Genetics of congenital heart diseases
Le Hasard et la nécessité

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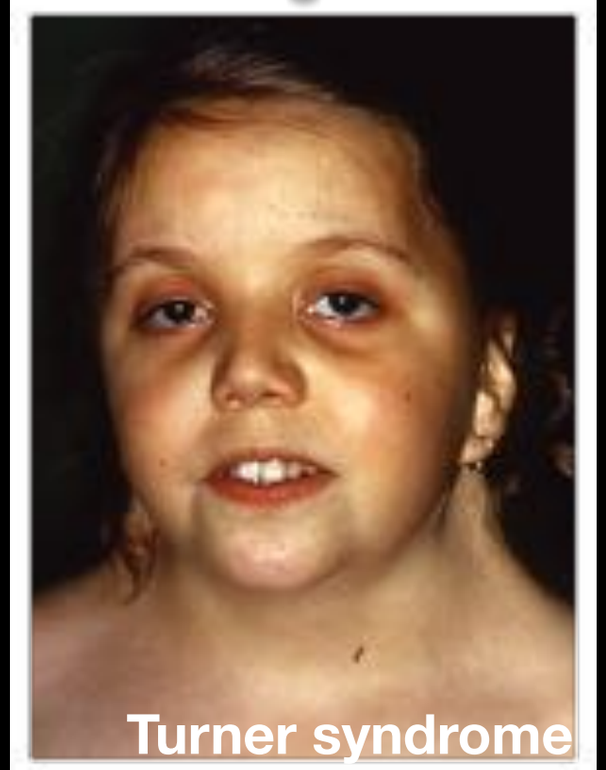
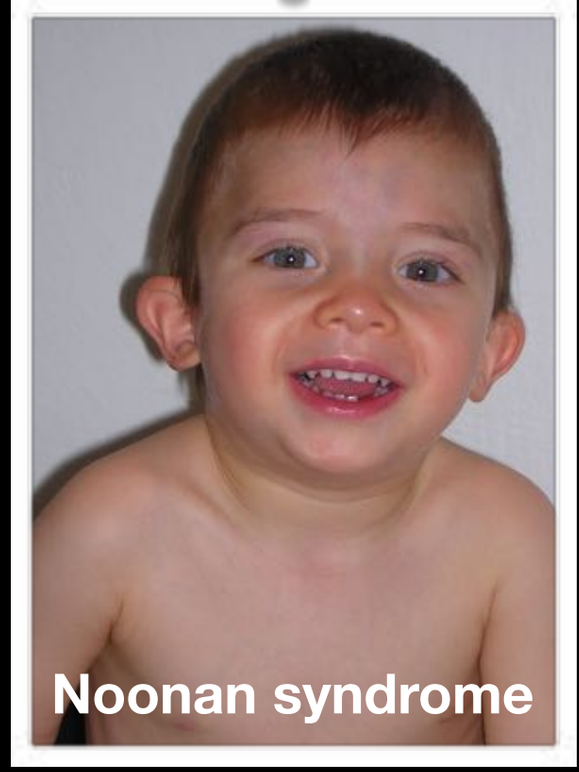
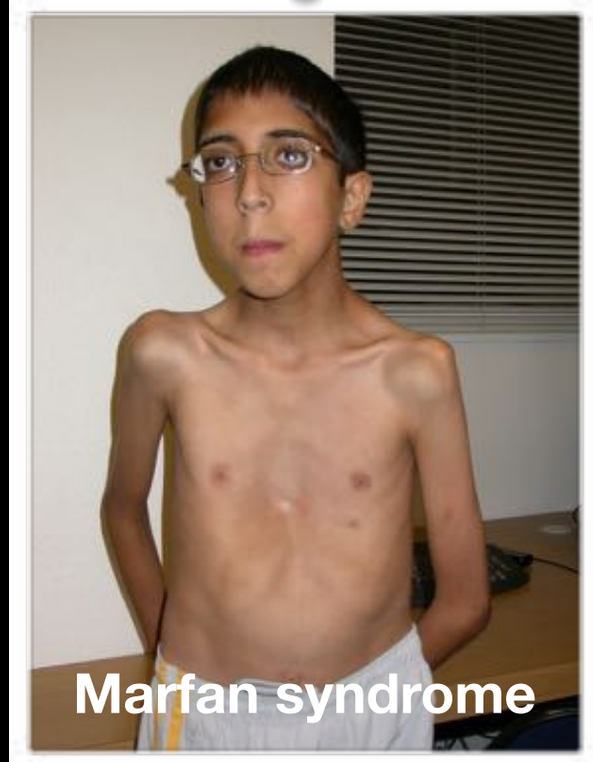
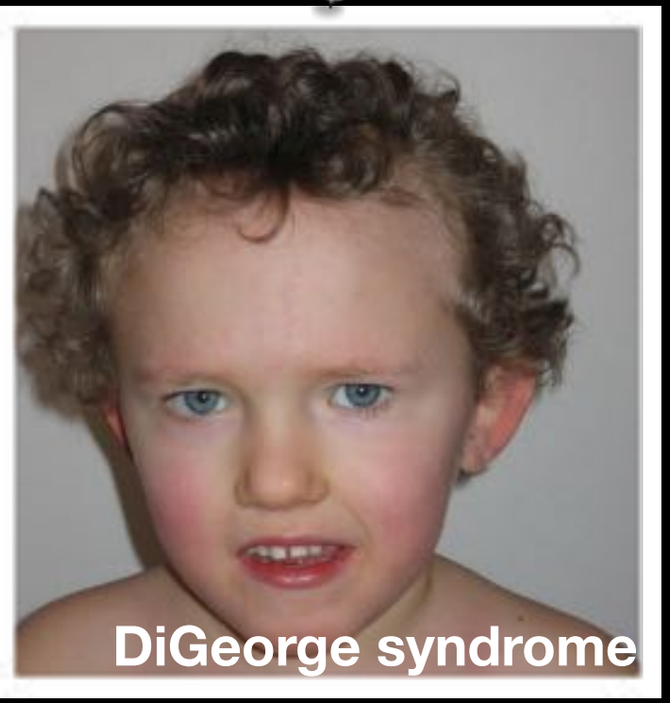
Centre de Référence Maladies Rares
Malformations Cardiaques Congénitales Complexes-M3C
Centre de Référence Maladies Rares
Maladies Cardiaques Héréditaires- CARDIOGEN

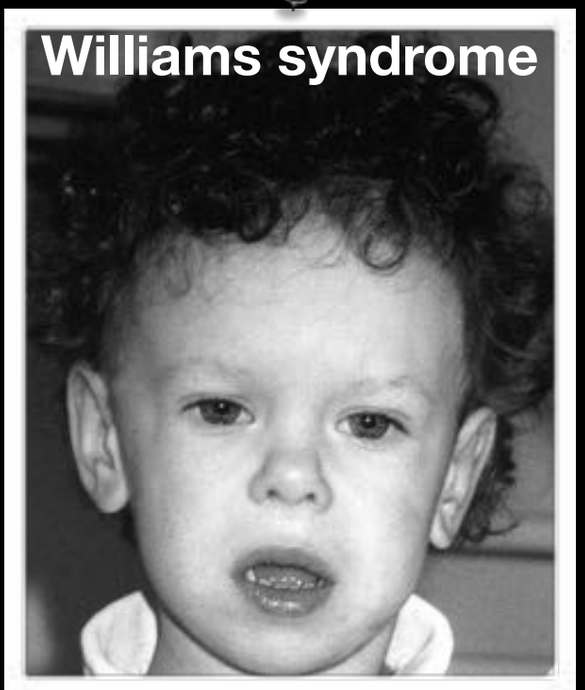
Old textbooks and clinical genetics

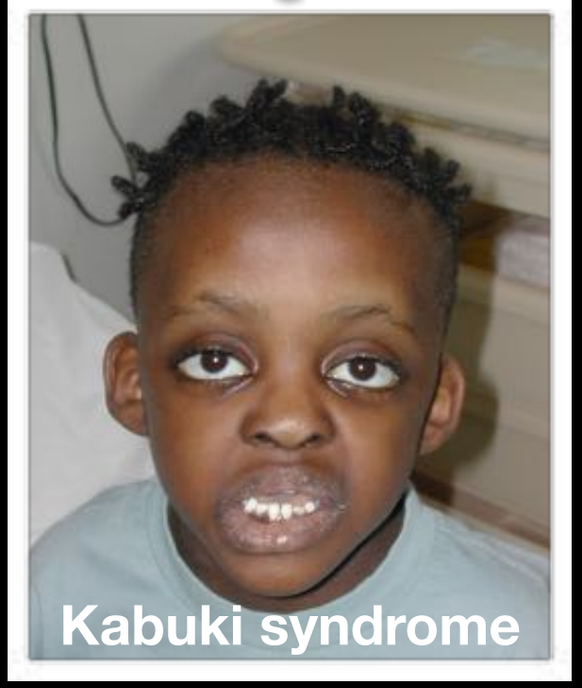


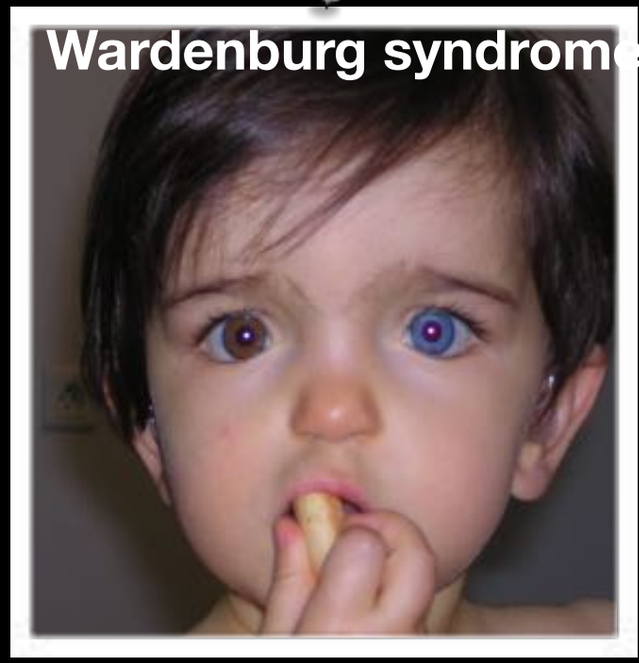




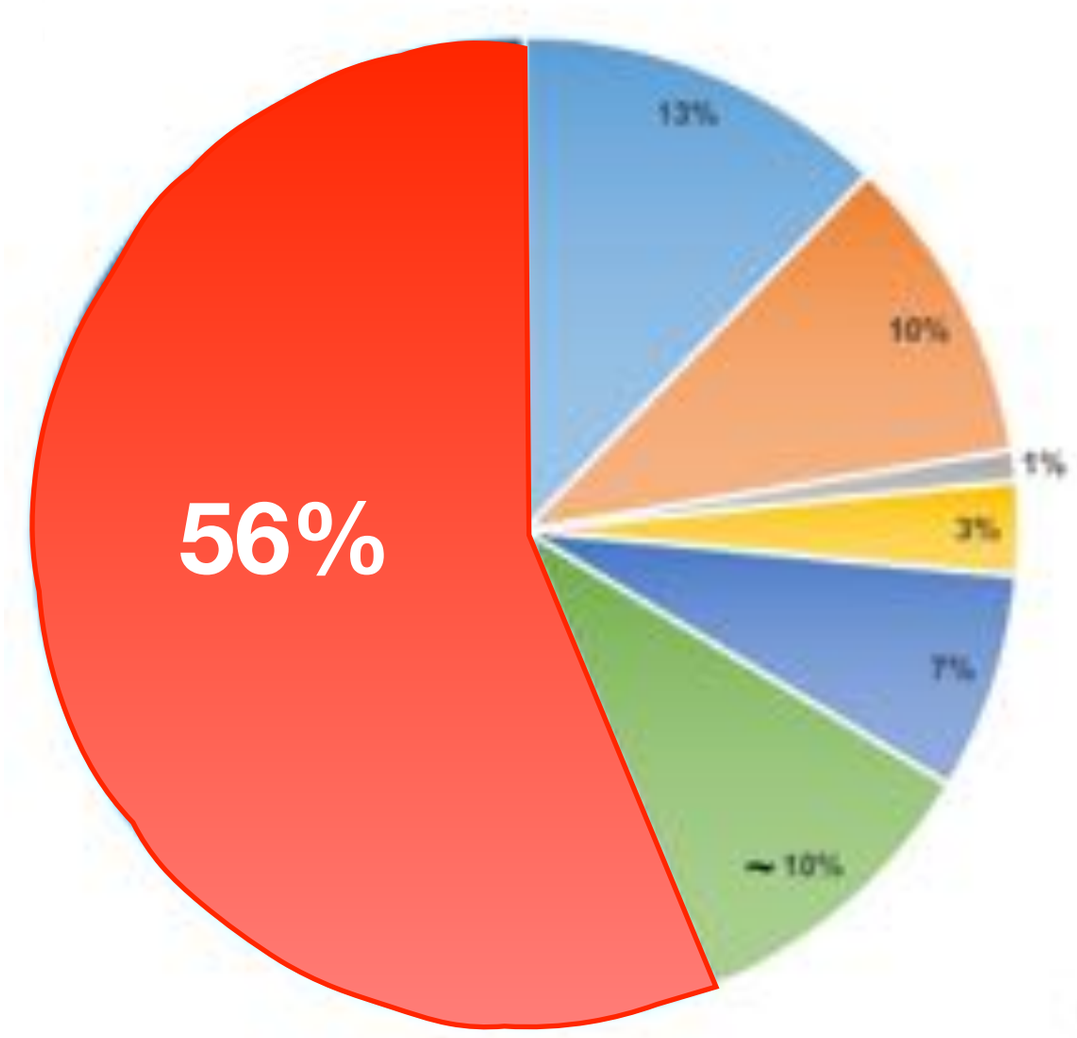








Percentages of known and unknown genetic causes of CHD



Aneuploidy

CNV

Known gene inherited

De novo chromatin CNV

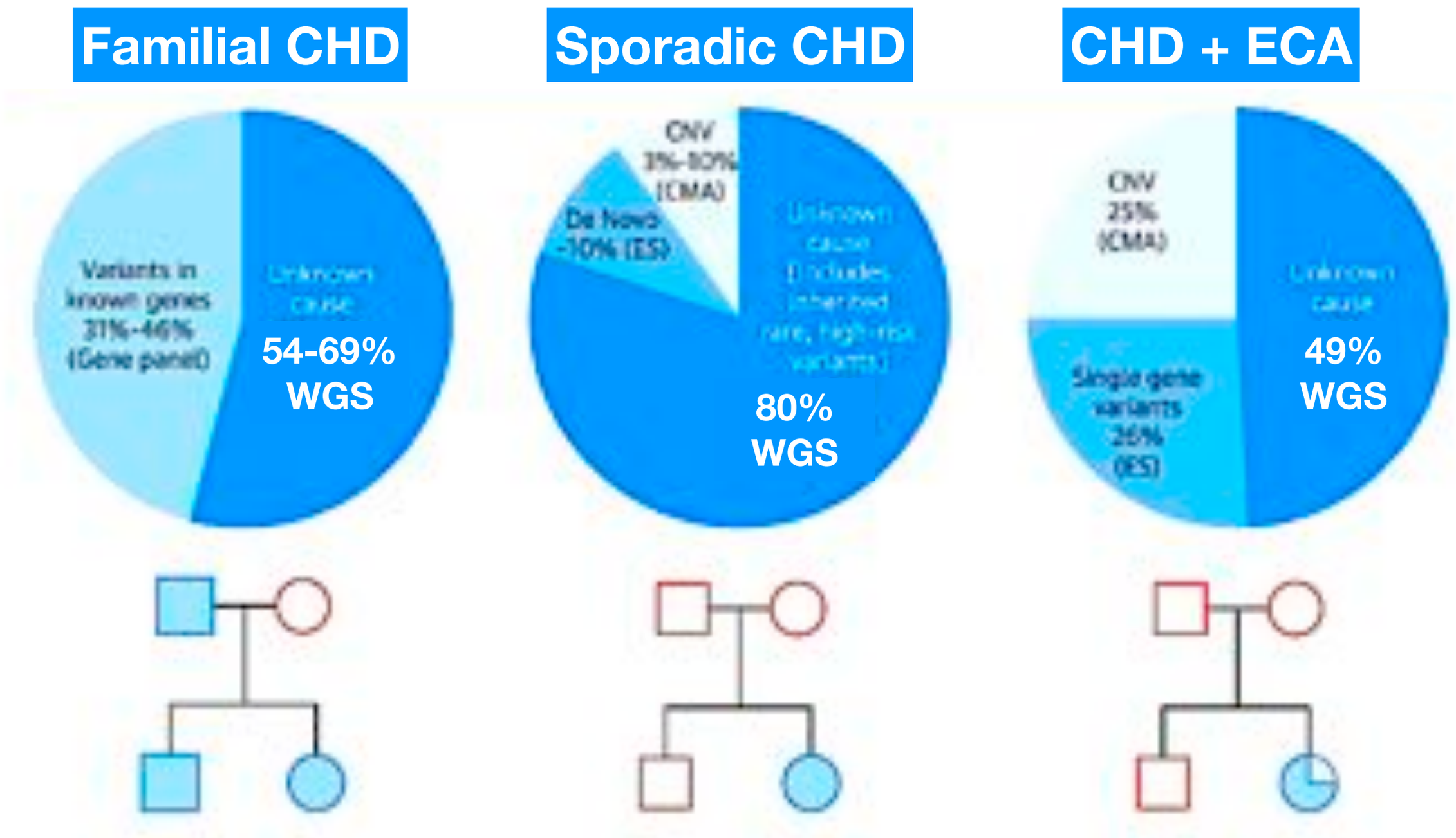
Other de novo SNV

Environmental

Unknown

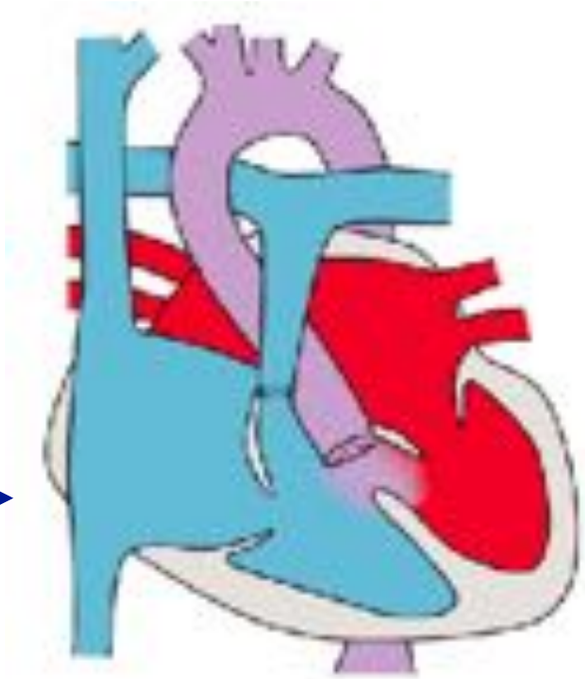
Zaidi S & Brueckner M. Circ Res 2017;120:923-40

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

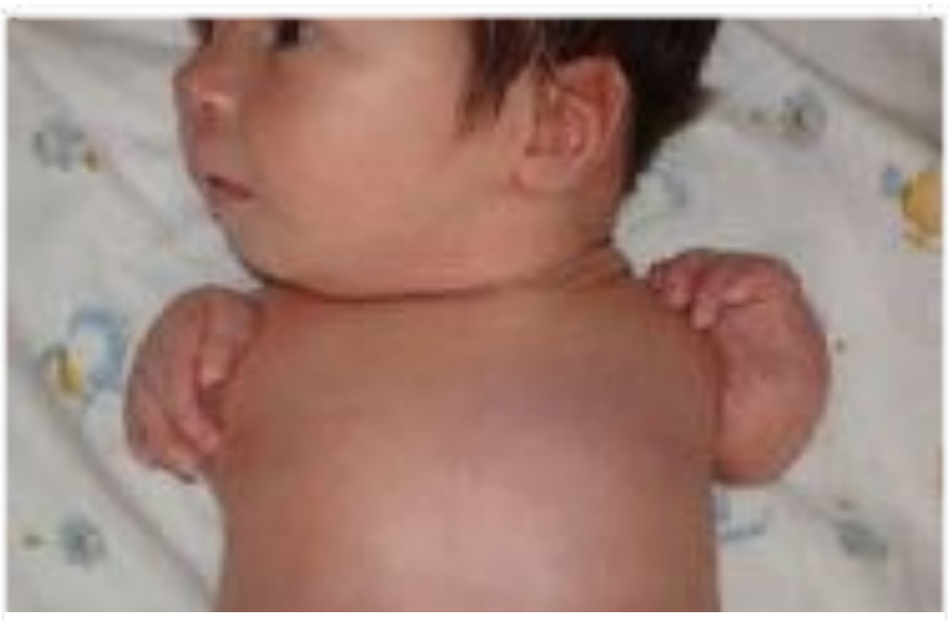


Blue GM et al. J Am Coll Cardiol 2017;69:859-70

Well-known risk factors for congenital heart diseases



A phenocopy is a variation in phenotype (generally referring to a single trait) which is caused by environmental conditions (often, but not necessarily, during the organism's development), such that the organism's phenotype matches a phenotype which is determined by genetic factors.



Thalidomide

Holt-Oram syndrome-*TBX5*

Half a century and the same old story !

Multifactorial Inheritance Hypothesis for the Etiology of Congenital Heart Diseases

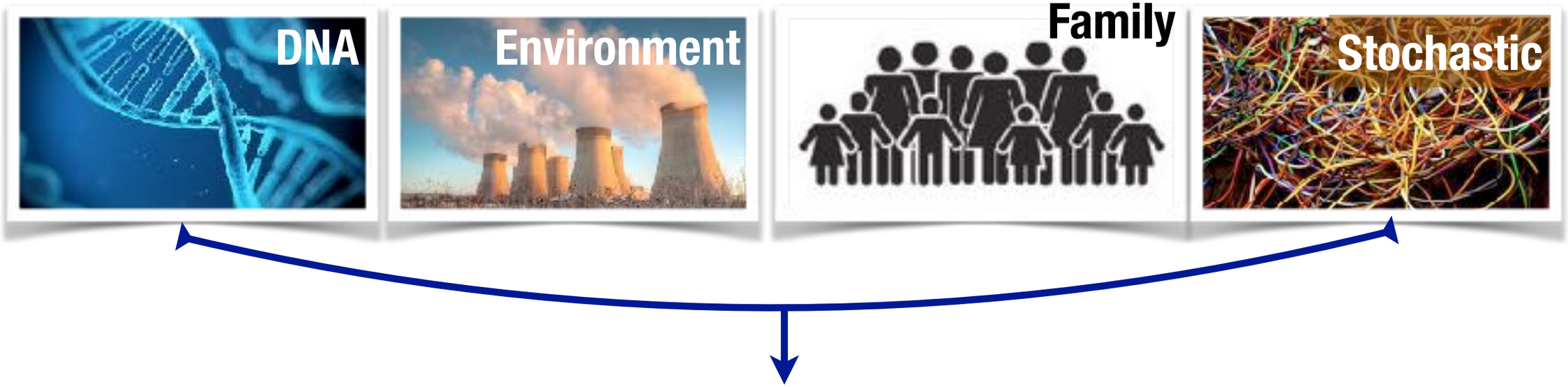
The Genetic-Environmental Interaction

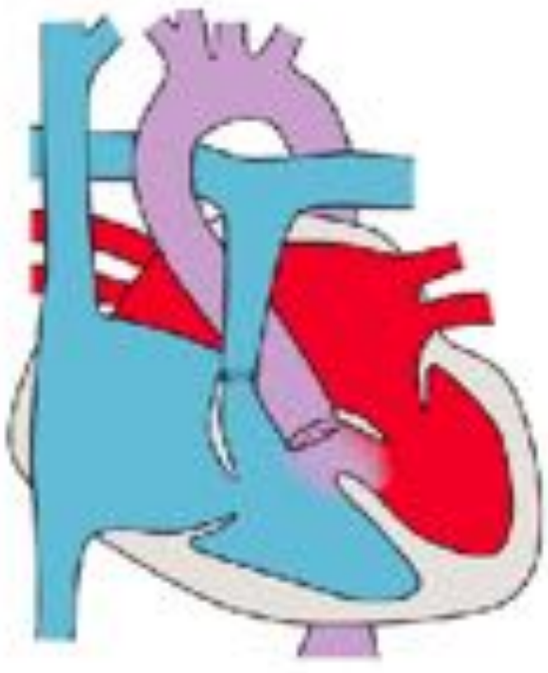
By JAMES J. NORA, M.D.

Circulation 1968


I WILL NOT EXPOSE THE IGNORANCE
OF THE FACULTY.
I WILL NOT EXPOSE THE IGNORANCE
OF THE FACULTY.

The multifactorial hypothesis





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

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

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

Twins	Dizygotic	Monochorionic Diamniotic	Monochorionic Monoamniotic
Concordance of CHD	**Identical** to concordance between sibs	**High but** Functional CHD Pulmonary stenosis in TTTS	**High but** Laterality defects related to the twining process
	Partly identical genome	*Fetal hemodynamics*	*Loss of laterality information*

Herskind AM et al. Circulation 2013

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

- ~~There is no genetic basis for CHD~~
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Nora JJ. Circulation 1968

Recurrence of CHDs in families

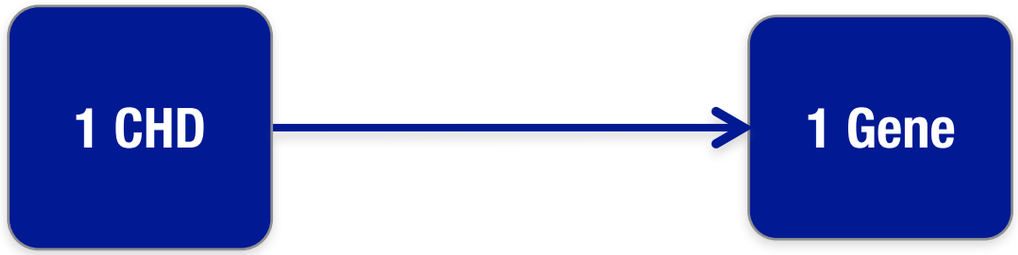
Same defect in affected members

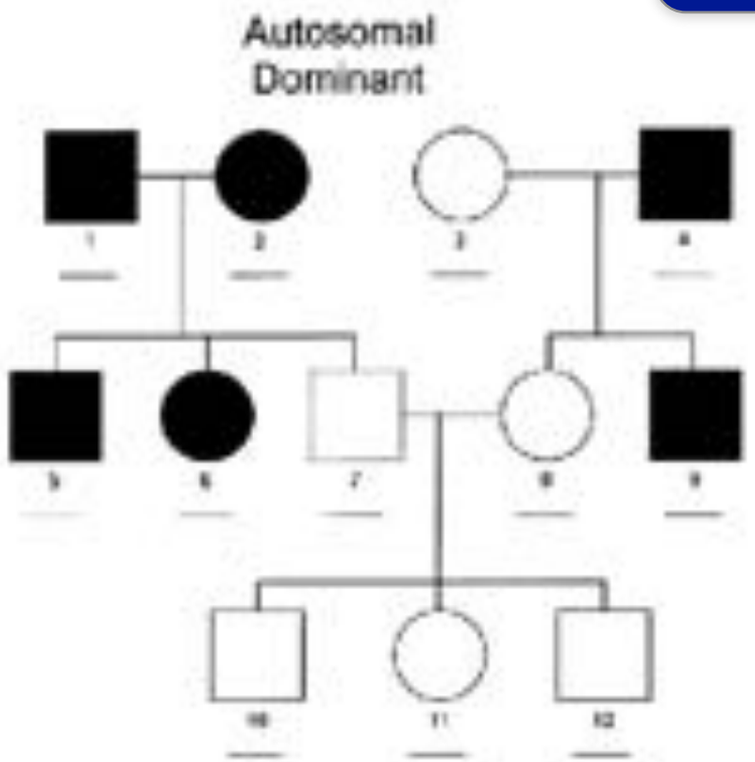
Heart defect phenotype in first degree relative	Relative risk
Heterotaxia	79.1
Conotruncal	11.7
AVSD	24.3
APVR	…
LVOTO	12.9
RVOTO	48.6
ASD	7.07
VSD	3.41
Overall same heart defect	8.15

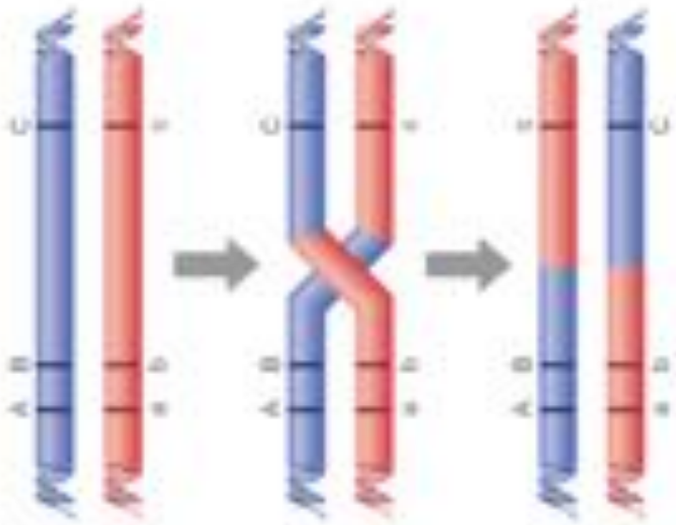
Recurrence of the same type can be due to inheritance of a single gene mutation

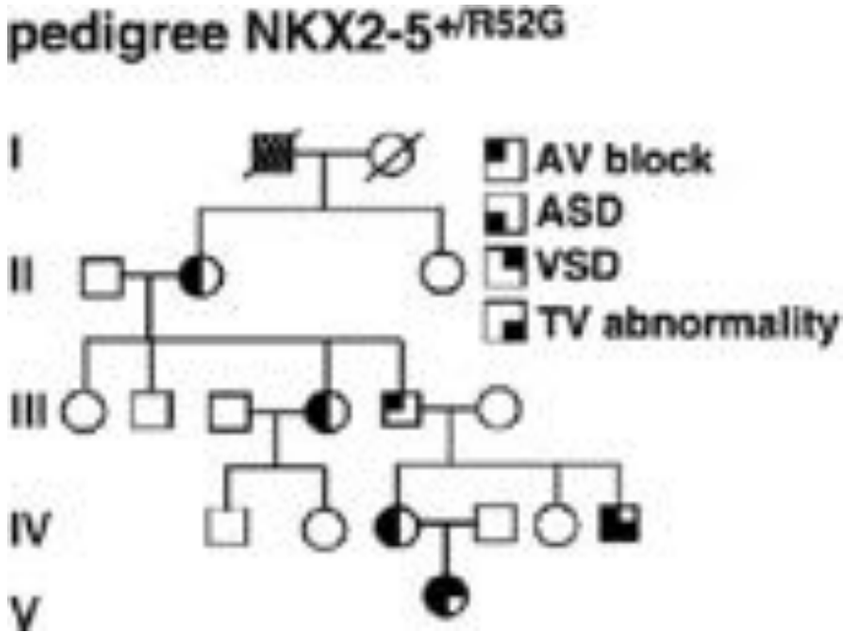
Oyen N et al. Circulation 2009

The monogenic hypothesis







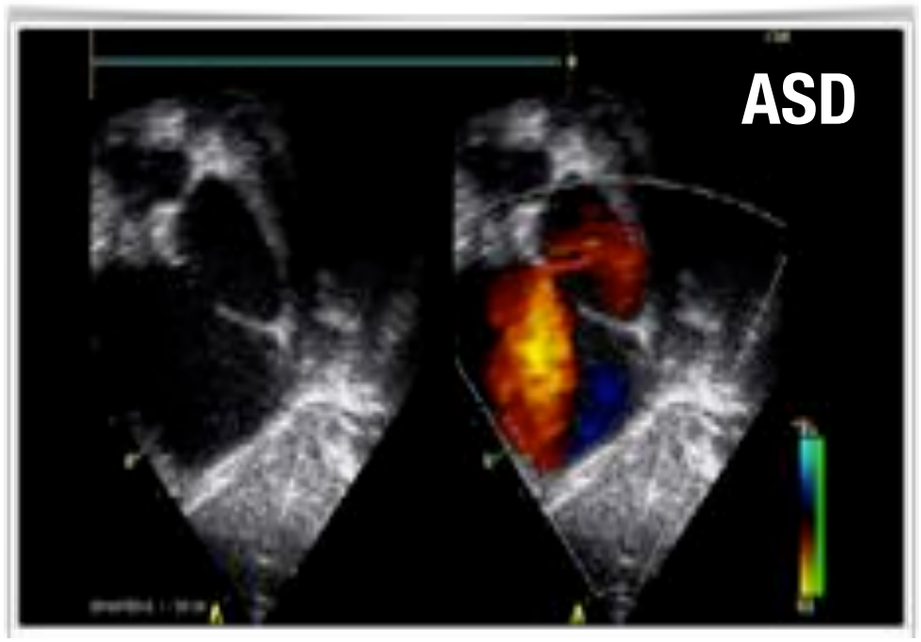


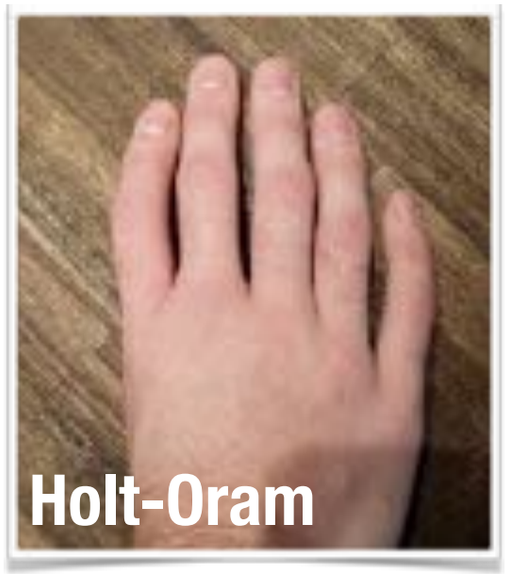
The positional cloning strategy

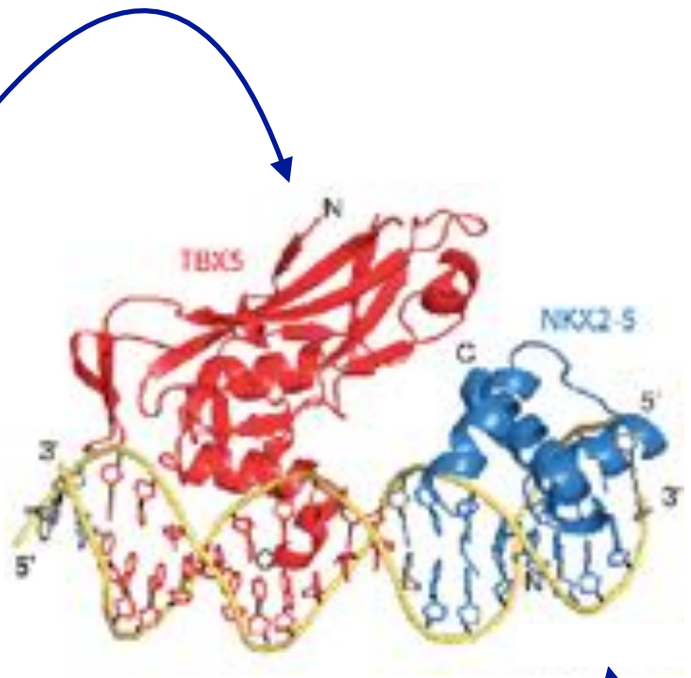
Genetic heterogeneity

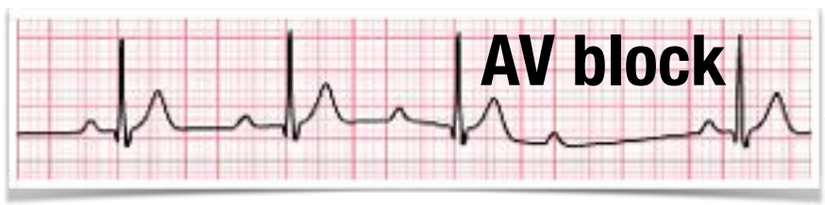
One cardiac phenotype-Different genotypes

1 CHD → **X Genes**

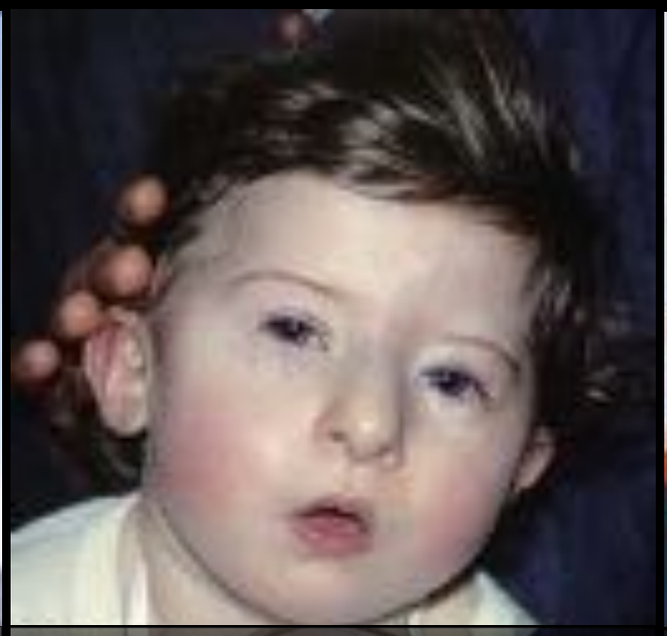
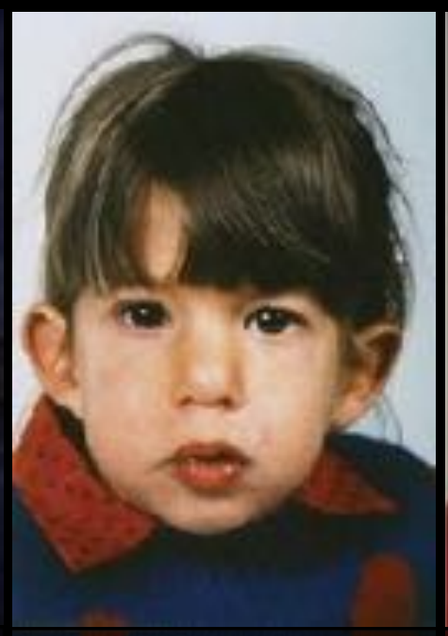






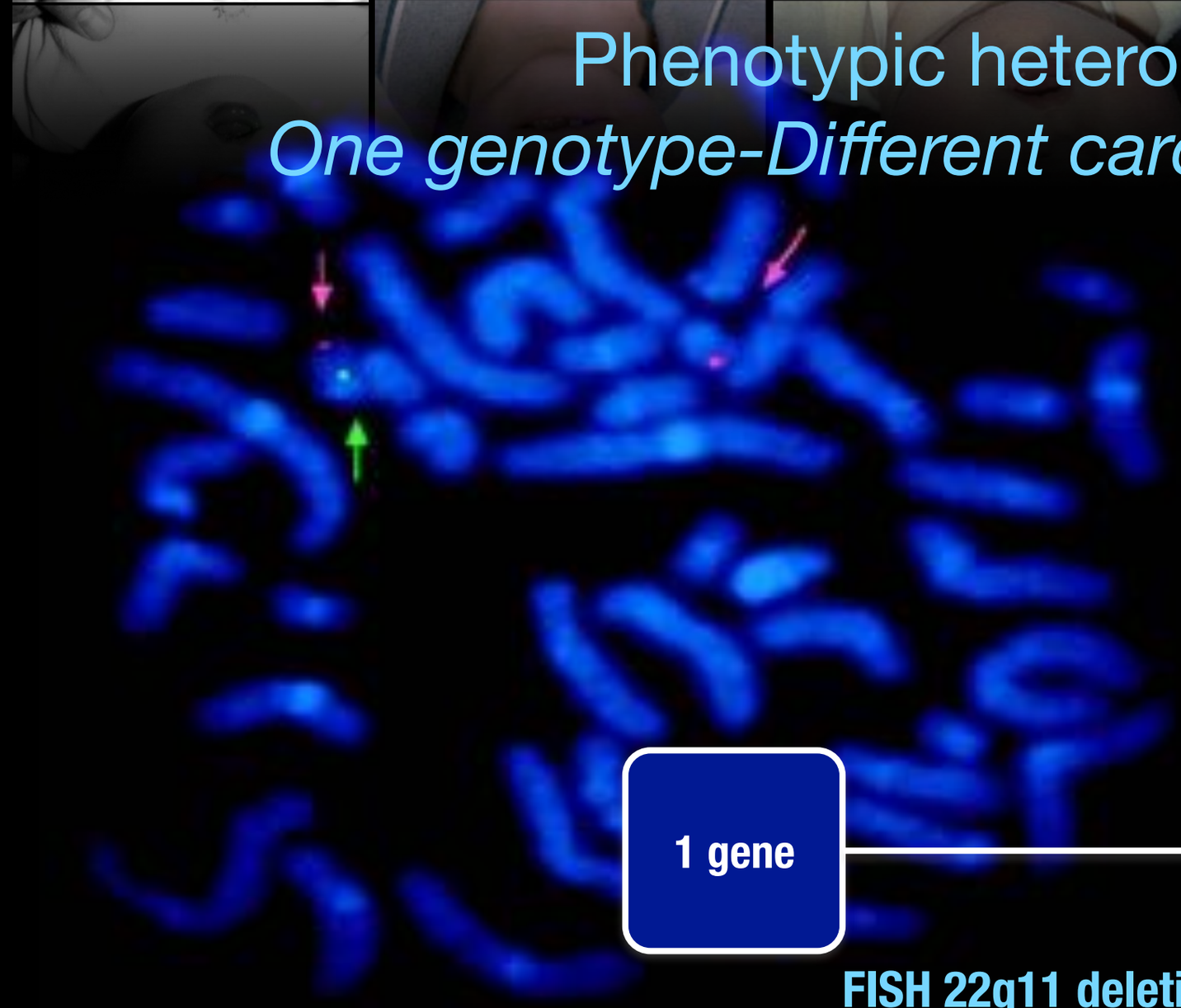


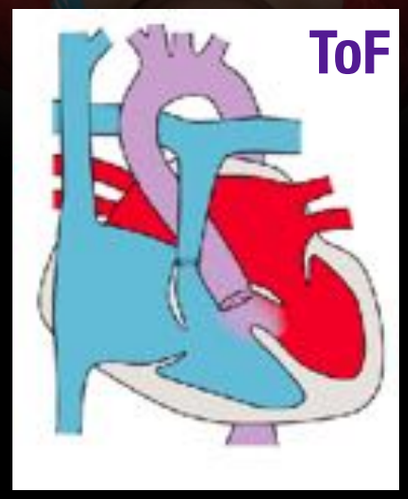


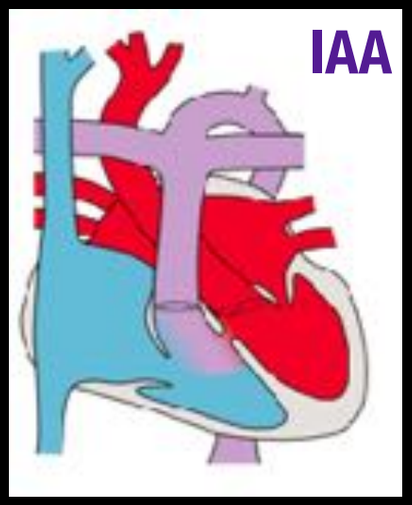




Phenotypic heterogeneity

One genotype-Different cardiac phenotypes





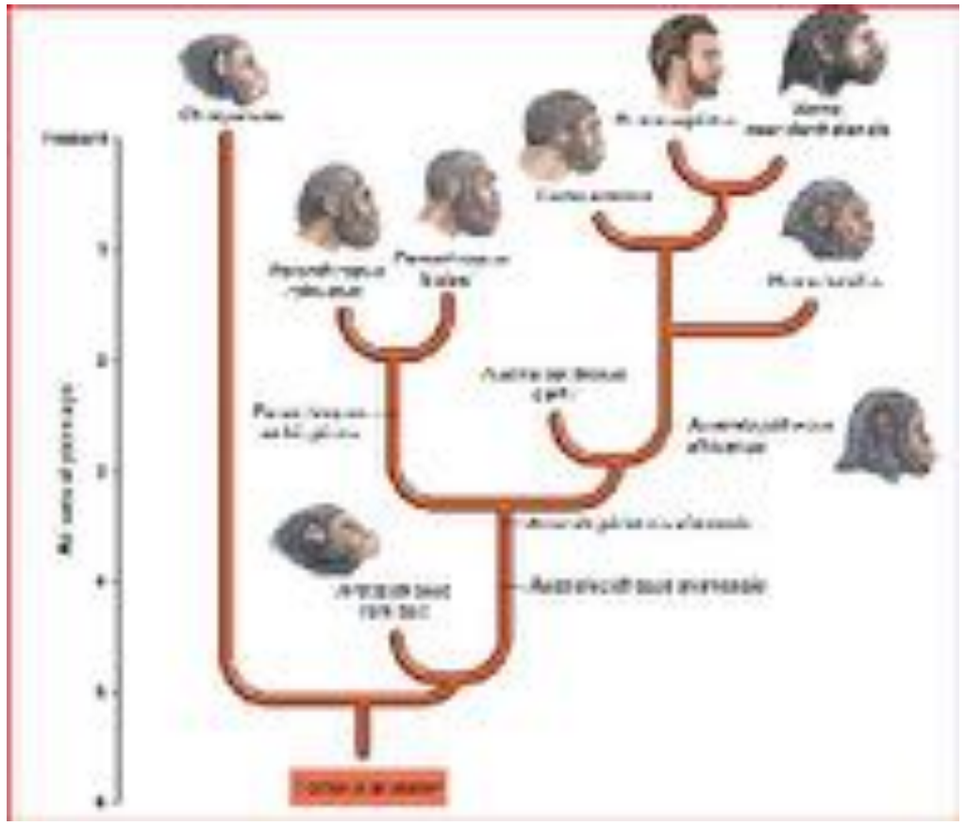


1 gene → X CHD

FISH 22q11 deletion

The mechanistic hypothesis

Darwin **or** **Carl von Linné**

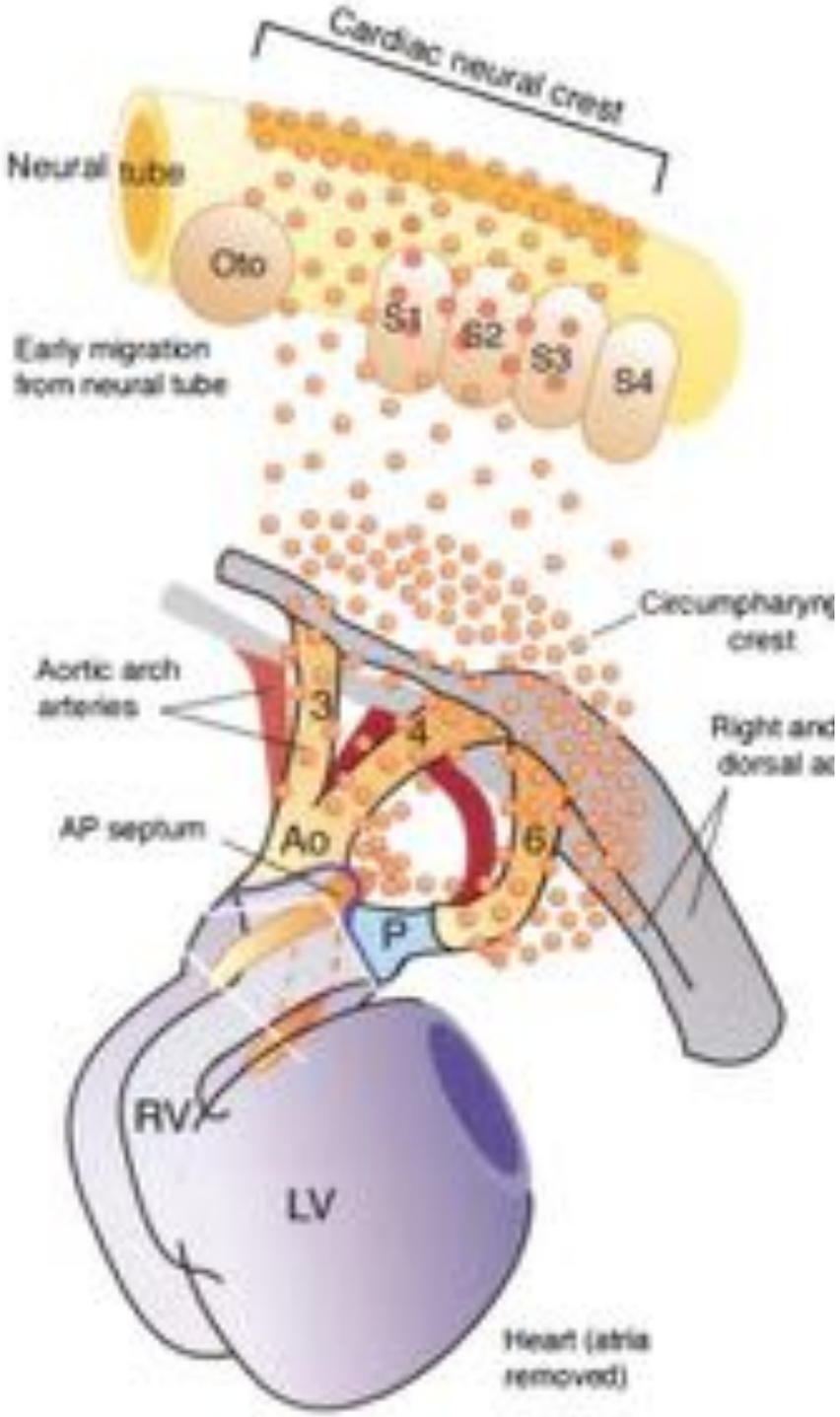


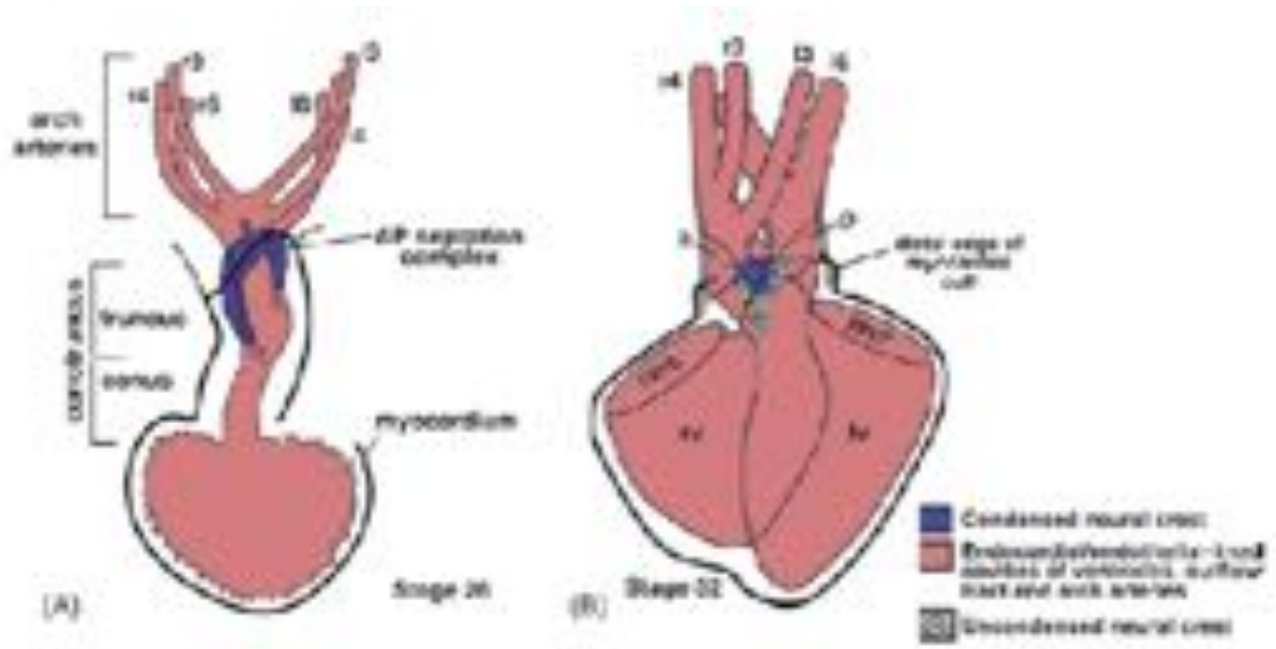
Phylogeny - Common ancestor

Classification according to morphological characteristics

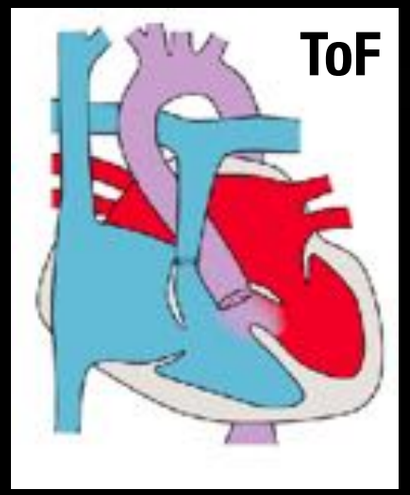
Migration of neural crest cells into the outflow tract

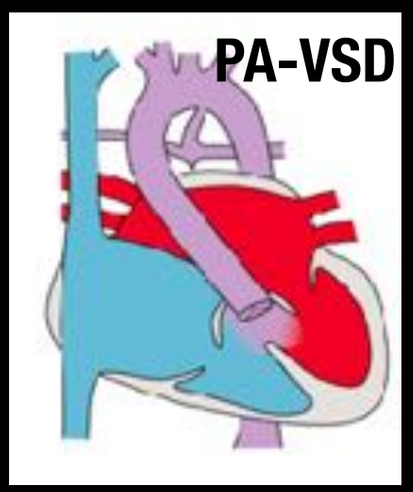
Darwin hypothesis example

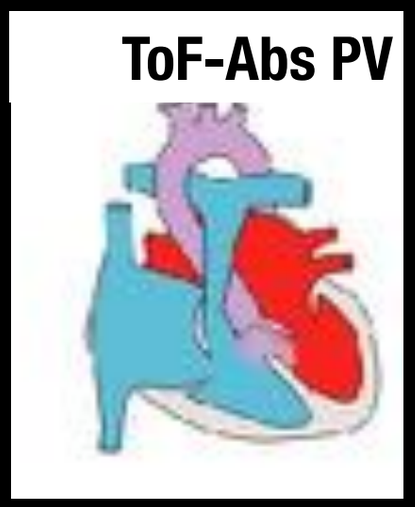


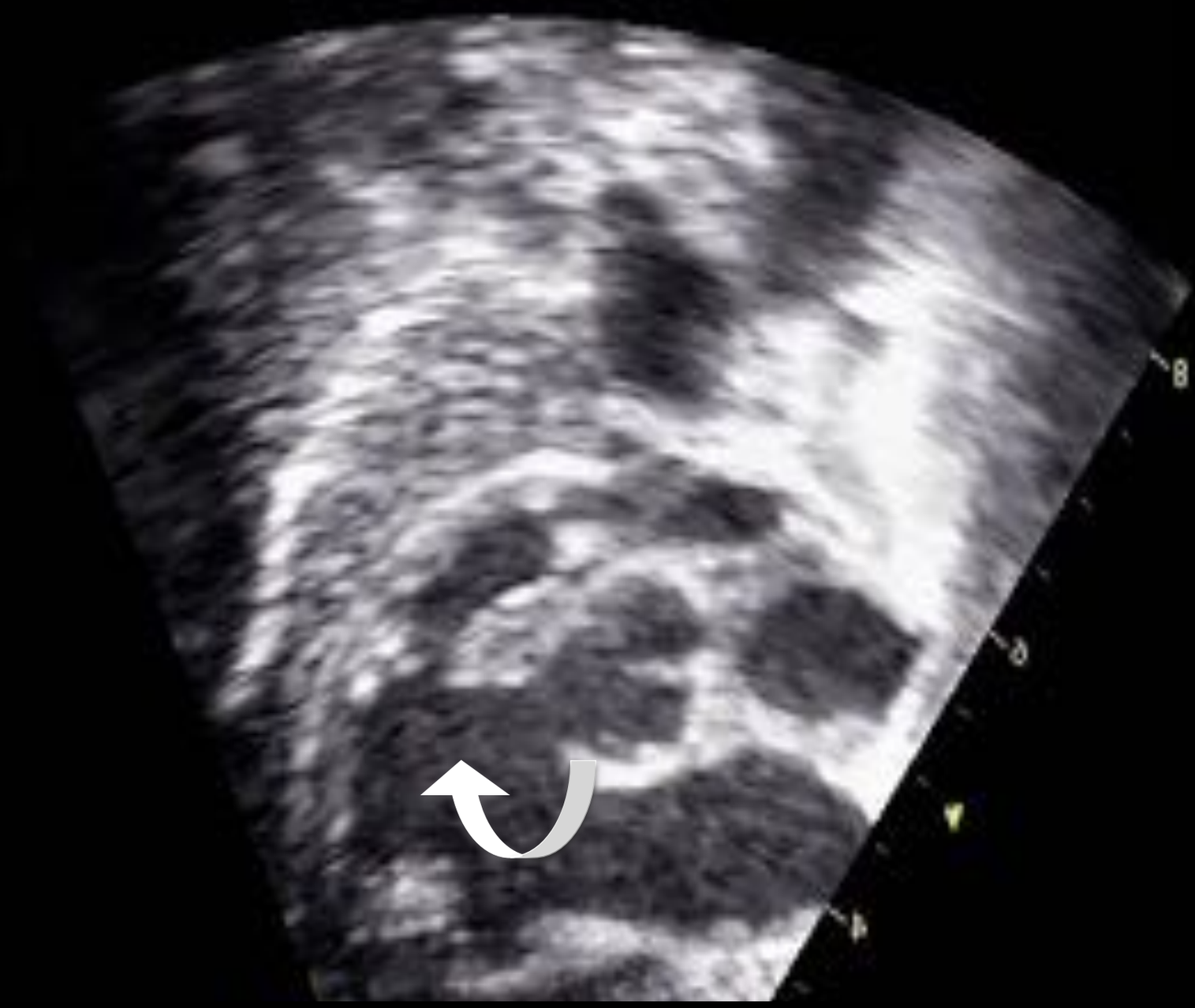


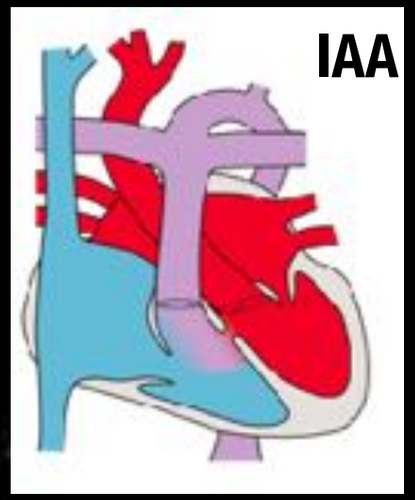
Hutson & Kirby 2007


ToF


PA-VSD


ToF-Abs PV

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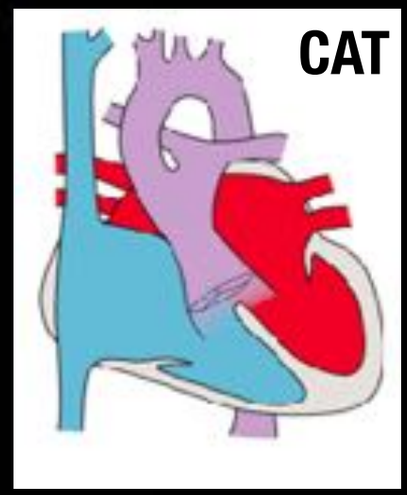
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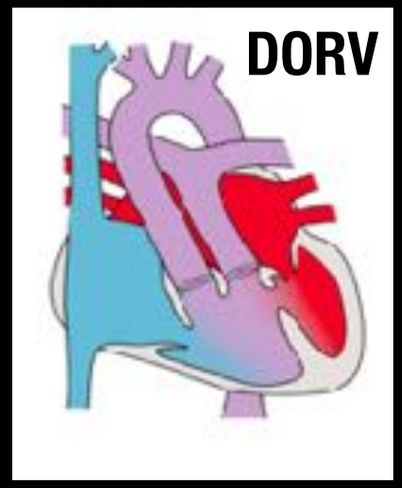

DORV

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

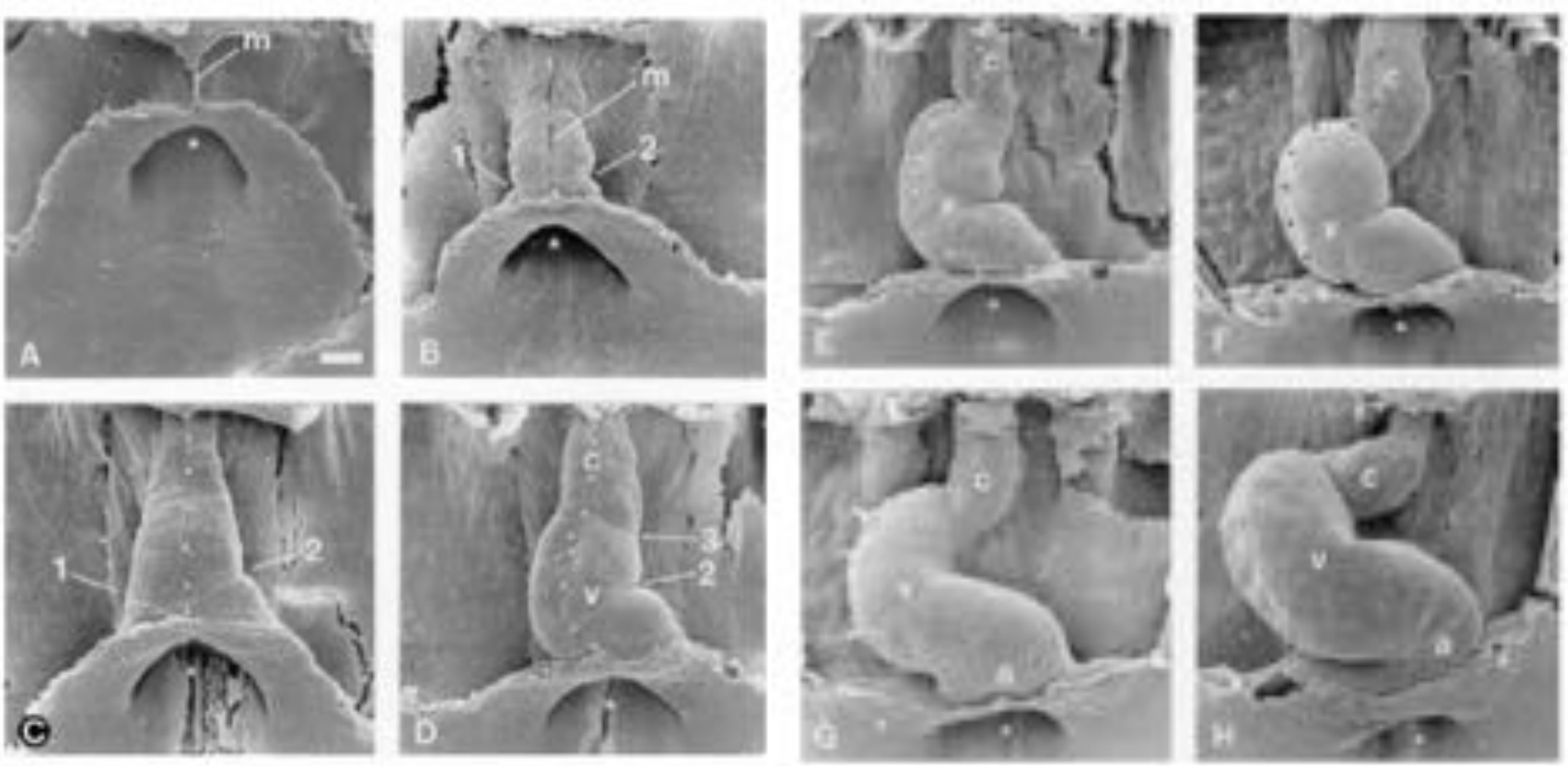
Heterotaxia

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

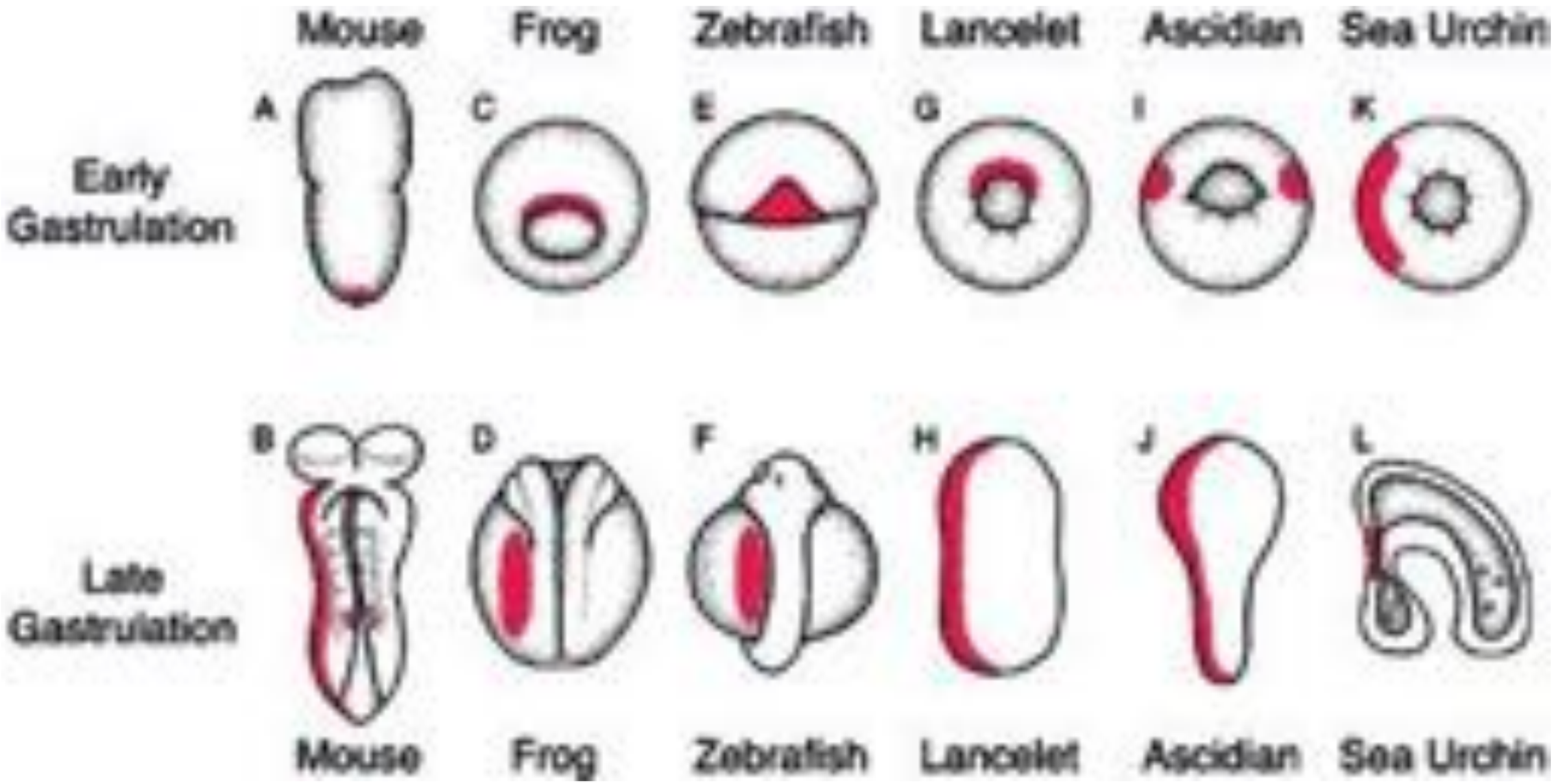
The mechanistic hypothesis

Carl von Linné hypothesis

1 Mechanism → **1 group of CHDs**



The example of laterality defects

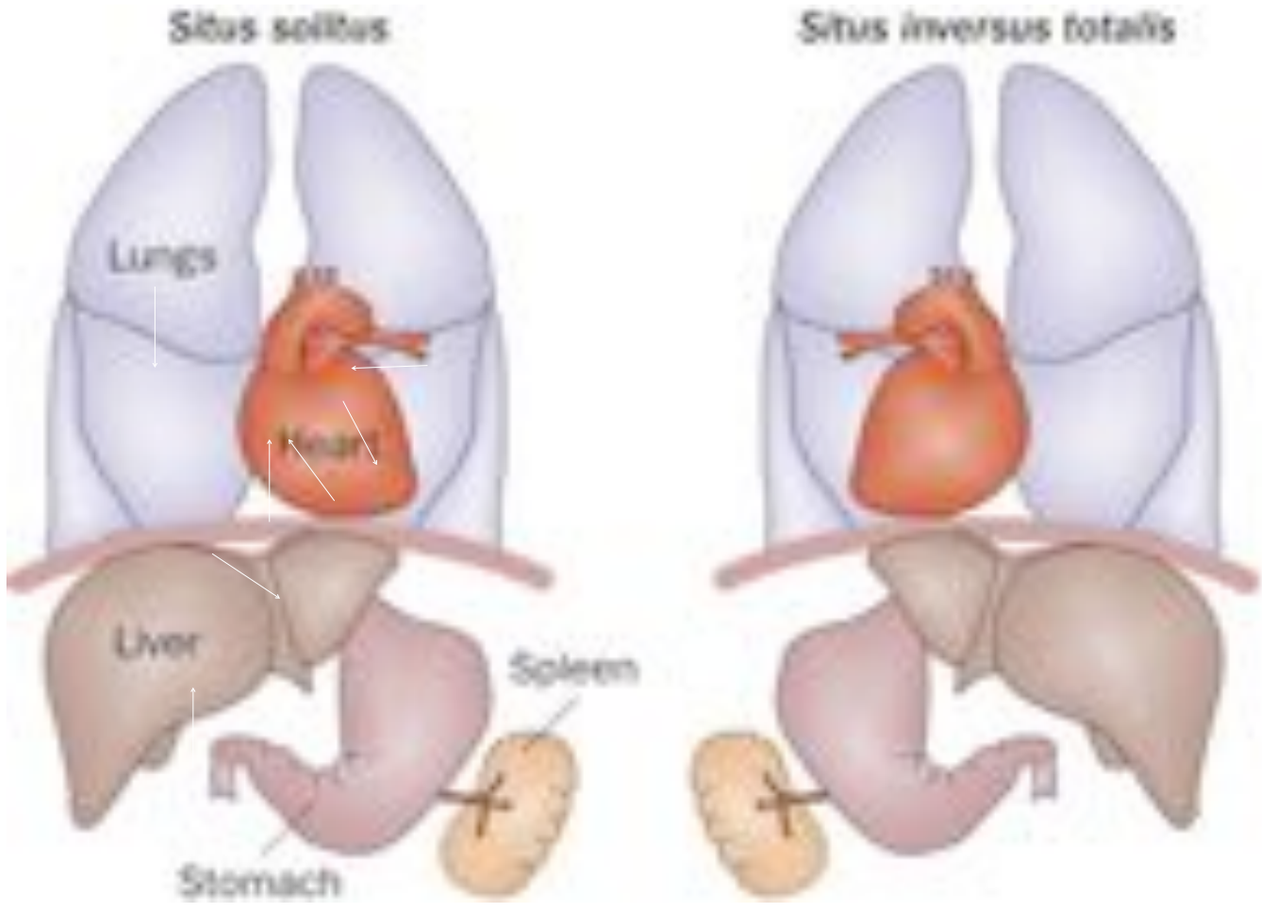


Expression of Nodal in different species is on the left side

What happens in the absence of left-right signaling ?

1.1/10,000 live births

3% of all Congenital Heart Diseases



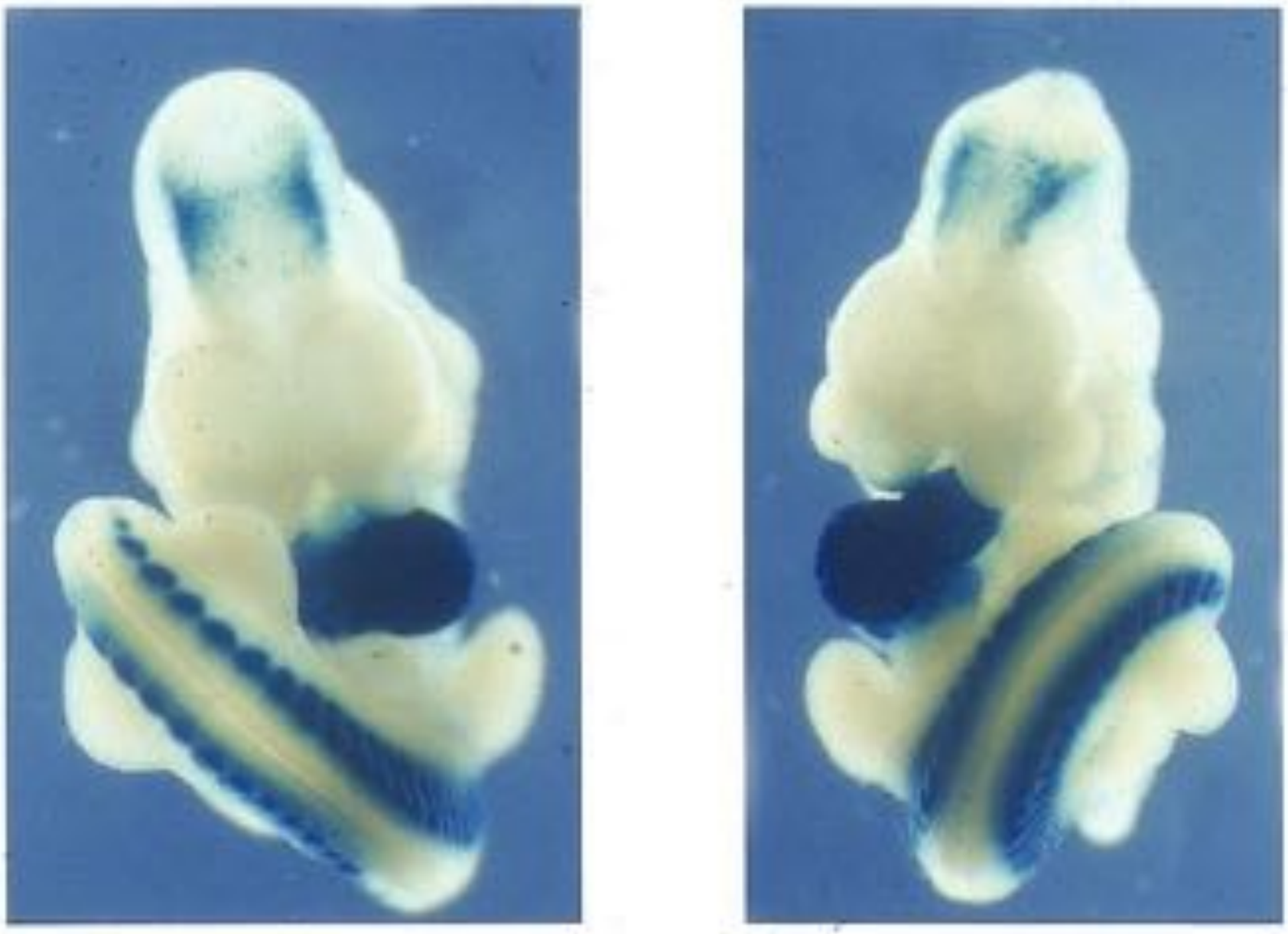
Impairment of Left/Right signaling

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

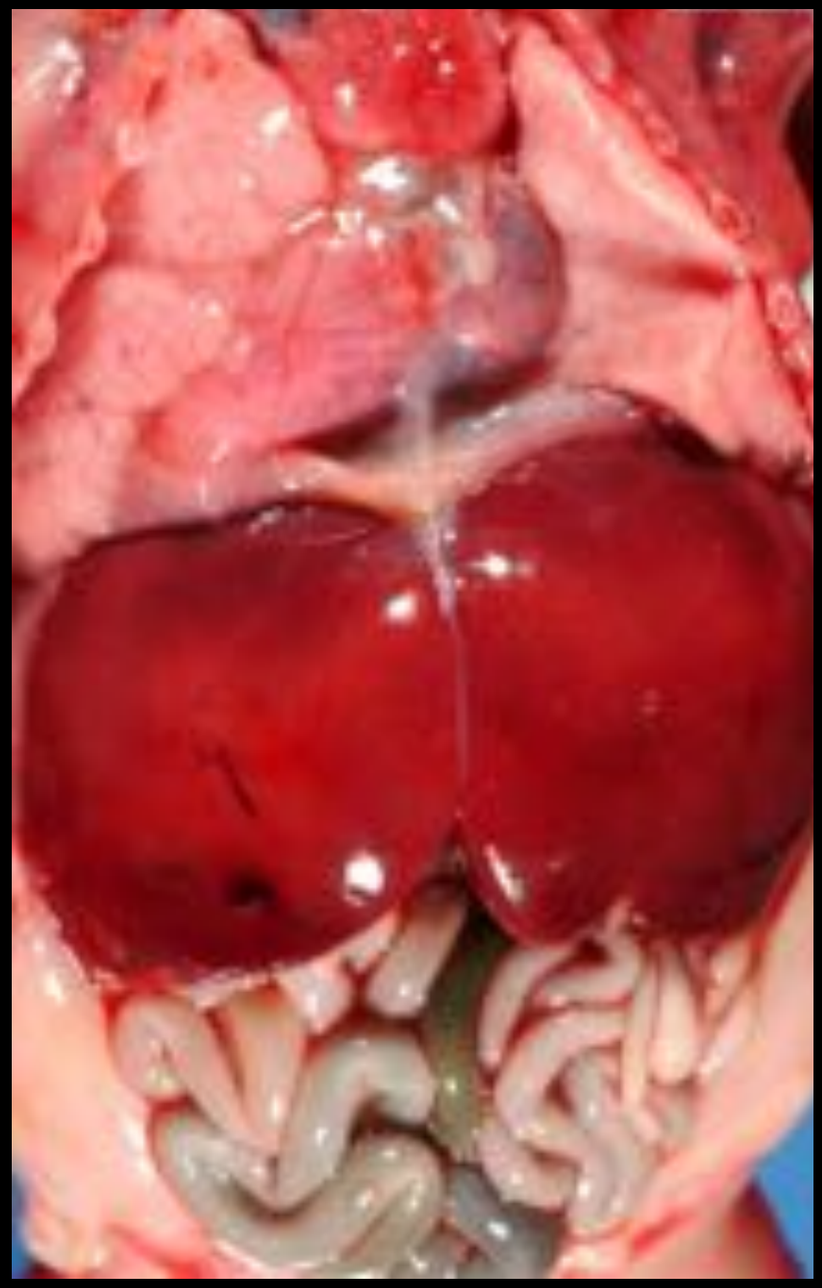
Lymnaea stagnalis

Absence of left-right signaling

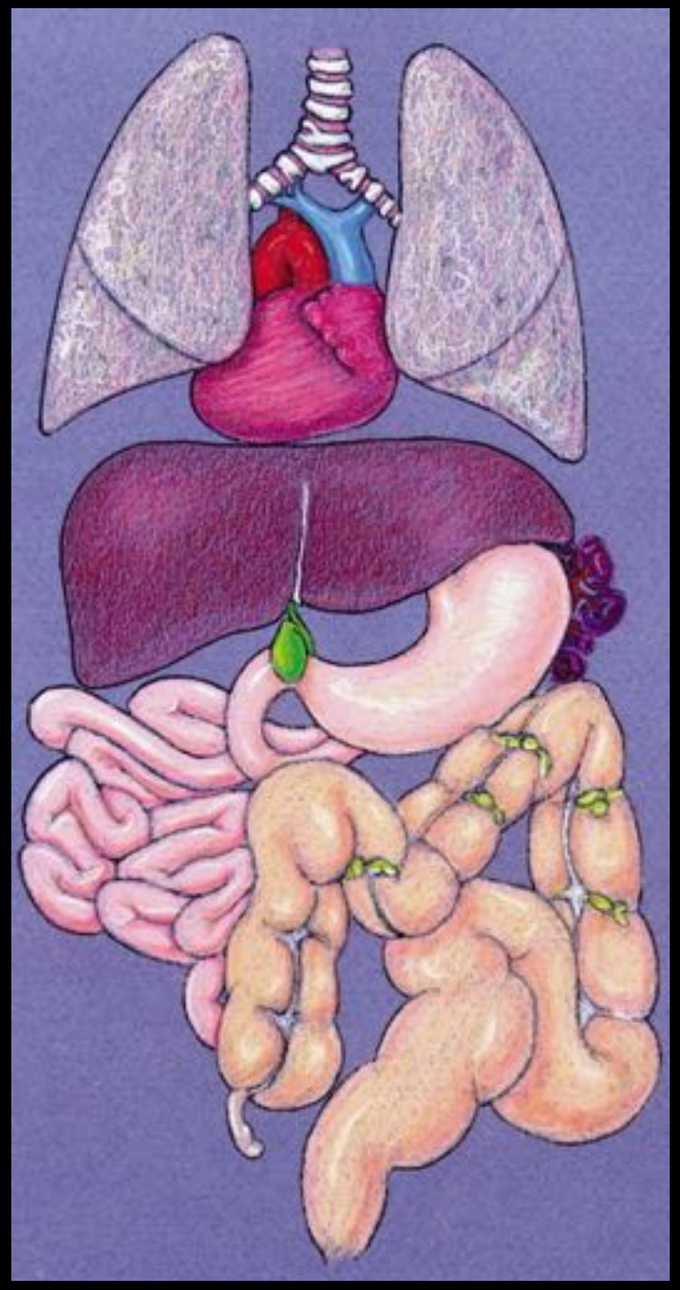
Inversion-mirror image, Isomerism-Heterotaxy

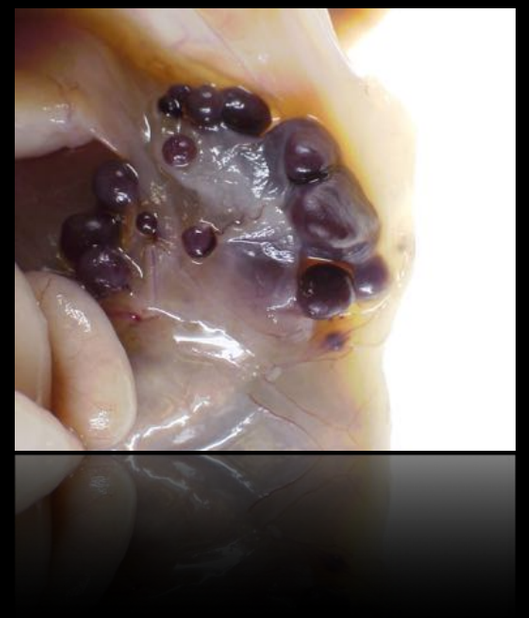
Isomerism is easy to understand for pair organs

Heterotaxy is abnormality of visceral asymmetry

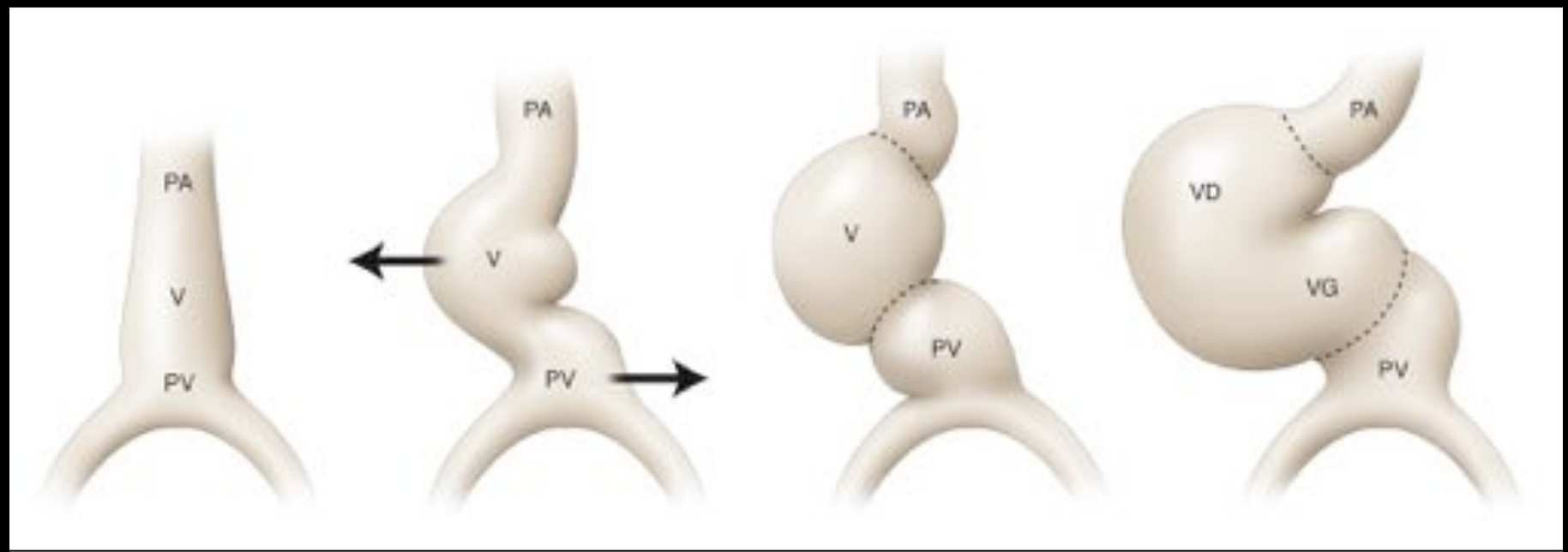


Right and left liver





Polysplenia

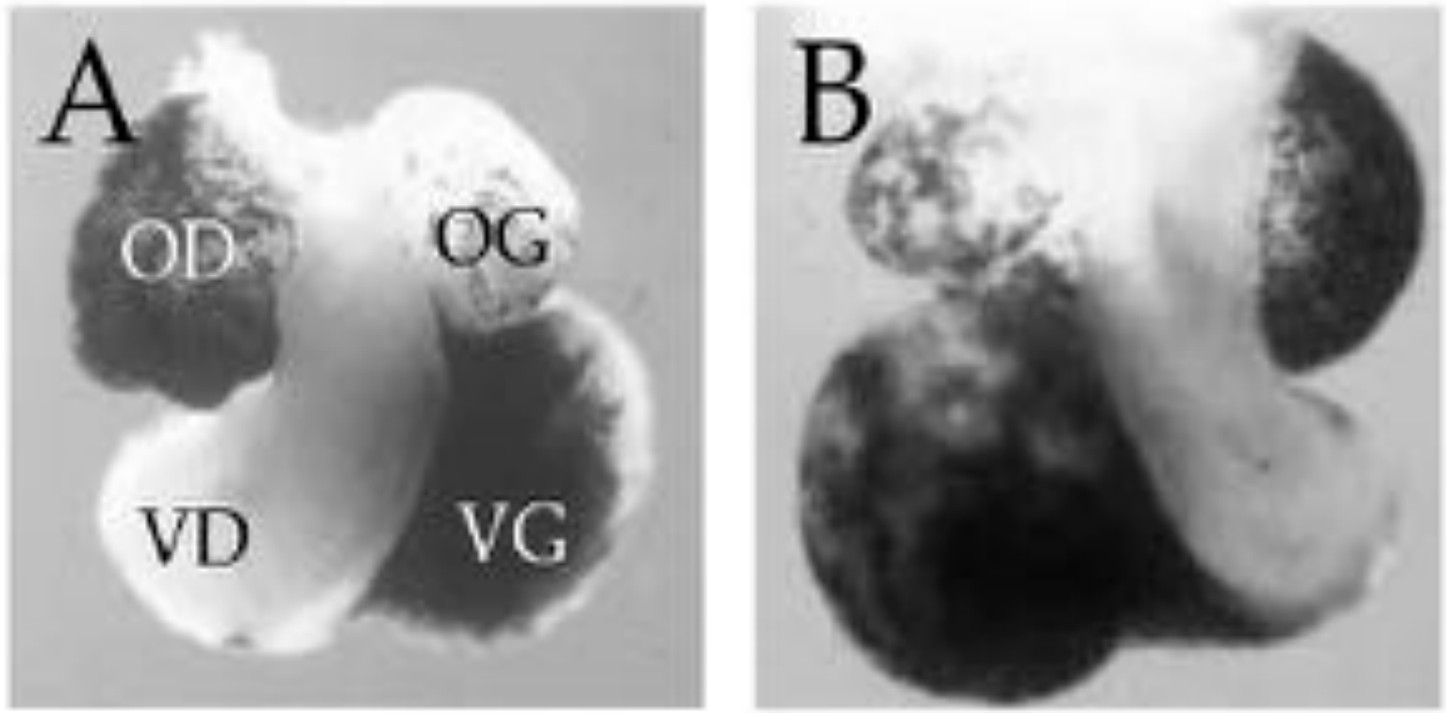


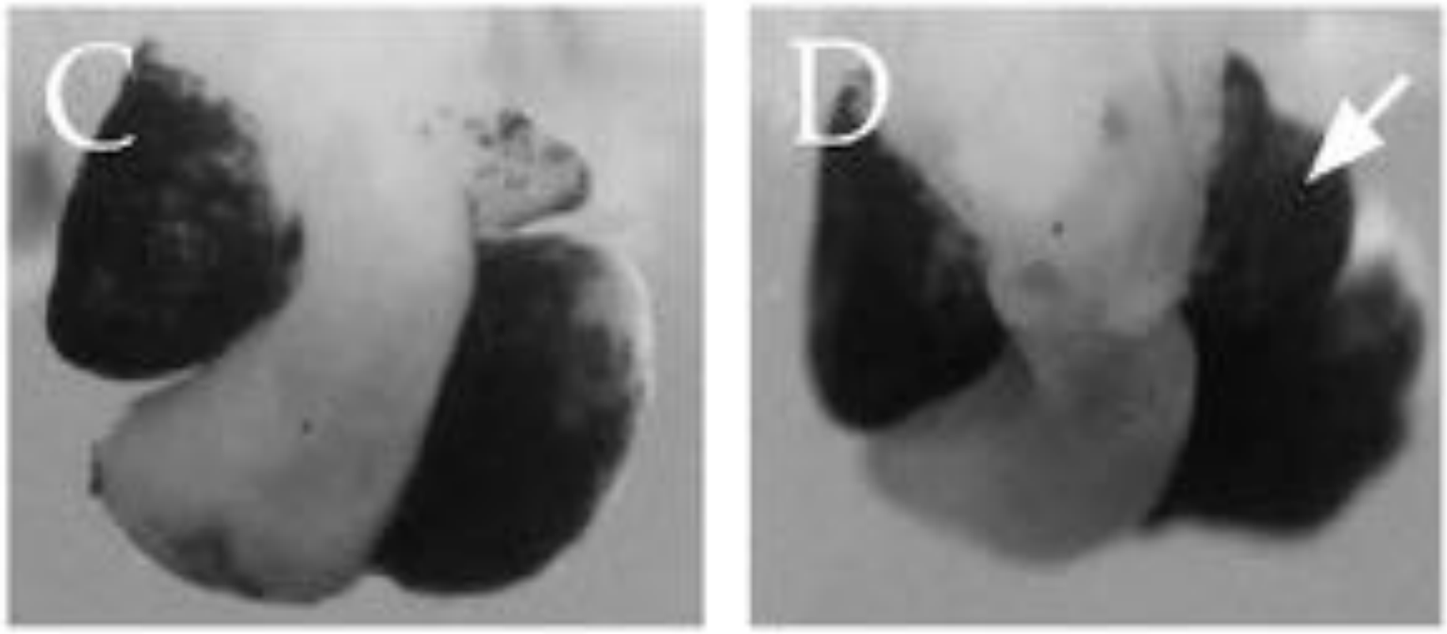
Right-sidedness and left-sidedness of cardiac structures are acquired during development, not present *de novo*

Transgenic mouse model for heterotaxy

E10.5

Situs inversus

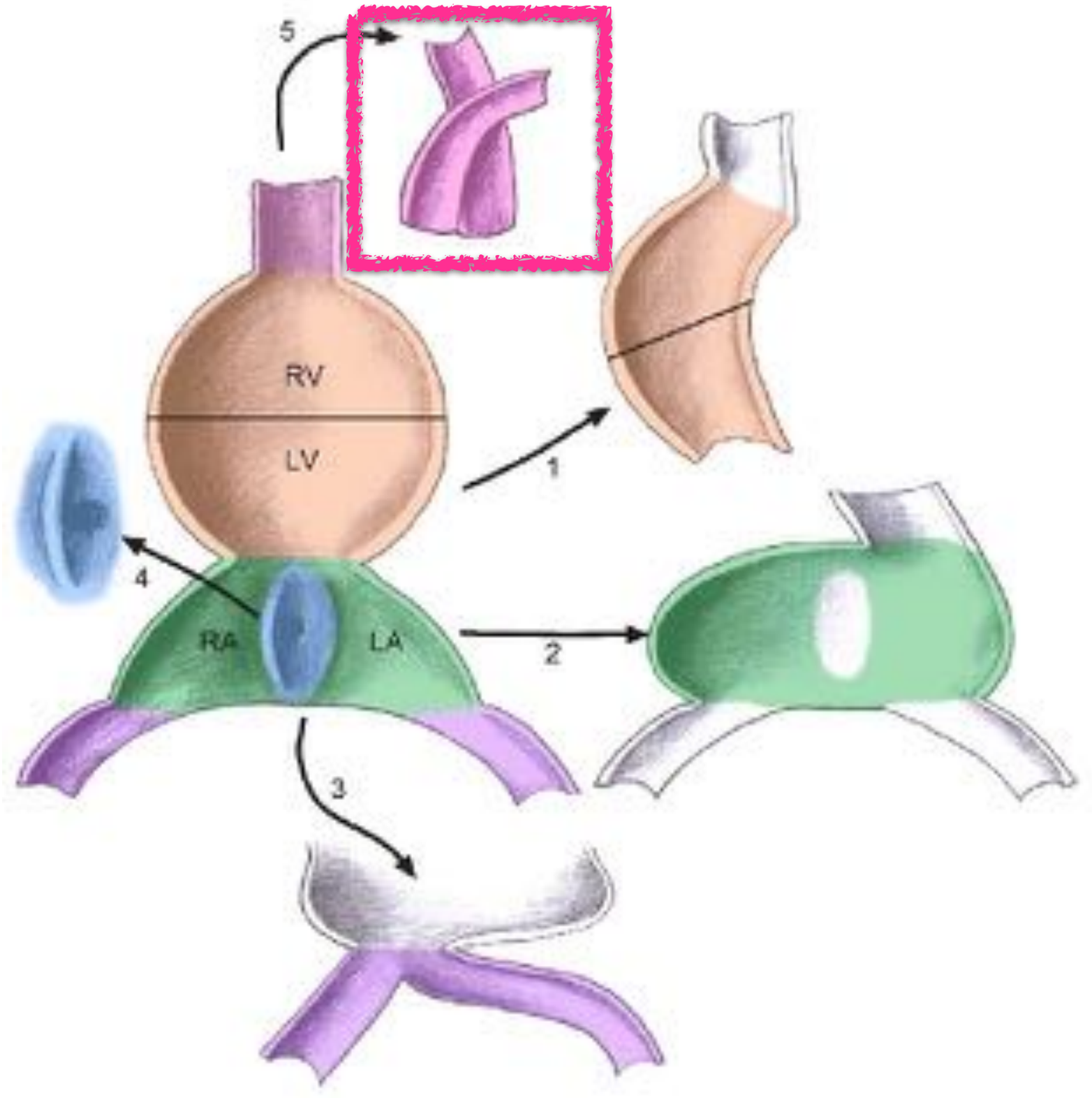




Situs solitus

Situs ambigus

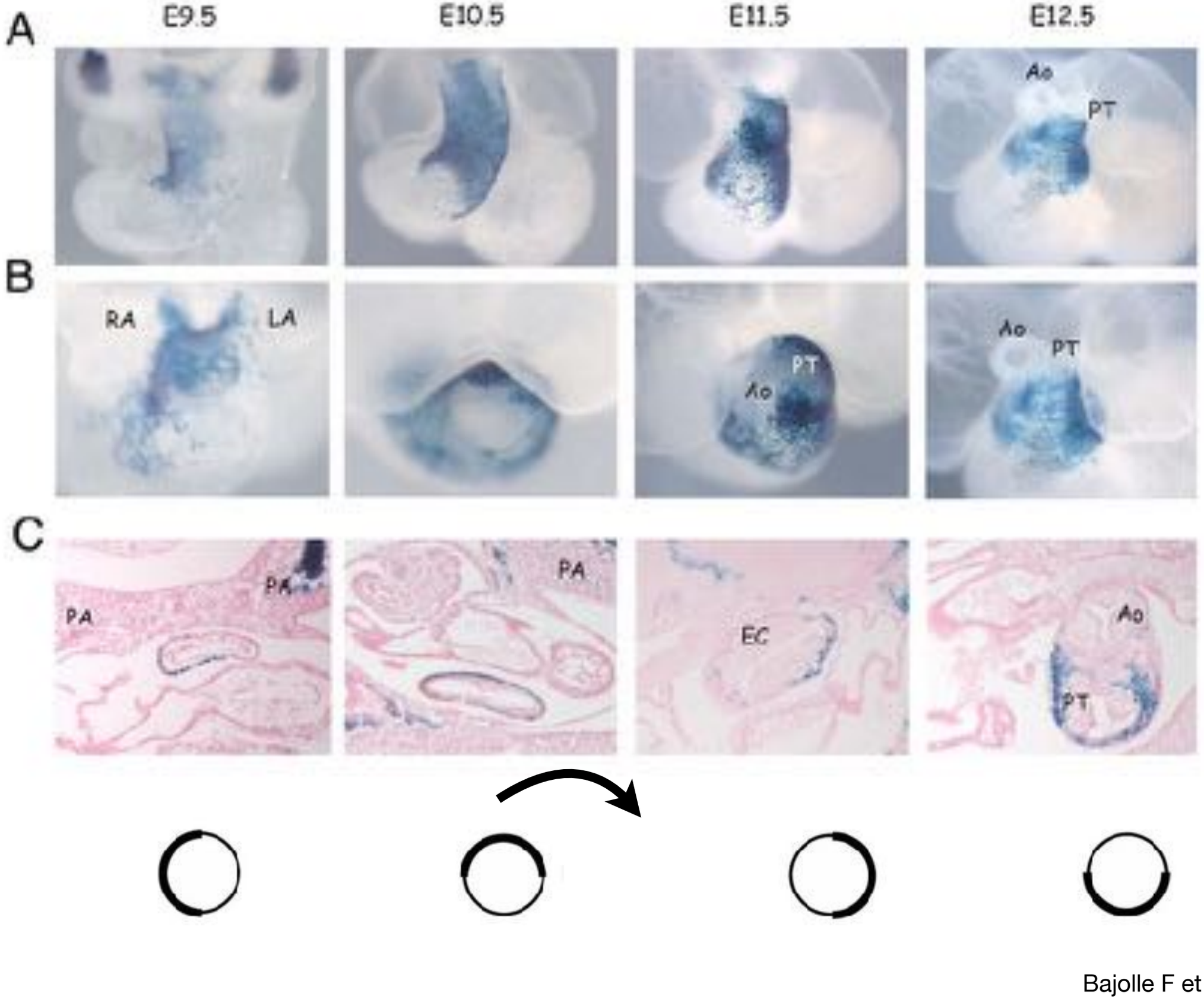
Mlc3f-2 X iv/iv



5 levels of asymmetry in the developing heart

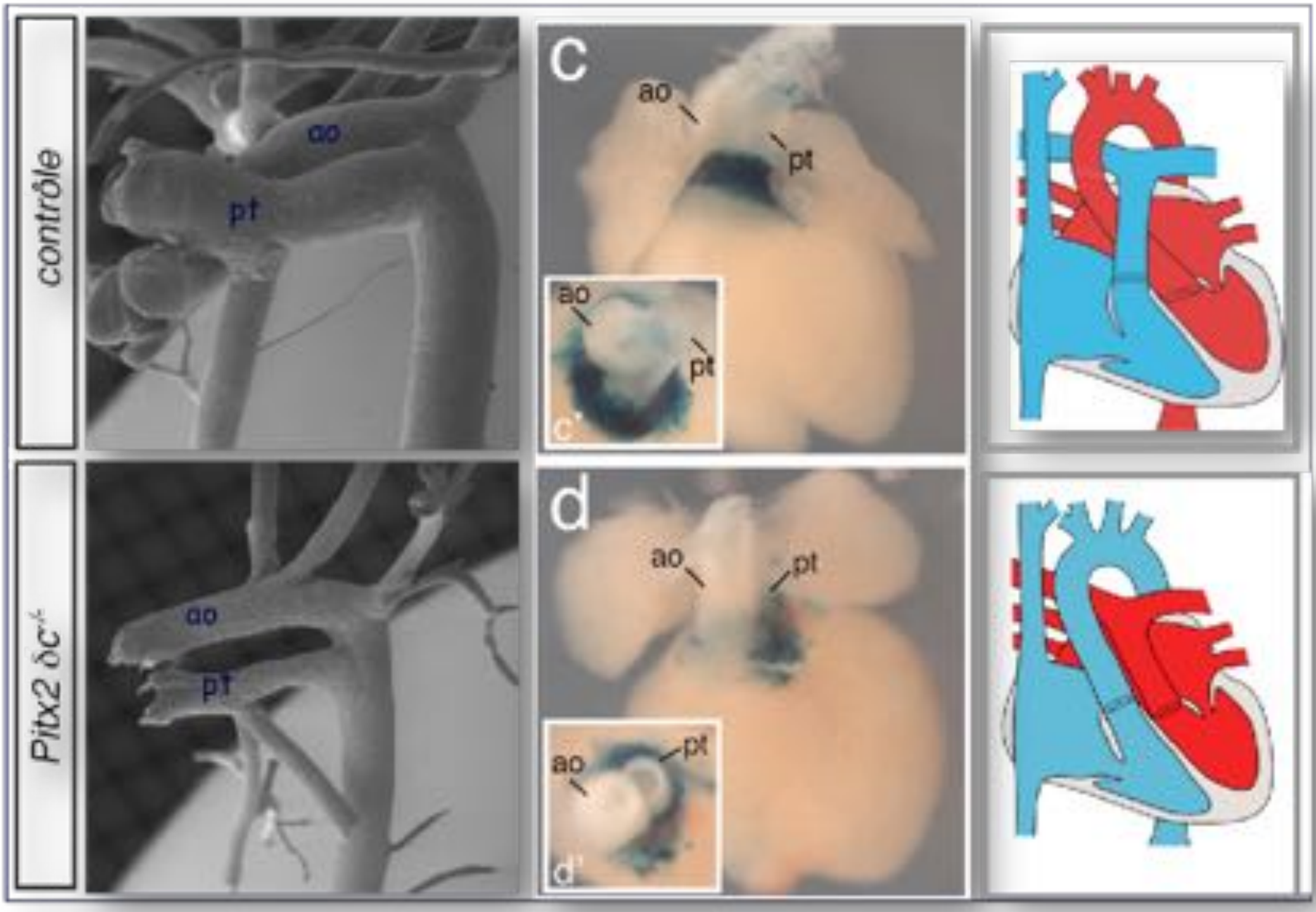
Nigel Brown

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

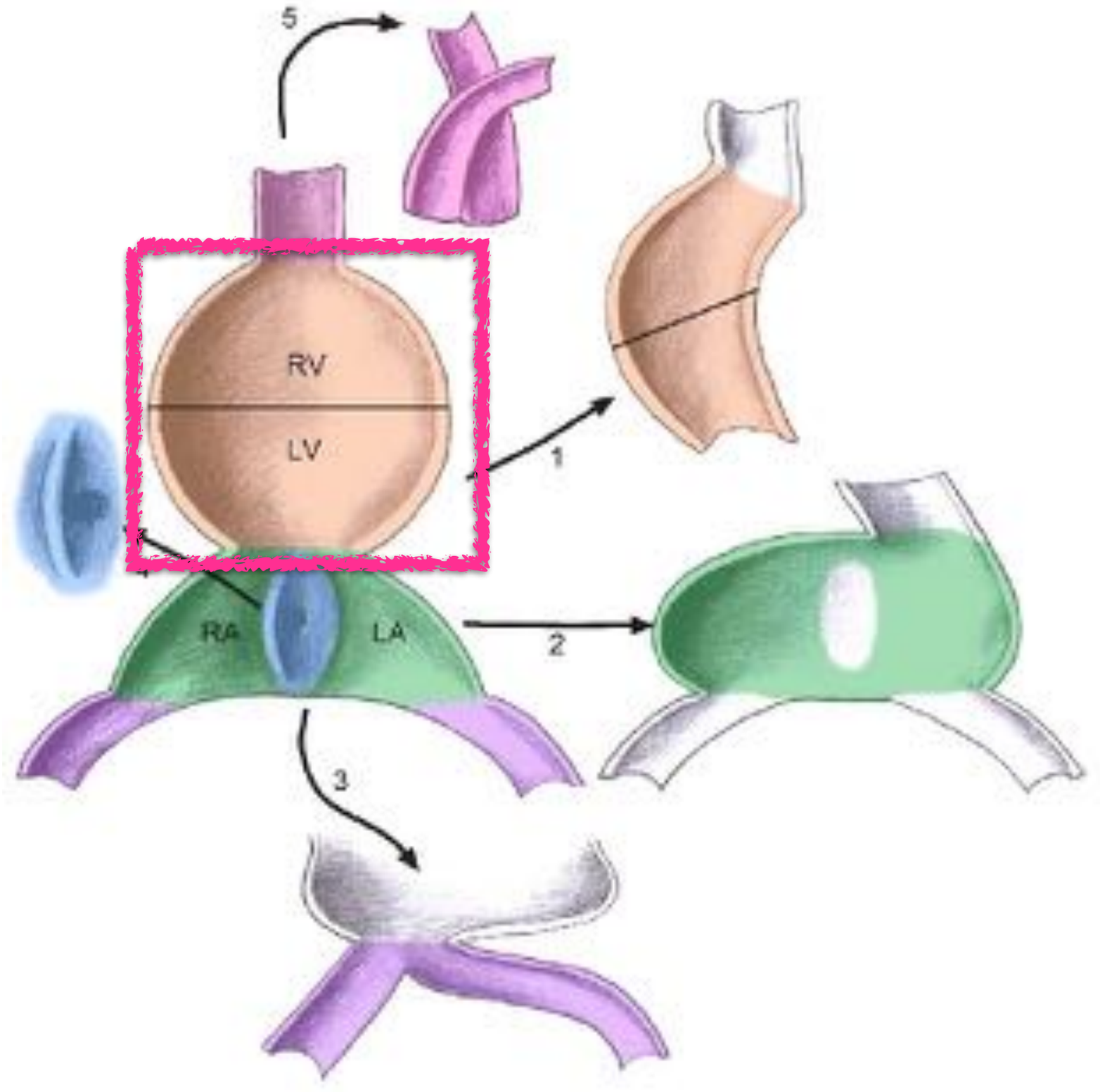
Alessandro De Luca,[1] Anna Sarkozy,[1,6] Federica Consoli,[1] Rosangela Ferese,[1] Valentina Guida,[1] Maria Lisa Dentici,[1] Rita Mingarelli,[1] Emanuele Bellacchio,[1] Giulia Tuo,[2] Giuseppe Limongelli,[3] Maria Cristina Digilio,[4] Bruno Marino,[5] Bruno Dallapiccola[1]

Heart 2010;**96**:673–677.

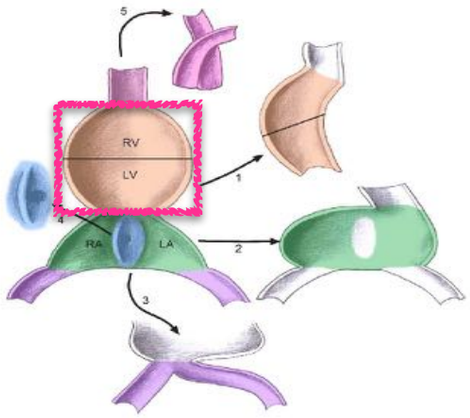
TGA is a laterality defect

It is not a conotruncal defect

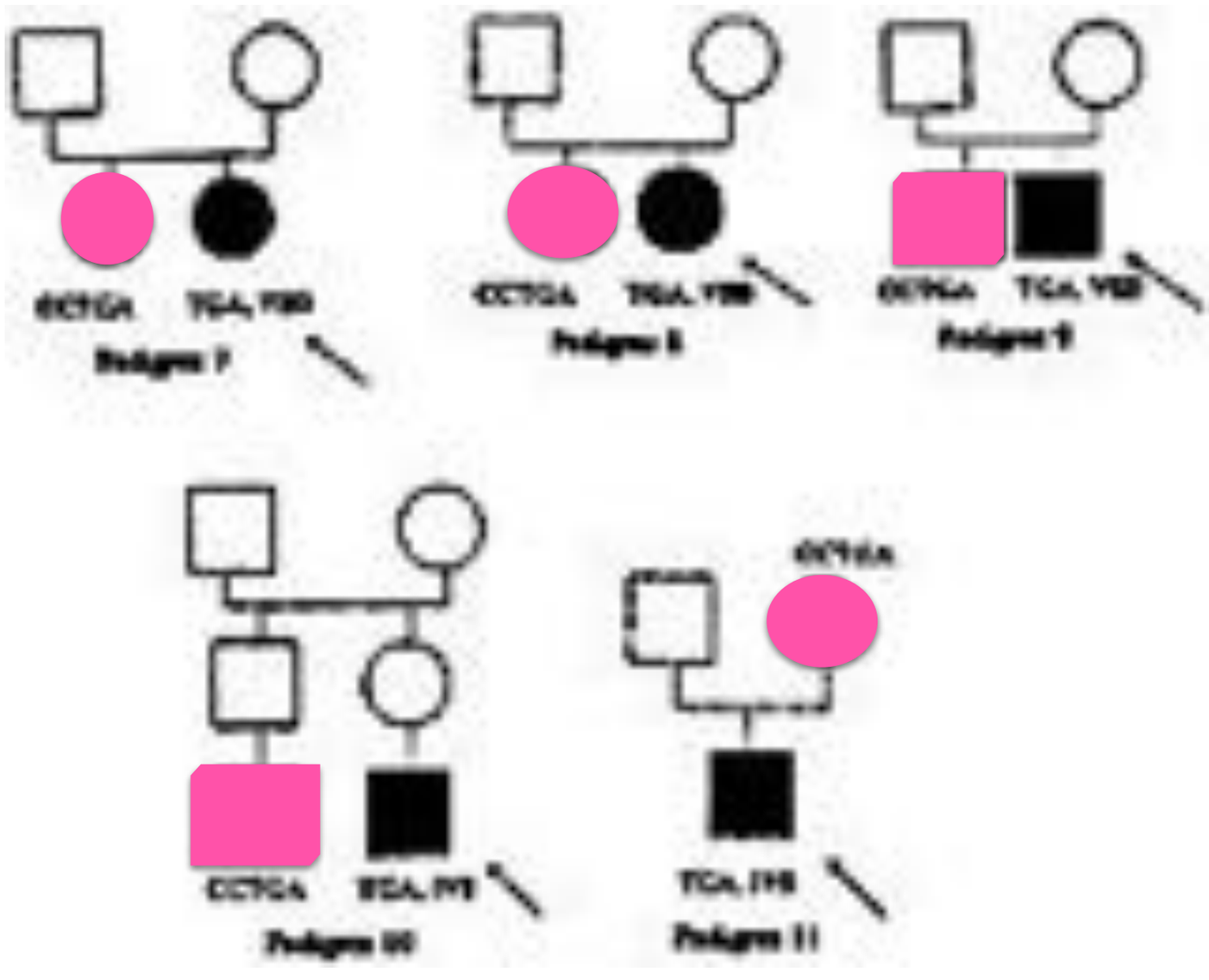
It is a laterality (rotation) restricted to a single segment of the developing heart



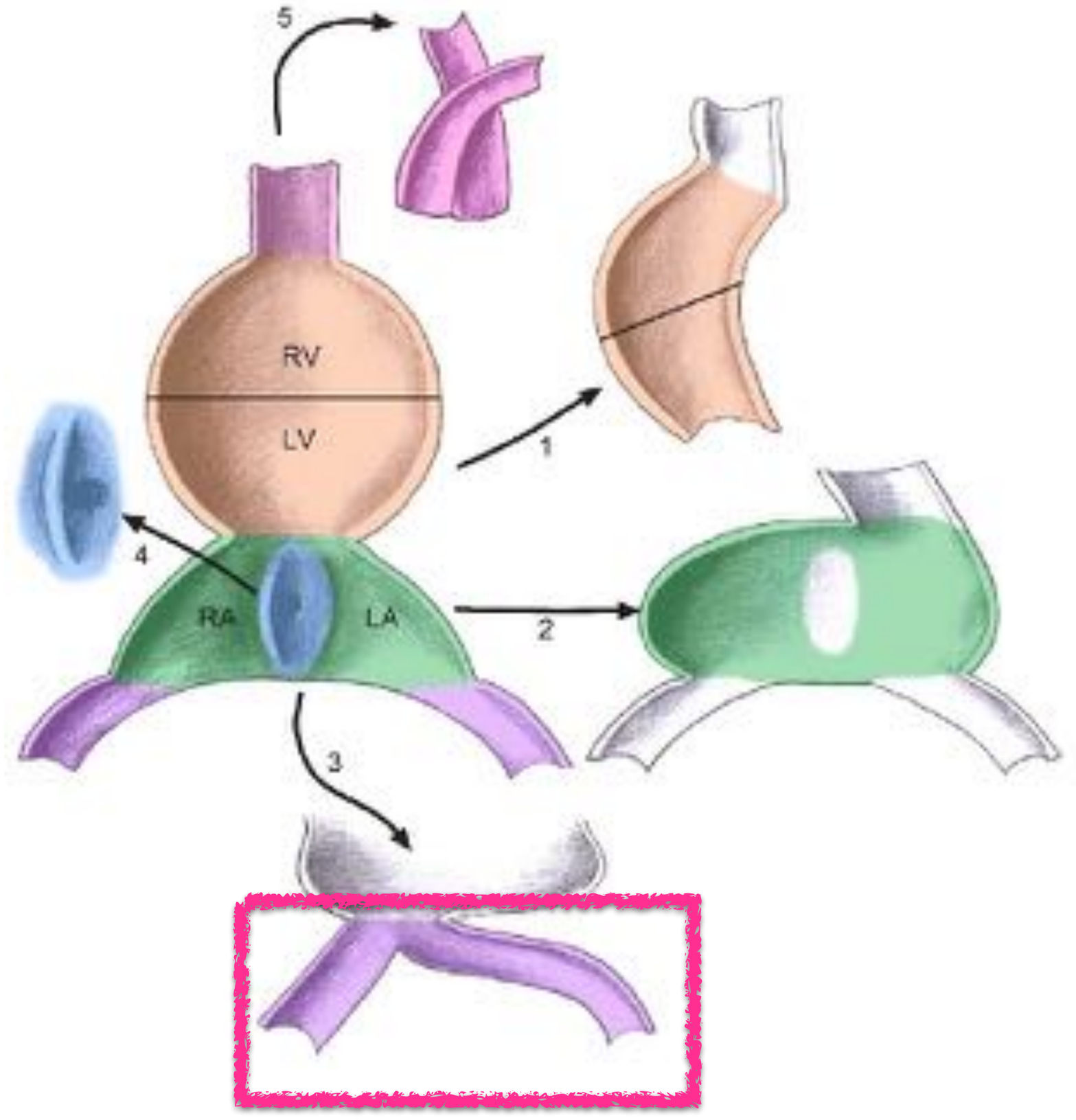
5 levels of asymmetry in the developing heart



Families TGA & CC-TGA

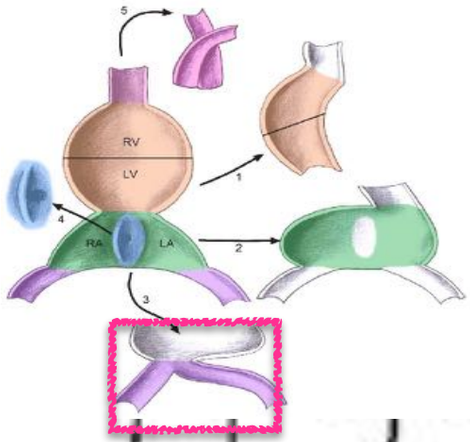


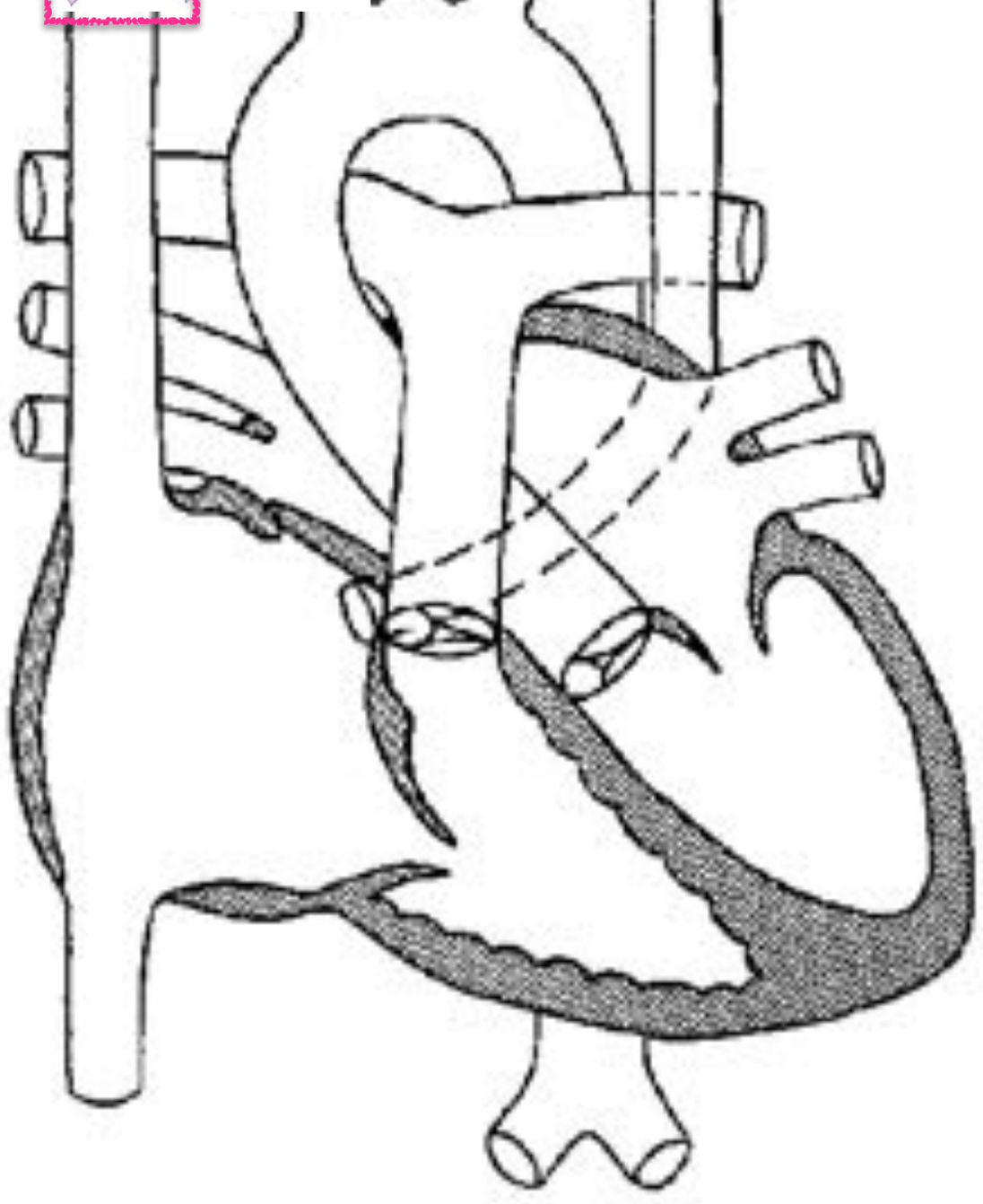
Digilio MC et al. Circulation. 2001;104:2809-2814.

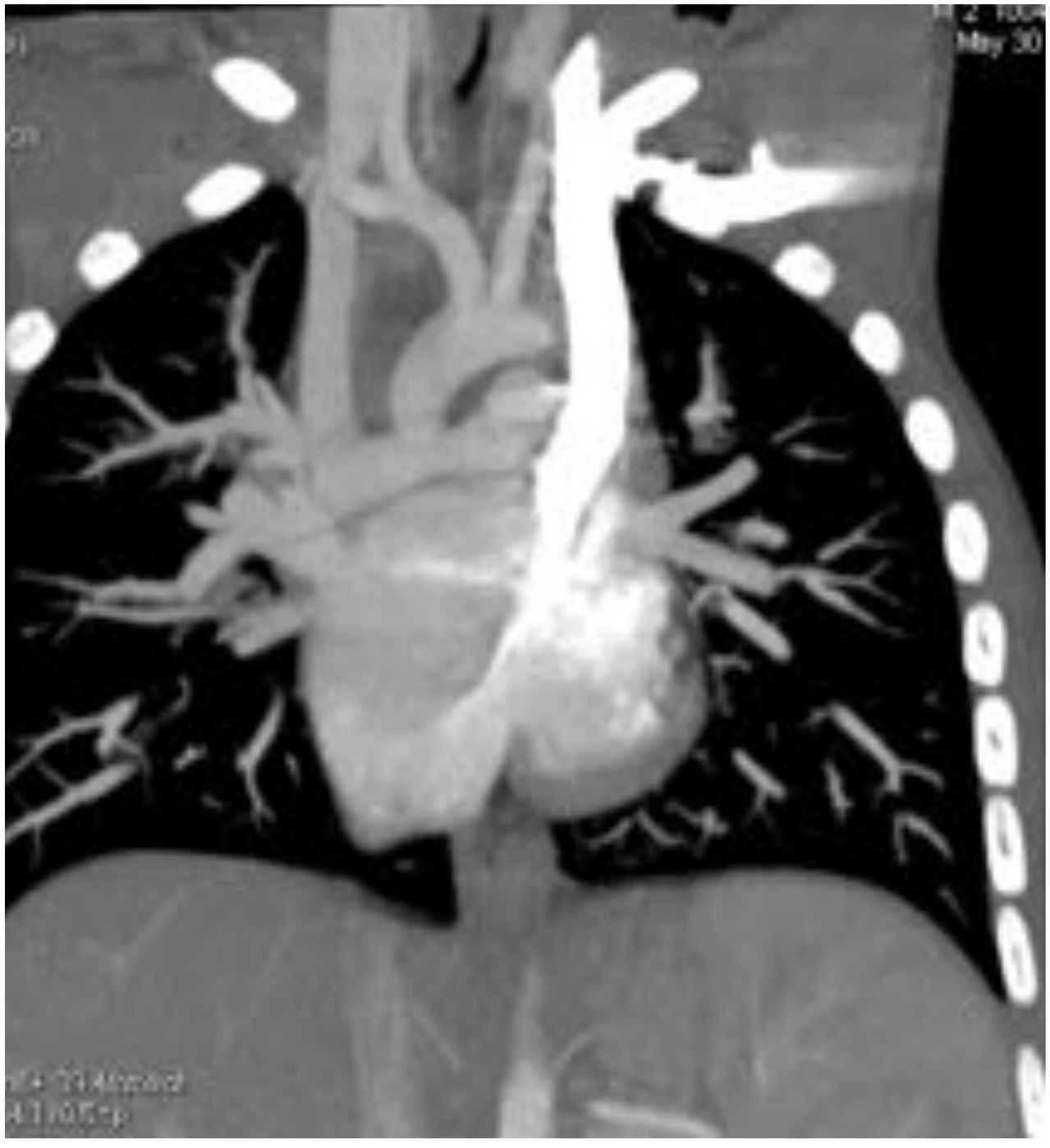


5 levels of asymmetry in the developing heart

Think out of the box







Left superior caval vein

What you see is not what it is ?

LSCV is a common finding

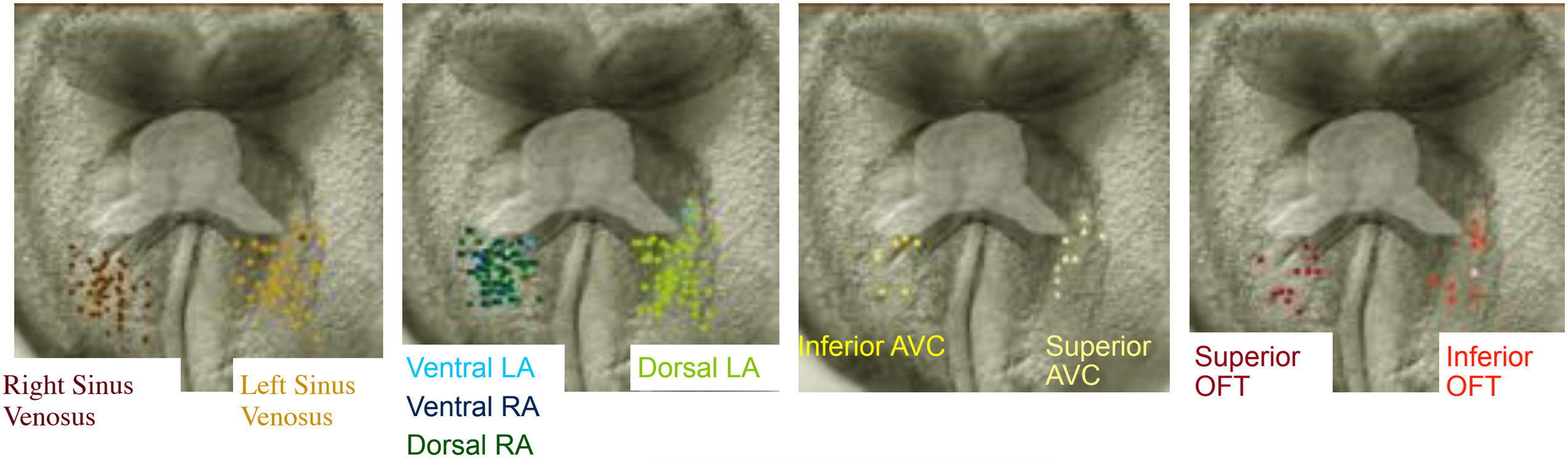
…that may be associated with aortic coarctation

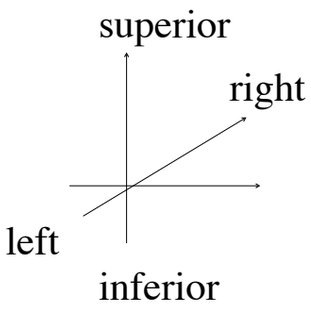
The clinical diagnosis is COARCTATION, the
« embryological » diagnosis is systemic vein maldevelopment

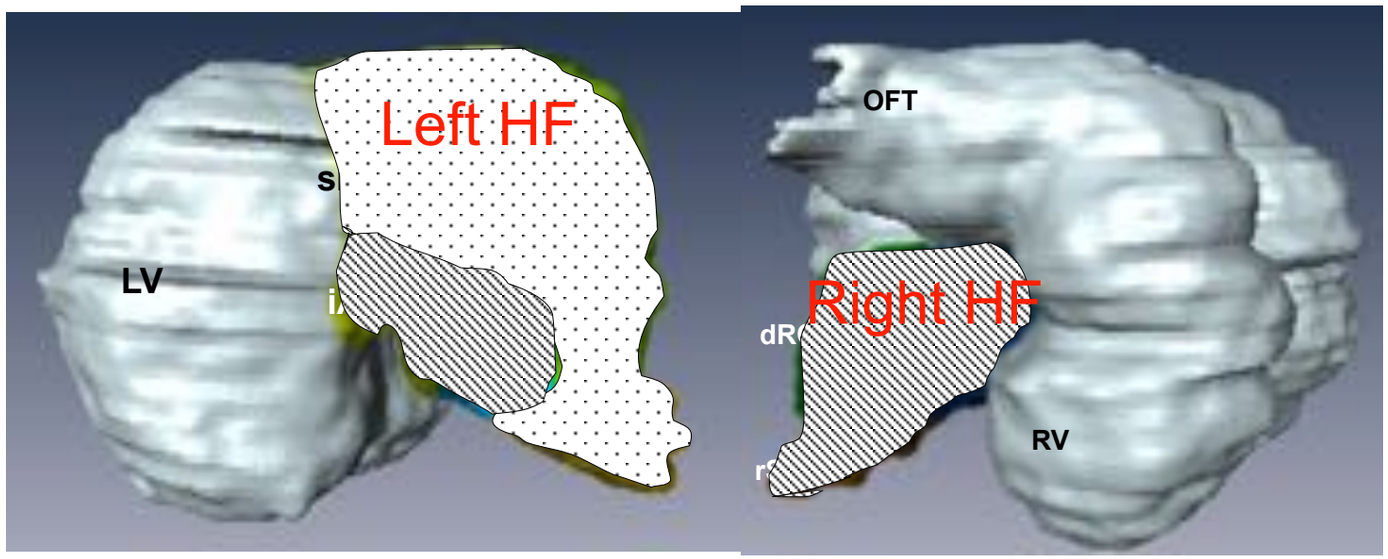
7 families in our data base with:

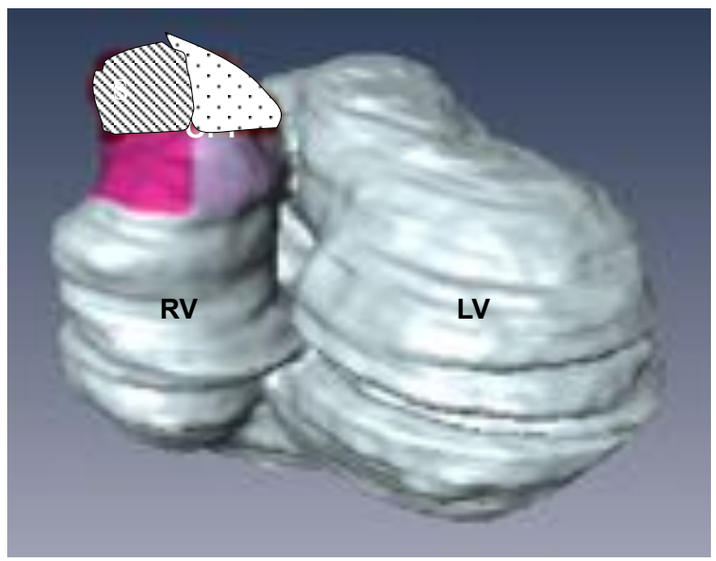
- Index case: let heart obstructive defect + LSCV
- Recurrence in siblings : laterality defects

Fate map of left-right heart precursors









Twisted left/right regionalisation of the heart

Dominguez et al., 2012

Courtesy Sigolène Meilhac

How to explain genetic heterogeneity ?

One malformation - different genes

Second heart field

-Abnormal contribution
-Abnormal proliferation

Neural crest cells

-Abnormal migration
-Abnormal proliferation

Myocardium

-Abnormal rotation
-Abnormal laterality

Endocardium

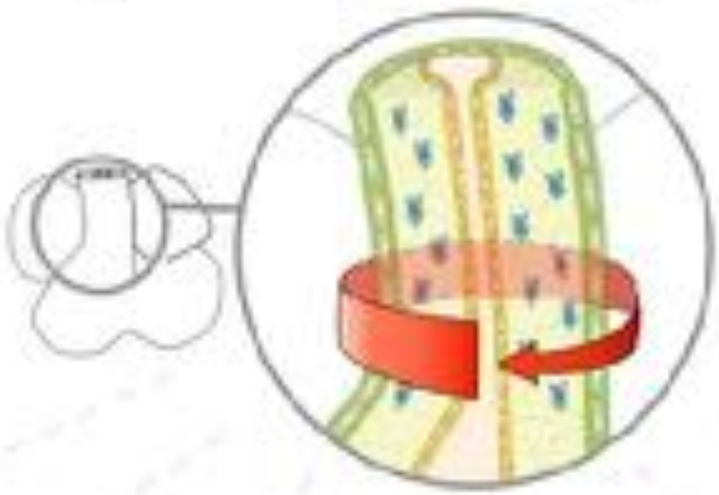
-Abnormal EM-transformation
-Abnormal proliferation

Elongation defect

Septation defect

Alignment defect

Cushion defect



Remodeling of
the outflow tract

Myocardium

Neural crest cells

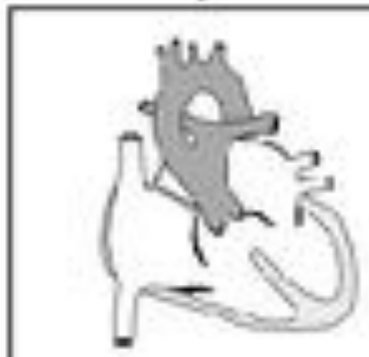
Endocardium

Endocardial cushions

TOF

TOF&PA

IAA

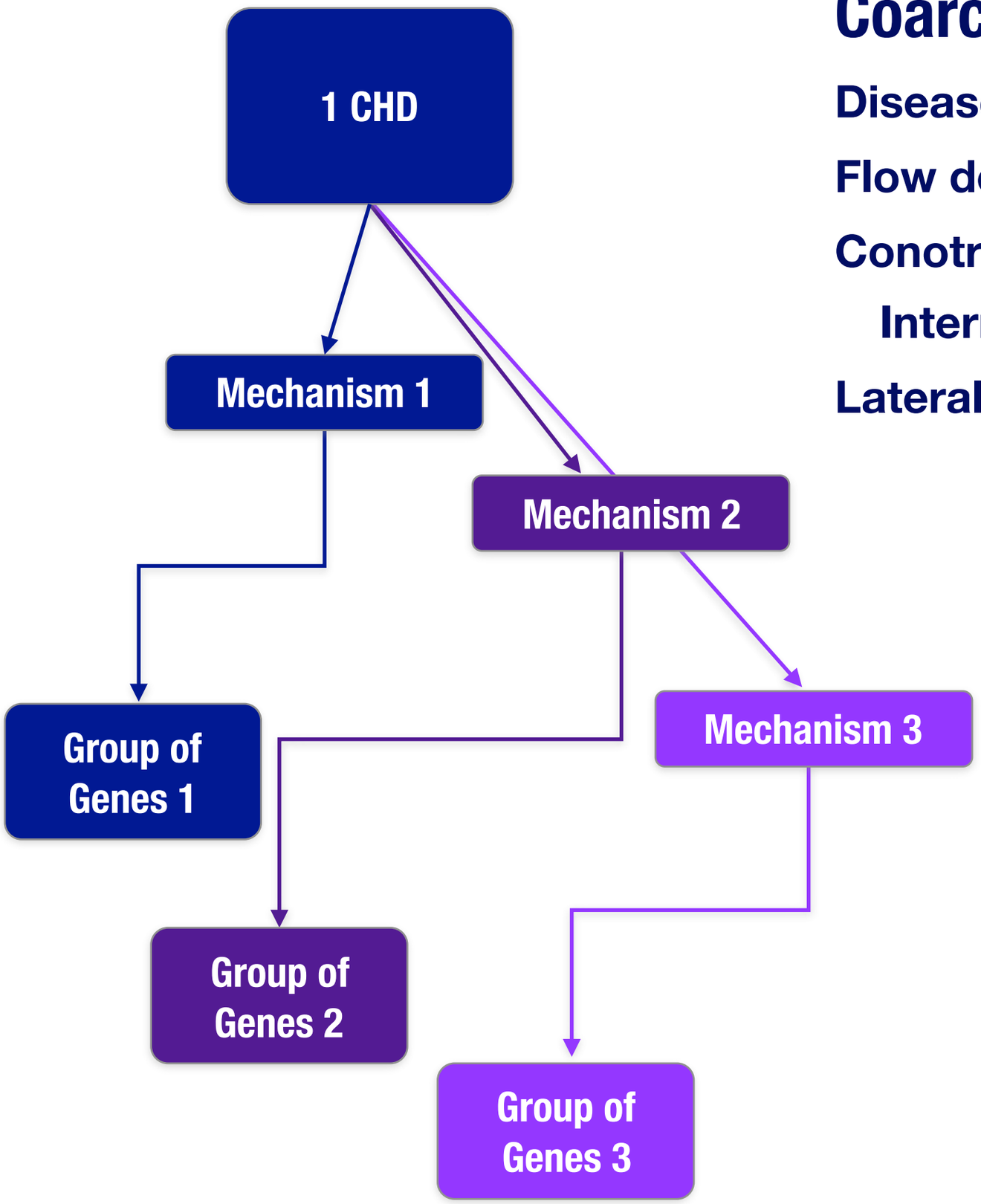


CAT

VSD

DORV

TGA



Coarctation of the aorta

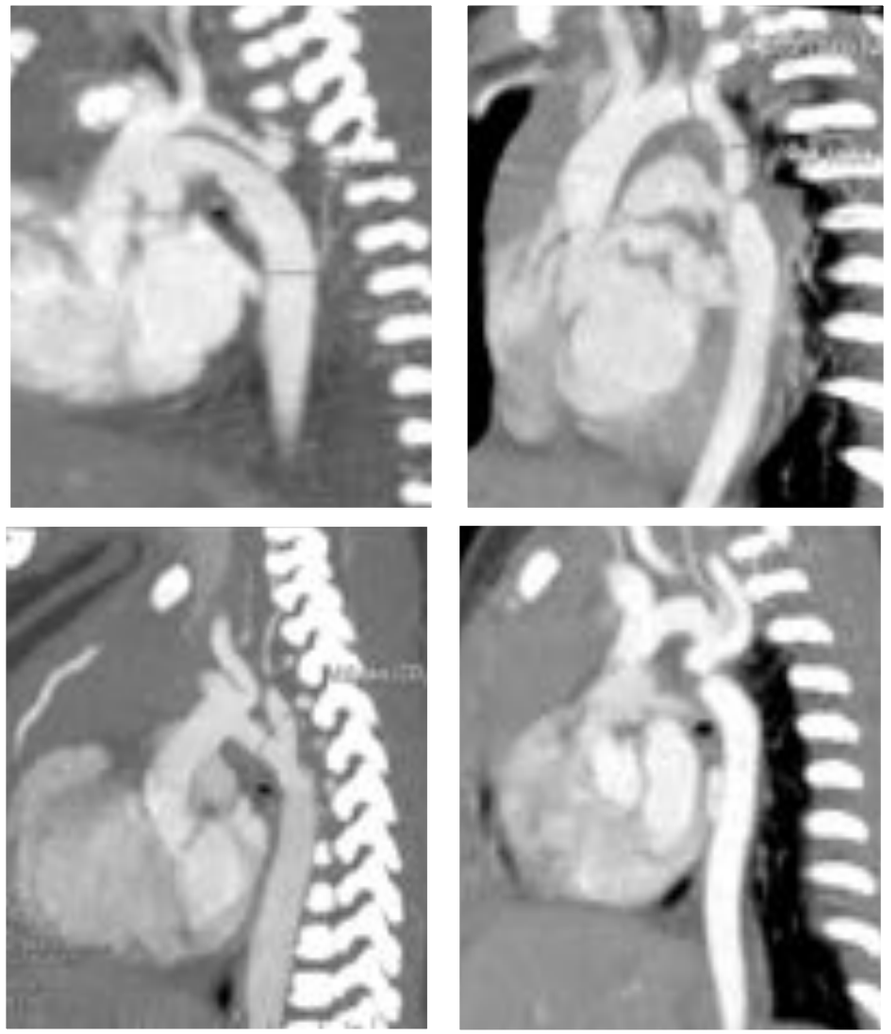
Disease of the aortic isthmus

Flow defect : spectrum of HLHS

Conotruncal defect

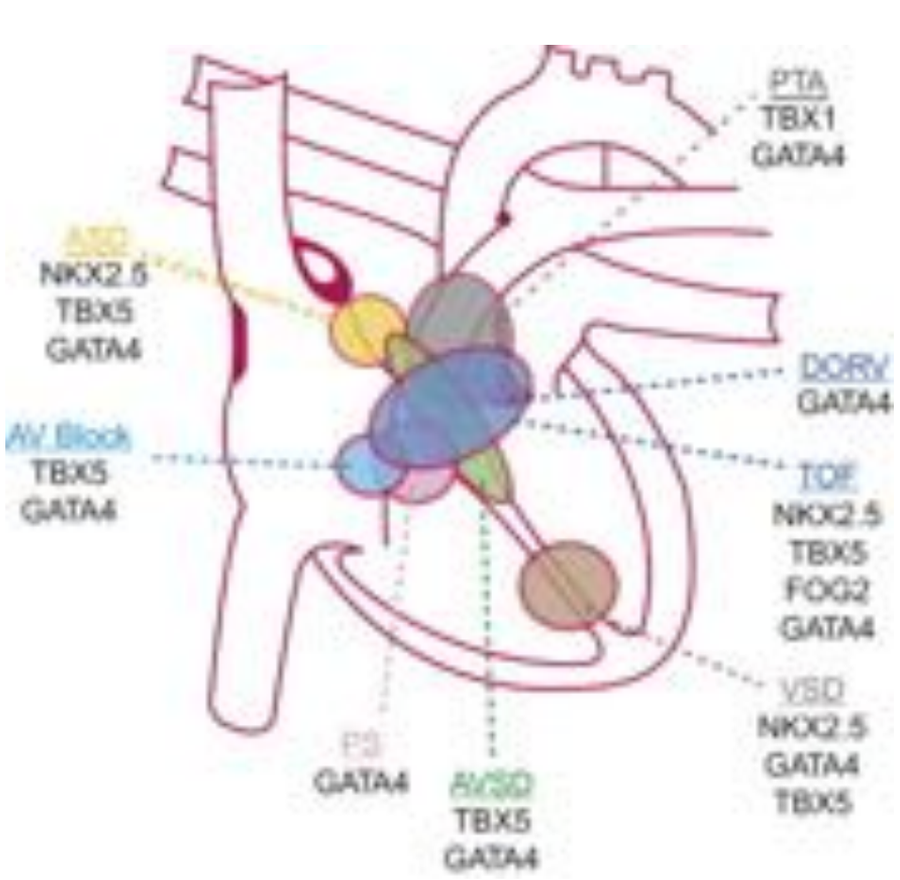
Interrupted aortic arch

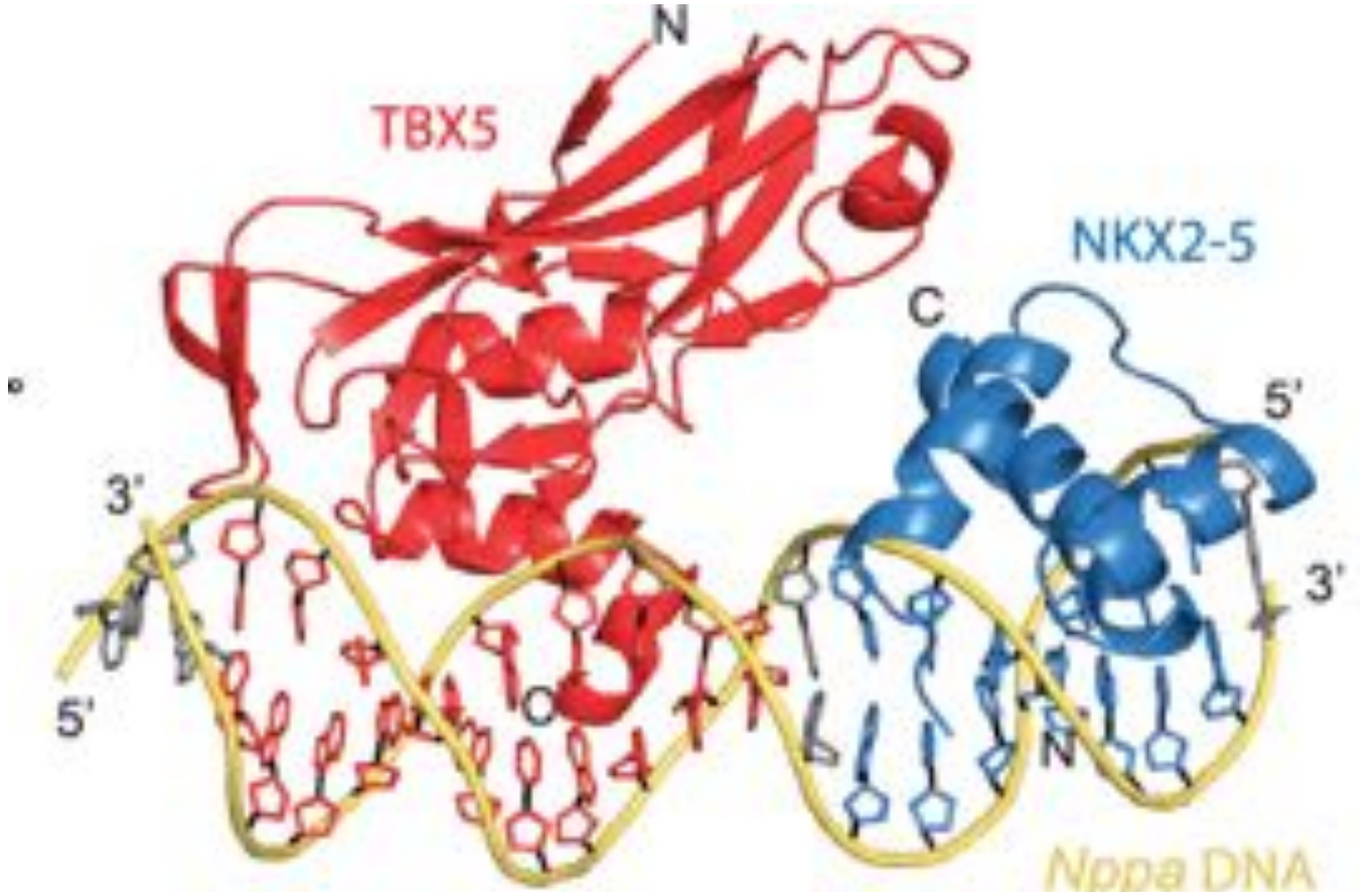
Laterality defect with persisting LSCV



Interdependency between transcription factors

Genome-scale cooperative interactions between cardiac transcription factors coordinate gene expression during cardiac differentiation and morphogenesis. Cooperative DNA binding depends on preferential motif arrangements and serves not only to activate lineage-appropriate genes, but also to prevent transcription factors from redistributing to other genomic sites and activate lineage-inappropriate genes.

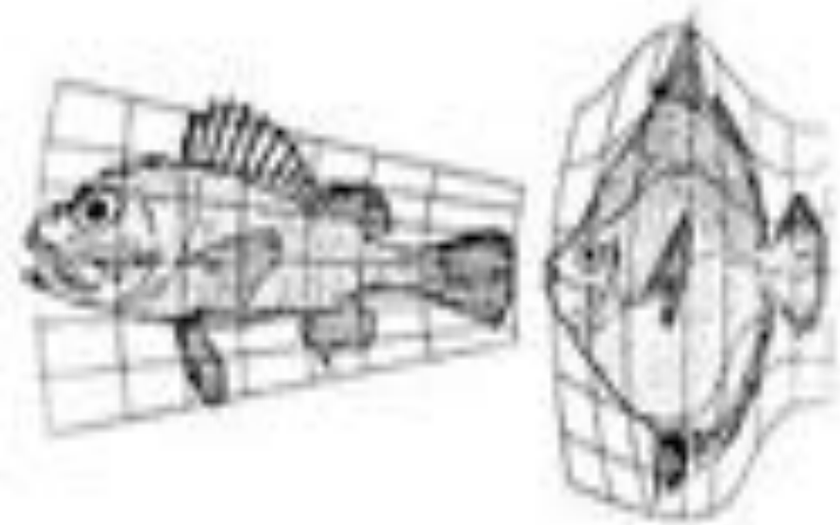


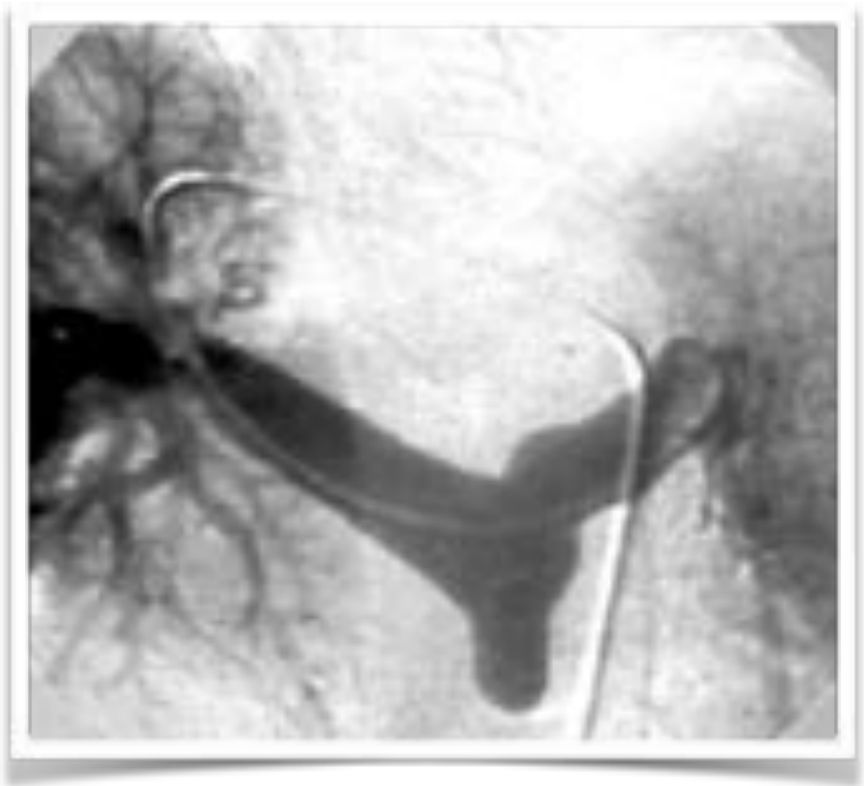


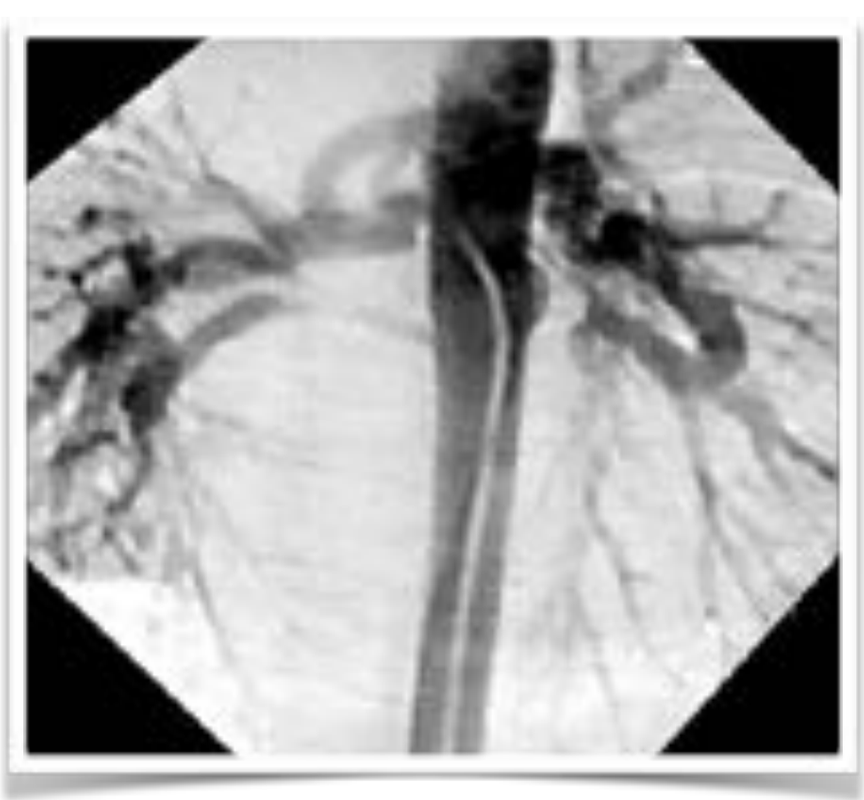
Luna-Zurita L, et al. Cell 2016

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

Genetic heterogeneity

Interdependency of molecules
involved in heart development

Familial CHD mutations

Different modes of inheritance
High penetrance
Variable clinical manifestations

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.

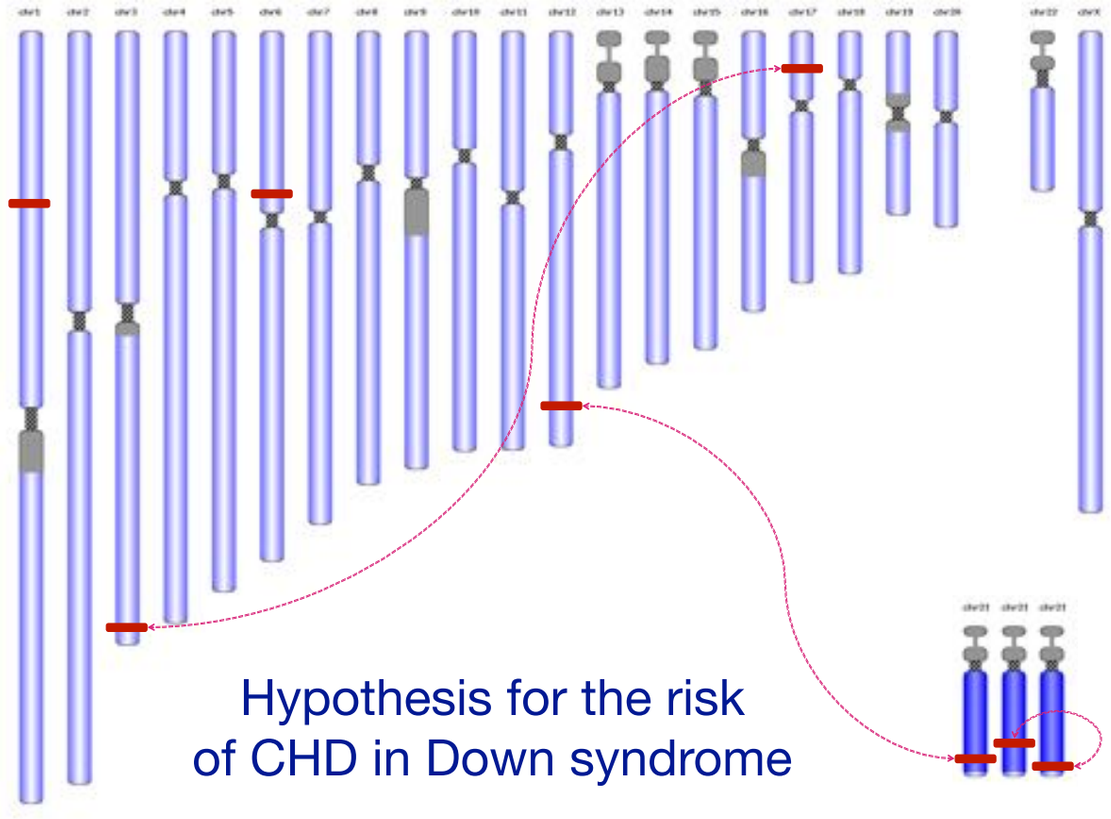
Fahed AC et al. Circ Res. 2013;112:707-720

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

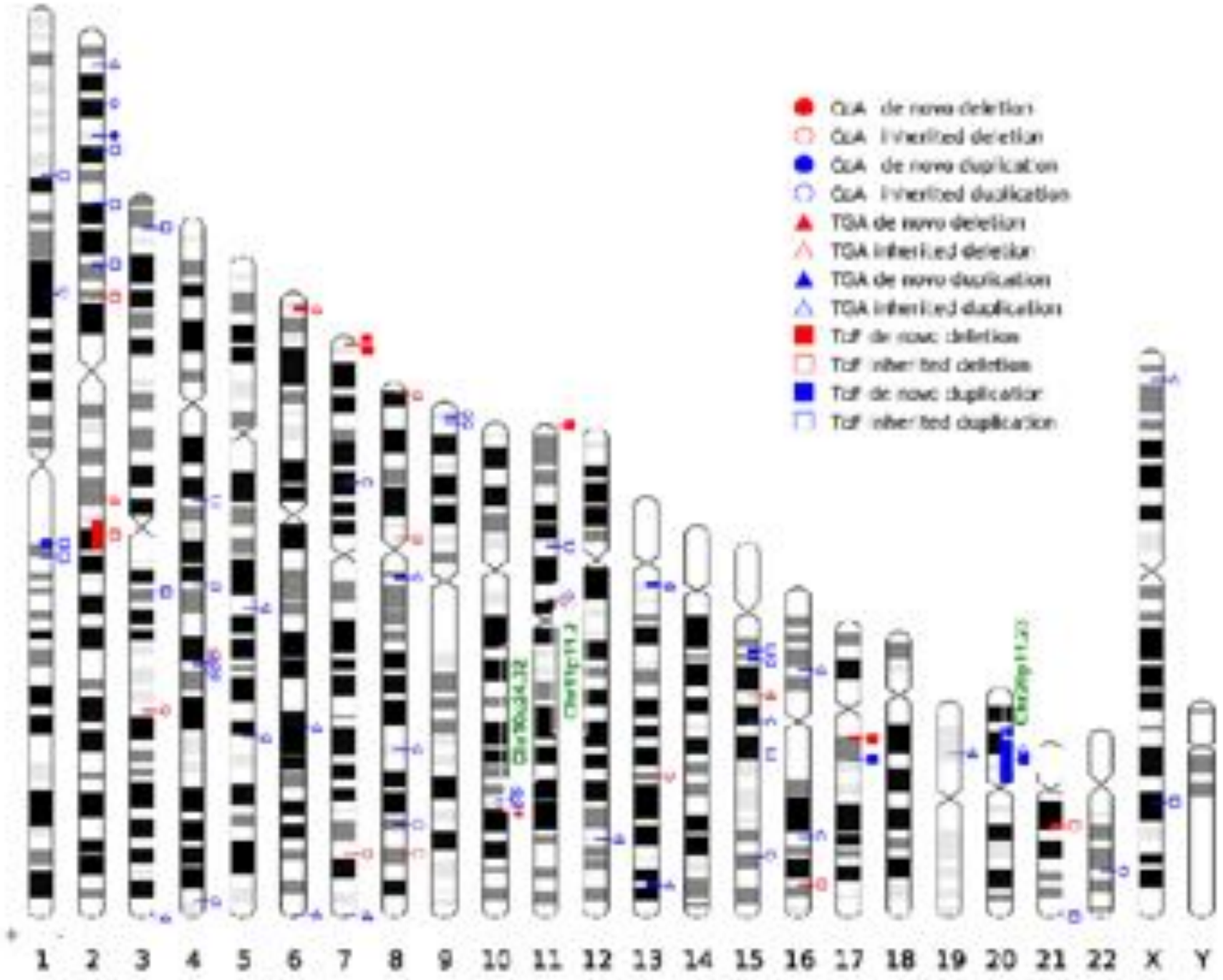


Genomic variability of chr21 (trisomic regions) may contribute to the CHD in Down syndrome.

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.

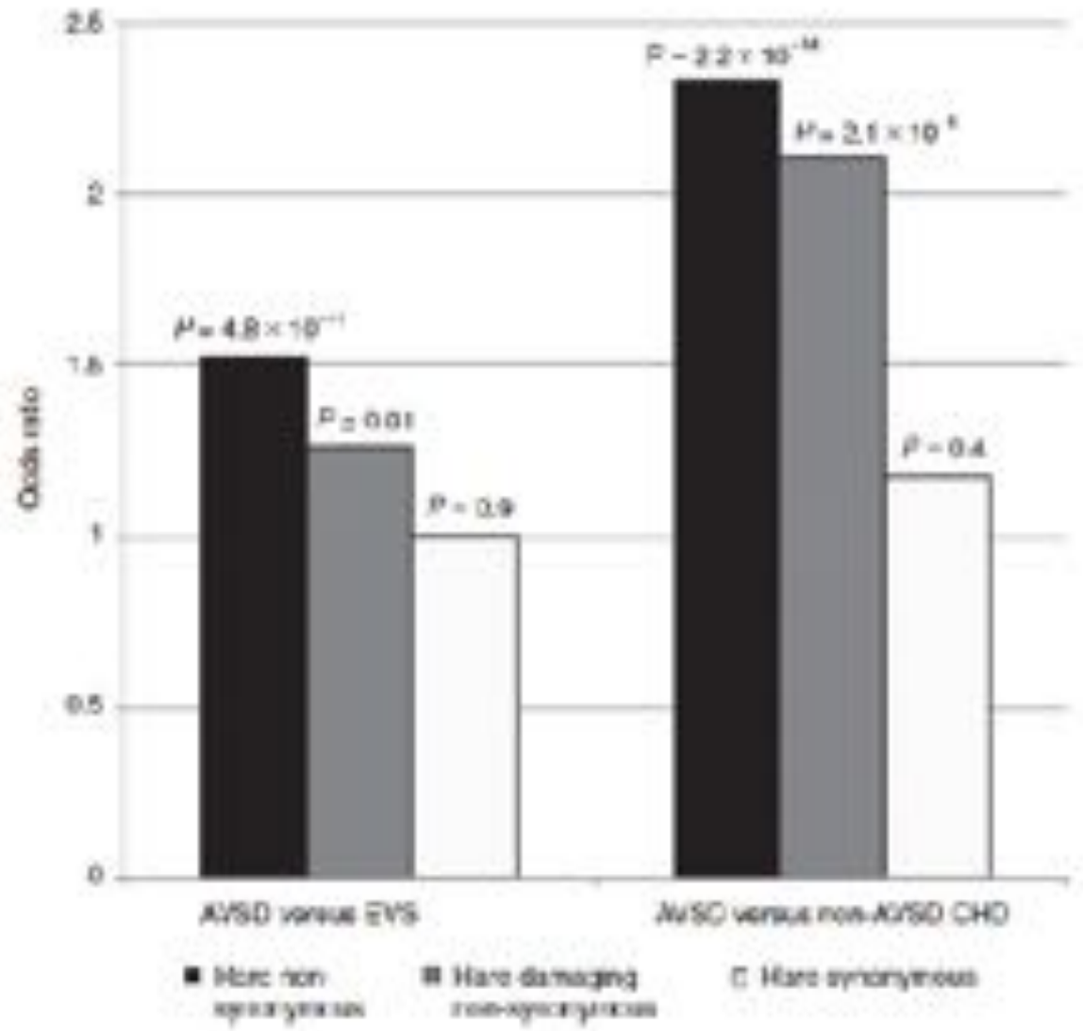


Sanchez-Castro M et al. Circ Cardiovasc Genet 2016;9:86-94

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

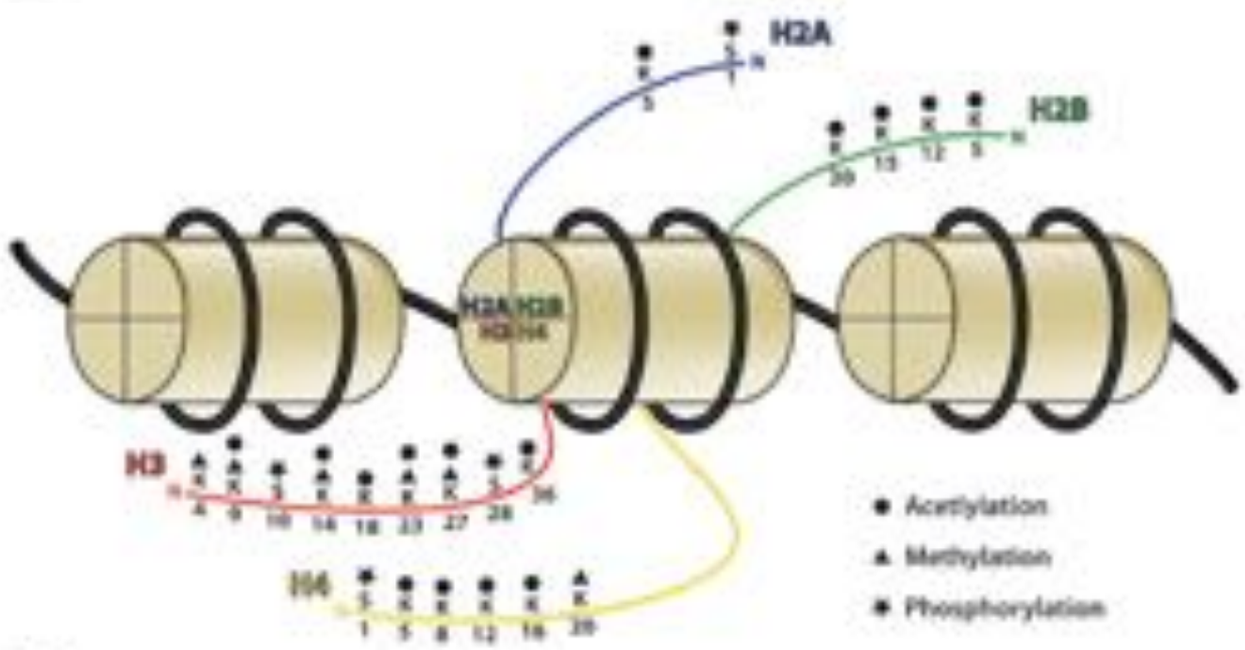
Total N/litters	Control 30/4		Diabetes 62/15		Control NAC 30/4		Diabetes NAC 43/7	
	n	%	n	%	n	%	n	%
Normal	30	100	26	41.9**	27	90	36	83.7††
Abnormal	0	0	36	58.1**	3	10	7	16.3††
ASD	0	0	19	30.6**	2	6.7	6	13.9†
VSD	0	0	25	40.3**	1	3.3	5	11.6††
AVSD	0	0	4	6.5	0	0	0	0
TGA	0	0	4	6.5	0	0	0	0
DORV	0	0	8	12.9*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

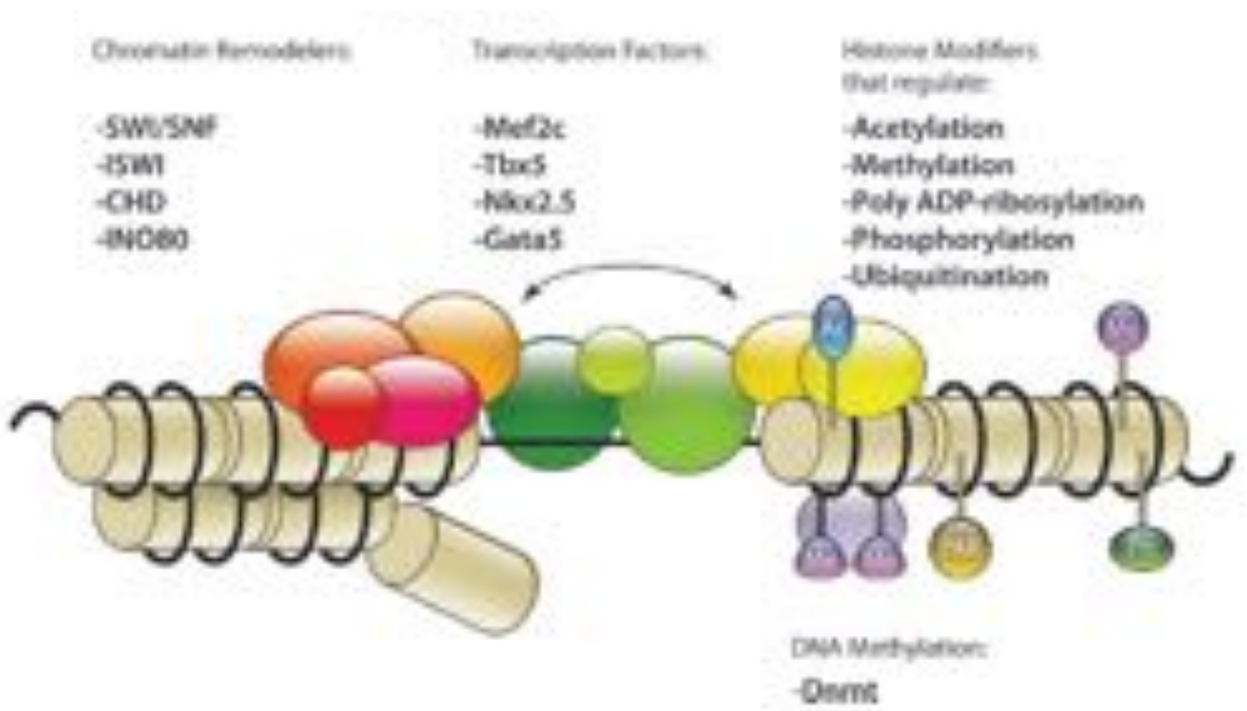
Moazzen et al. Cardiovascular Diabetology 2014

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.

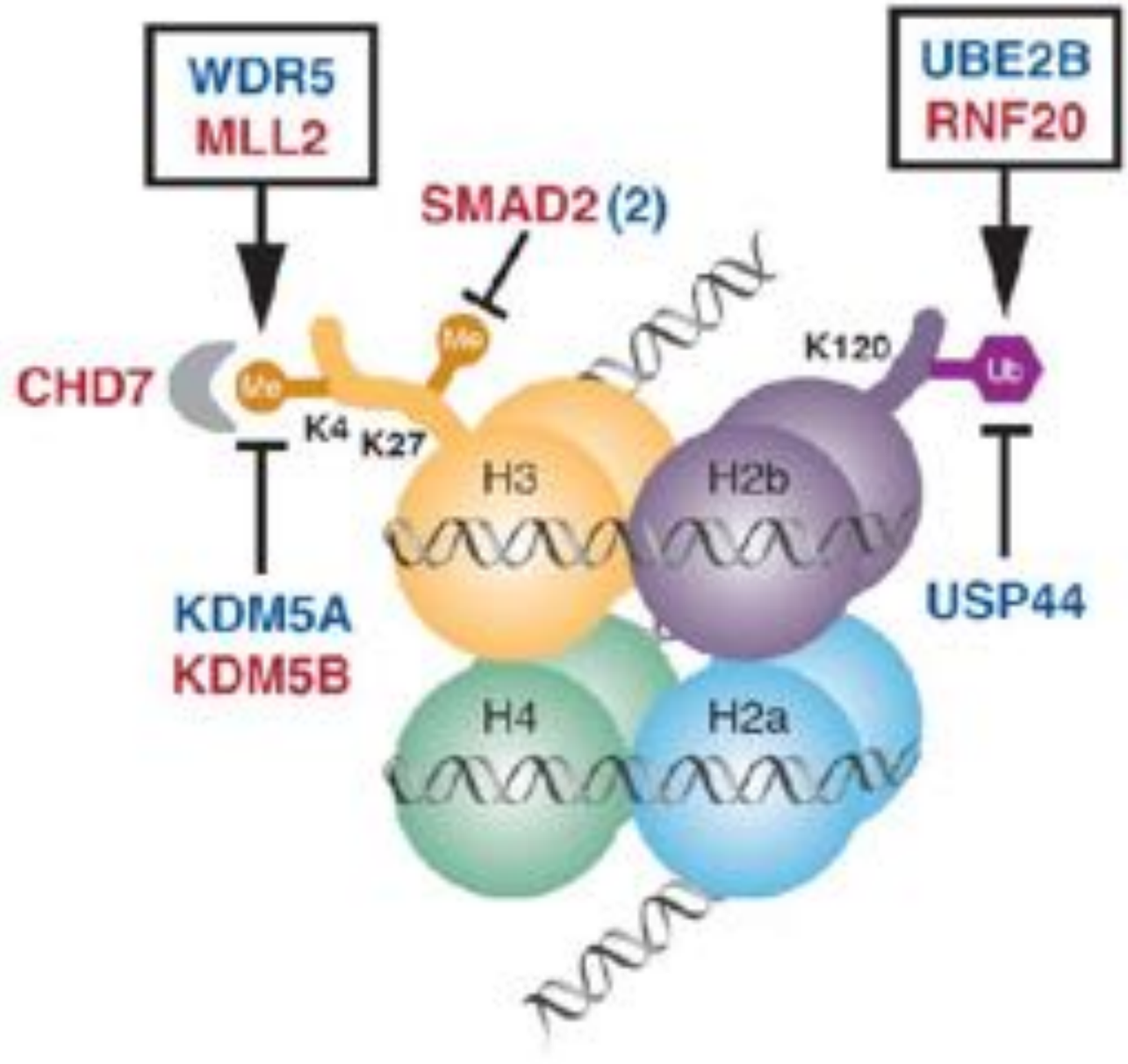
Rôle of maternal aging

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

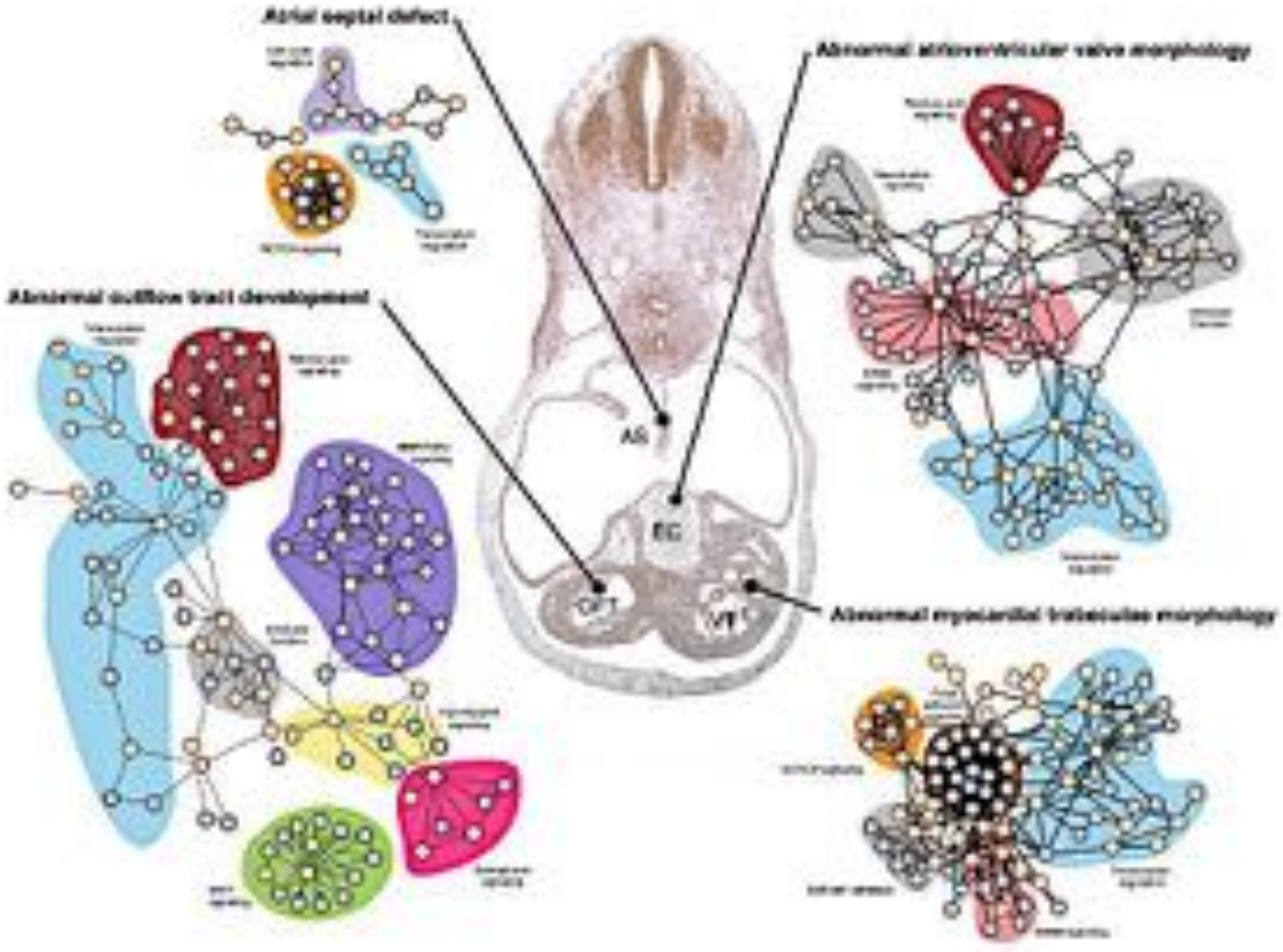
De novo mutations in histone modifying genes in congenital heart disease



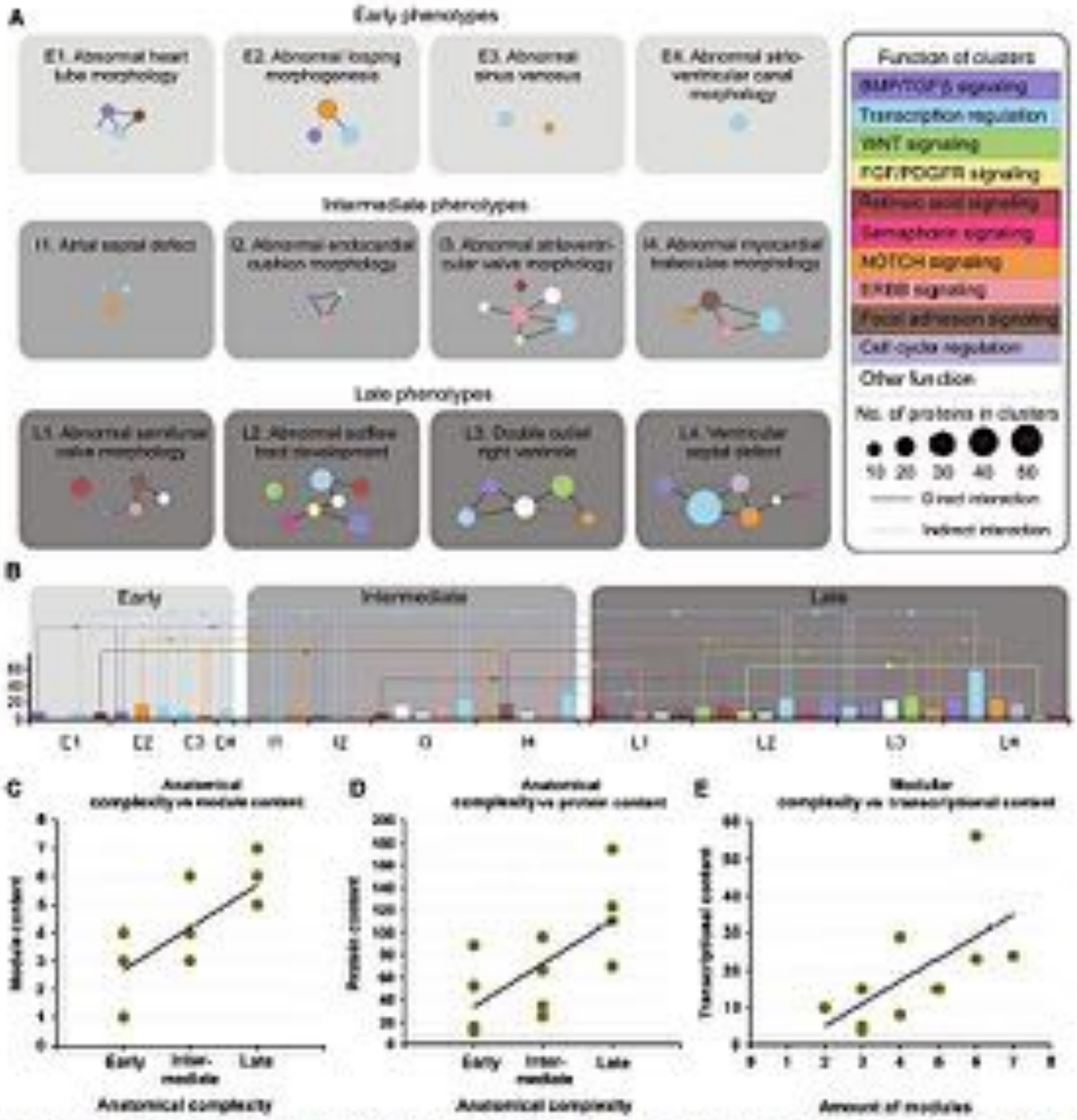
De novo mutations in the H3K4 and H3K27 methylation pathways

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

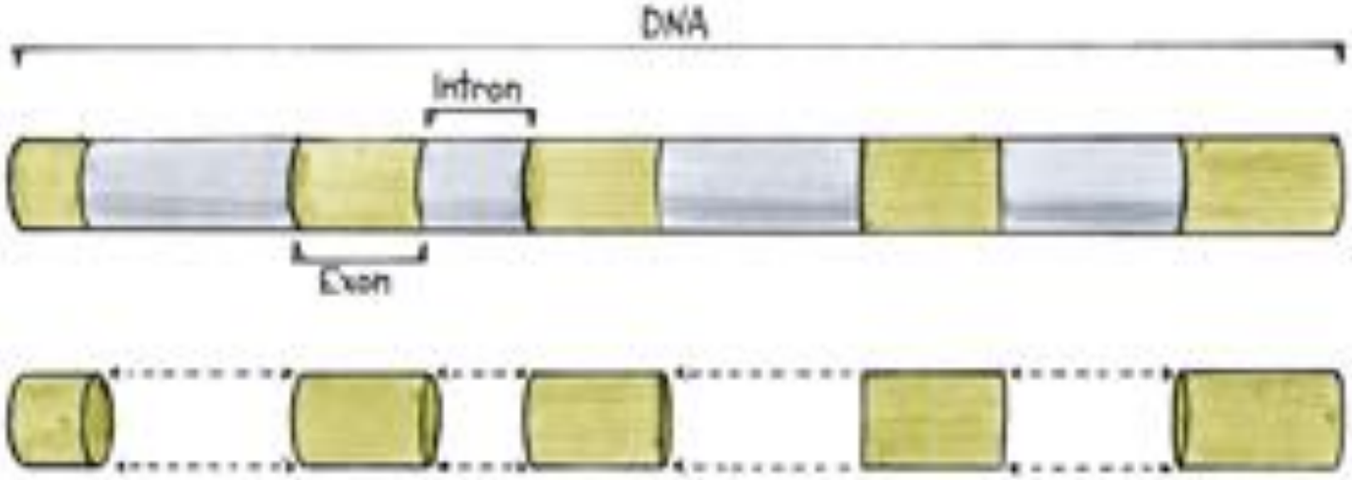
Biological networks in CHDs

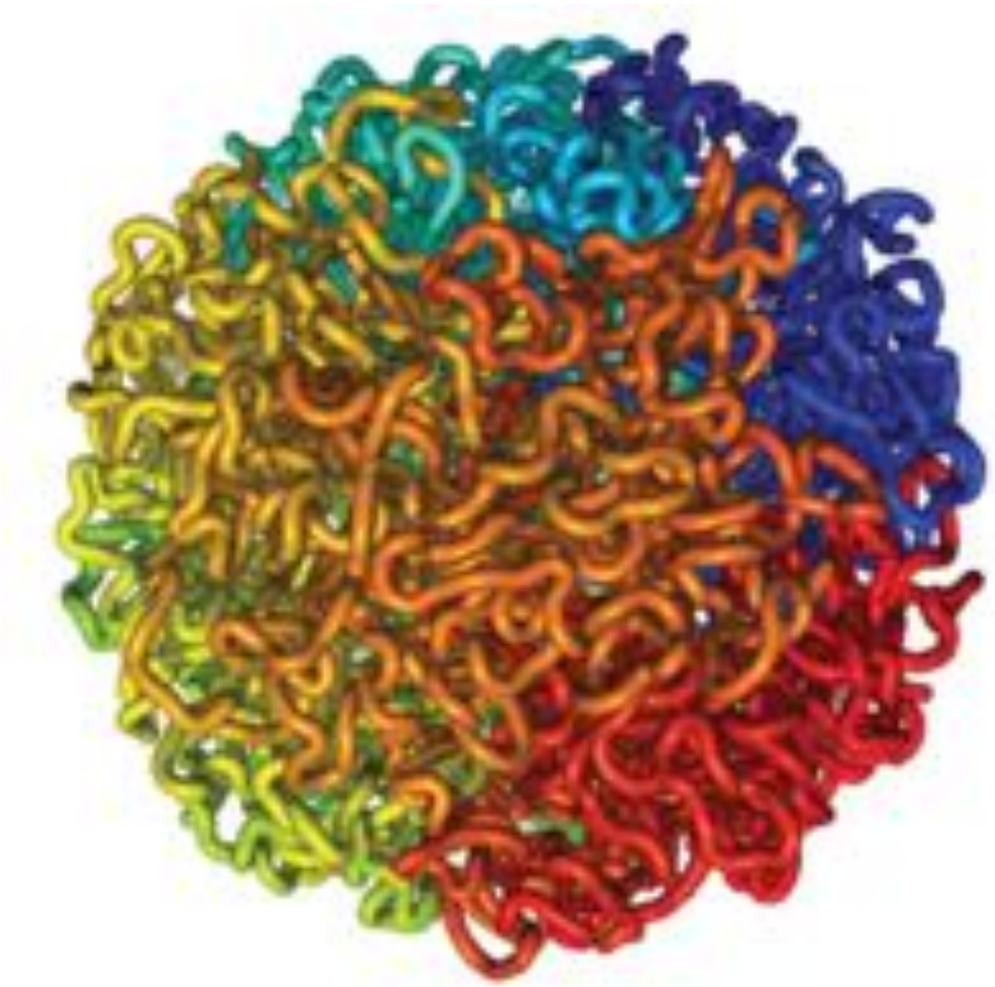


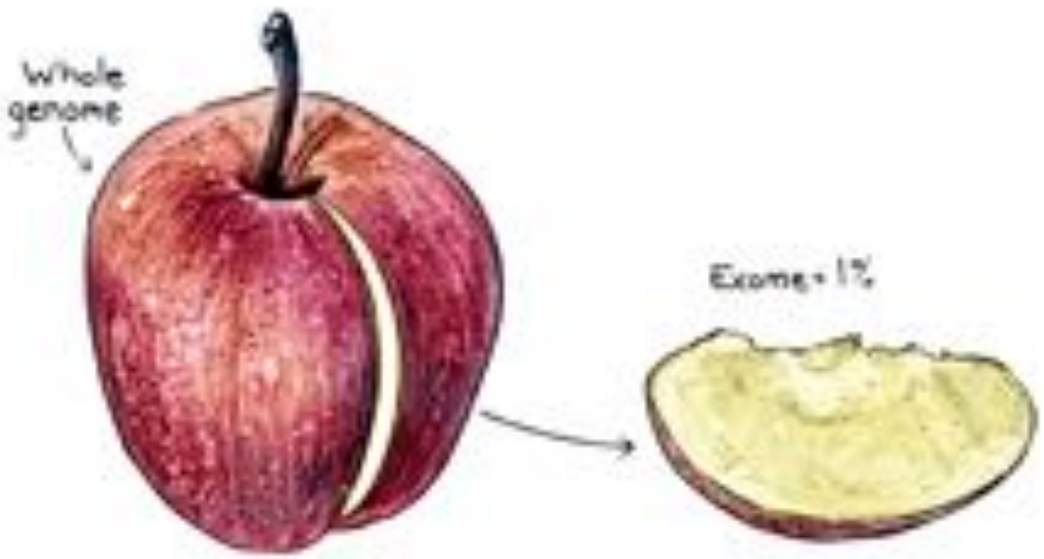
Overview of the molecular organization of heart development

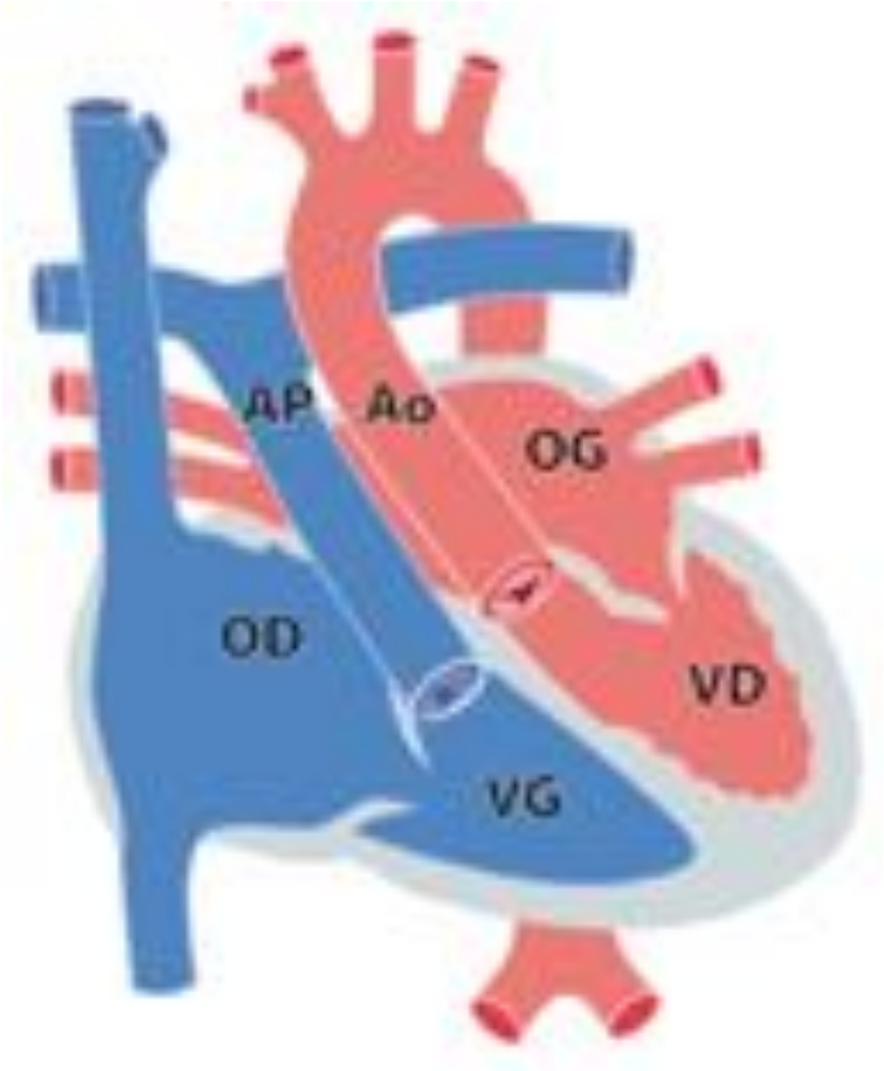


Non-coding DNA ?









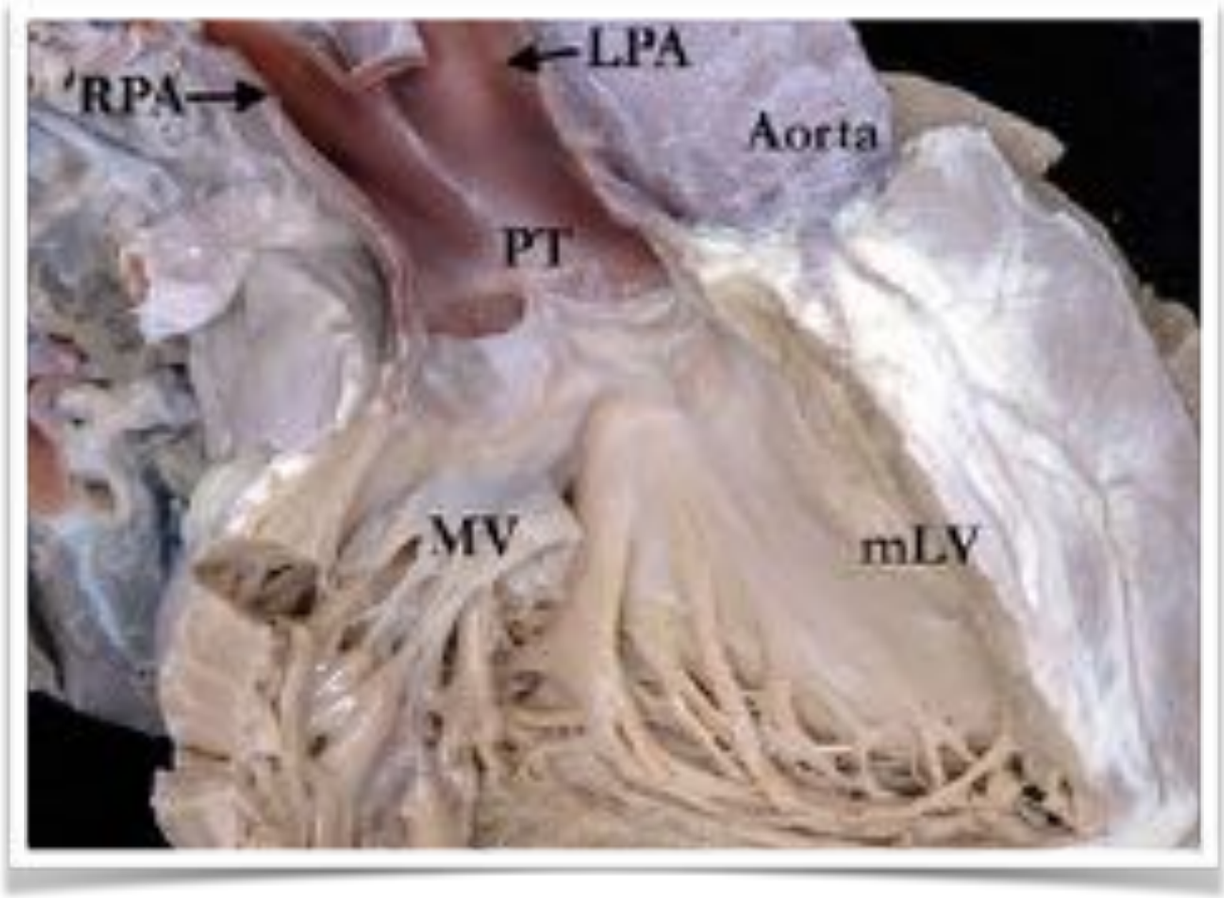
The Double Discordance genome program

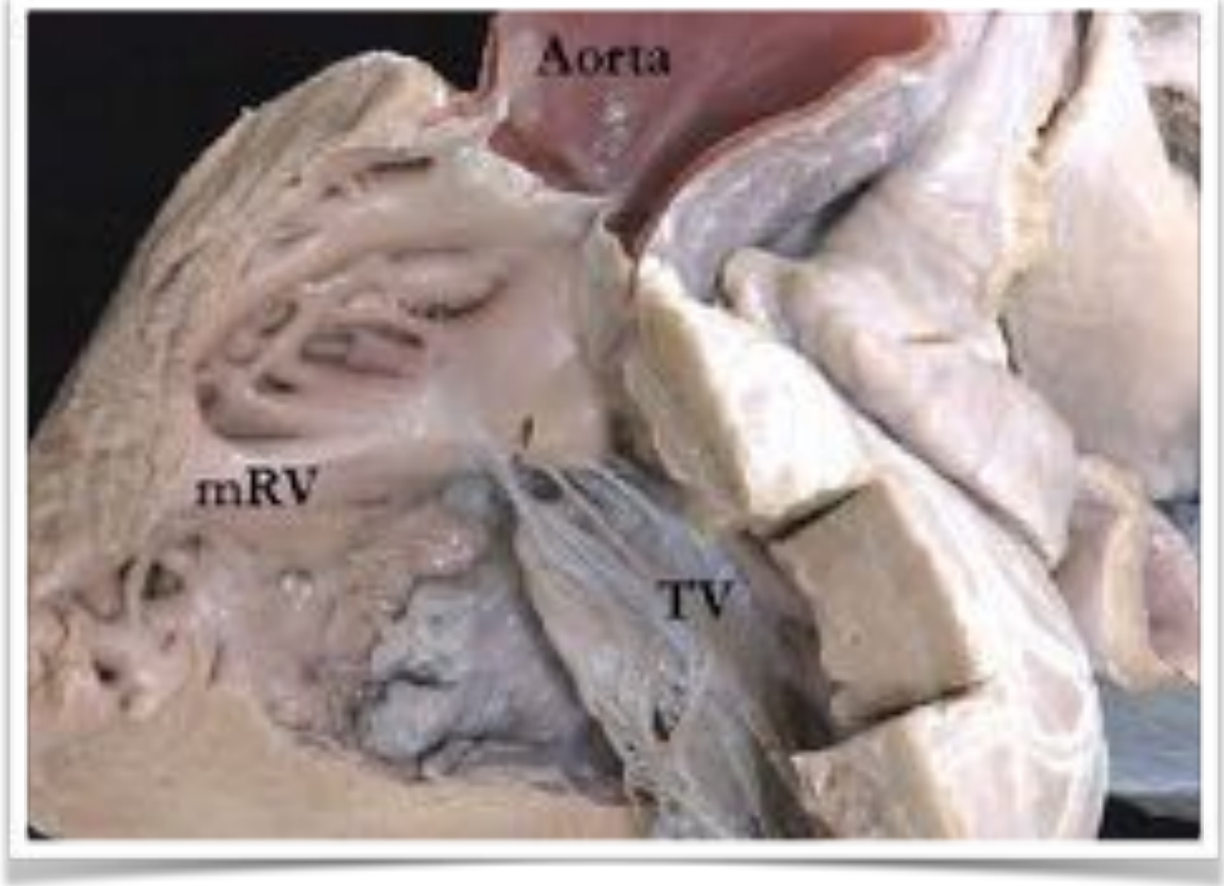
Stanilas Lyonnet
Sigolène Meilhac
Damien Bonnet-Fanny Bajolle

M3C

FRANCE GÉNOMIQUE

imagine
INSTITUT DES MALADIES GÉNÉTIQUES





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

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- 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

Thank you