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Centre de Référence Maladies Rares

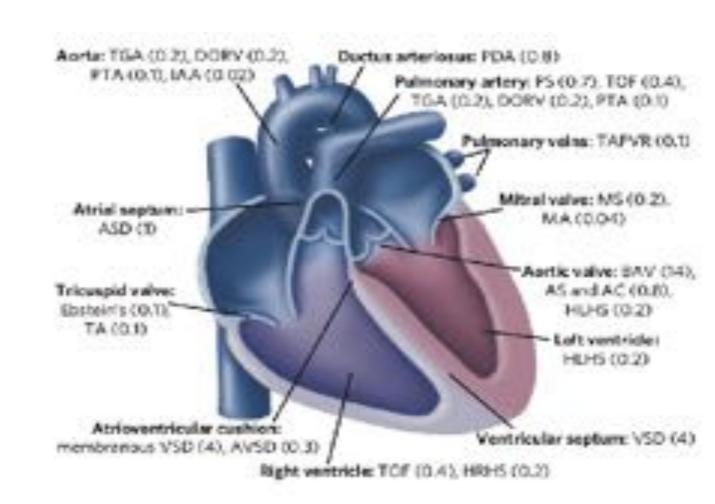
Malformations Cardiaques Congénitales Complexes-M3C

Centre de Référence Maladies Rares

Maladies Cardiaques Héréditaires- CARDIOGEN

# Congenital heart diseases

- Incidence: 8/1000 live-births
- 28%: associated anomalies600 entries in OMIM
- Genetic counseling is a challenge as survival is now the rule



The developmental genetics of congenital heart disease

Benoit G. Bruneau

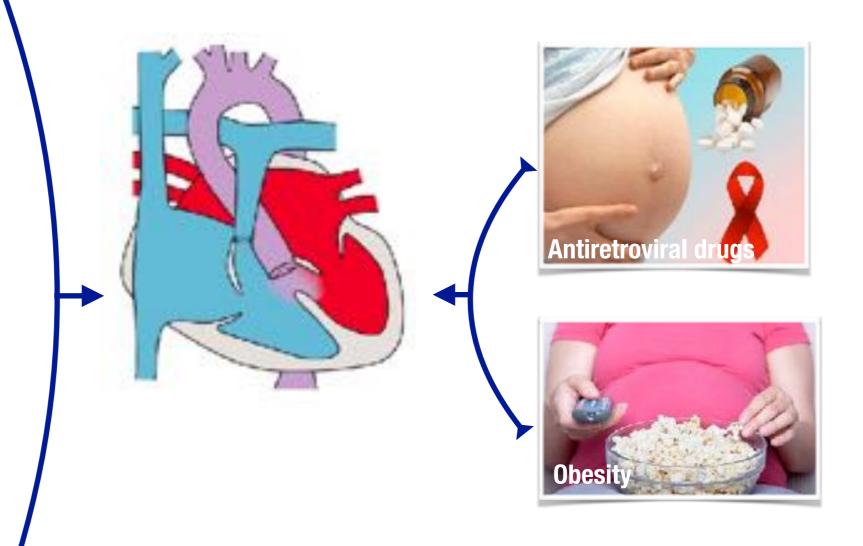
#### Well-known risk factors for congenital heart diseases





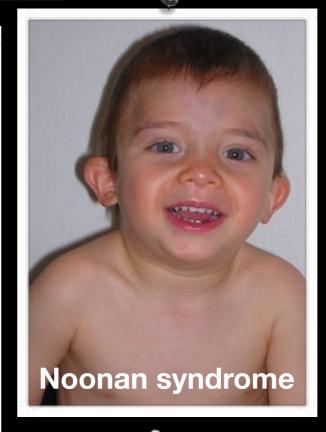




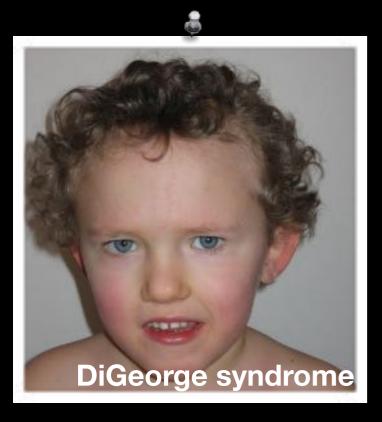


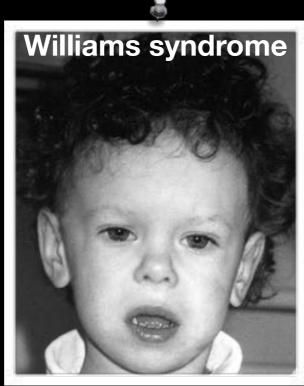




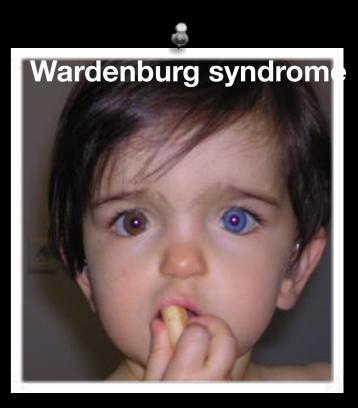








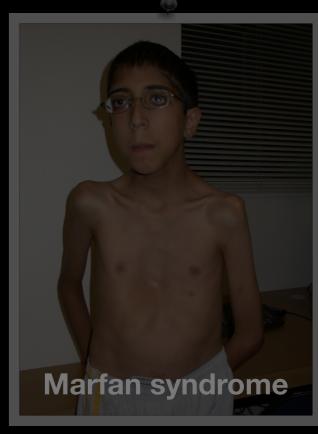


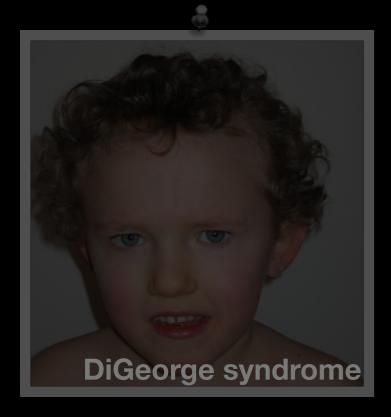


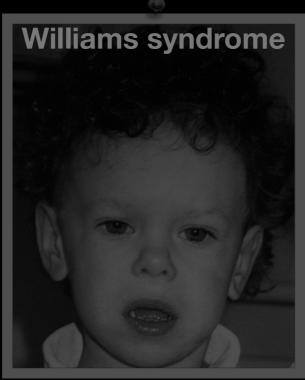


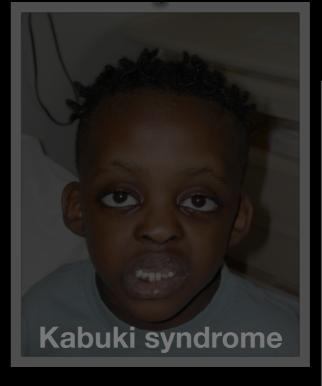




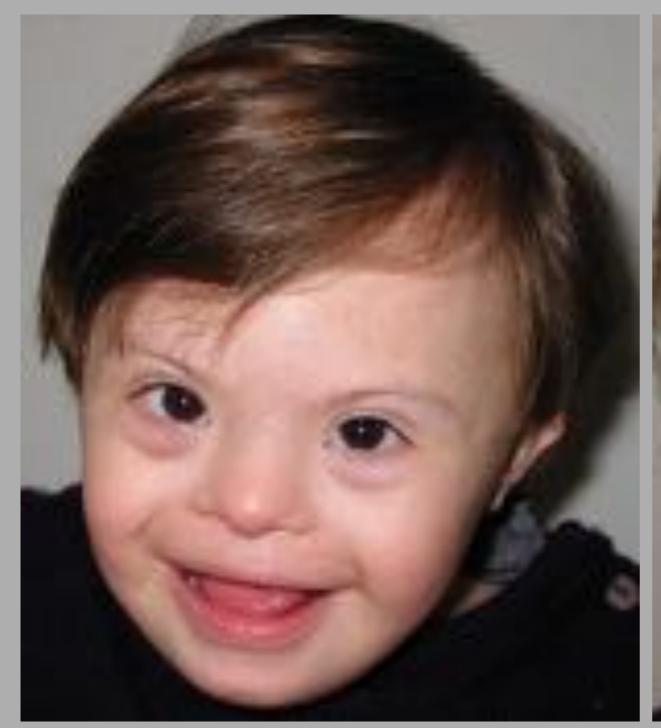






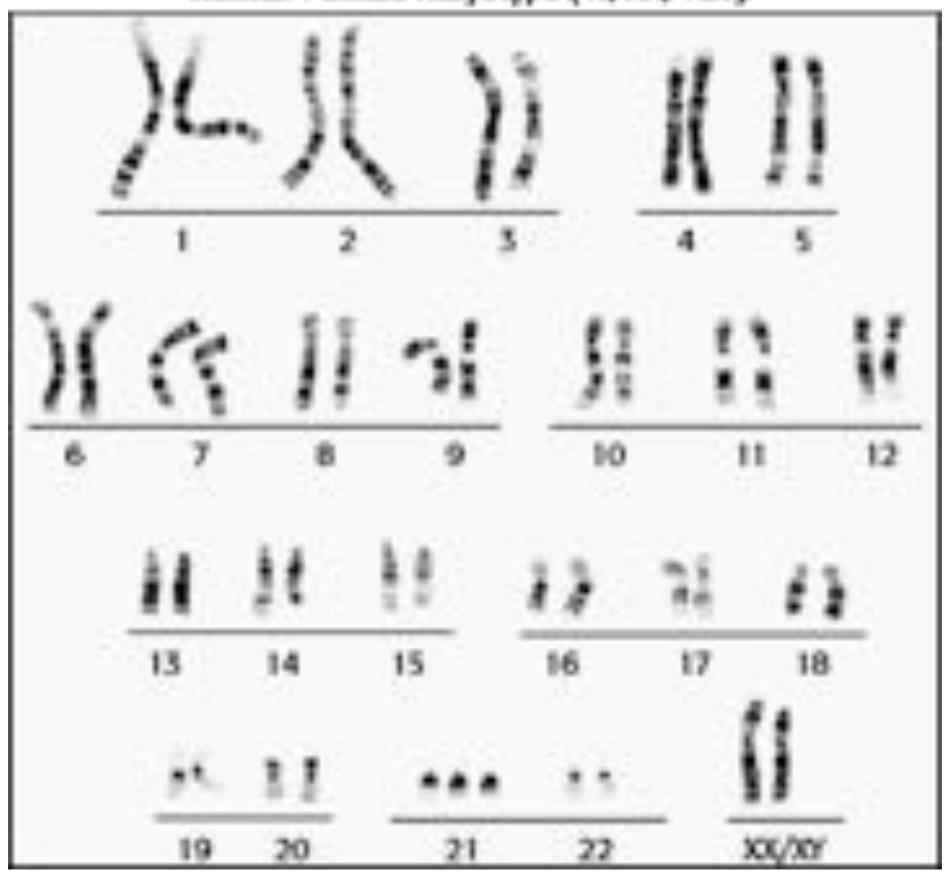






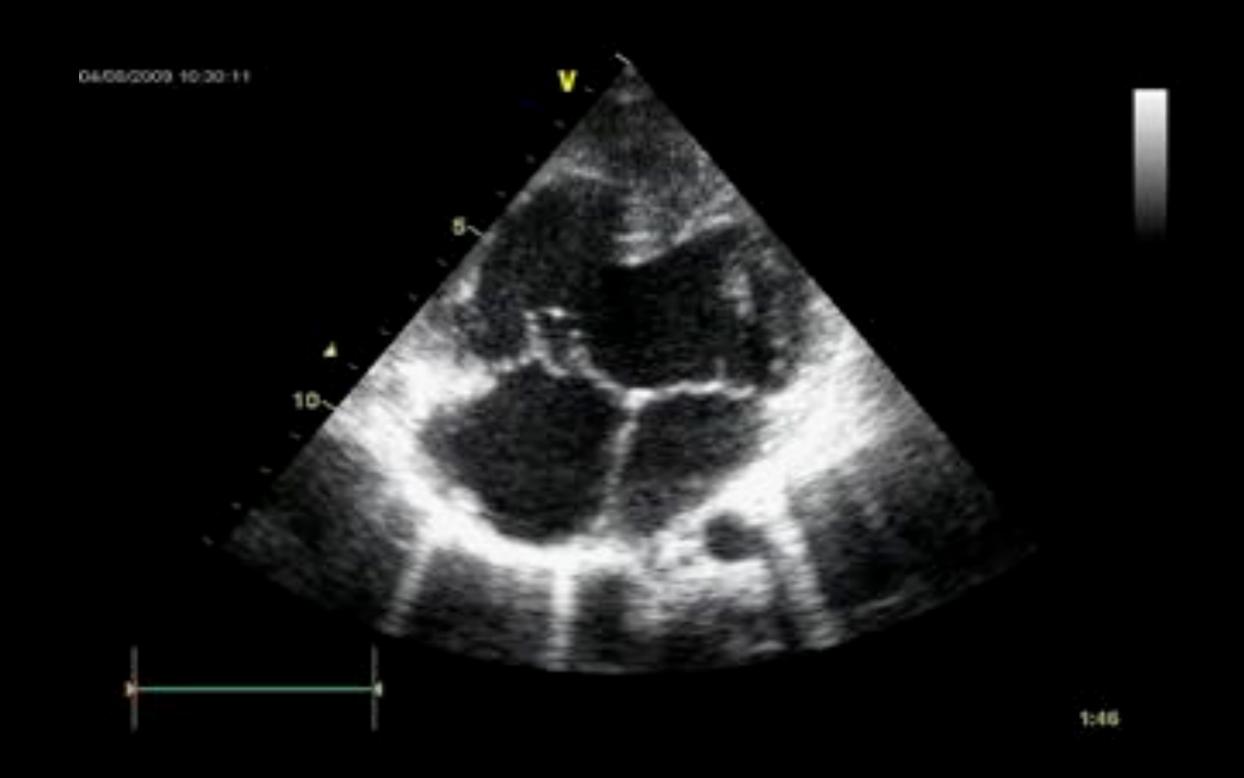


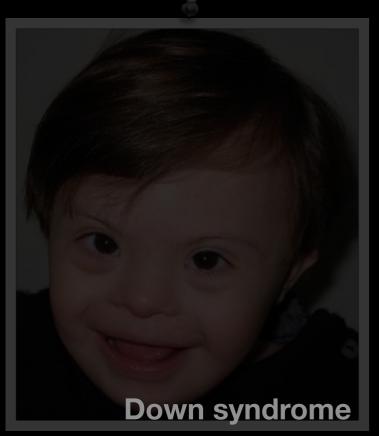
#### Human Female Karyotype (47, XX, +21)



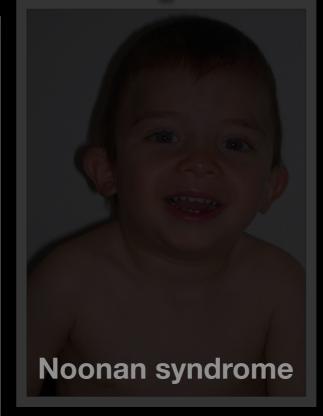


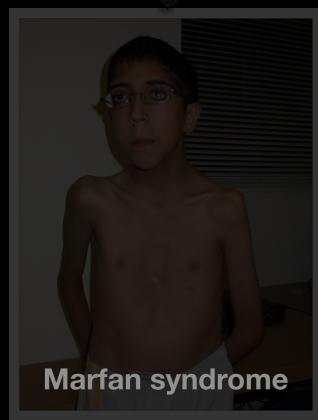
# CIV d'admission

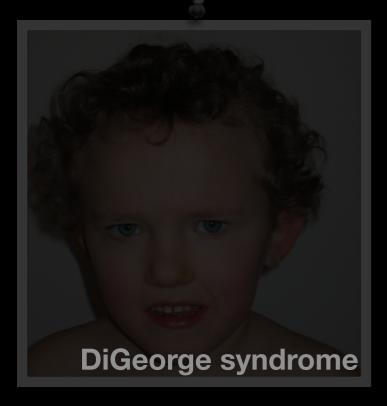


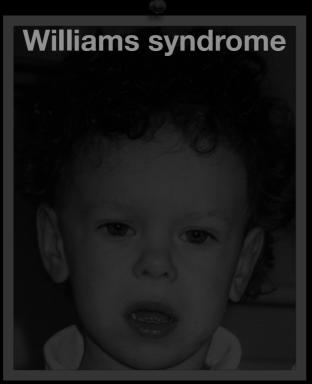














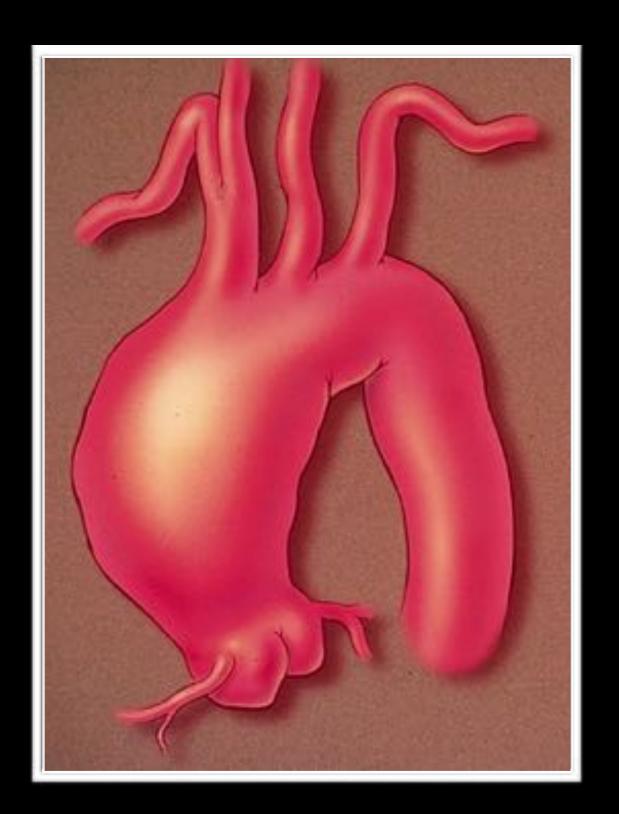


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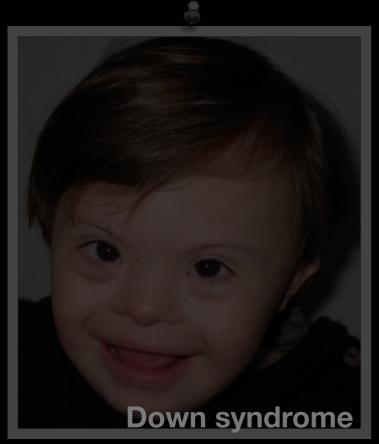




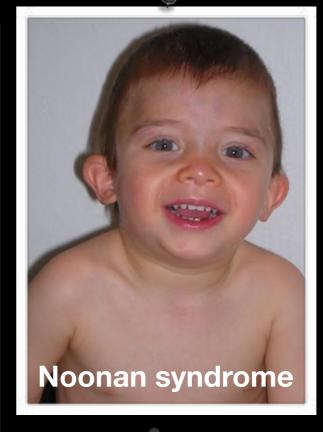
Syndrome de Bonnevie Ulrich



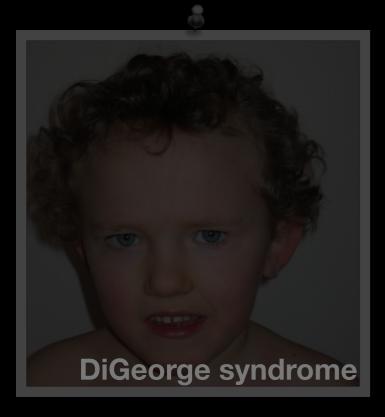


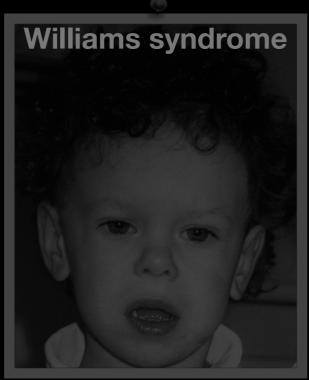


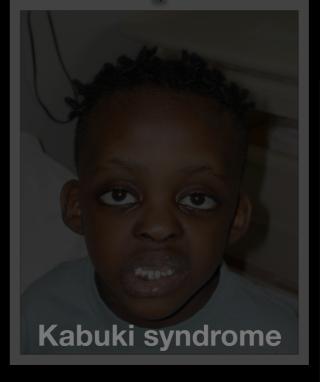


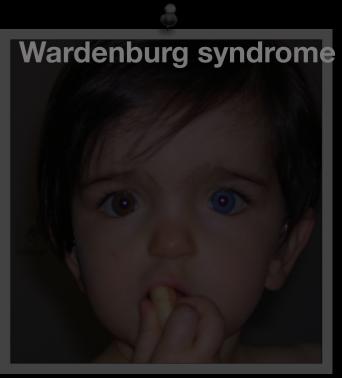




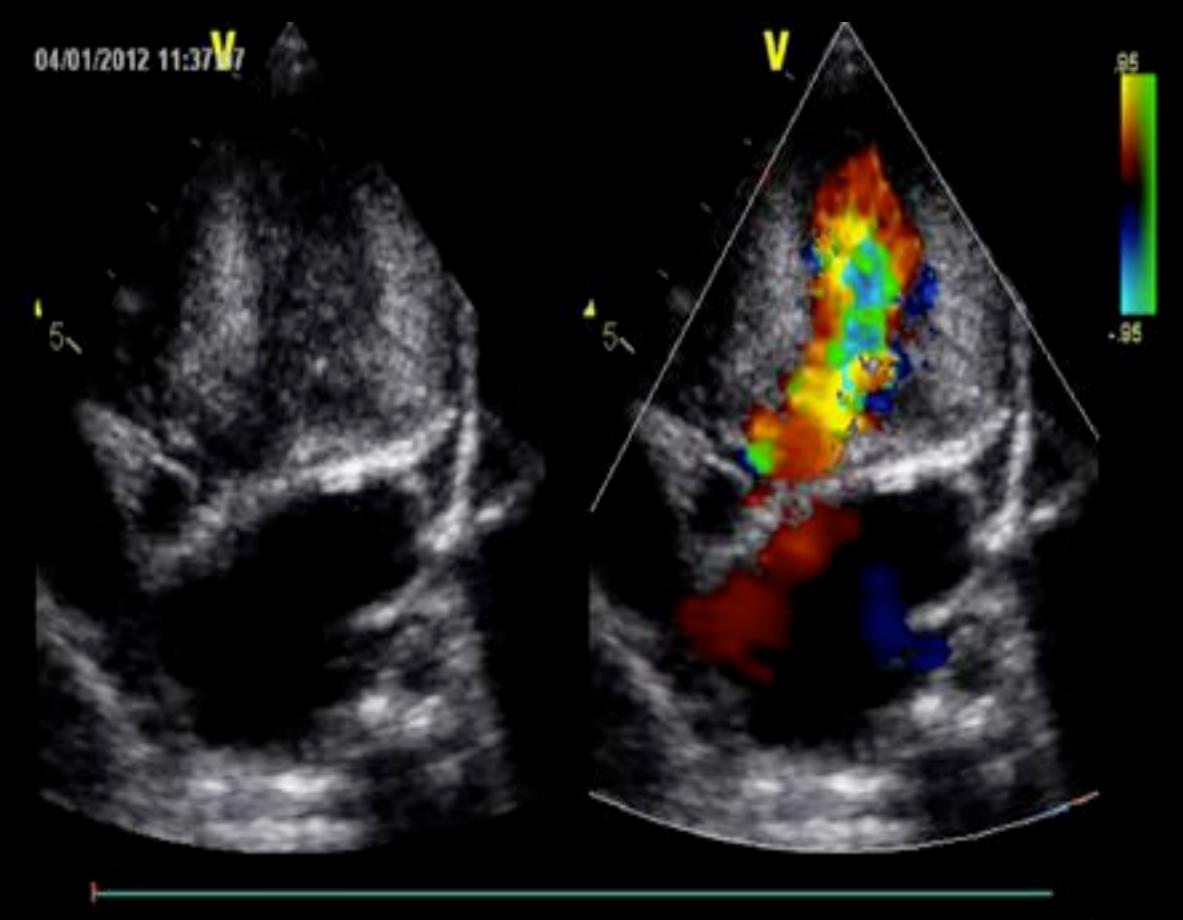


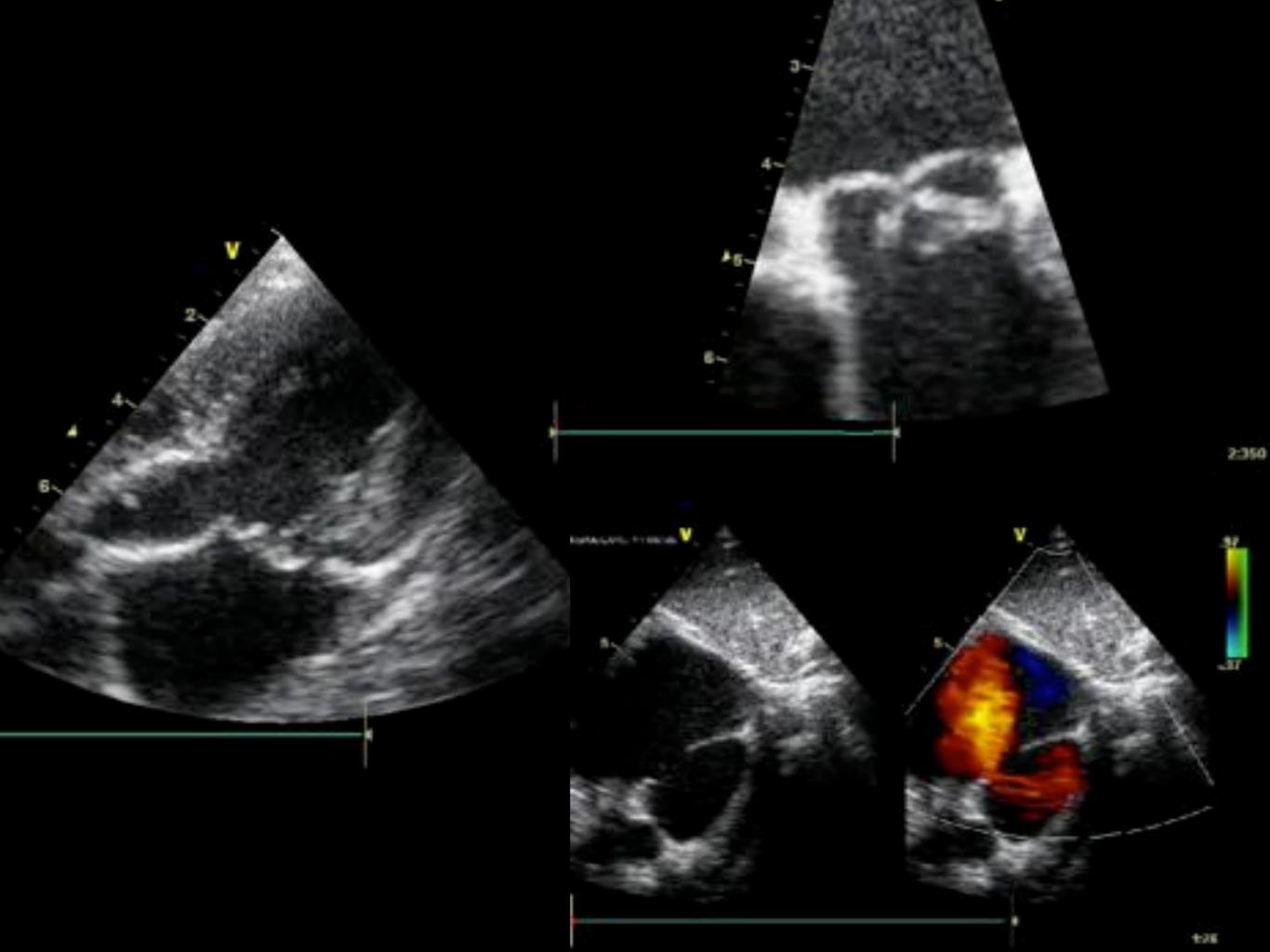










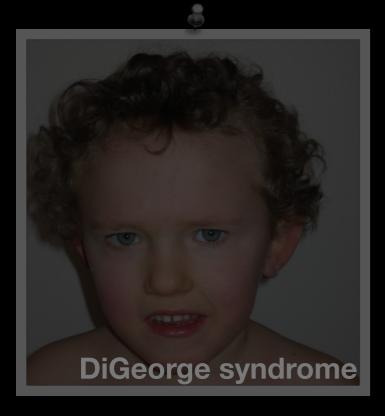


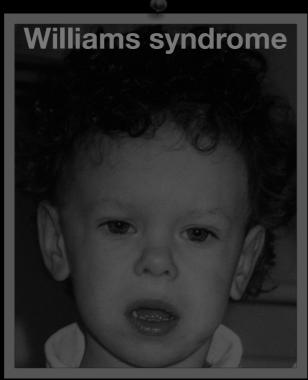


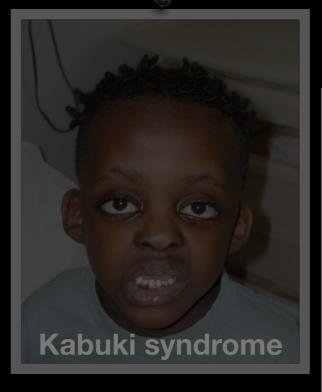




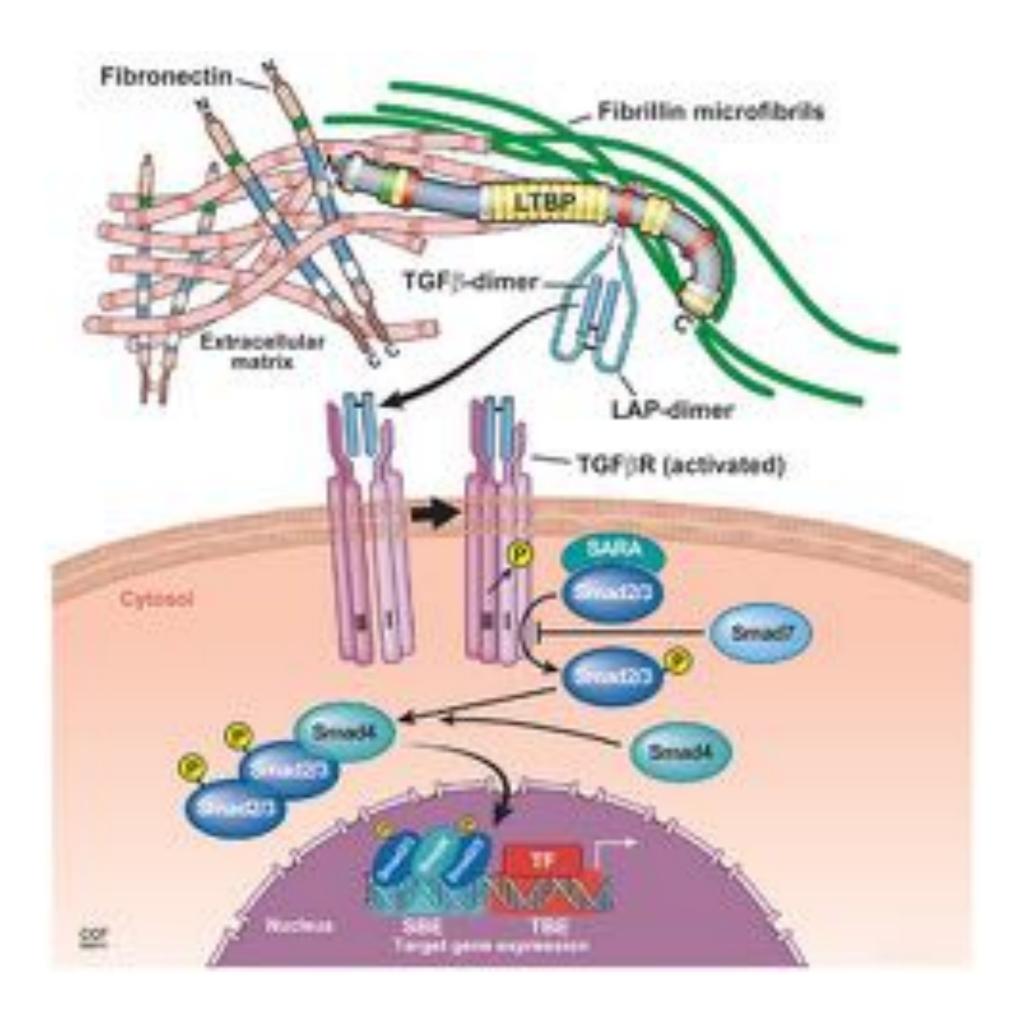


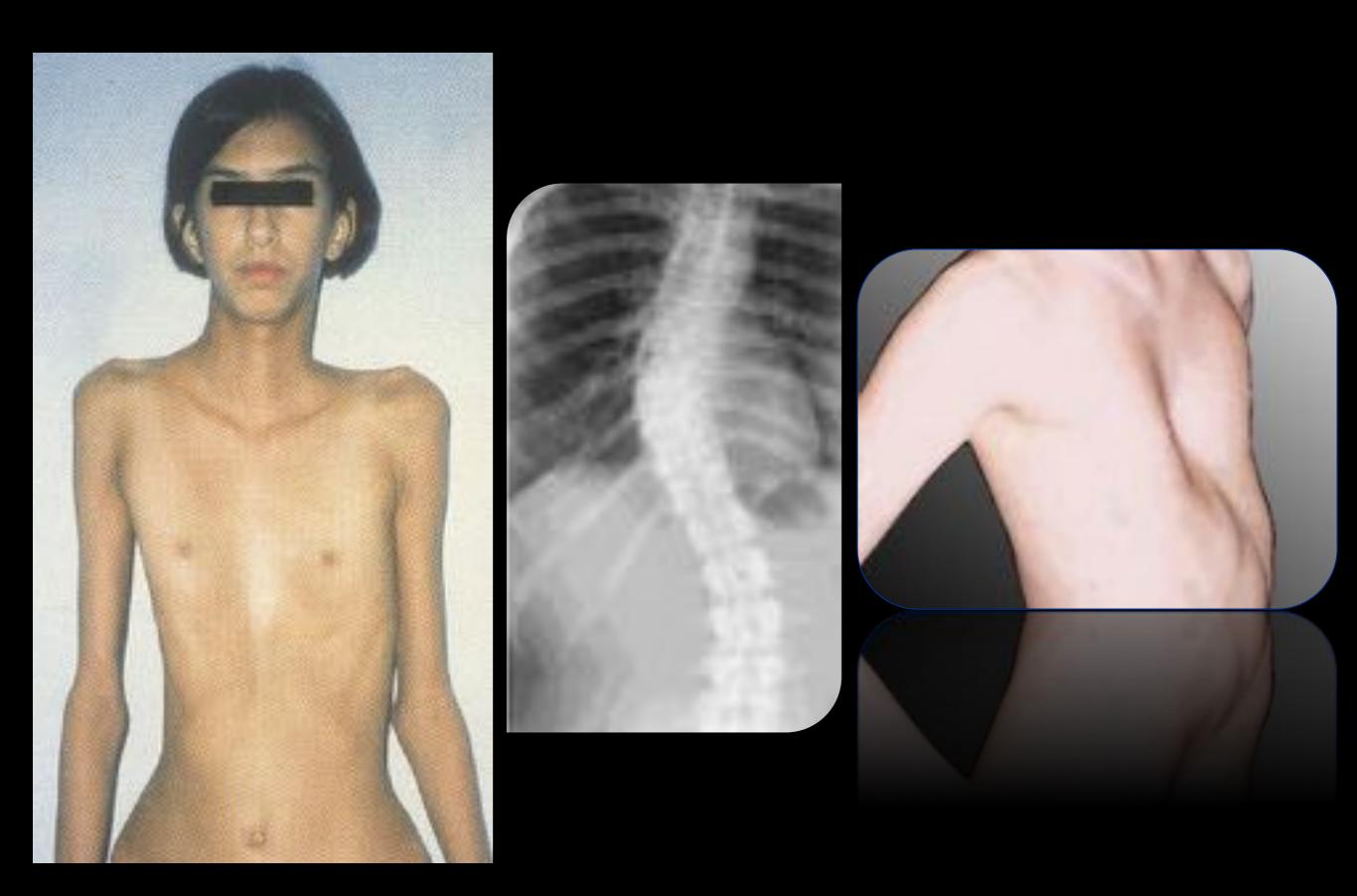






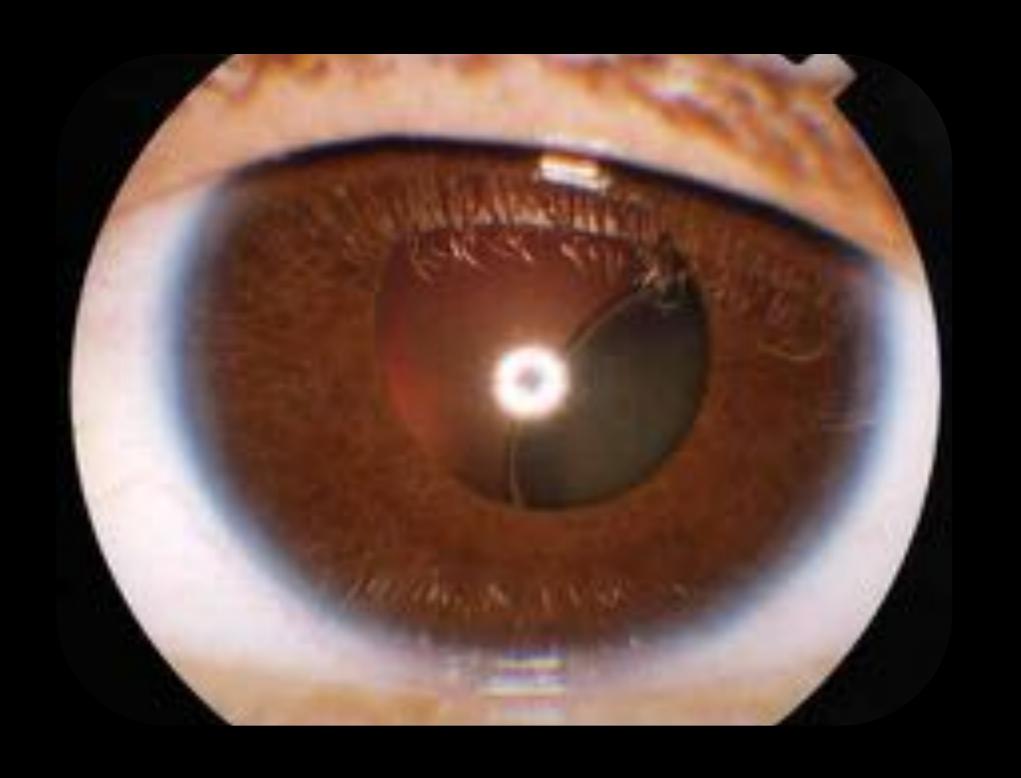


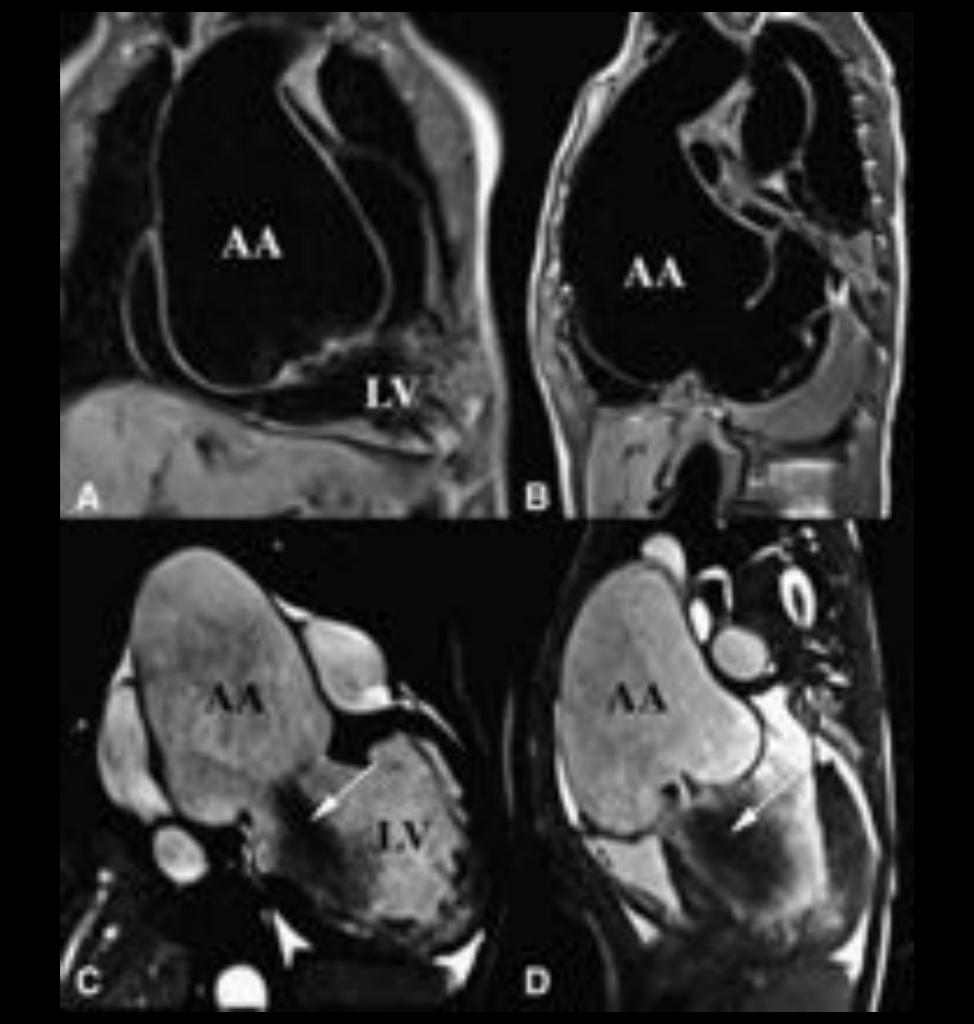




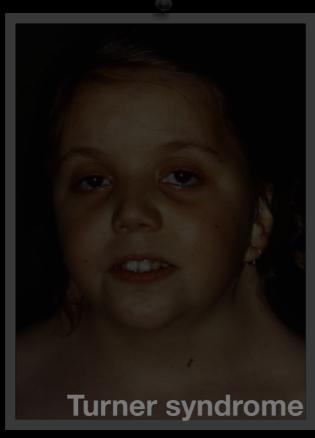








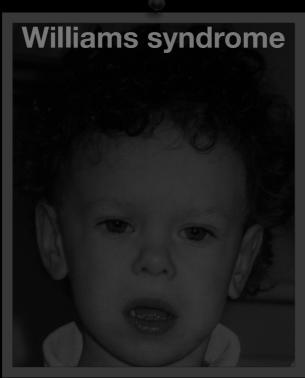


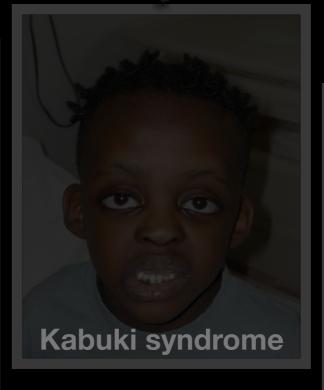




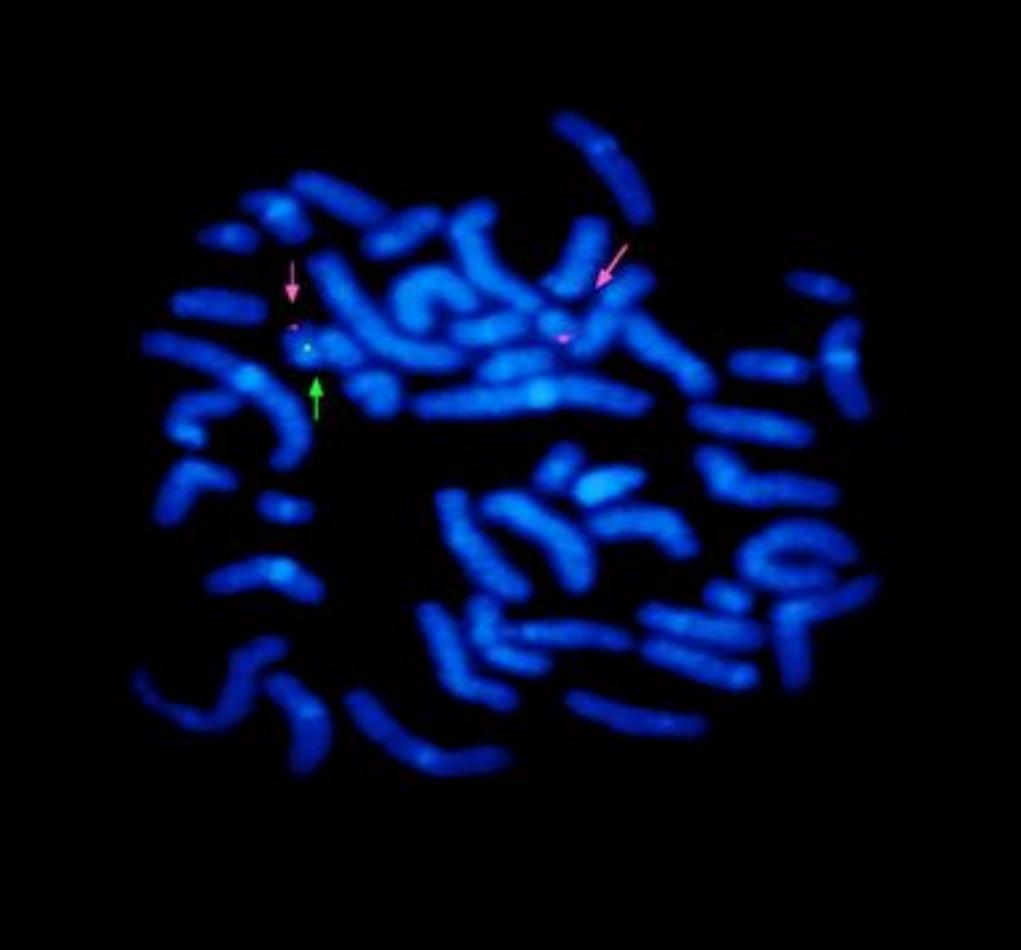


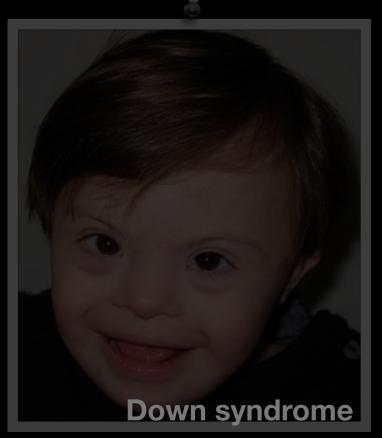




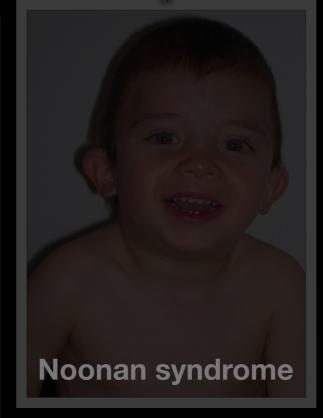


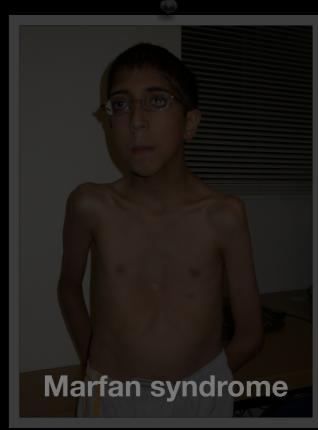


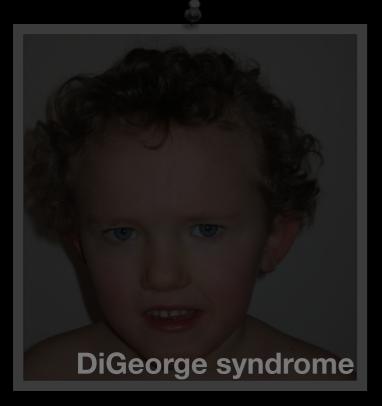


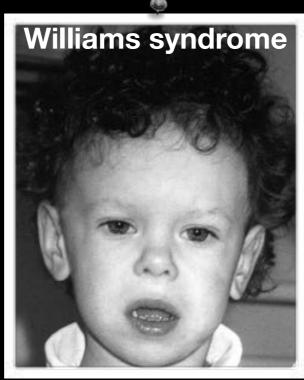


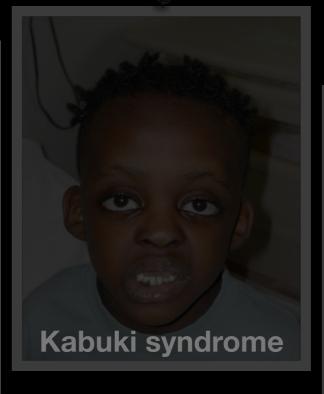




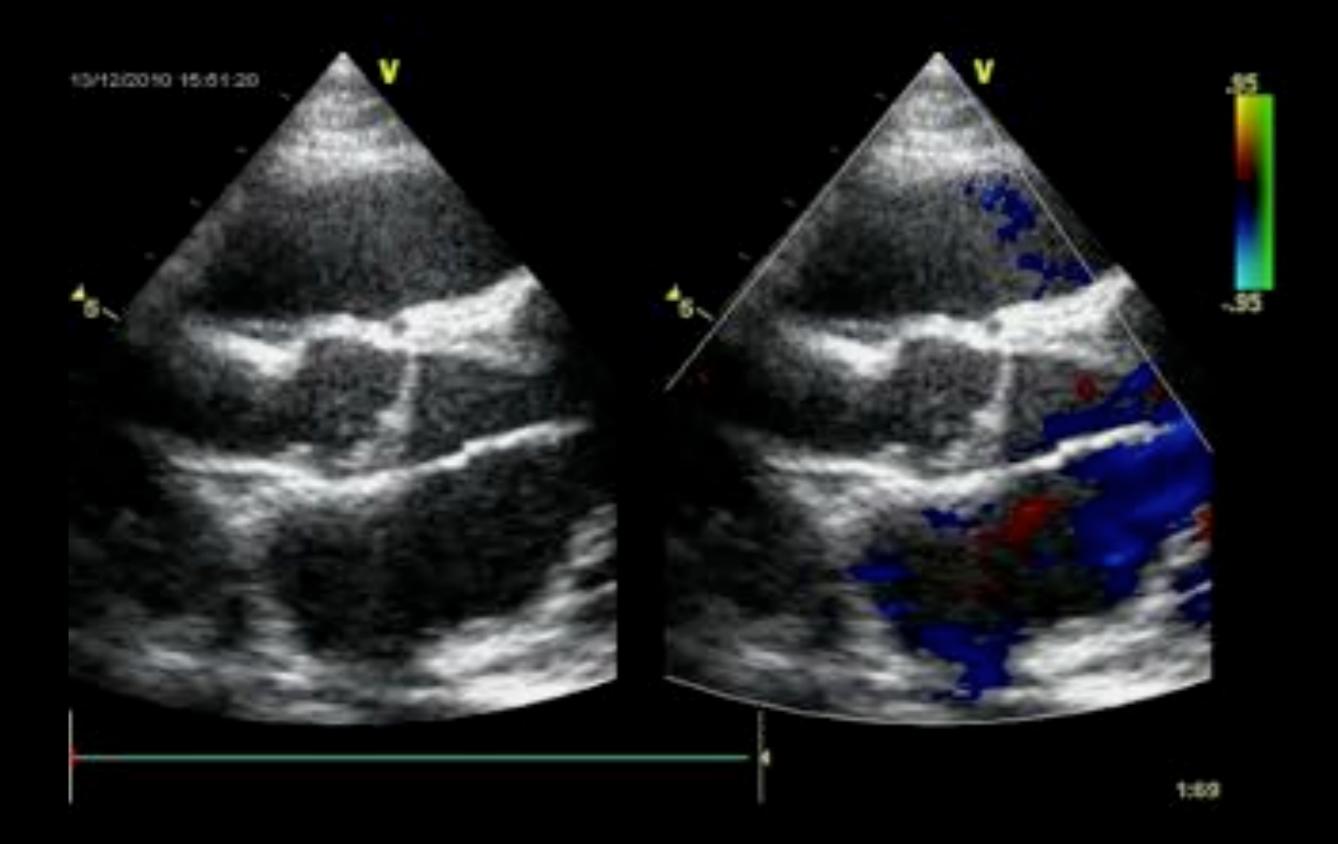




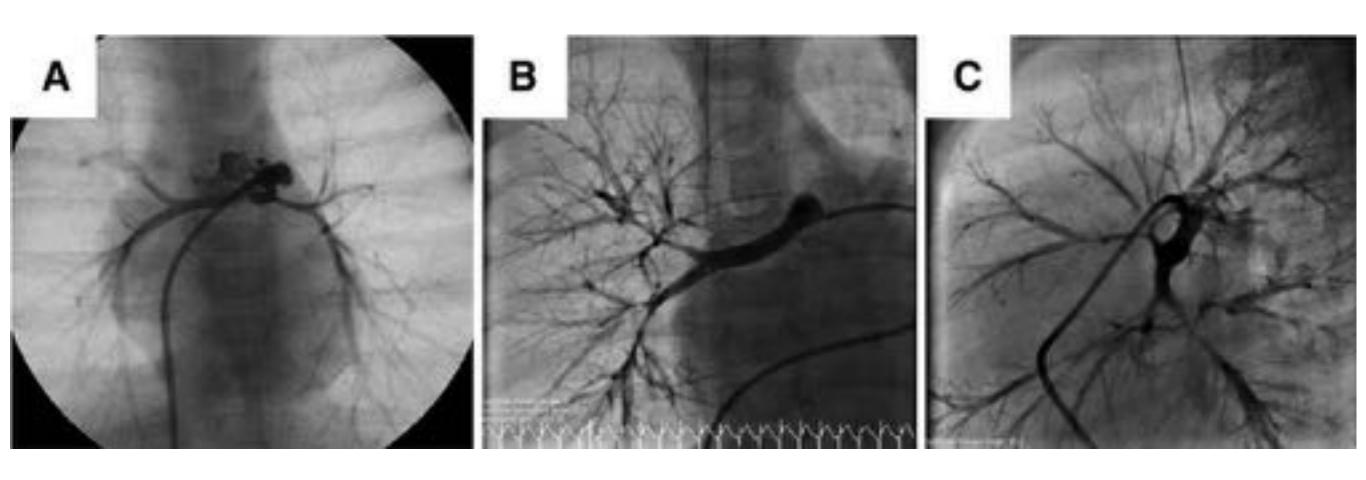




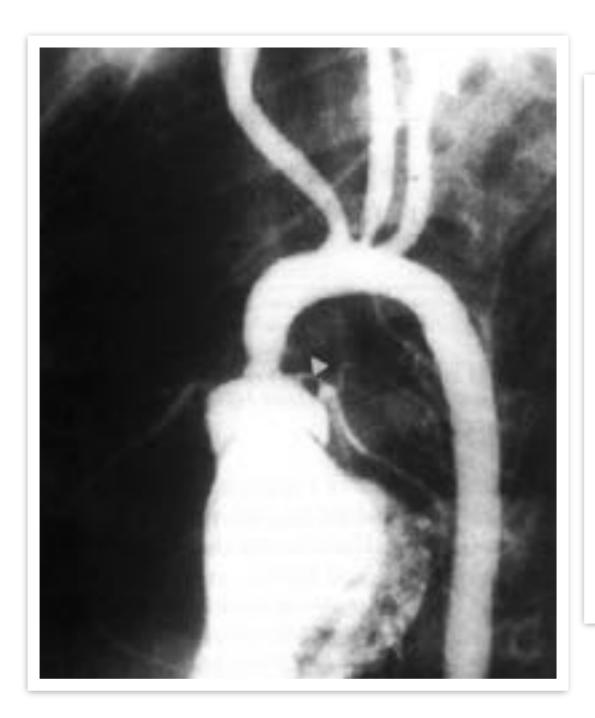




## Peripheral pulmonary arterial stenosis

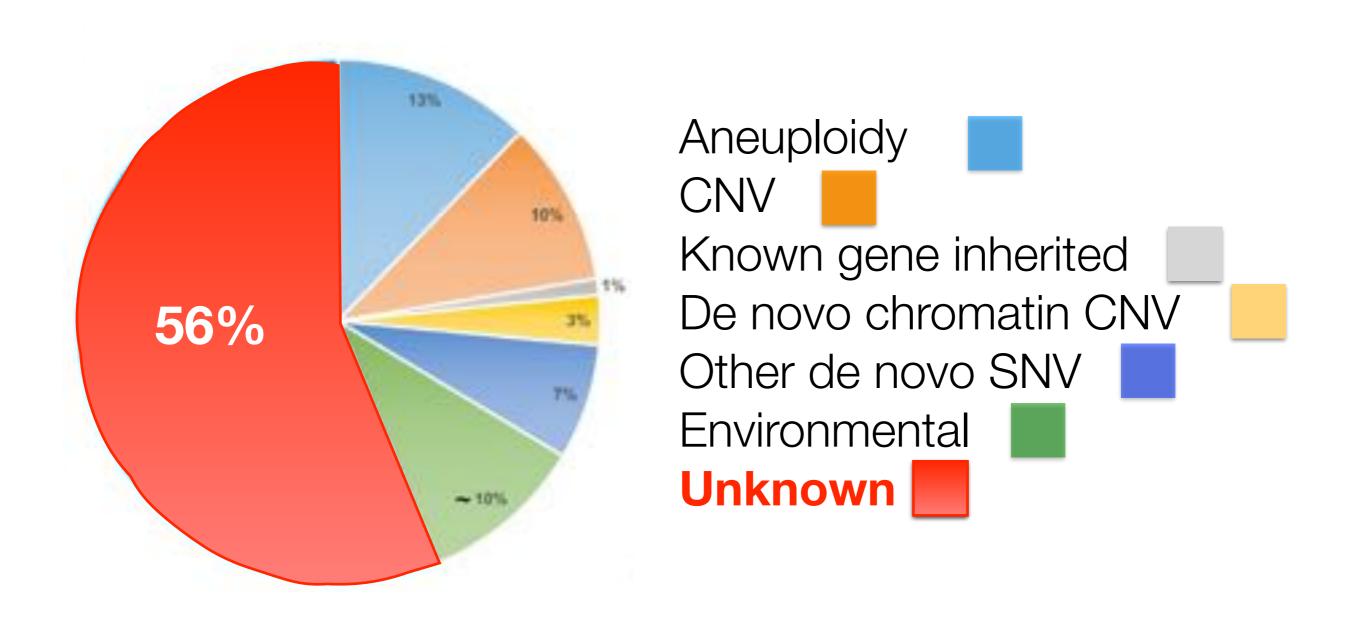


### Coronary artery abnormalities in WS





#### Percentages of known and unknown genetic causes of CHD

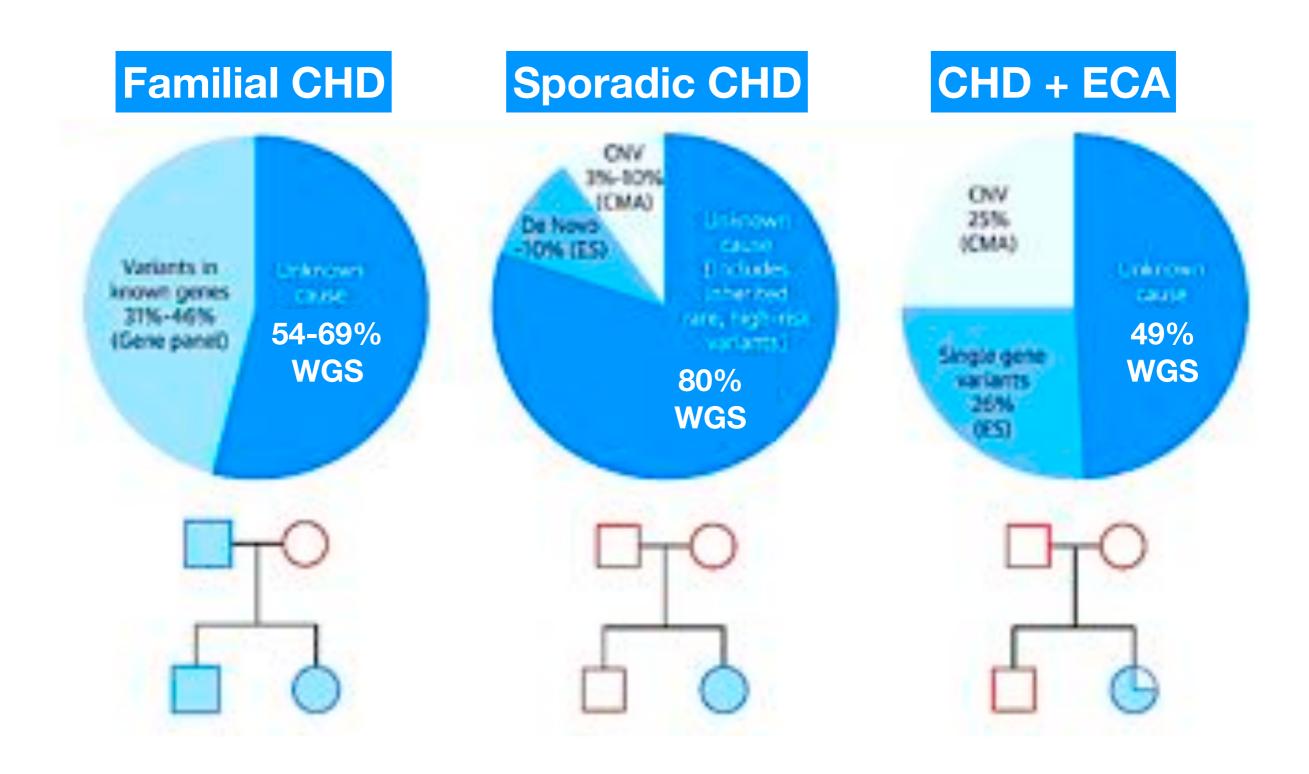


# Malformations Cardiaques Congénitales

- Désordres génomiques (Copy Number Variation): 5-15 %
- CNVs : 5% en cas de tétralogie de Fallot isolée (Greenway et al. Nat Genet 2009)
- CNVs: 15 % en cas de cardiopathies isolées (Soemedi R et al. Am J Hum Genet 2012)
- CNVs: 25-35% en cas de cardiopathies syndromiques (Cooper et al. Nat Genet 2011)
- Altérations géniques connues : 5 %
- Modèle multifactoriel : 85% avec héritabilité h² = 35

# Twin Concordance Studies (MZ Vs DZ registers) Vs Vs Vs CHD – DZ : 2 %

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



# Half a century and the same old story!

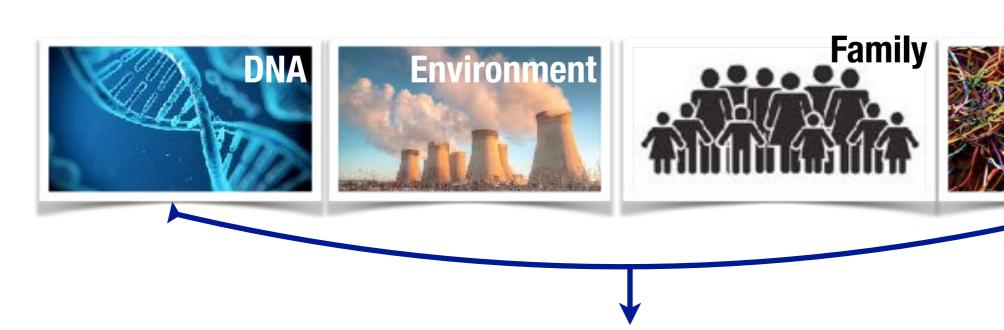
## Multifactorial Inheritance Hypothesis for the Etiology of Congenital Heart Diseases

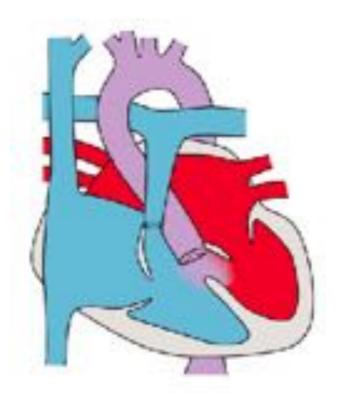
The Genetic-Environmental Interaction

By James J. Nora, M.D.

Circulation 1968

# The multifactorial hypothesis





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

- There is no genetic basis for CHD
- Gross chromosomal aborrations 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

# 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

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

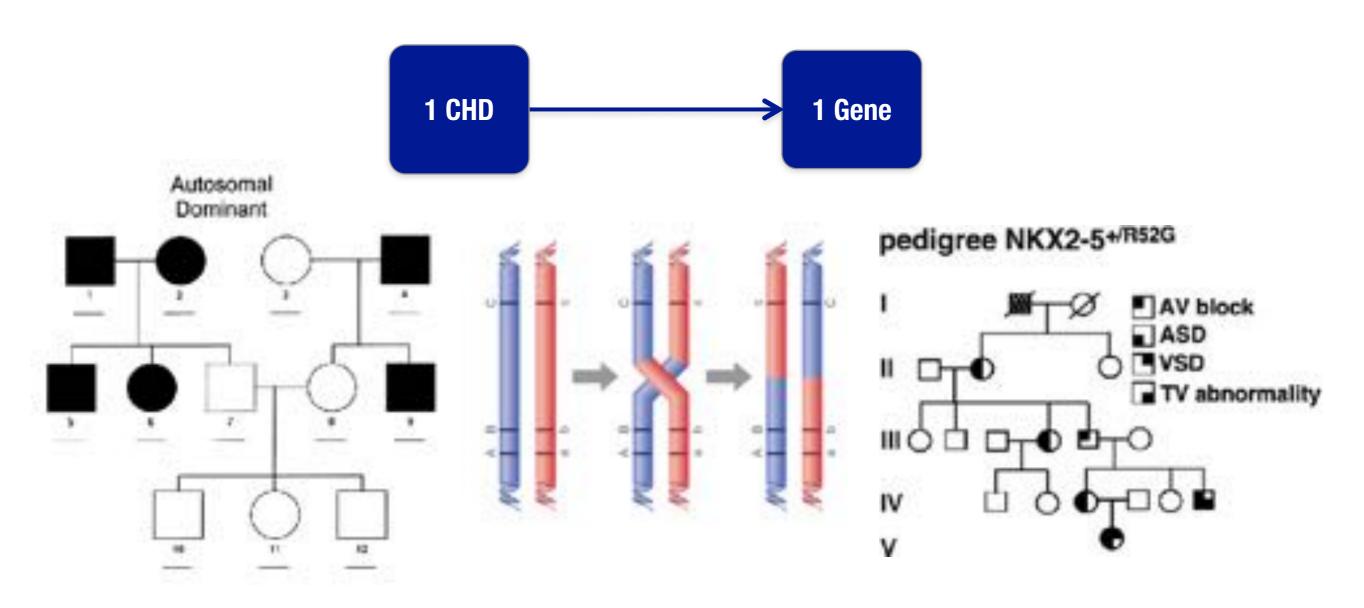
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# Recurrence of CHDs in families Same defect in affected members

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

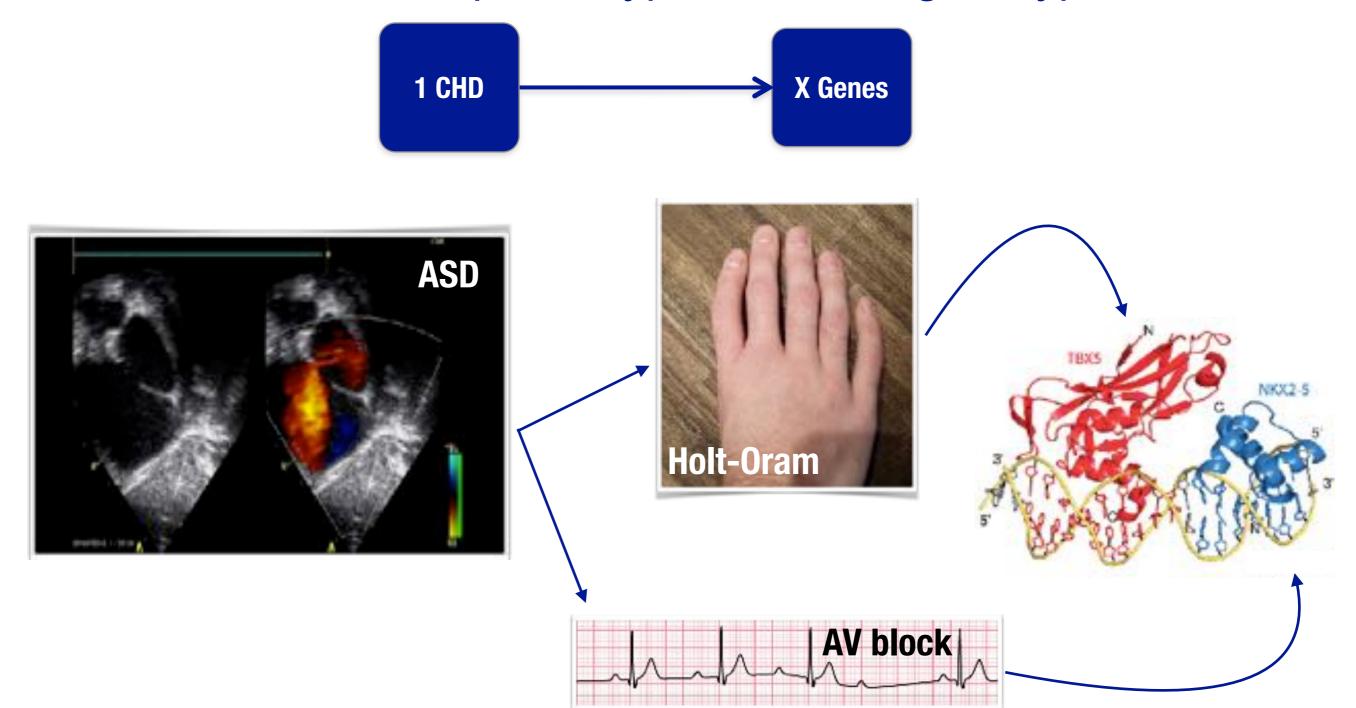
Recurrence of the same type can be due to inheritance of a single gene mutation

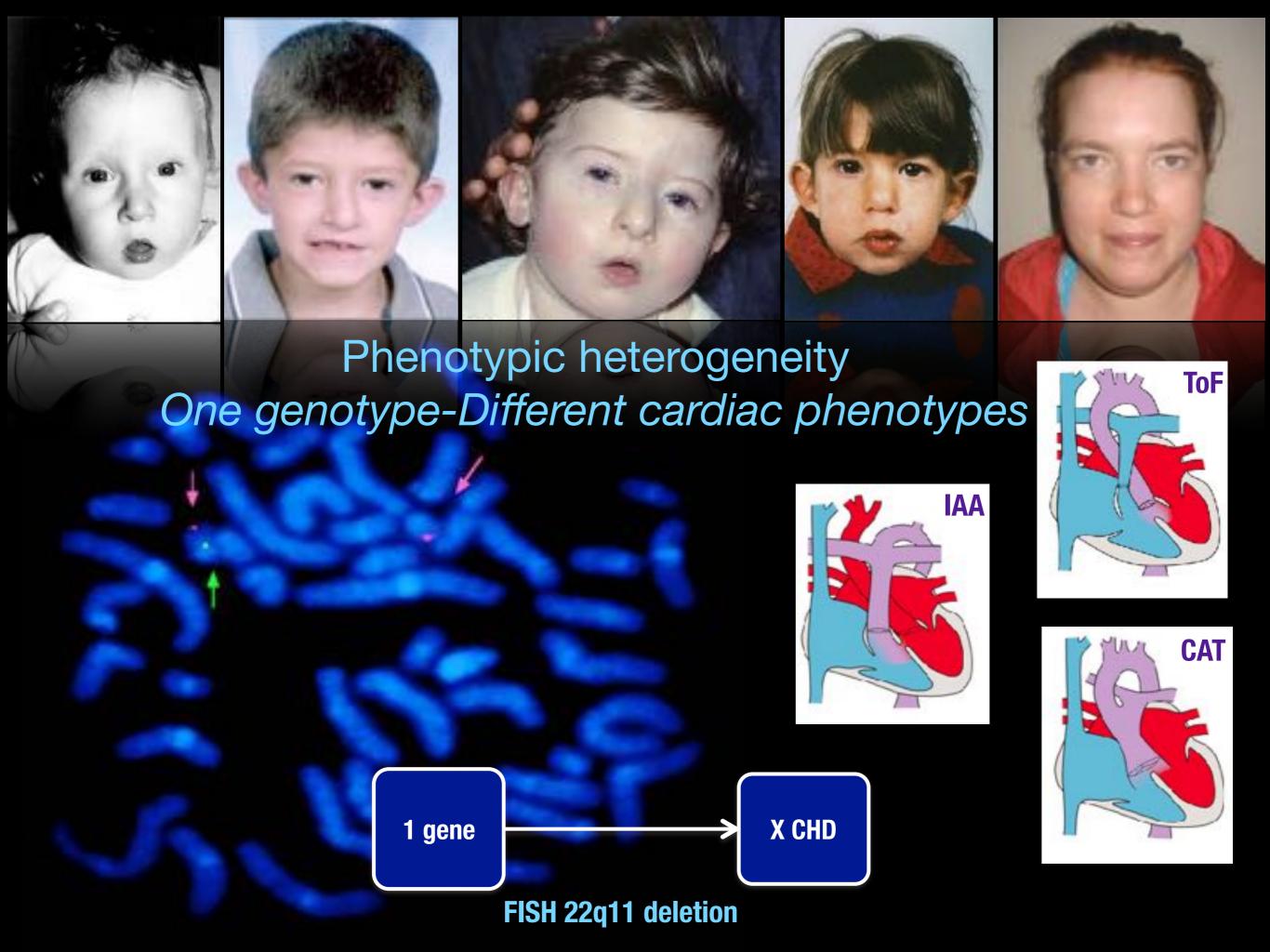
## The monogenic hypothesis



The positional cloning strategy

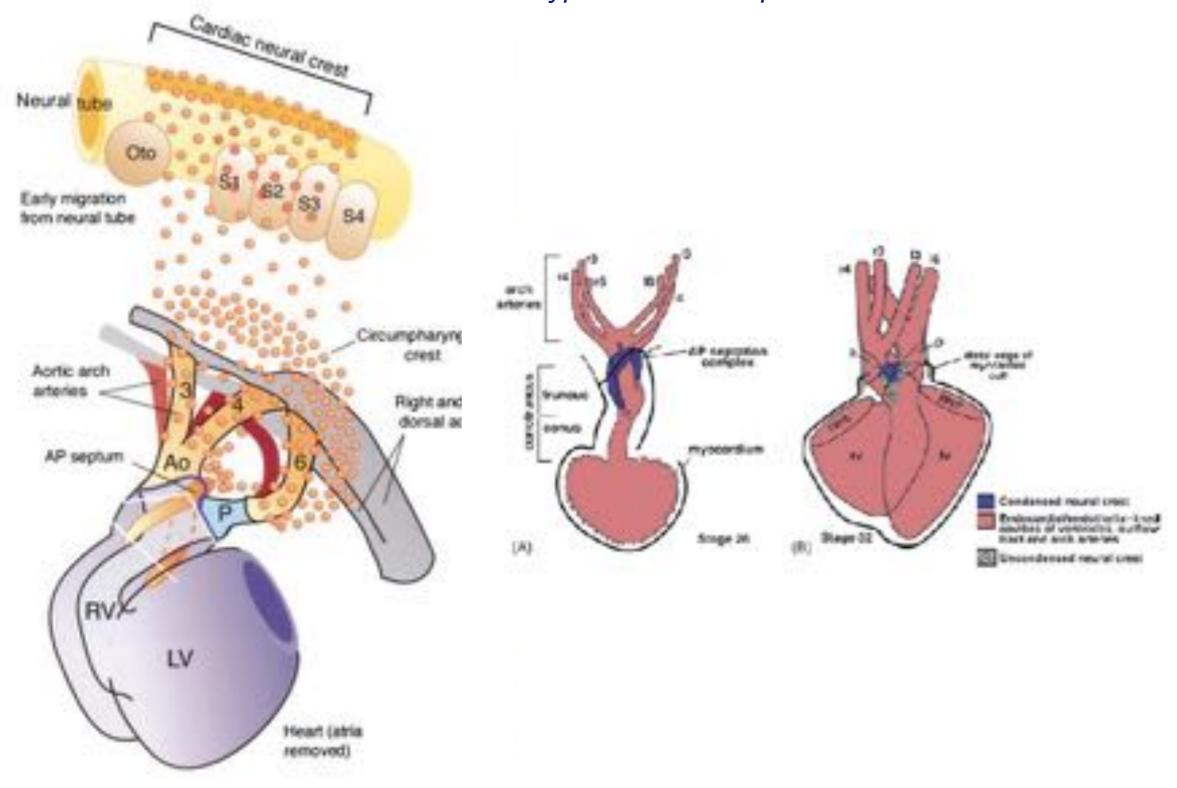
# Genetic heterogeneity One cardiac phenotype-Different genotypes

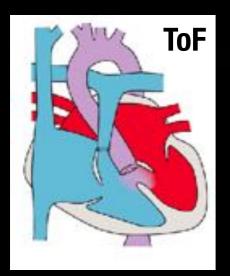


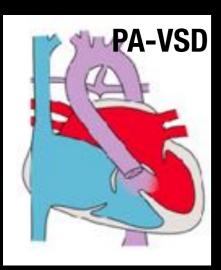


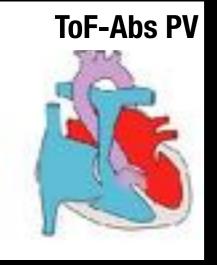
## Migration of neural crest cells into the outflow tract

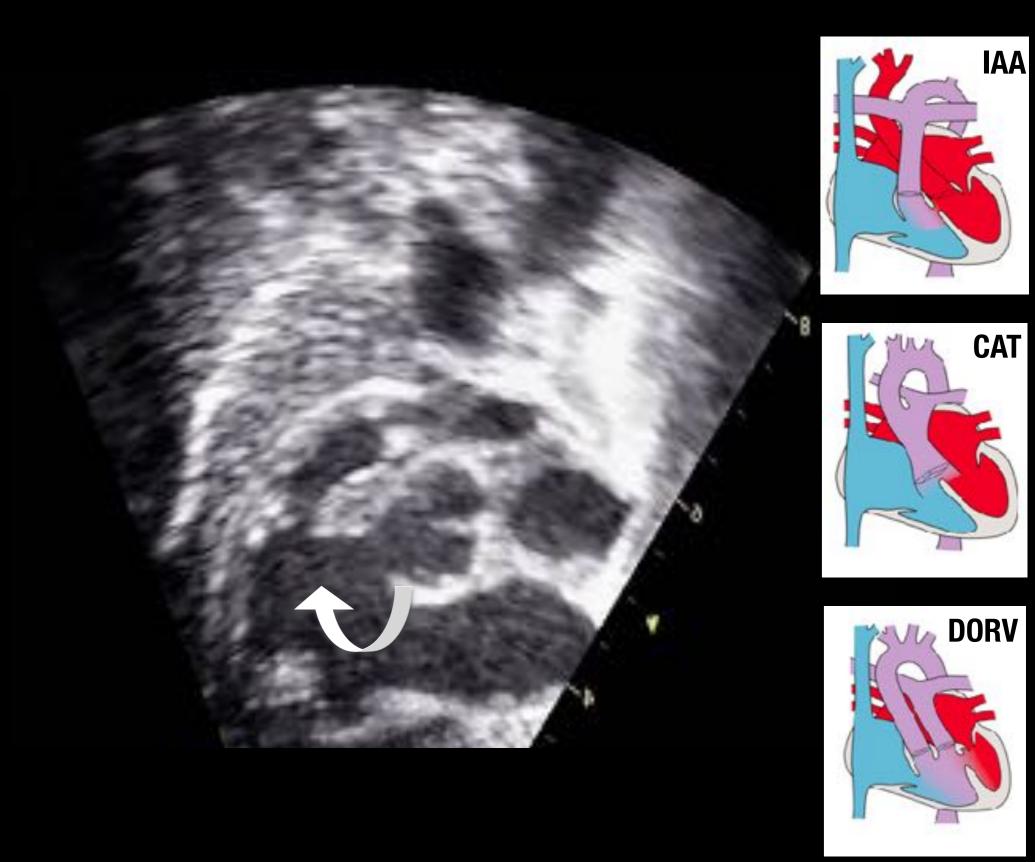
Darwin hypothesis example











### 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

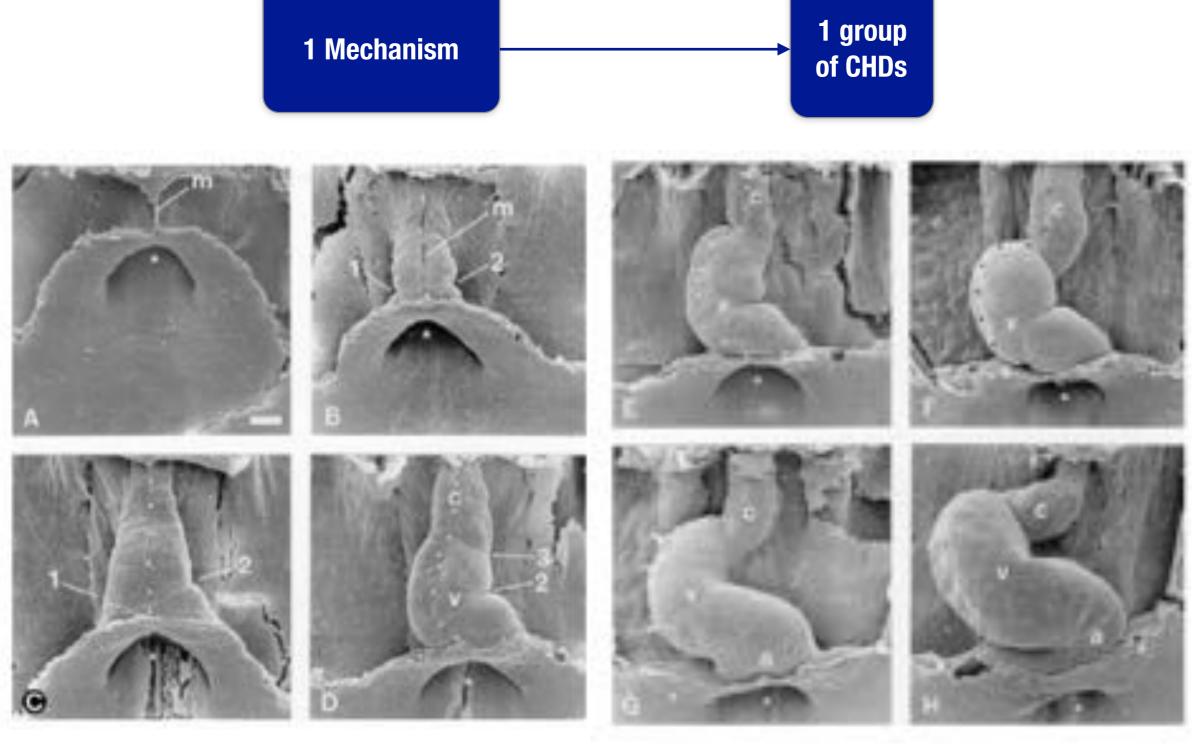
Atrioventricular septal defects

Looping anomalies-laterality defects

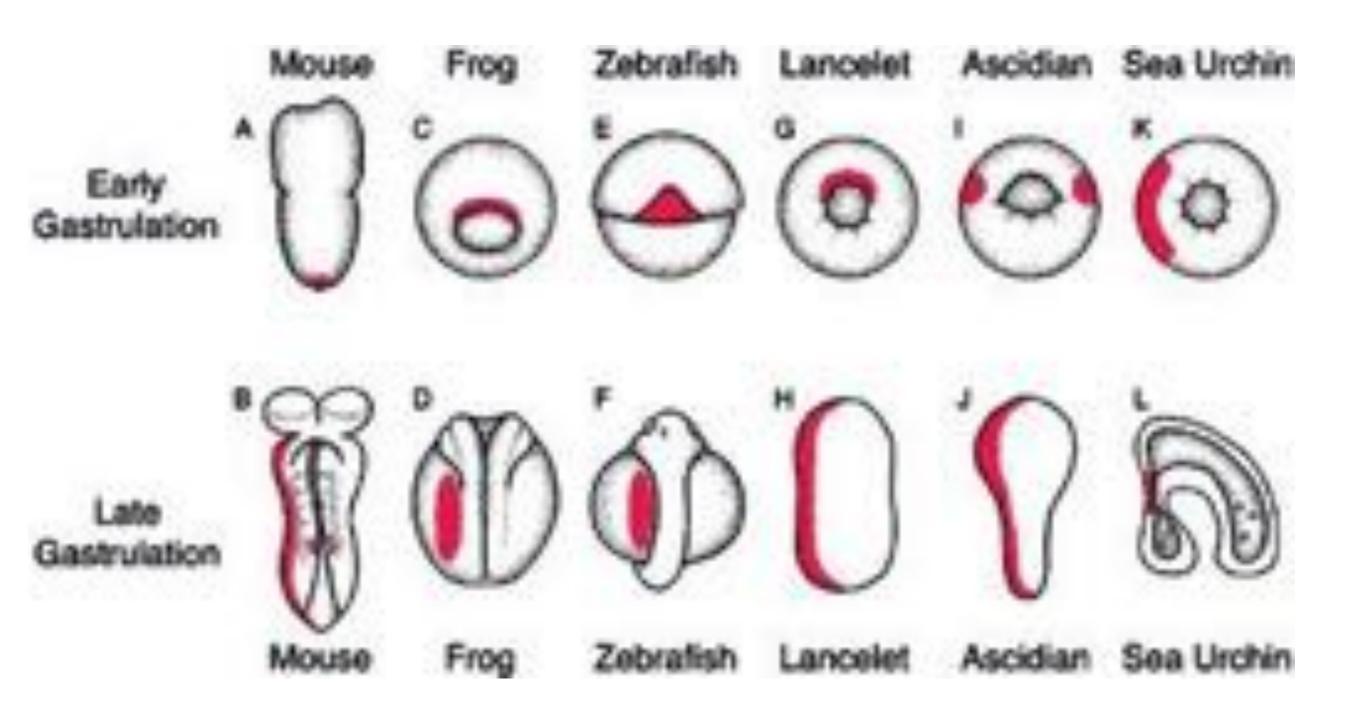
Heterotaxia

# The mechanistic hypothesis

Carl von Linné hypothesis



The example of laterality defects

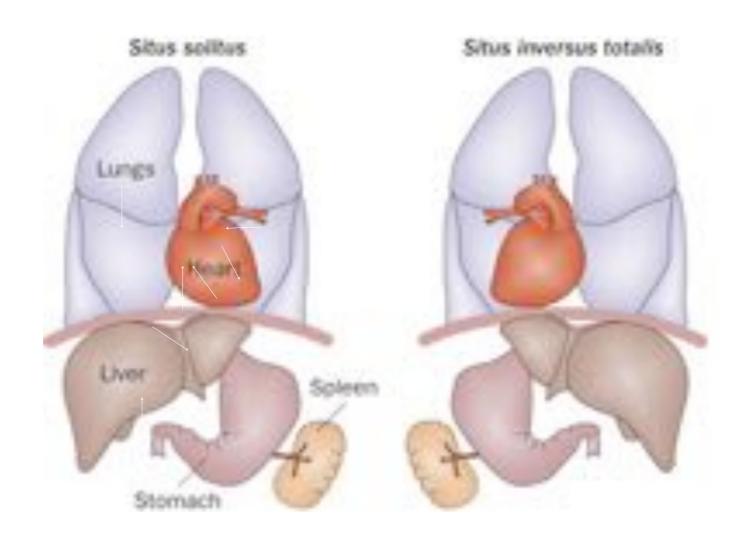


**Expression of Nodal in different species is on the left side** 

# 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

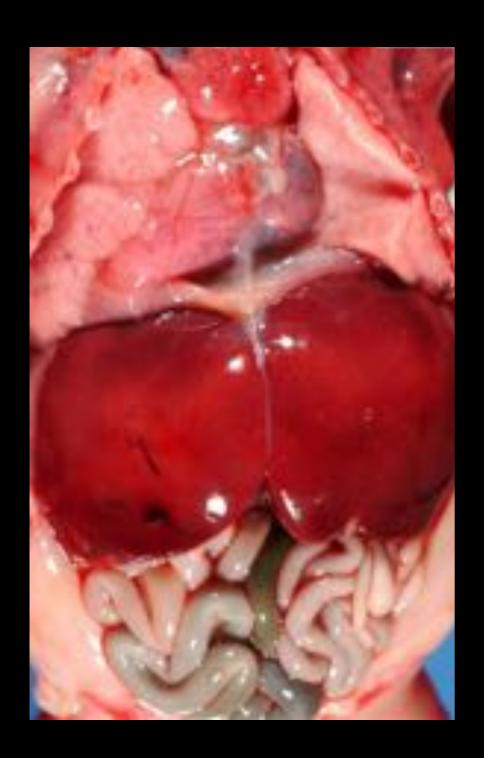


Lymnaea stagnalis

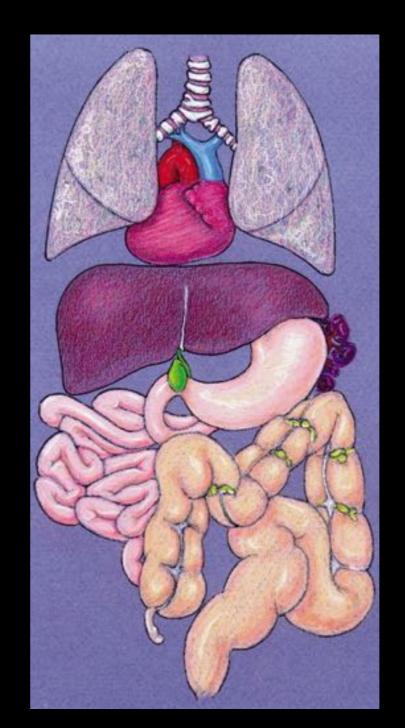
# Absence of left-right signaling Inversion-mirror image, Isomerism-Heterotaxy



Isomerism is easy to understand for pair organs Heterotaxy is abnormality of visceral asymmetry



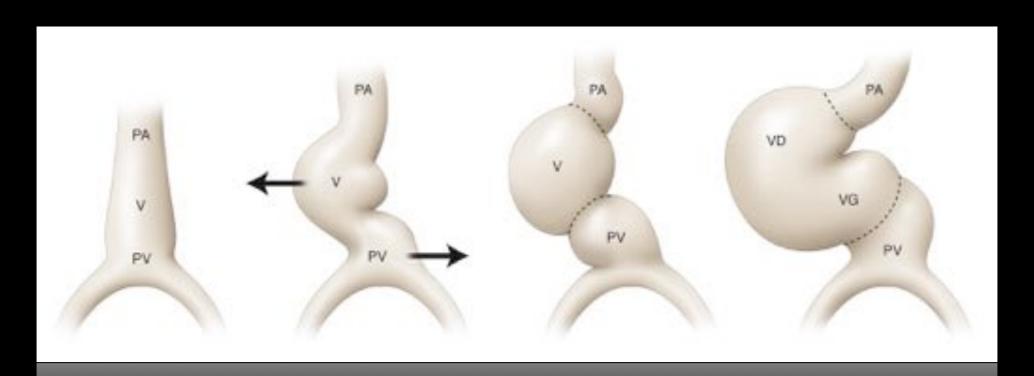
Right and left liver





Polysplenia



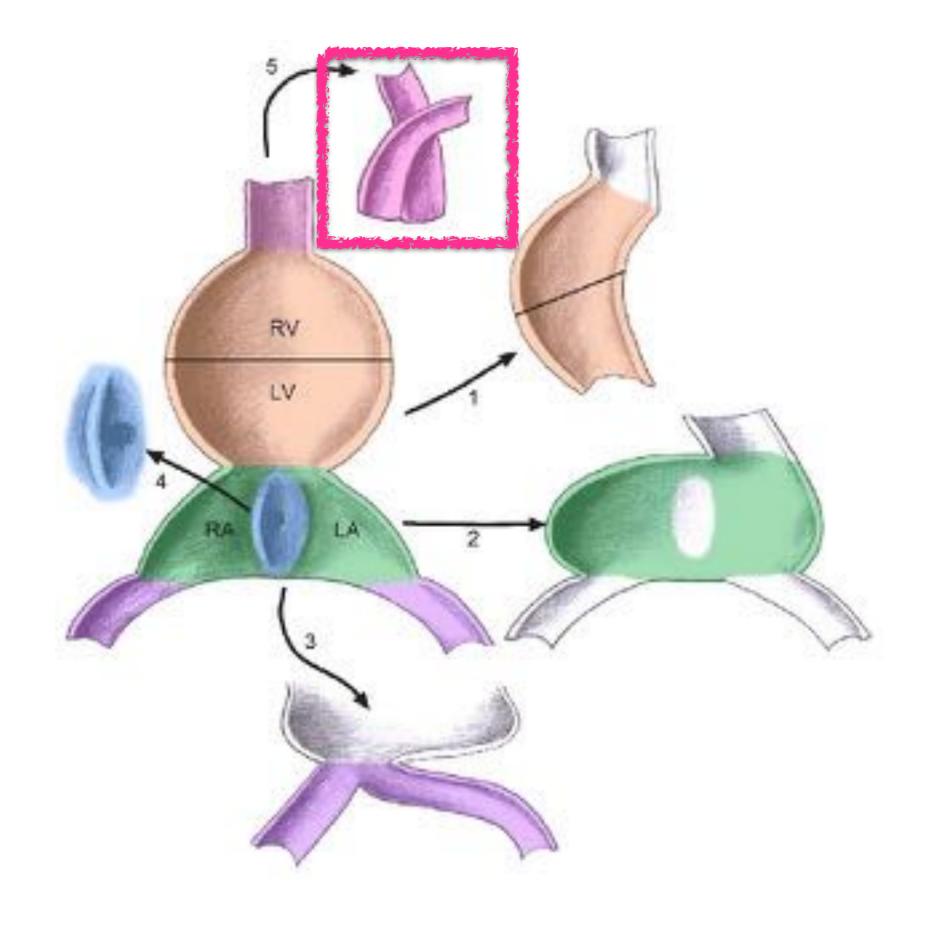


Right-sidedness and left-sidedness of cardiac structures are acquired during development, not present *de novo* 

## Transgenic mouse model for heterotaxy

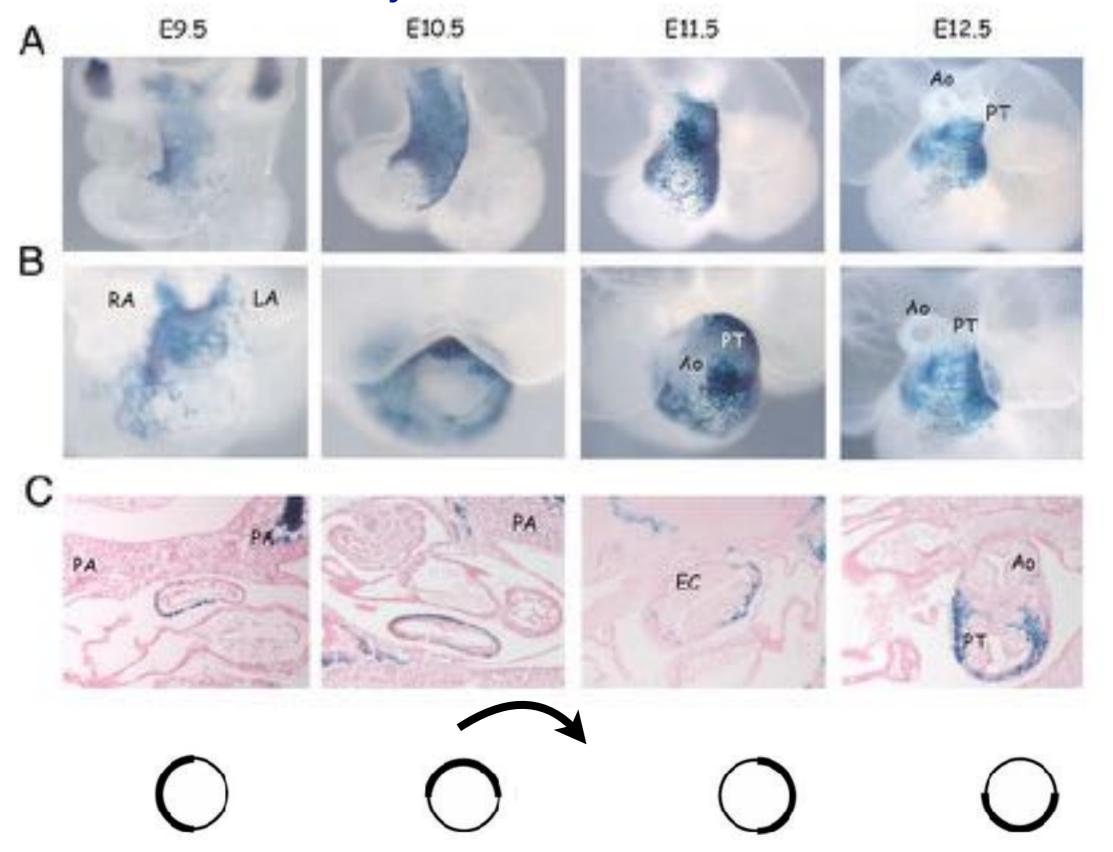
Situs inversus E10.5 VG VD Situs solitus Situs ambigus

Mlc3f-2 X iv/iv

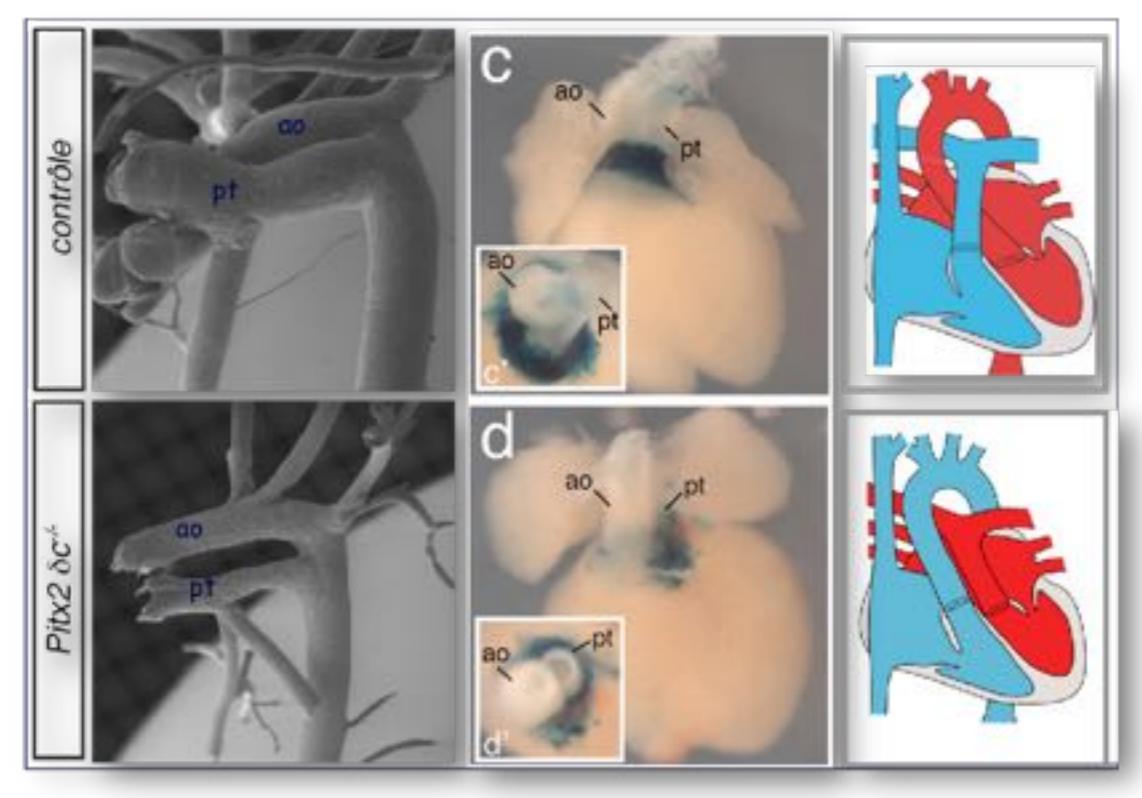


5 levels of asymmetry in the developing heart

## Rotation of the myocardium in cardio sensor mouse



### 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

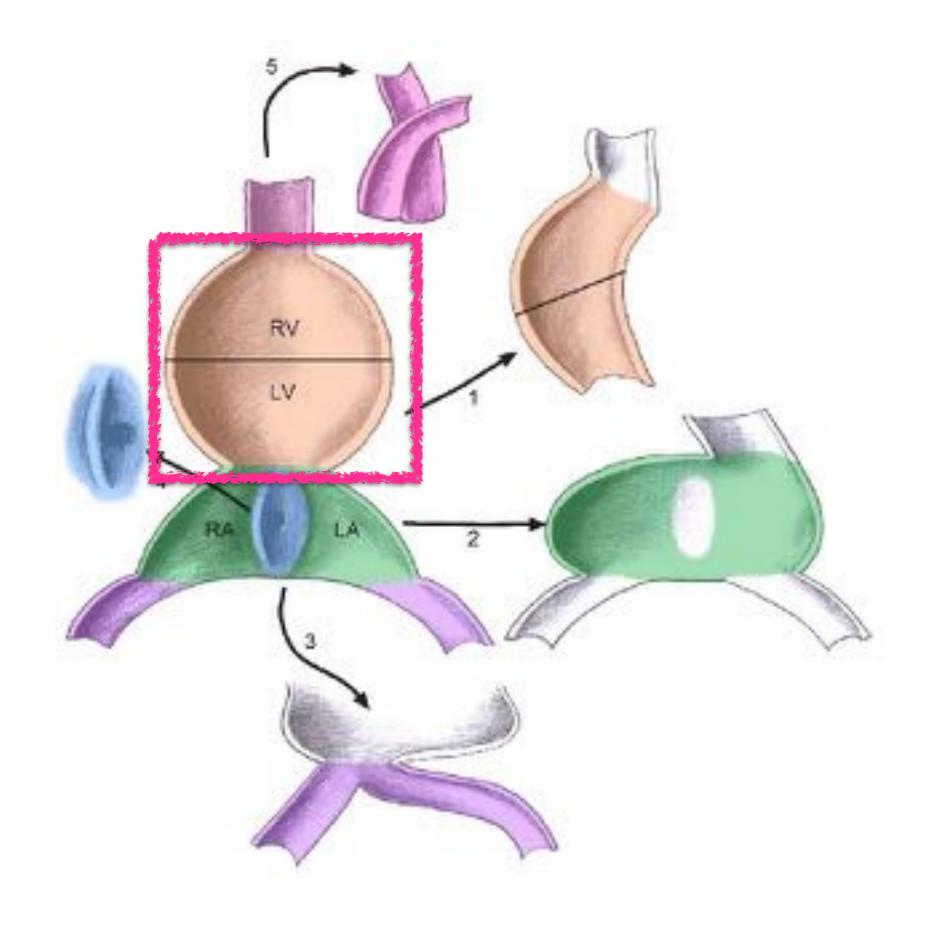
# Familial transposition of the great arteries caused by multiple mutations in laterality genes

Alessandro De Luca, Anna Sarkozy, Federica Consoli, Rosangela Ferese, Salentina Guida, Maria Lisa Dentici, Rita Mingarelli, Emanuele Bellacchio, Giulia Tuo, Giuseppe Limongelli, Maria Cristina Digilio, Bruno Marino, Heart 2010;96:673—677.

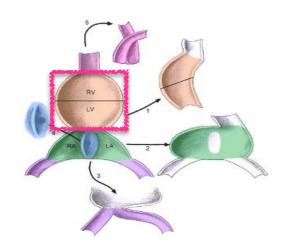
## TGA is a laterality defect

It is not a conotruncal defect

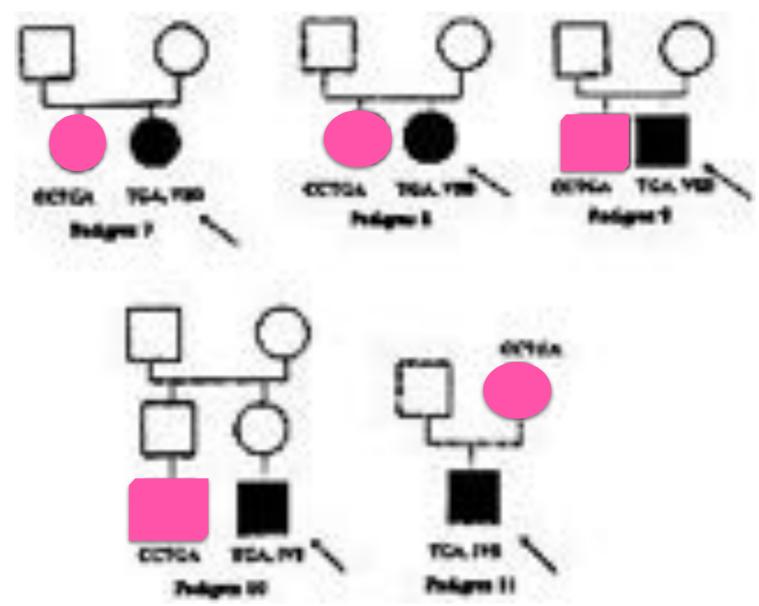
It is a laterality (rotation) restricted to a single segment of the developing heart



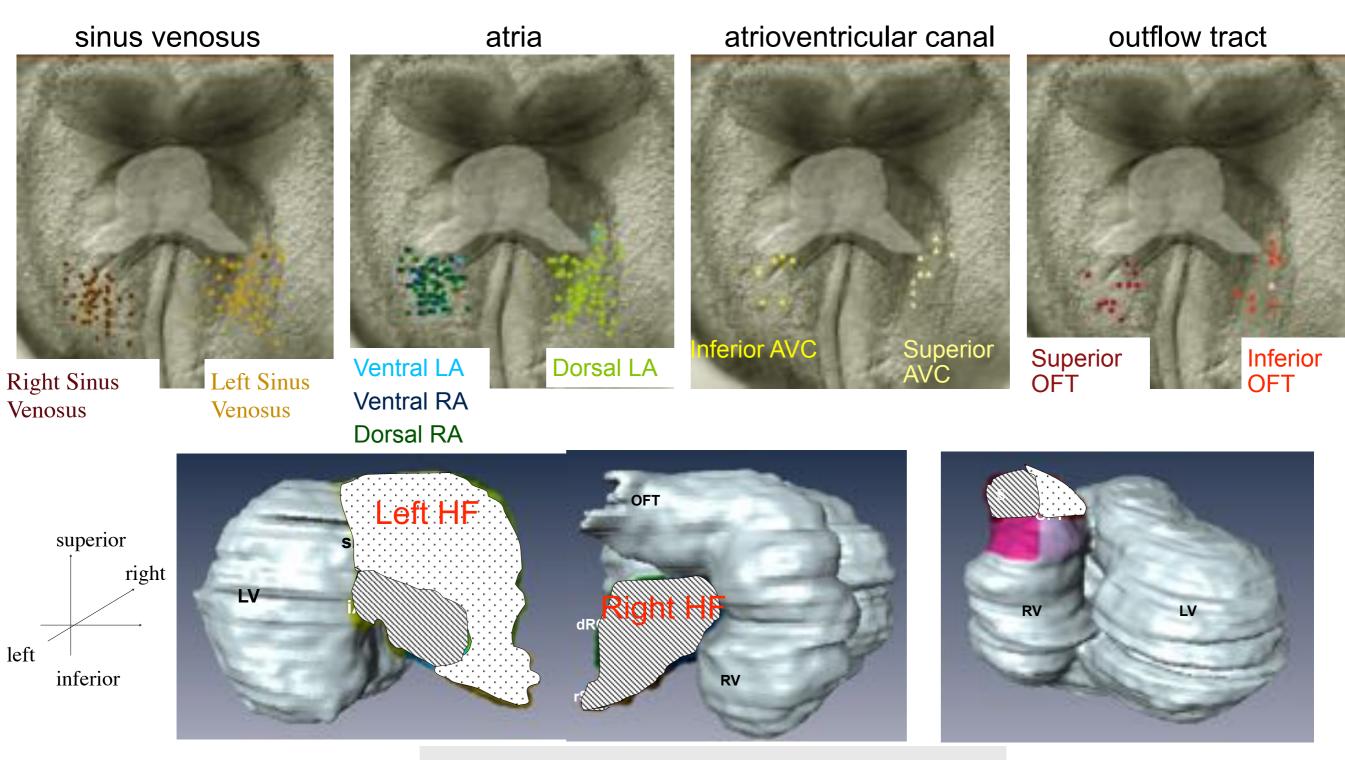
5 levels of asymmetry in the developing heart



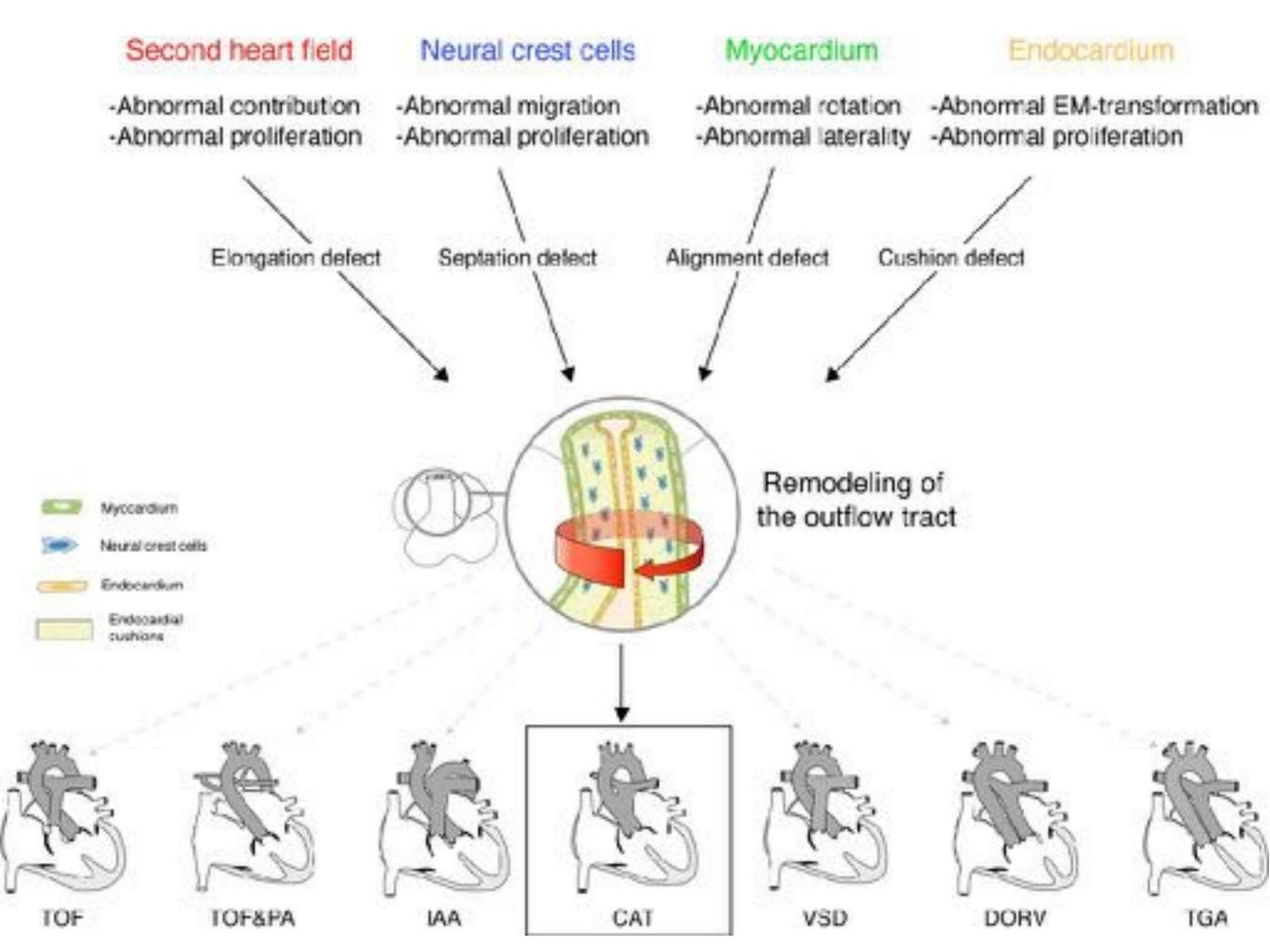
# Families TGA & CC-TGA

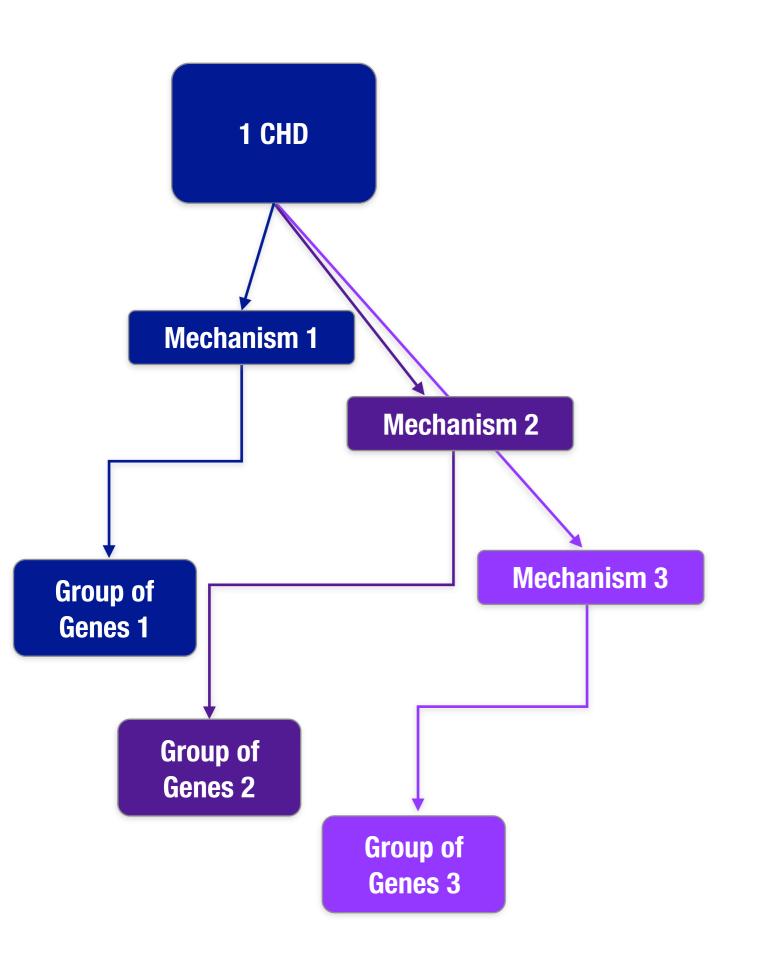


### Fate map of left-right heart precursors



Twisted left/right regionalisation of the heart





#### **Coarctation of the aorta**

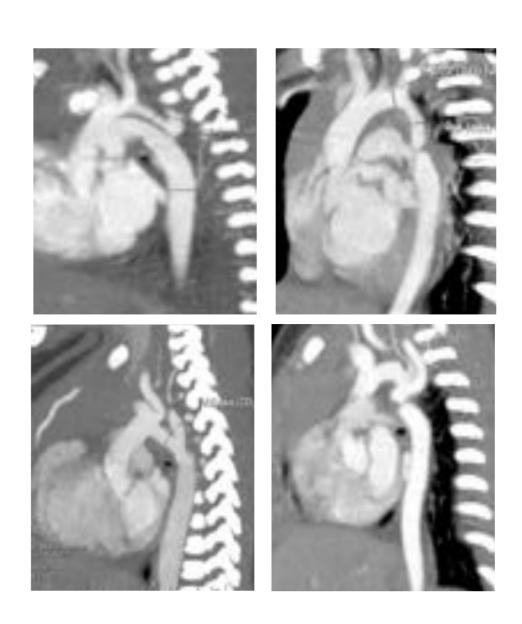
Disease of the aortic isthmus

Flow defect : spectrum of HLHS

Conotruncal defect

Interrupted aortic arch

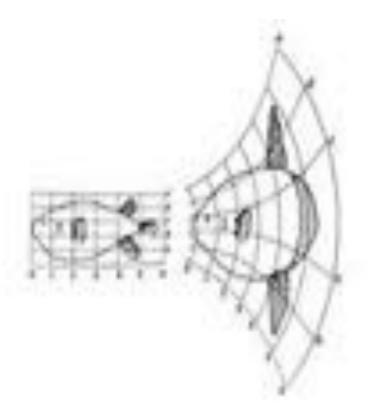
Laterality defect with persisting LSCV



# 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.

#### 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

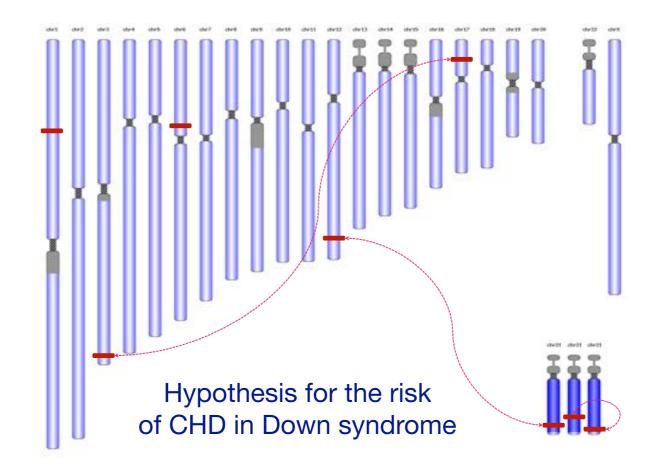
Somatic mutations during cardiac development

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

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

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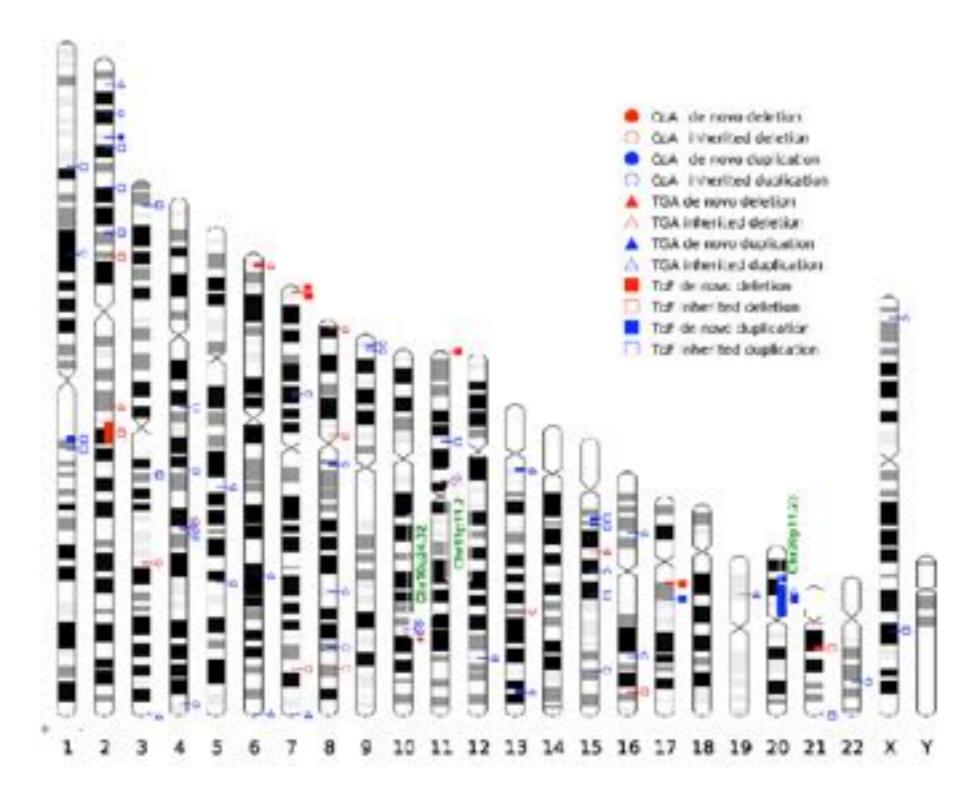
# A multigenic model for the development of CHD in trisomy 21 with effects of several genetic variants



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.

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



# 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

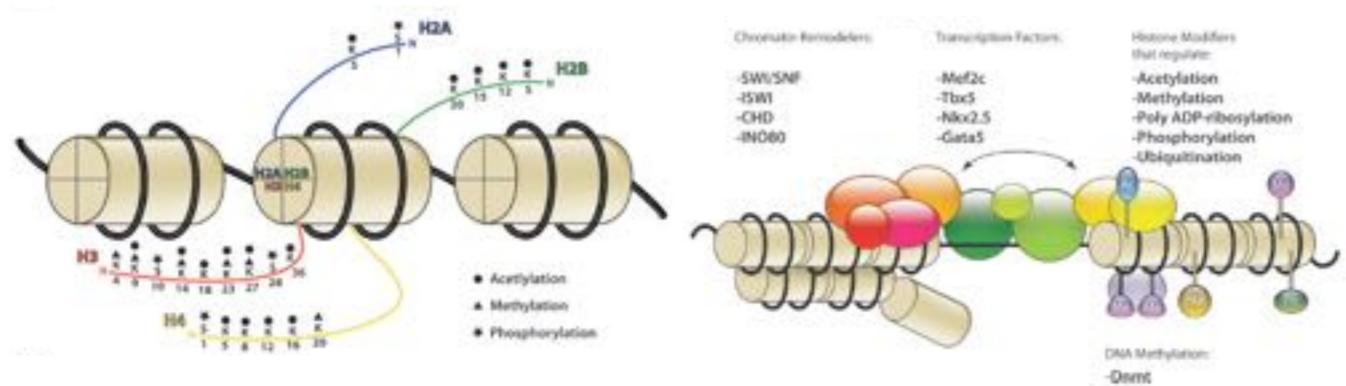
Total N/litters	Control 30/4		Diabetes 62/15		Control NAC 30/4		Diabetes NAC 43/7	
	n	96	n	%	n	%	n	%
Normal	30	100	26	41.9**	27	90	36	83.711
Abnormal	0	0	36	58.1**	3	10	7	16.311
ASD	0	0	19	30.6**	2	6.7	6	13.9†
VSD	0	0	25	40.3**	1	3.3	5	11.611
AVSD	0	0	4	6.5	0	0	0	0
TGA	0	0	4	6.5	0	0	0	0
DORV	0	0	8	12.9*	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.

#### Prevention of CHD in human

Nucleosome structure

Interactions between chromatin regulators and transcription factors to control gene expression



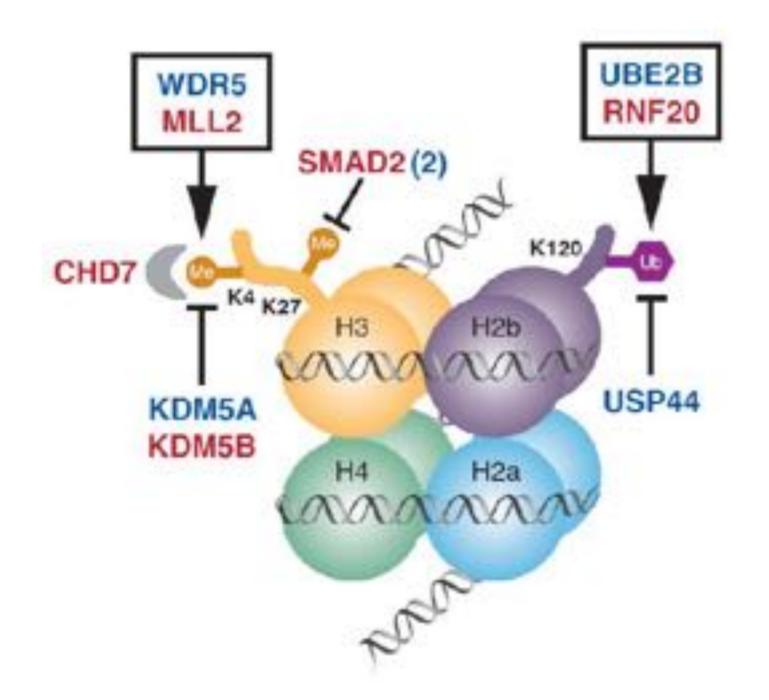
- Chromatin regulation is an epigenetic mechanism that controls gene expression and function without changes in the DNA sequence.
- Chromatin remodelers use energy derived from ATP hydrolysis to change chromatin architecture.
- Histones are covalently modified to modulate access of transcription factors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

Rôle of maternal aging

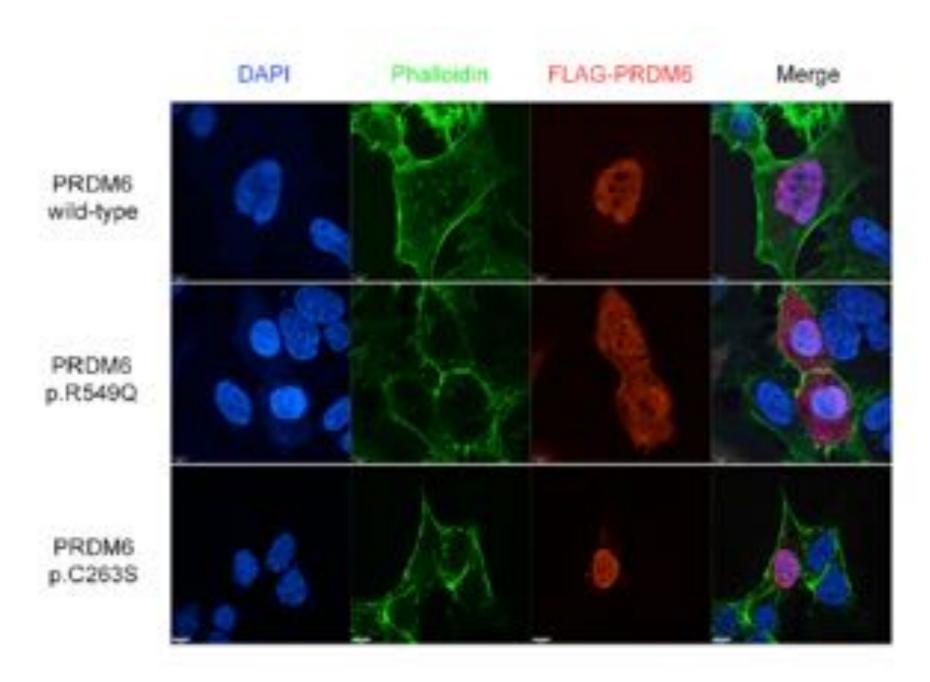
Prevention with folic acid

Function of known genes such as CHD7

# De novo mutations in histone modifying genes in congenital heart disease

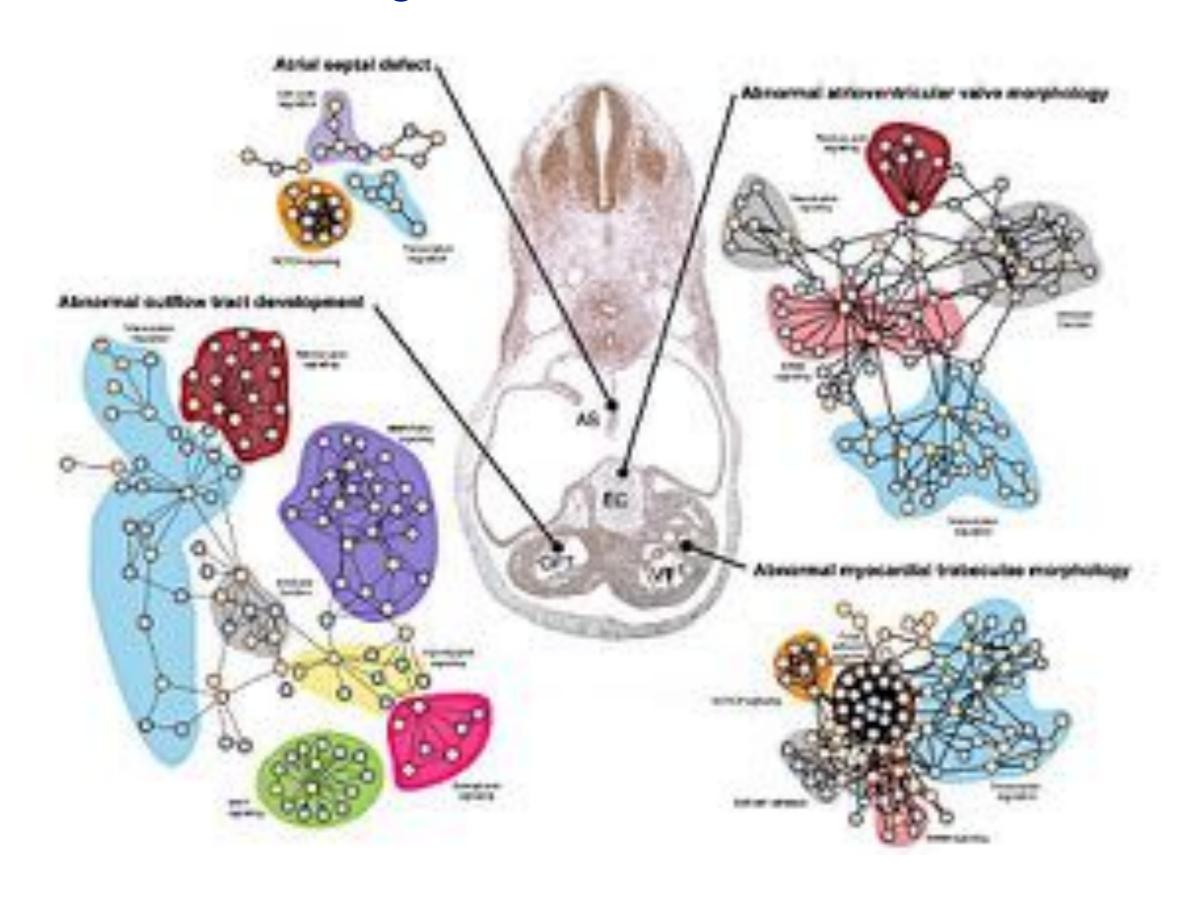


De novo mutations in the H3K4 and H3K27 methylation pathways

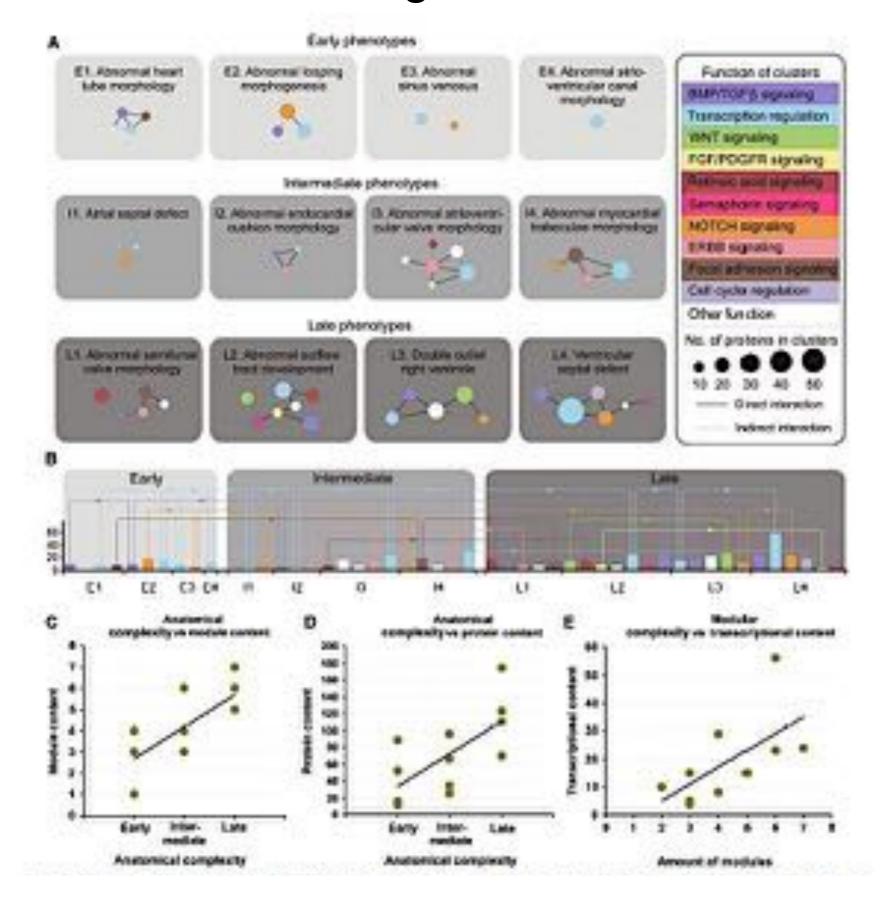


PRDM6 mutations are underlying genetic causes of nonsyndromic isolated PDA in humans and implicates the wild-type protein in epigenetic regulation of ductus remodeling.

# 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 each 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

