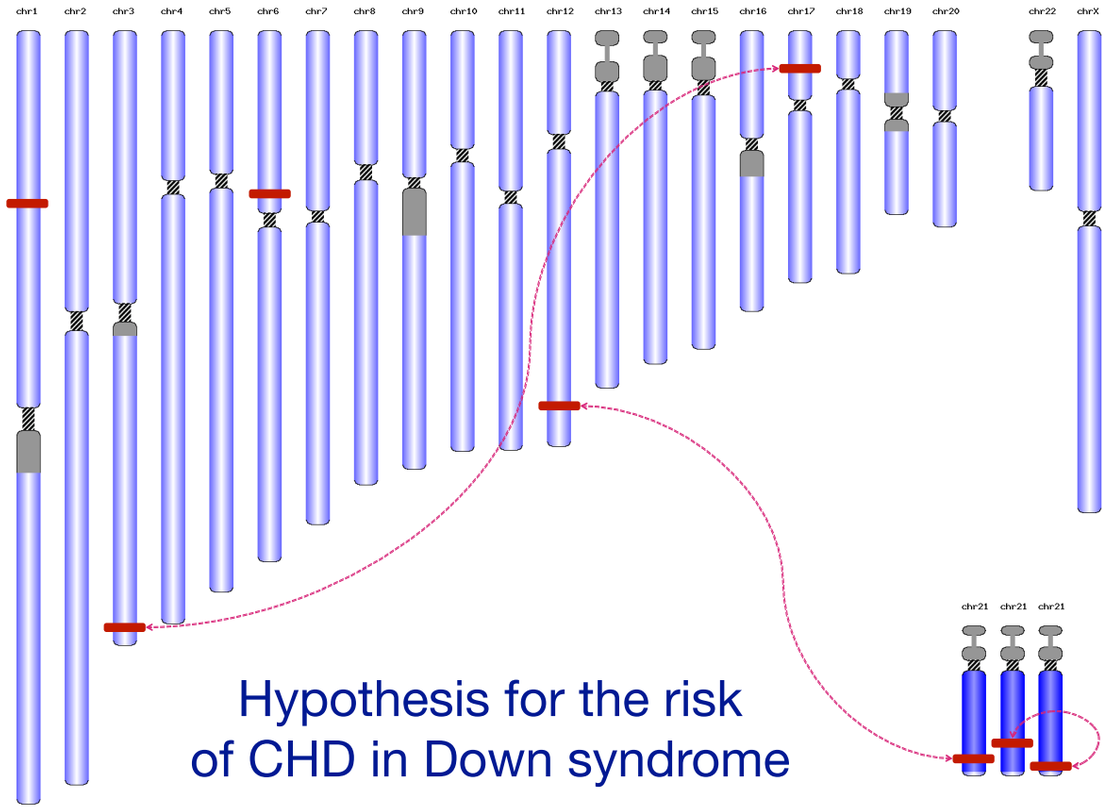
Fahed AC et al. Circ Res. 2013;112:707-720

The four hypotheses relevant for the genetic basis of congenital heart diseases

- ~~There is no genetic basis for CHD~~
- ~~Gross chromosomal aberrations are responsible for the majority of CHD~~
- ~~Single gene mutations are the main cause for CHD~~
- Congenital heart disease are a heterogeneous category of developmental anomalies, representing in most cases the multifactorial inheritance of threshold characters, the expression of which is the product of genetic-environment interaction

Nora JJ. Circulation 1968

A multigenic model for the development of CHD in trisomy 21 with effects of several genetic variants

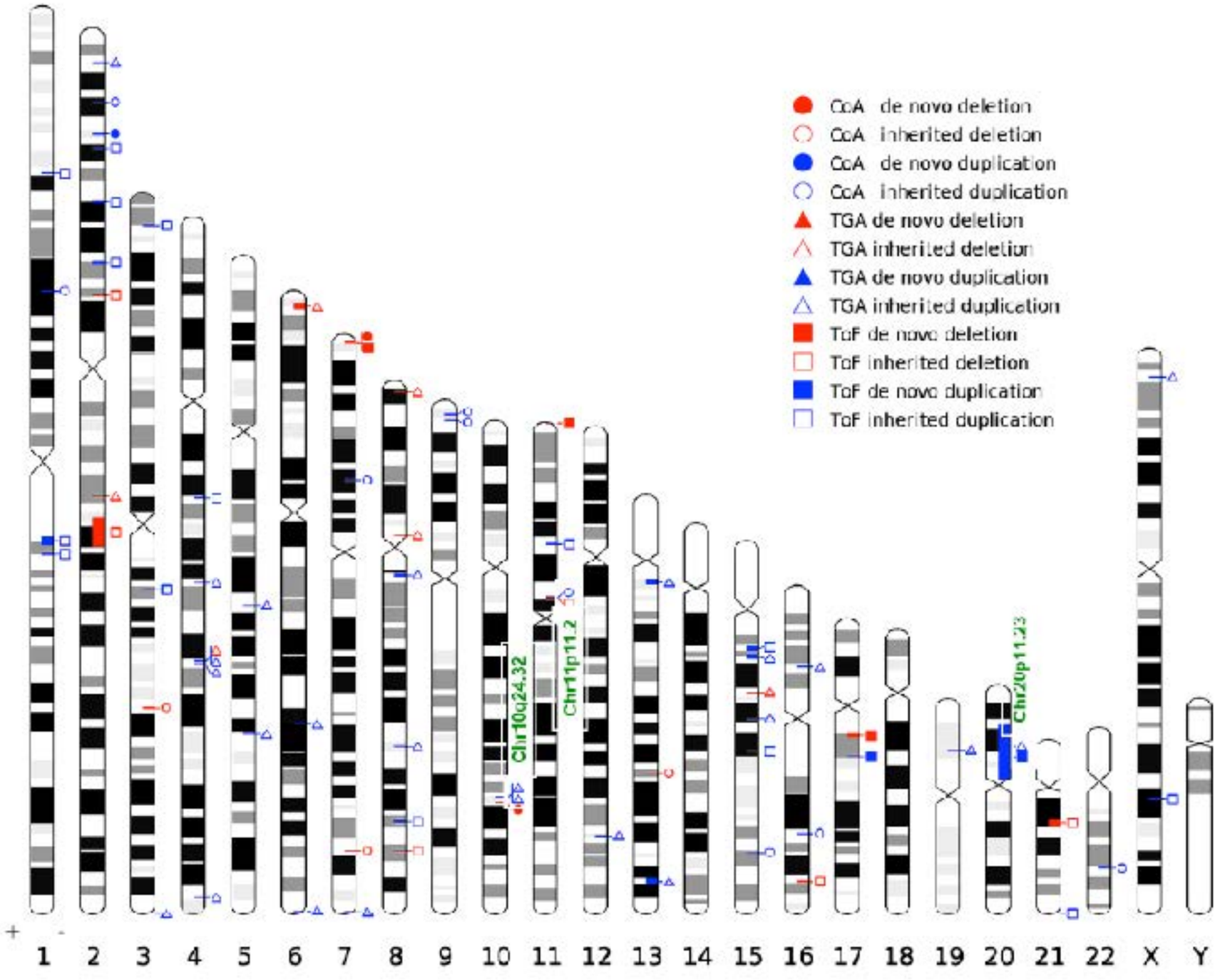


Genomic variability of chr21 (trisomic regions) may contribute to the CHD in Down syndrome.

The CHD risk of Down syndrome is determined not only by trisomy 21 but also the genome-wide interaction of specific alleles.

Sallani MR et al. Genome Res. 2013 Sep;23(9):1410-21

Cardiac malformations are not because of a global change in genomic content, but rather from altered dose of specific genes.

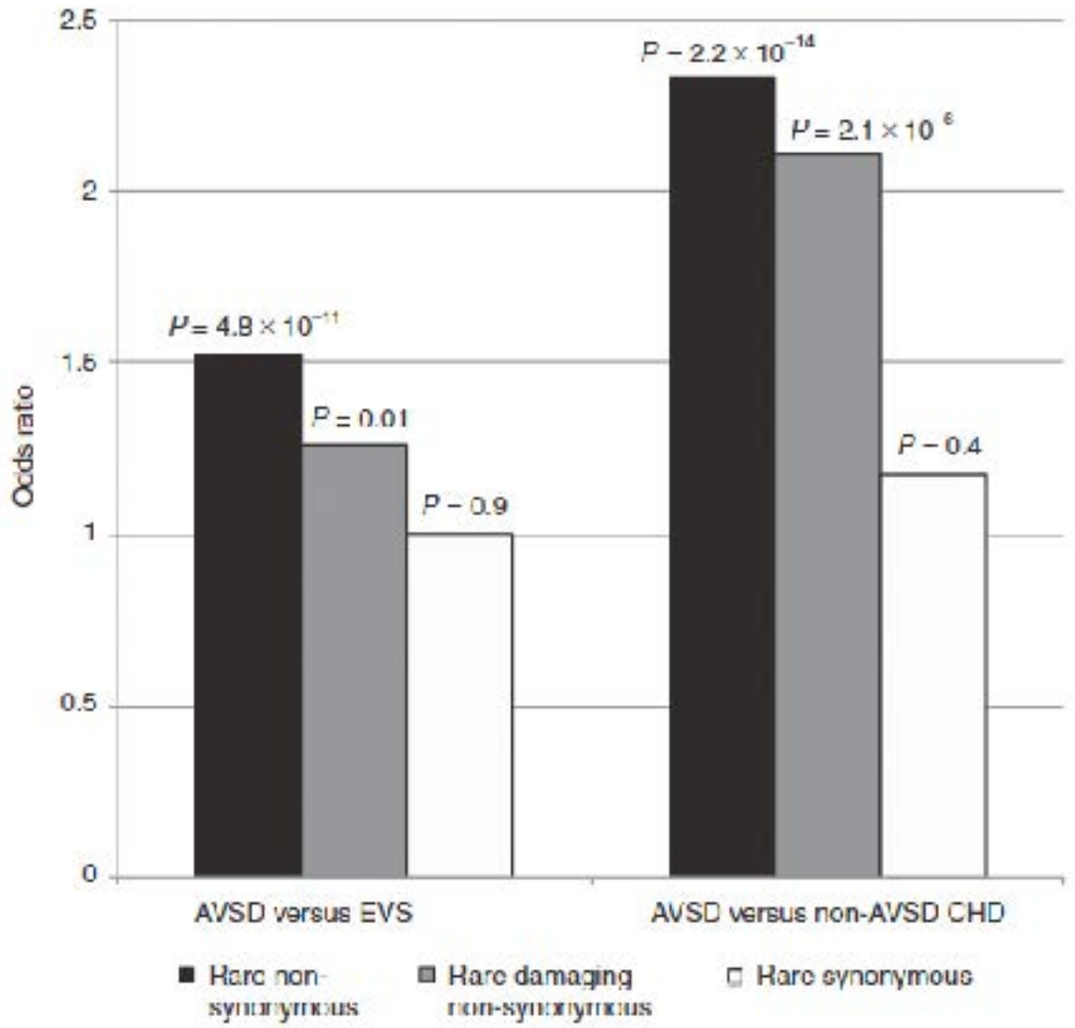


Sanchez-Castro M et al. Circ Cardiovasc Genet 2016;9:86-94

Exome sequencing identifies rare variants in multiple genes in atrioventricular septal defect

Whole-exome sequencing was performed in 81 unrelated probands with AVSD to identify potentially causal variants in a comprehensive set of 112 genes with strong biological relevance to AVSD.

Mutations in genes with strong biological relevance to AVSD, including syndrome-associated genes, can contribute to AVSD, even in those with isolated heart disease.



D'Alessandro LCA et al. Gen Med 2015

Role of epigenetic
Prevention of CHD in animal models

Rate of CHD in the offspring of diabetic and control females with and without N-acetylcysteine (NAC) treatment

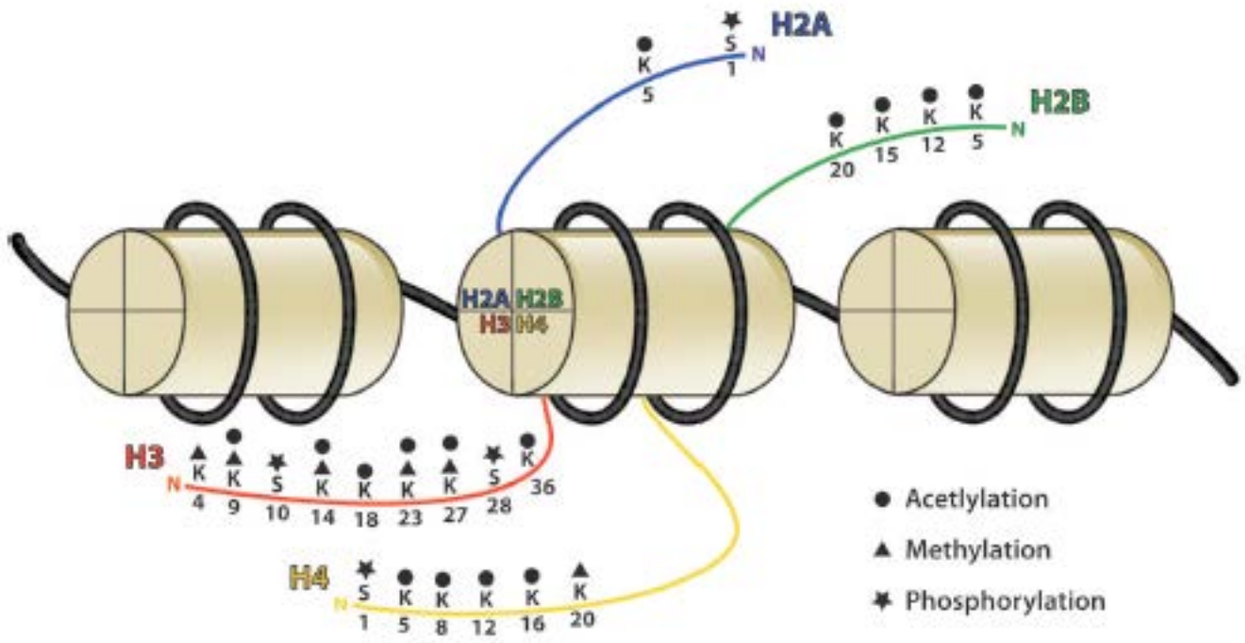
Total N/litters	Control 30/4		Diabetes 62/15		Control NAC 30/4		Diabetes NAC 43/7	
	n	%	n	%	n	%	n	%
Normal	30	100	26	41.9**	27	90	36	83.7††
Abnormal	0	0	36	58.1**	3	10	7	16.3††
ASD	0	0	19	30.6**	2	6.7	6	13.9†
VSD	0	0	25	40.3**	1	3.3	5	11.6††
AVSD	0	0	4	6.5	0	0	0	0
TGA	0	0	4	6.5	0	0	0	0
DORV	0	0	8	12.9*	0	0	3	6.9
TOF	0	0	3	4.8	0	0	0	0

Data were analyzed by Chi-square test. *$P < 0.05$, **$P < 0.001$ vs. untreated control, †$P < 0.05$, ††$P < 0.001$ vs. untreated diabetes. ASD, atrial septal defect; VSD, ventricular septal defect; AVSD, atrioventricular septal defect; TGA, transposition of great arteries; DORV, double outlet right ventricle; TOF, Tetralogy of Fallot.

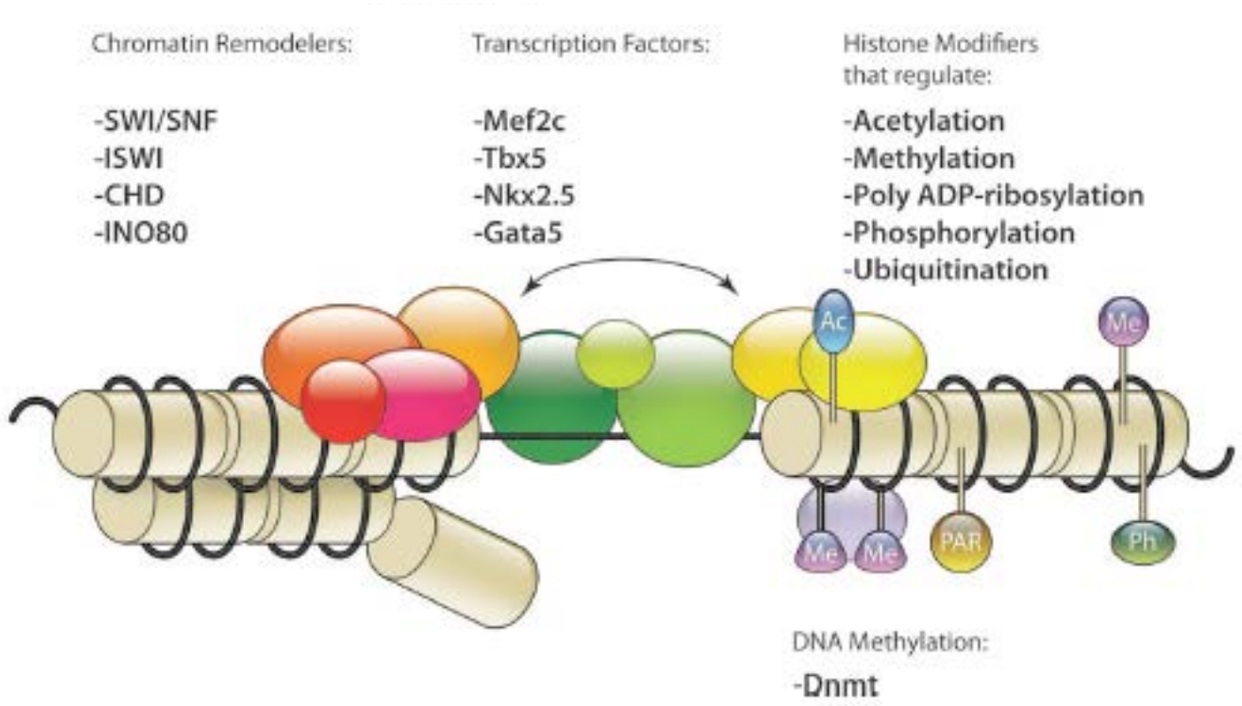
Moazzen et al. Cardiovascular Diabetology 2014

Prevention of CHD in human

Nucleosome structure



Interactions between chromatin regulators and transcription factors to control gene expression



- Chromatin regulation is an epigenetic mechanism that controls gene expression and function without changes in the DNA sequence.
- Chromatin remodelers use energy derived from ATP hydrolysis to change chromatin architecture.
- Histones are covalently modified to modulate access of transcription factors to genomic loci.
- DNA can be methylated to control transcription.

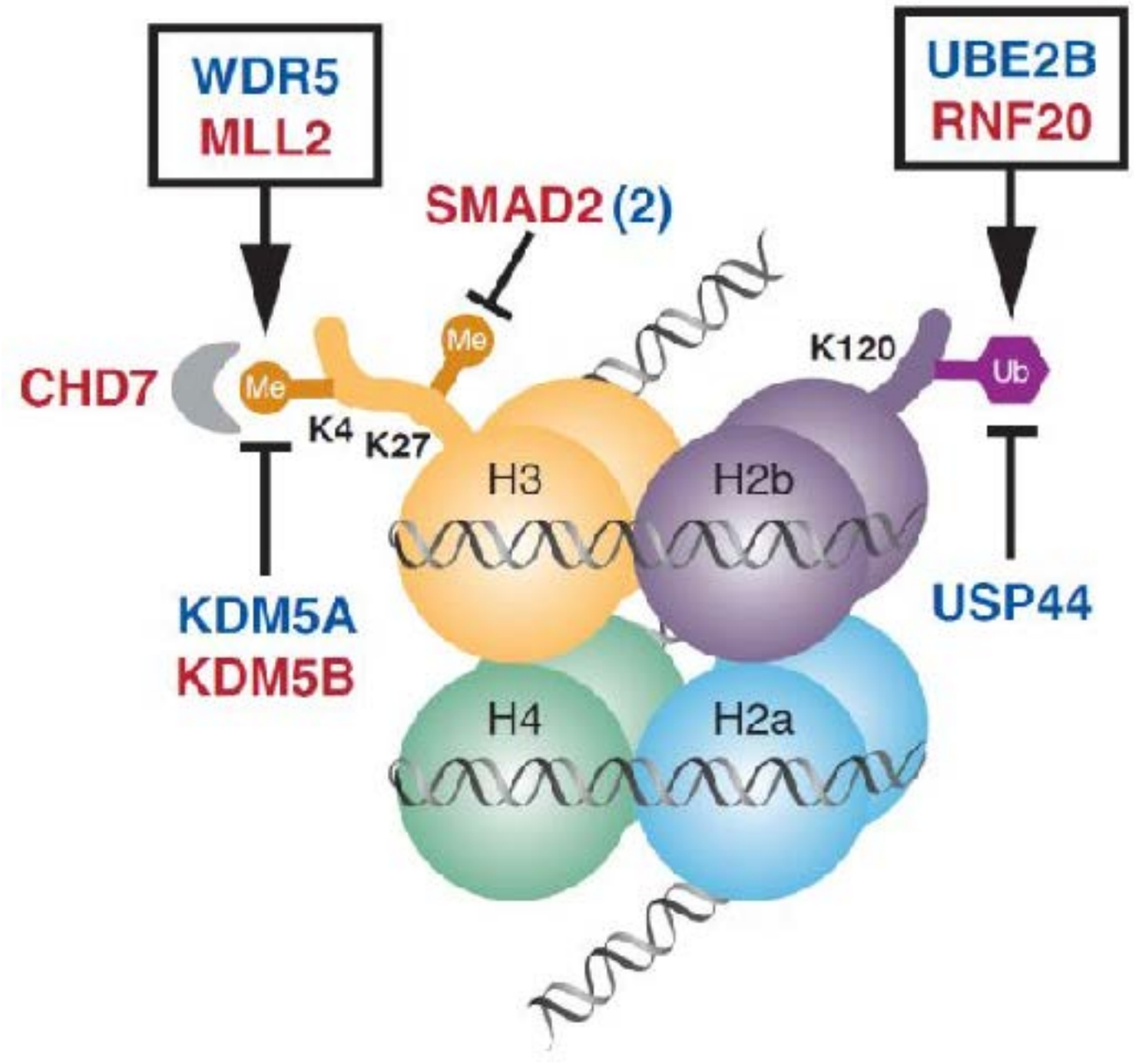
Rôle of maternal aging

Prevention with folic acid

Function of known genes such as CHD7

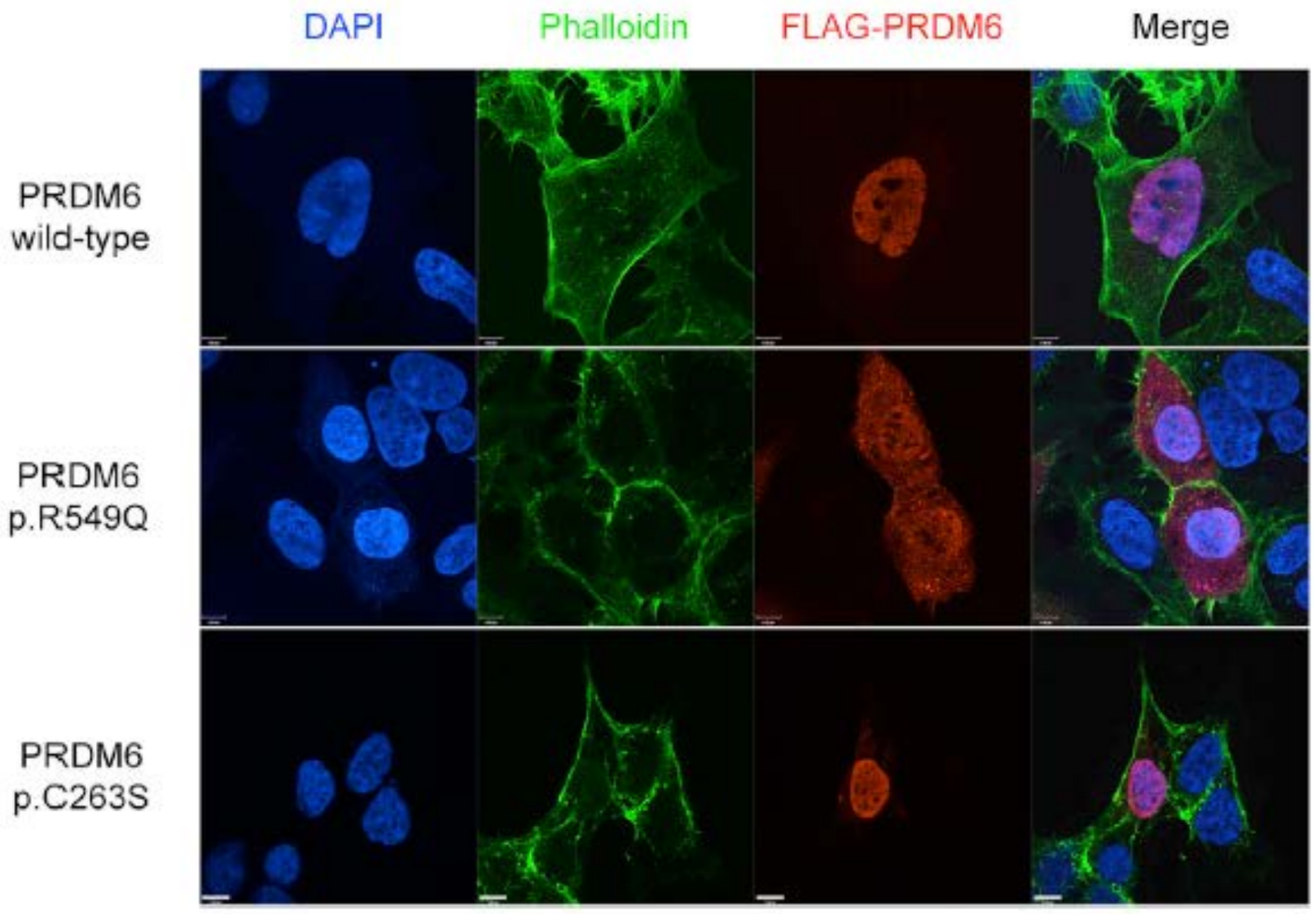
Linask KK. J Pregnancy 2013
Han P et al. Circ Res. 2011;108:378-396

De novo mutations in histone modifying genes in congenital heart disease



De novo mutations in the H3K4 and H3K27 methylation pathways

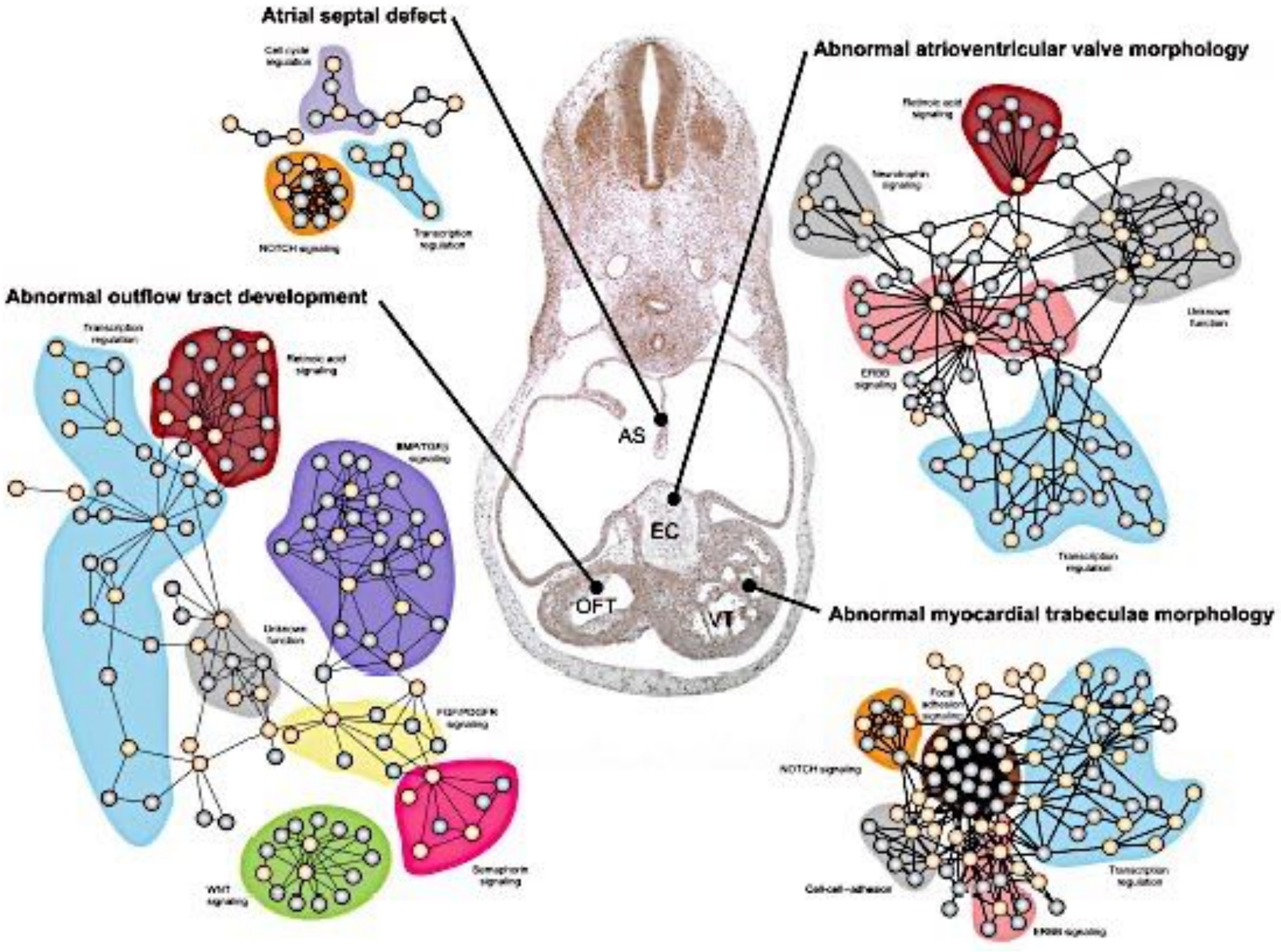
Zaidi S et al. Nature. 2013; 498: 220–223.



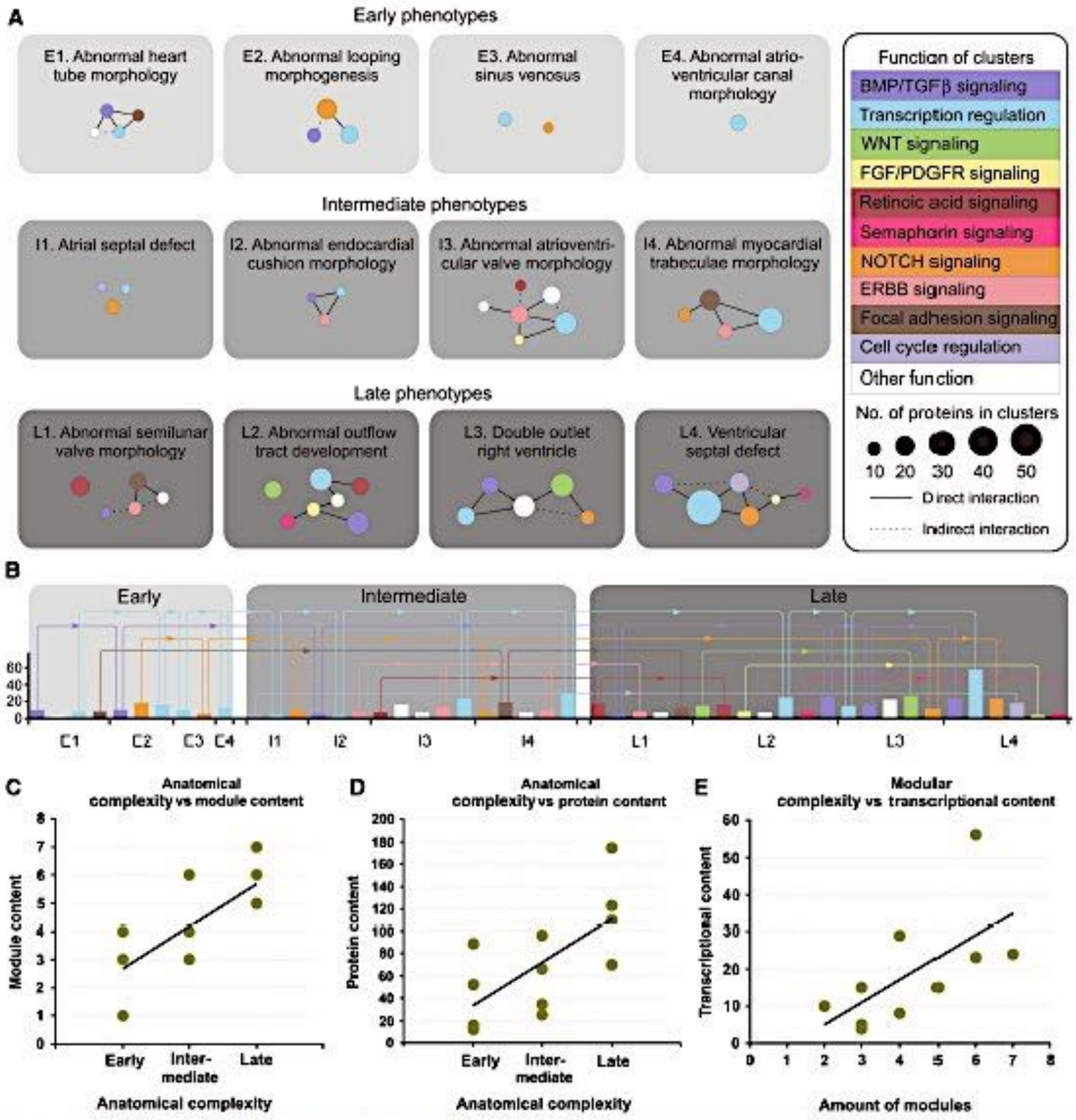
PRDM6 mutations are underlying genetic causes of nonsyndromic isolated PDA in humans and implicates the wild-type protein in epigenetic regulation of ductus remodeling.

Li N et al. Am J Hum Genet 2016;98:1082-91

Biological networks in CHDs



Overview of the molecular organization of heart development



The four hypotheses relevant for the genetic basis of congenital heart diseases

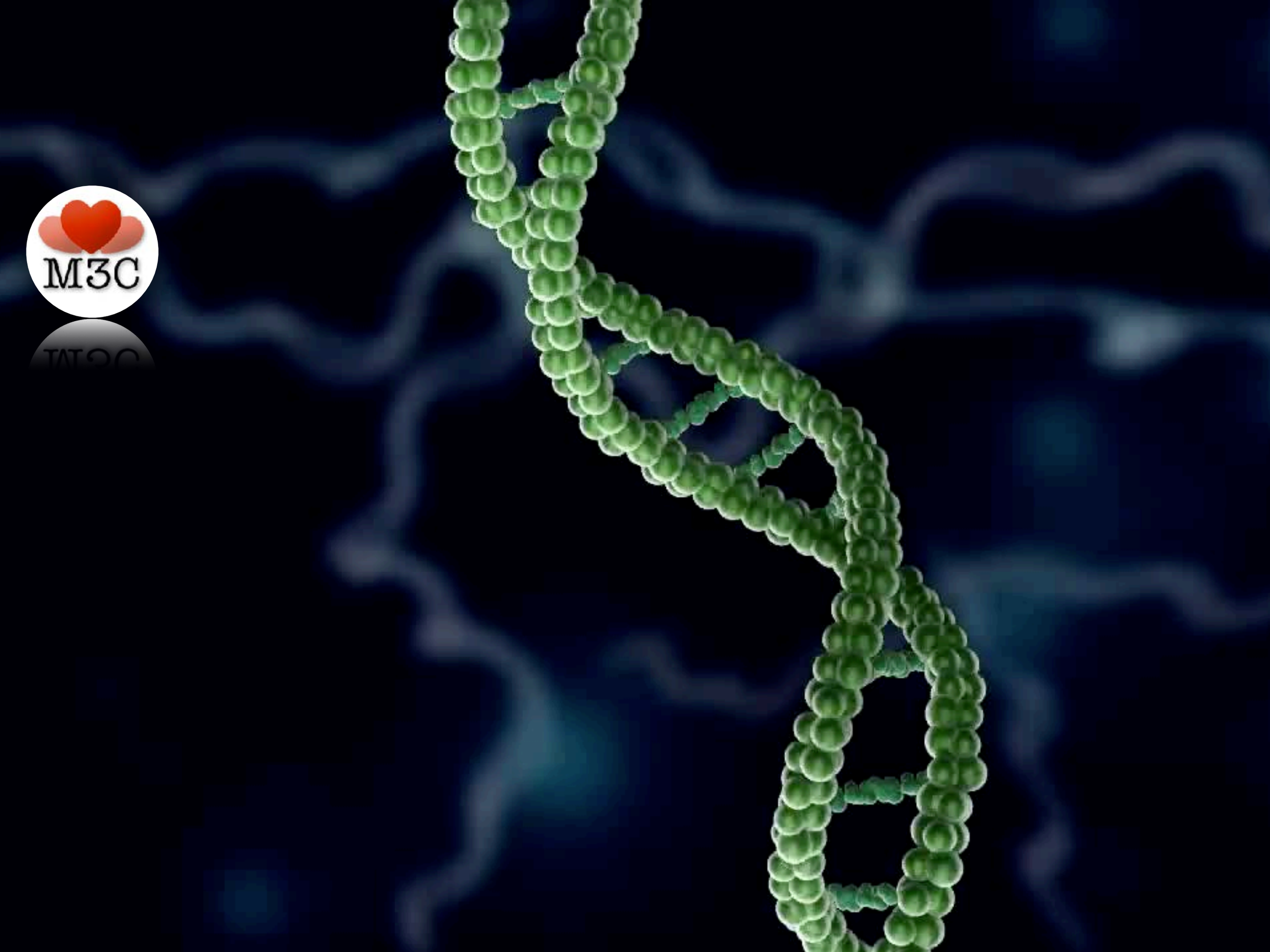
- There is ~~no~~ genetic basis for CHD

- Gross chromosomal aberrations are responsible for the ~~majority of CHD~~ minority of CHD

- Single gene mutations are ~~the main cause for CHD~~ a rare cause for CHD

- Congenital heart disease are a heterogeneous category of developmental anomalies, with polygenic inheritance, with the expression of « CHD genes » being the product of genetic-environment interaction

Nora JJ. Circulation 1968


M3C