Génétique des cardiopathies congénitales

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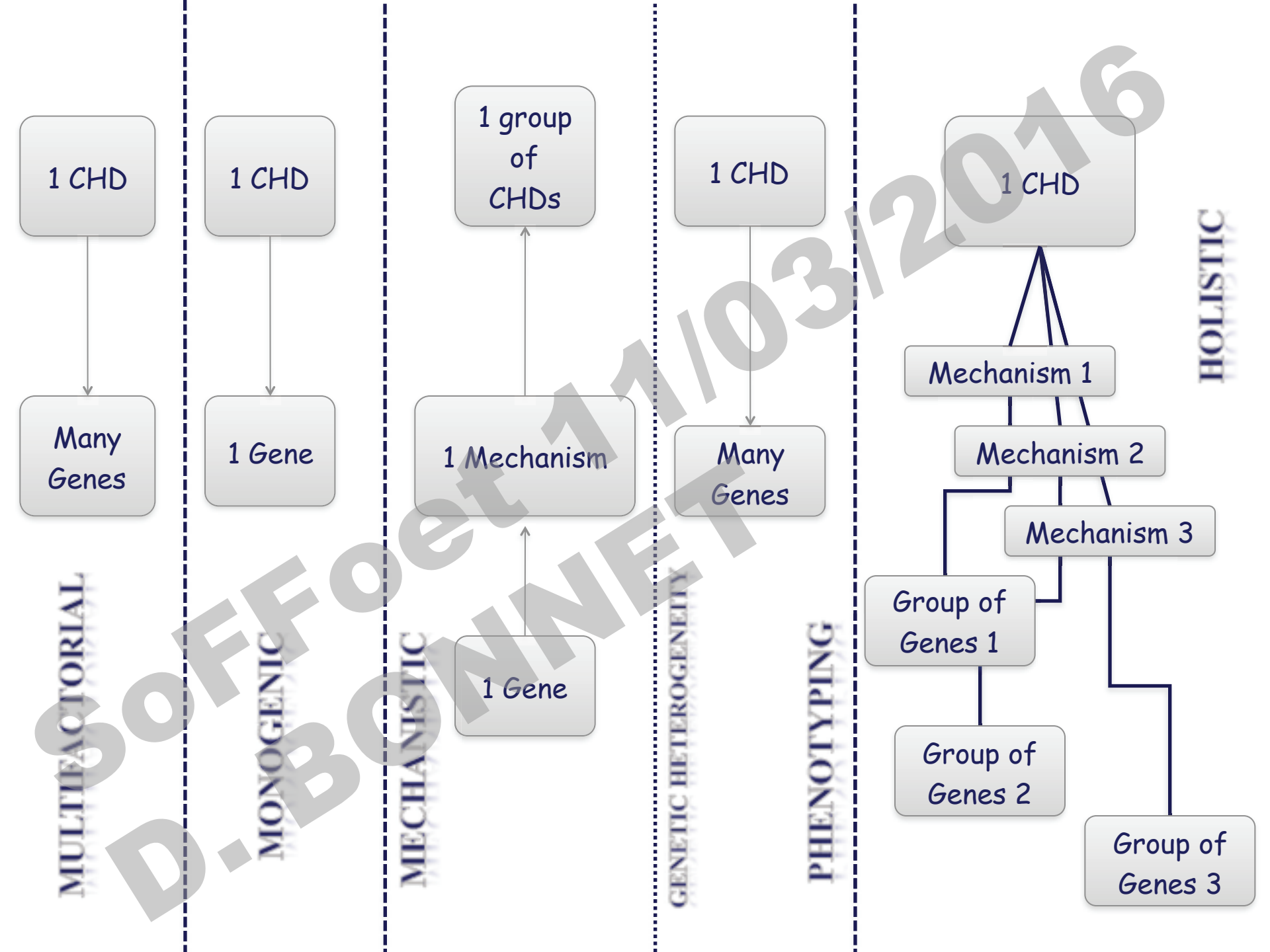
1 CHD
Many Genes
MULTIFACTORIAL
1 CHD
1 Gene
MONOGENIC
1 group of CHDs
1 Mechanism
1 Gene
MECHANISTIC
1 CHD
Many Genes
GENETIC HETEROGENEITY
PHENOTYPING
1 CHD
Mechanism 1
Mechanism 2
Mechanism 3
Group of Genes 1
Group of Genes 2
Group of Genes 3
HOLISTIC
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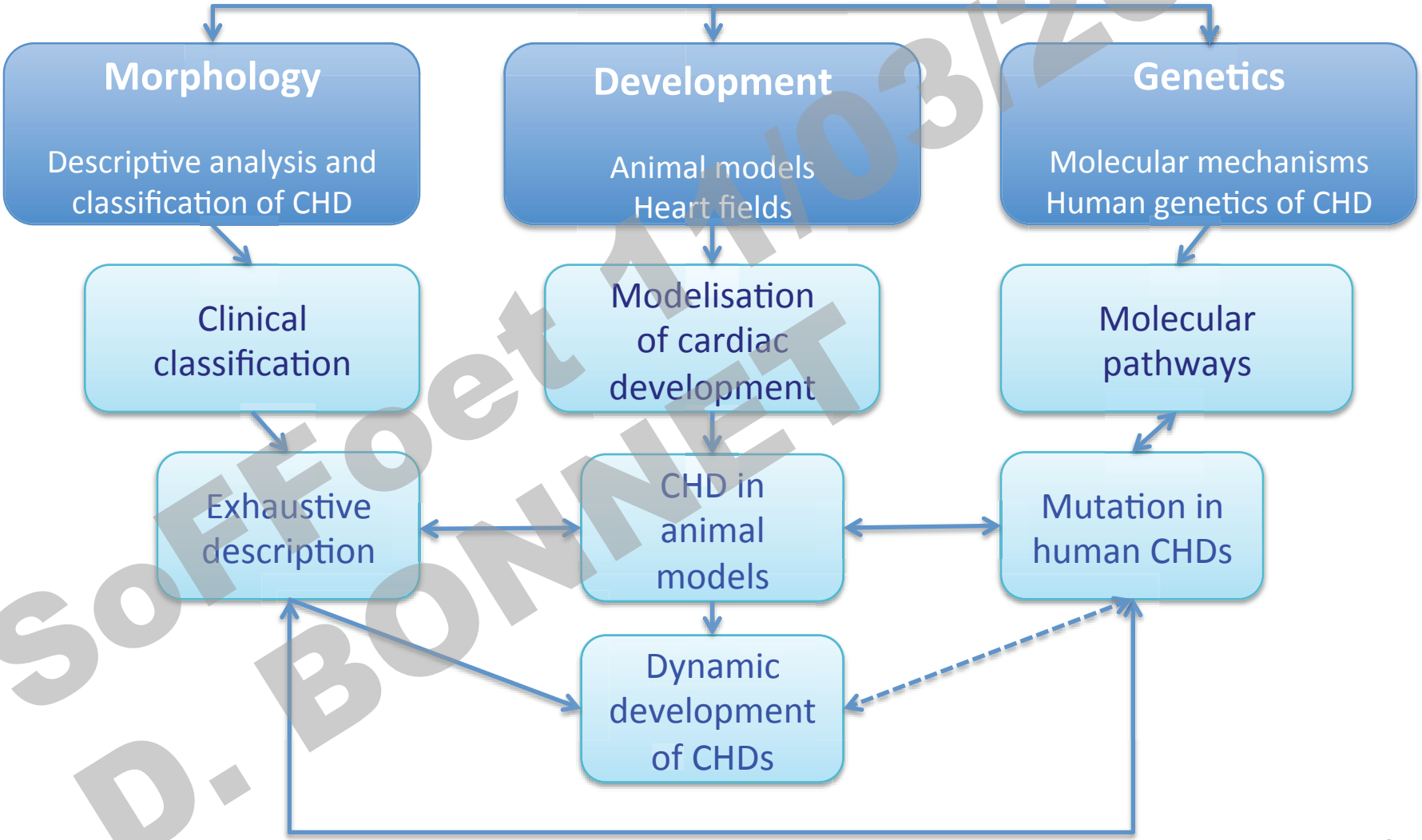
Holistic approach to genetics of human CHD



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The glass half empty

- Multiple experimental animal models
  - perturbing selected molecules that function in the dev. pathways involved in myocytes specification, differentiation, or cardiac morphogenesis
- The precise genetic, epigenetic, or environmental basis for these perturbations in humans remains poorly understood

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How to bridge this knowledge gap ?

- Genome-wide analysis in rare mendelian CHD families
- Sequencing candidate genes in CHD cohorts

Three notable insights

1. Human CHD mutations impact a heterogeneous set of molecules that orchestrate cardiac development
2. CHD mutations often alter gene/protein dosage
3. Identical pathogenic CHD mutation cause a variety of distinct malformations, implying that higher order interactions account for particular CHD phenotype

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Mendelian inheritance of CHD

- Hundreds of mutations identified (autosomal dominant or X-linked)
- Reduced reproducibility fitness and early mortality should lead to negative selection of human CHD mutations
- AD de novo mutation should lead to a high recurrence rate
- In the danish population, only 2.2% of CHD patients had a first degree relative with a CHD challenging the idea that de novo, dominant mutations is the prevailing model
- Other hypotheses:
  - somatic mutations in the developing heart
  - multiple variants collectively cause CHD (ex *NKX2.5)*

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Oyen N et al. Circulation 2009; 120:295-301

Structural mutations in CHD

- Chromosomal anomalies and CHD
- Trisomy 21 : 40-50% CHD
- Prototypic CHD : AVSD
- Some defects strikingly underrepresented (TGA)

CHD are not due to a global change in genomic content, but rather from altered dose of specific gene

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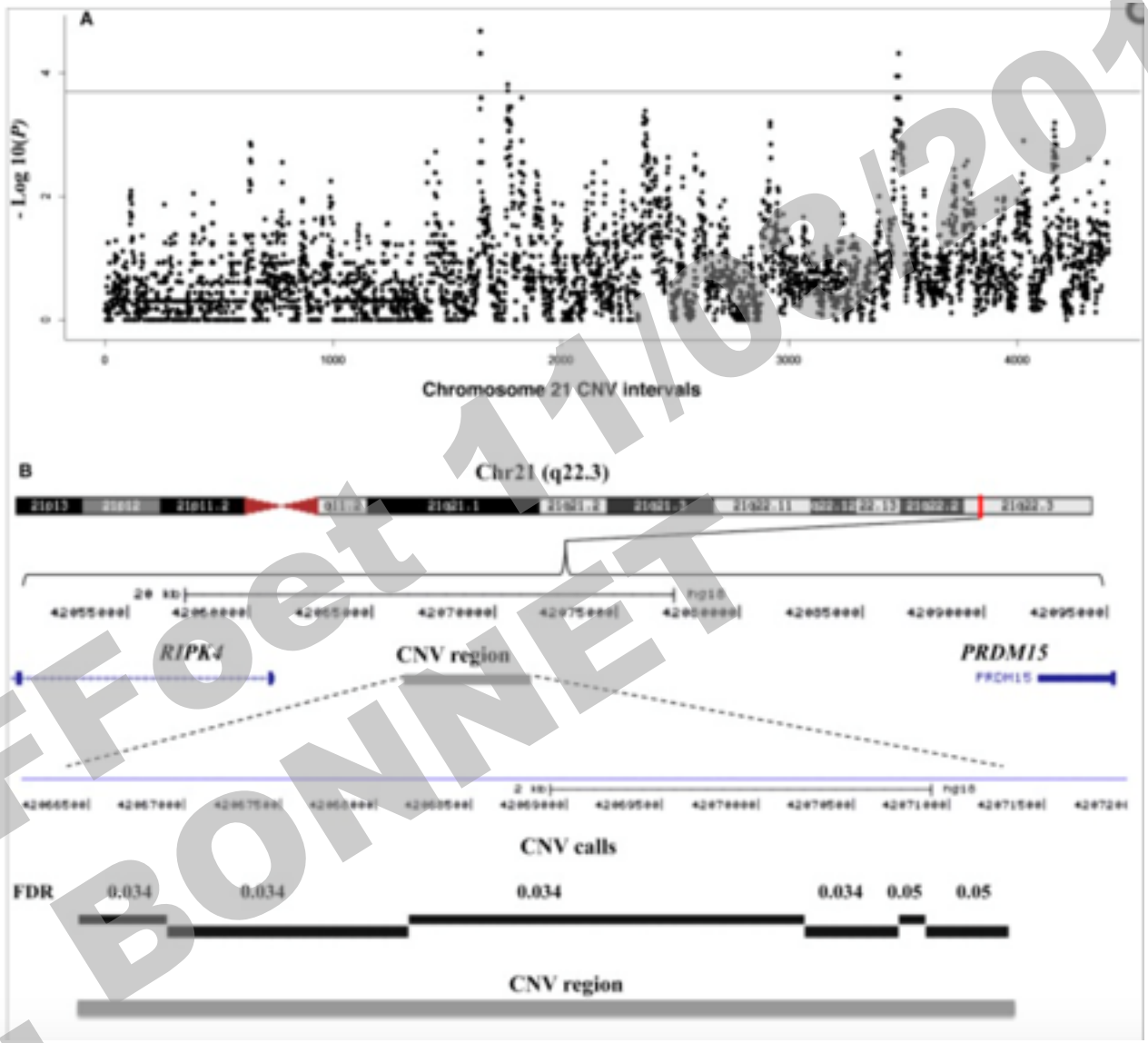

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Sailani MR,et al. The complex SNP and CNV genetic architecture of the increased risk of congenital heart defects in Down syndrome. Genome Res. 2013 Sep;23(9):1410-21.

CNV and syndromic CHDs

CNVs alter the dosage of contiguous genes

- a 3MB CNV on chromosome 22q11
  - altered dosage of *TBX1* that promotes cell proliferation in the second heart field
- 7q23 and *ELN*

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Table 2. Copy Number Variations (CNVs) Associated With Recurrent Cases of Nonsyndromic CHD

Locus	Size Range (Kbp)	No of Cases	Inheritance	CNV	No of Genes	Genes*	Phenotype	Reference(s)
1q21.1	418–3,981	21	De novo, inherited, n/a	Gain, loss	3–45	PRKAB2, FMO5, CHD1L, BCL9, ACP6, GJA5, CD160, PDZK1, NBPF11, FMO5, GJA8	TOF, AS,CoA, PA, VSD	23, 63, 64, 66, 70
3p25.1	175–12,380	3	De novo, inherited	Gain	2	RAF J, TMEM40	TOF	63, 72
3q22.1–3q26.1	680–32134	3	Inherited, n/a	Gain, loss	0–300	FOXL2, NPHP3,FAM62C, CEP70, FAIM, PIK3CB, FOXL2, BPESC1	DORV, TAPVR, AVSD	69, 70, 71
4q22.1	45	2	De novo	Gain	1	PPM1K	TOF	63, 23
5q14.1-q14.3	4,937–5454	2	Inherited, de novo	Gain	41103	EDIL3, VCAN, SSBP2, TMEM167A	TOF	23, 64
5q35.3	264–1777	4	De novo, n/a	Gain	19–38	CNOT6, GFPT2, FLT4, ZNF879, ZNF345C, ADAMTS2, NSD1	TOF	23, 70
7q11.23	330–348	2	N/a	Gain	5–8	FKBP6	HLH, Ebstein's	70
8p23.1	67–12,000	10	N/a	Gain, loss	4	GATA4, NEIL2, FDFT1, CSTB, SOX7	AVSD, VSD, TOF, ASD, BAV	23, 70
9q34.3	190–263	3	De novo	Loss	2–9	NOTCH1, EHMT1	TOF, CoA, HLH	63, 70
11p15.5	256–271	2	N/a	Gain	13	HRAS	DILV, AS	70
13q14.11	555–1430	3	N/a, de novo	Gain	7	TNFSF11	TOF, TAPVR, VSD, BAV	64, 69
15q11.2	238–2,285	12	N/a	Loss	4	TUBGCP5, CYFIP1, NIPA2, NIPA1	CoA, ASD, VSD, TAPVD, complex left-sided malformations	23
16p13.11	1414–2903	3	N/a	Gain	11–14	MYH11	HLH	70
18q11.1–18q11.2	308–6118	2	N/a	Gain	1–28	GATA6	VSD	70
19p13.3	52–805	3	N/a, de novo	Gain, loss	1–34	MIER2, CNN2, FSTL3, PTBP1, WDR18, GNA11, S1PR4	TOF	64, 23
Xp22.2	509–615	2	N/a	Gain	2–4	MID1	TOF, AVSD	64

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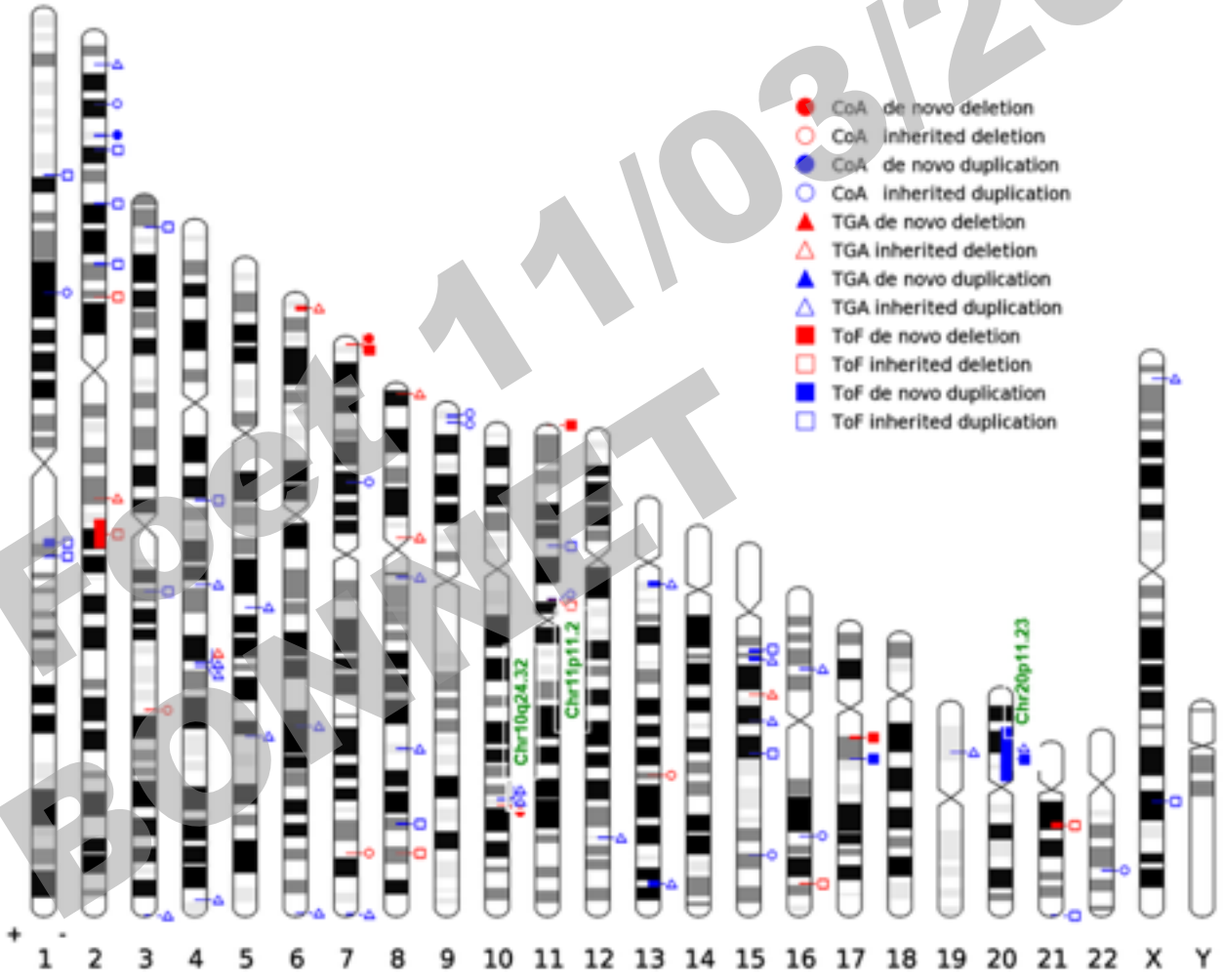
Search for Rare Copy-Number Variants in Congenital Heart Defects Identifies Novel Candidate Genes and a Potential Role for FOXC1 in Patients With Coarctation of the Aorta



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

CNVs and identification of new genes for CHD

- *TAB2 story*
  - CHD CNV recurrent at 6q23: 100 genes
  - analysis for dosage sensitivity using morpholinos in zebrafish and monitoring cardiac development
  - a translocation in a CHD family
- Developmental network using bioinformatics repositories of biological interactions identified the Wnt pathway as an important player in cardiac morphogenesis

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Point mutations in CHD

- Linkage analysis
- Exome and genome sequencing
  - rare, deleterious non synonymous SNPs
  - Argument for pathogenicity in CHD
    - statistically significant cosegregation in CHD families
    - identification of recurrent non synonymous SNPs that occur de novo in sporadic unrelated cases of CHDs
    - genetic complementation
    - recapitulation of CHD in model organisms

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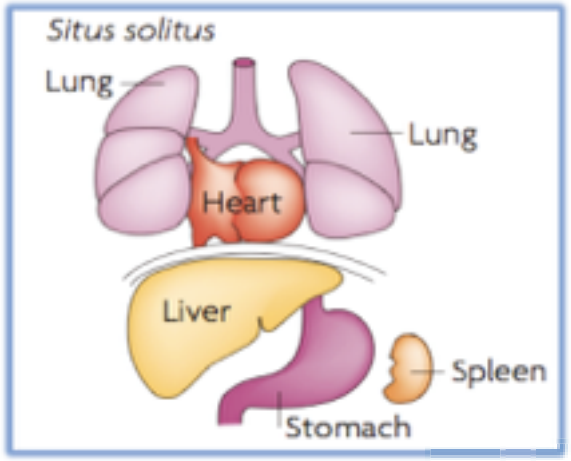
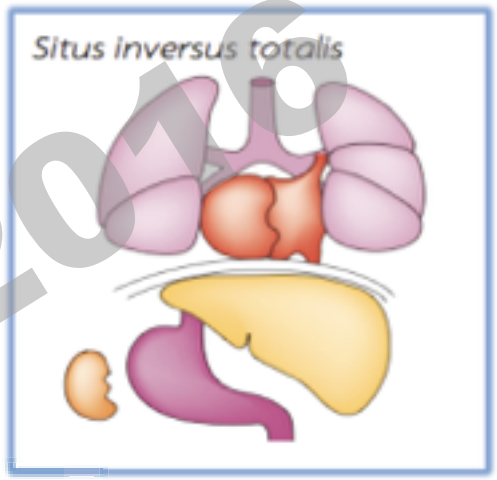
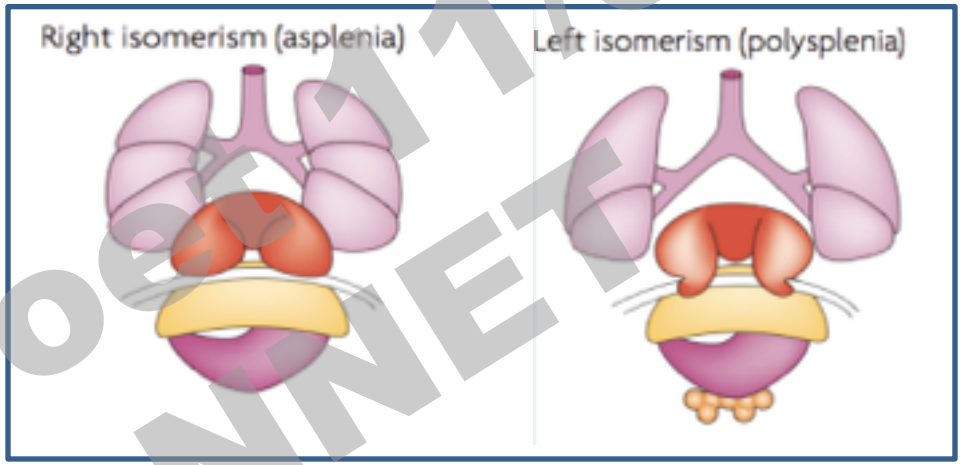


Human Heterotaxy

Abnormal arrangement
of
thoracic and visceral organs





1/10,000 live births

Heterotaxy Spectrum (Situs Ambigus)

Prognosis depends on the cardiac phenotype

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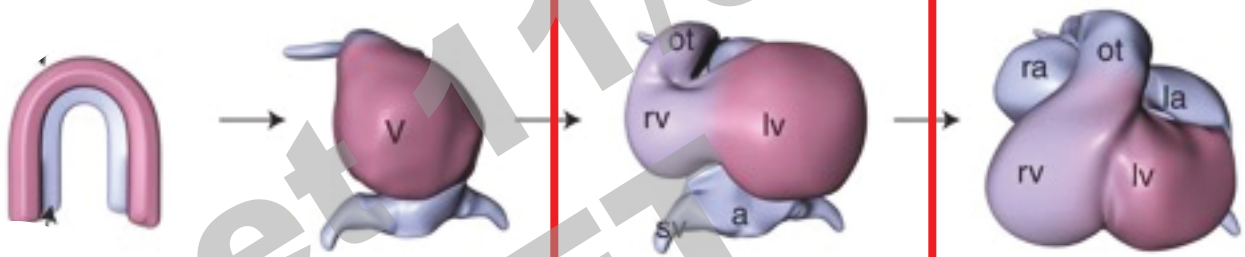
courtesy A Guimier, C. Gordon

Adapted from Fliegauf et al. Nat Rev Mol Cell Biol 2007

The Heart is the First Organ to Break the Bilateral L/R Symmetry of the Embryo



Stage:	Cardiac crescent	Linear heart tube	Looping heart	Chamber formation
Mouse embryo day:	E7.5	E8	E9	E10
Human embryo day:	Day 15	Day 20	Day 28	Day 32

Early chambers form
Looping to the right

Heterotaxy ~ 3% of all Congenital Heart Defect cases

Adapted from Bruneau BG. Nature 2008

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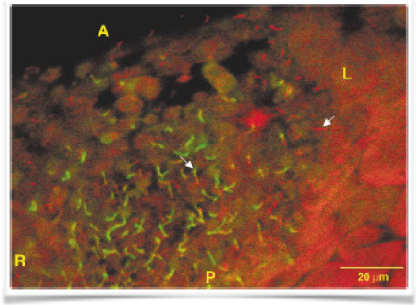
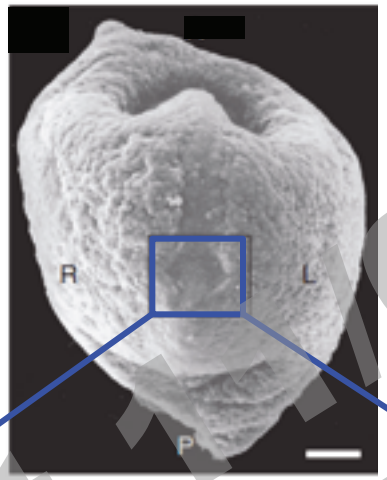
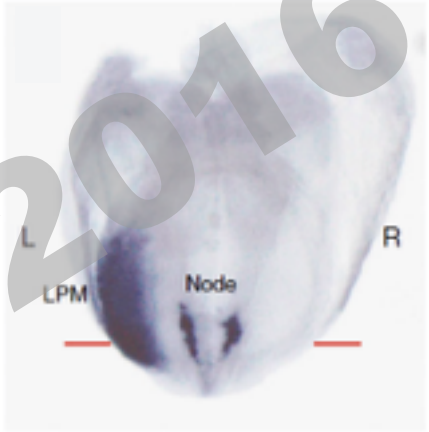
Left/Right Determination

Kartagener syndrome
is a
Primary ciliary dyskinesia

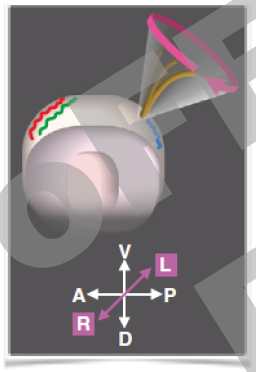


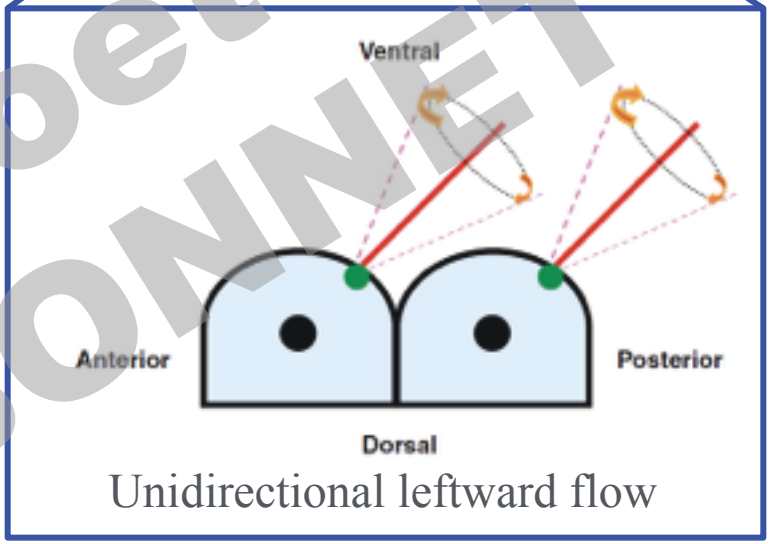


Primitive Node



Asymmetric expression of the ***Nodal*** signaling pathway





Unidirectional leftward flow

ZIC3 (XLR)

CFC1
ACVR2B
LEFTY2
NODAL
GDF1
CITED2
PITX2
…

None of these genes represent more than 1% of HT cases

Shiratori and Hamada. ***Development*** 2006

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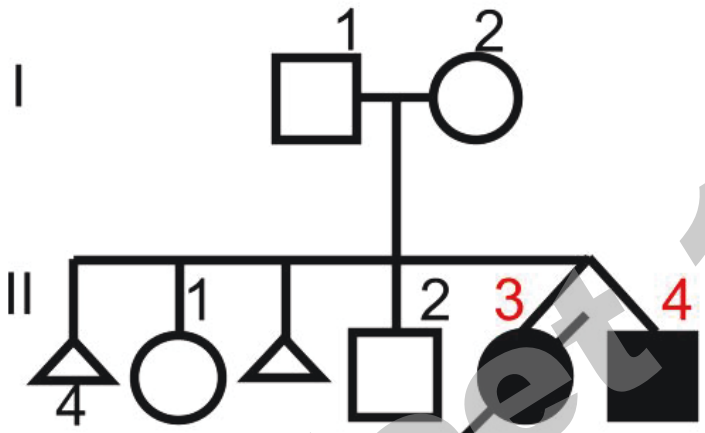
Two families with recurrent heterotaxy

Family 1

Whole Exome Sequencing

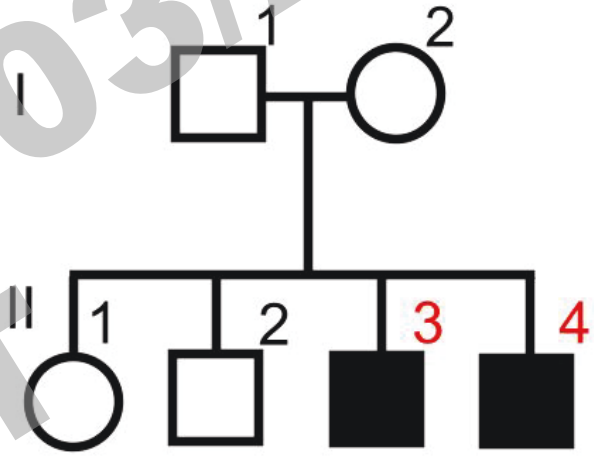


partial **APVR**, **hypoplastic left V, dextrocardia**	Abnormal AV connection, subarterial VSD, RAA
Intestinal malrotation polysplenia	Situs ambiguus

Family 2

S.Kingsmore, Children's Mercy, USA

Whole Genome Sequencing



TGA, DORV, valvar pulmonary stenosis, **dextrocardia**	Total **APVR, ASD, VSD, TGA**, valvar pulmonary atresia
Situs ambiguus (spleen, liver)	Situs ambiguus (spleen, liver and stomach)

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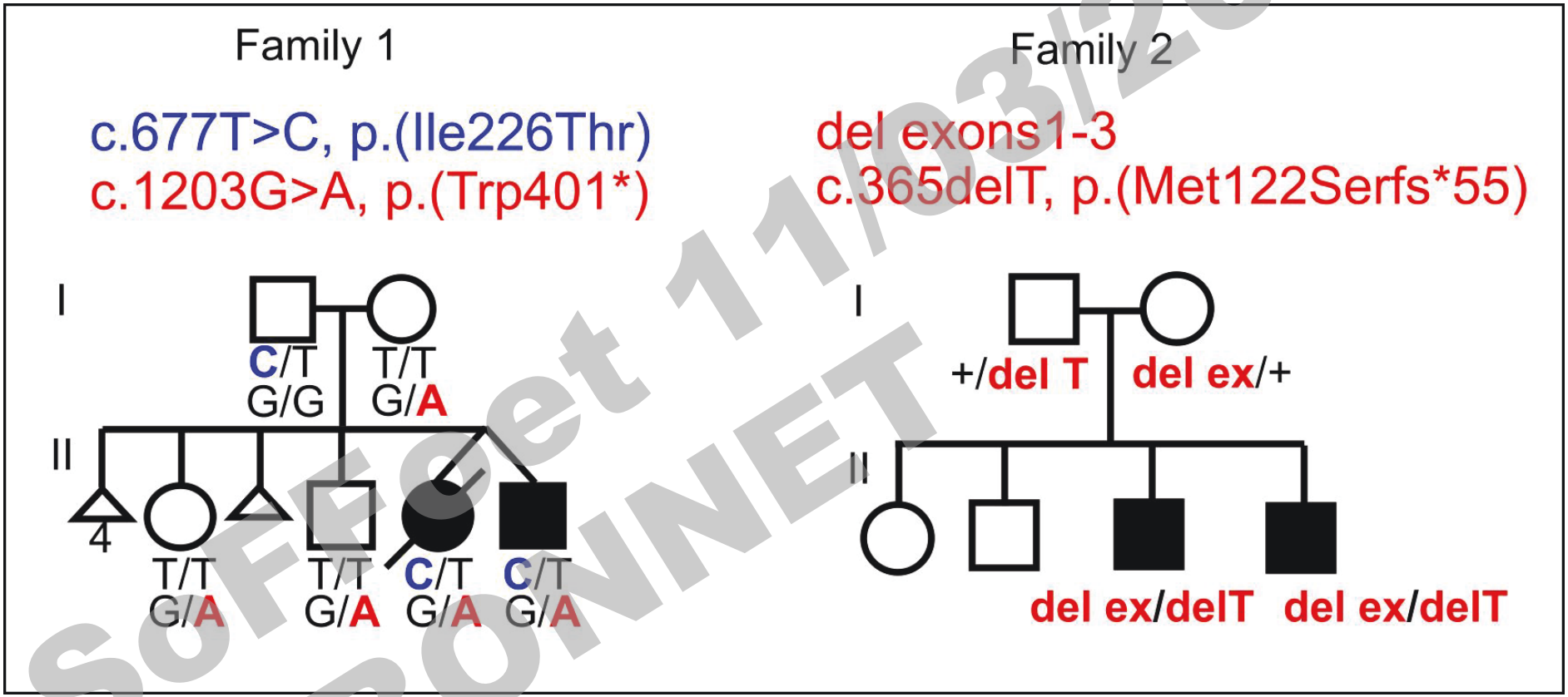
Compound Heterozygous Alterations in the MMP21 (Matrix Metallopeptidase 21) Gene



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Mouse model

Two Mmp21 ENU-mutant mice

Cecilia Lo, Pittsburgh University, USA

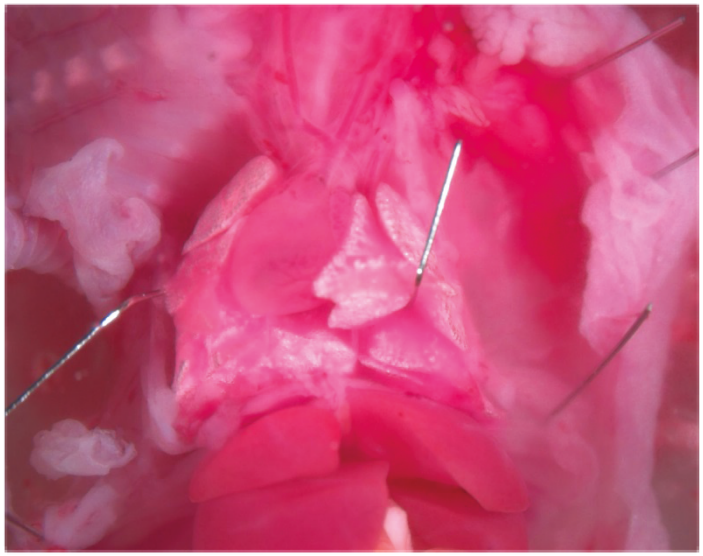
ENU screen for recessive cardiovascular development anomalies

Strain Name: C57BL/6J-*Mmp21*^{b2b873Clo}/J

- p.W177L
- p.Y325N

Homozygote mutants for *mmp21* missense mutations exhibit

- **Heterotaxy**
- **Cardiovascular defects :**
  - dextrocardia
  - transposition of the great arteries
  - tricupid atresia (IIc)
  - ventricular septal defect



Li Y. et al. Nature 2015

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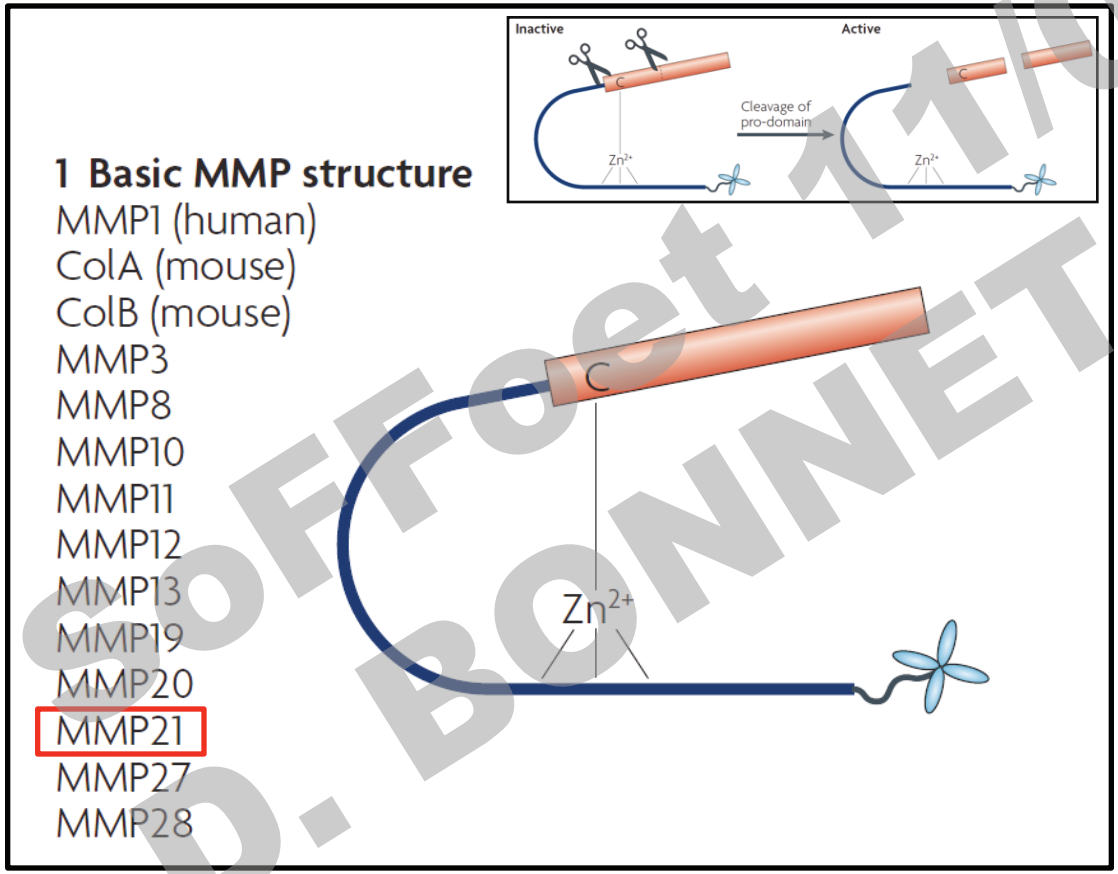
MMP21 is a Member of the MMP Family

Zinc-dependent endopeptidases involved in ECM degradation
Involved in various diseases, tissue remodeling…



MMP genes	Mutant mouse phenotypes
Mmp7	Innate immunity
Mmp9	Bone development Vas. remodeling
Mmp13	Bone remodeling
Mmp14	Lethality Bone remodeling

Page-McCaw et al. Nat Rev Mol Cell Biol 2007

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Enzyme	ECM substrates	Structure
MMP7	Collagen IV, gelatin, fibronectin, laminin, elastin, transferrin, casein	S Propeptide Catalytic Zn
MMP26	Collagen IV, fibrinogen, fibronectin, gelatin, pro-MMP9	
MMP1	Collagens, entactin, PGs, ovostatin, MMP2, MMP9, pro-MMP9	S Propeptide Catalytic Zn HPX Linker region
MMP3	Collagens, gelatin, aggrecan, laminin, elastin, casein, osteonectin, fibronectin, ovostatin, entactin, plasminogen, pro-MMP9	
MMP8	Collagens, fibronectin, PGs	
MMP10	Collagens, gelatin, casein, elastin, fibronectin	
MMP12	Collagen IV, elastin, gelatin, casein, fibronectin, vitronectin, laminin, entactin, fibrinogen	
MMP13	Collagens, tenascin, plasminogen, aggrecan, fibronectin, osteonectin, MMP9	
MMP19	Collagen I and IV, gelatin, fibronectin, laminin	
MMP20	Amelogenin	
MMP11	Collagens, laminin, elastin, fibronectin, casein, PGs	Furin-like cleavage S Propeptide Catalytic Zn HPX
MMP21	N.D.	
MMP28	Casein	
MMP2	Collagens (IV, V, VII, X), gelatin, elastin, fibronectin	S Propeptide Catalytic Zn HPX Fibronectin-like motif
MMP9	Collagens (IV, V, VII, X), gelatin, elastin, fibronectin	
MMP17	Gelatin, fibrinogen, pro-MMP2	S Propeptide Catalytic Zn HPX GPI
MMP25	Collagen IV, gelatin, fibronectin, pro-MMP2, pro-MMPp	

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Bonnans et al. Nature Rev Mol Cell Biol 2014

MMP21 Screening by NGS in a Replication Cohort

	264
HT cases Extra cardiac and/or cardiac laterality defects	**154**
Isolated CHDs (tetralogy of Fallot, truncus arteriosus…)	**110**

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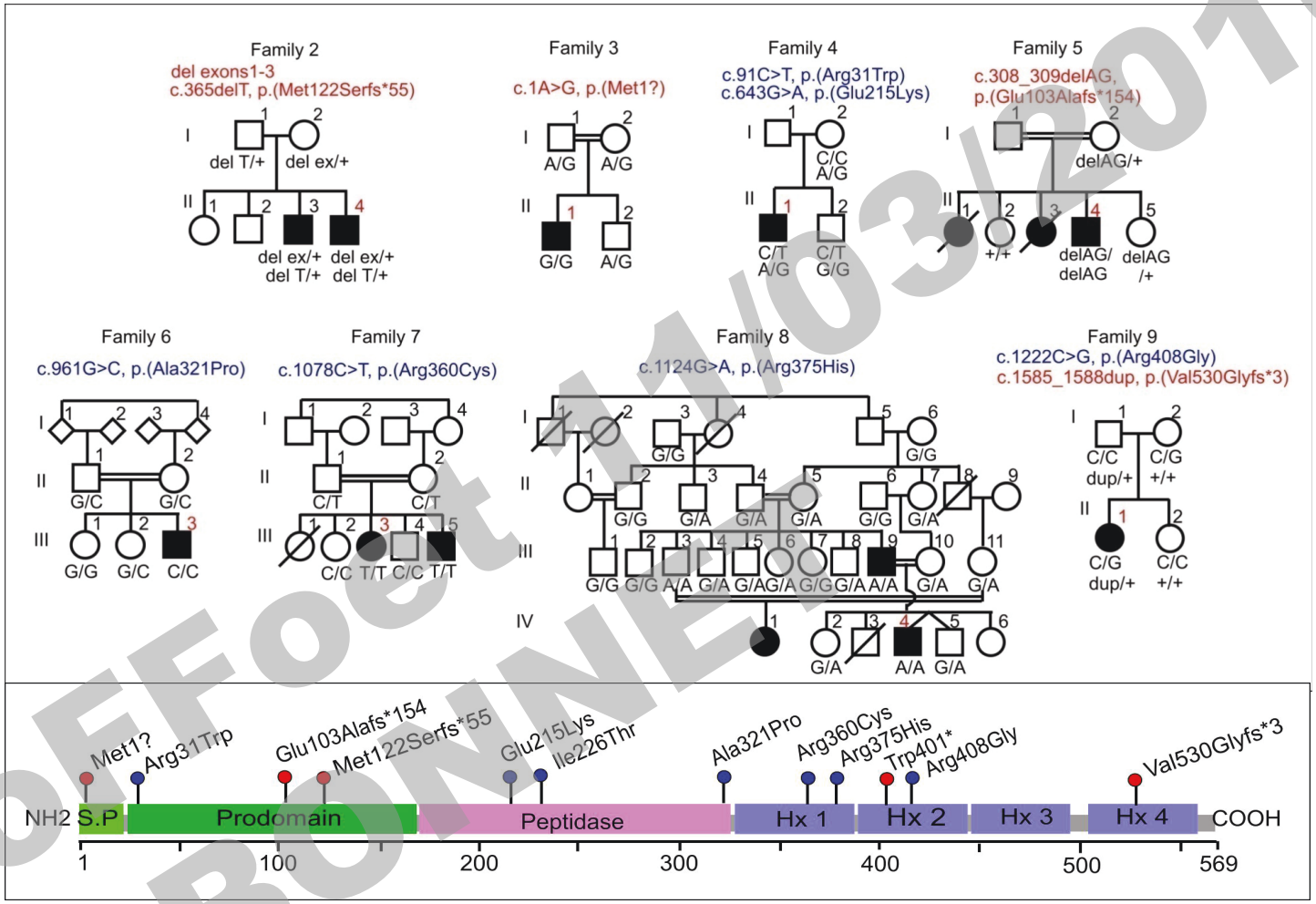
MMP21 Mutations in 7 other Affected Families



MMP21 mutations account for ~ 6 % of non syndromic HT cases

Guimier A, et al Nat Genet 2015

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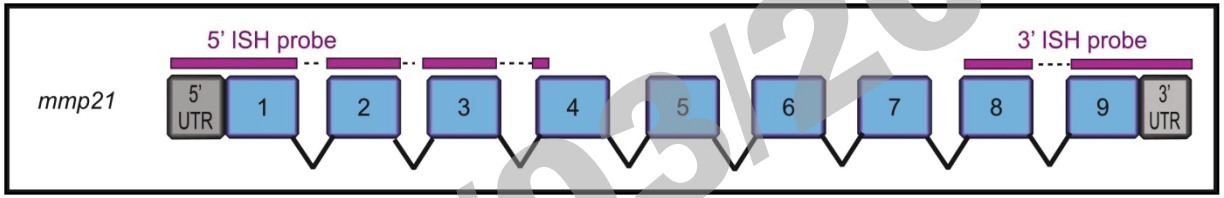
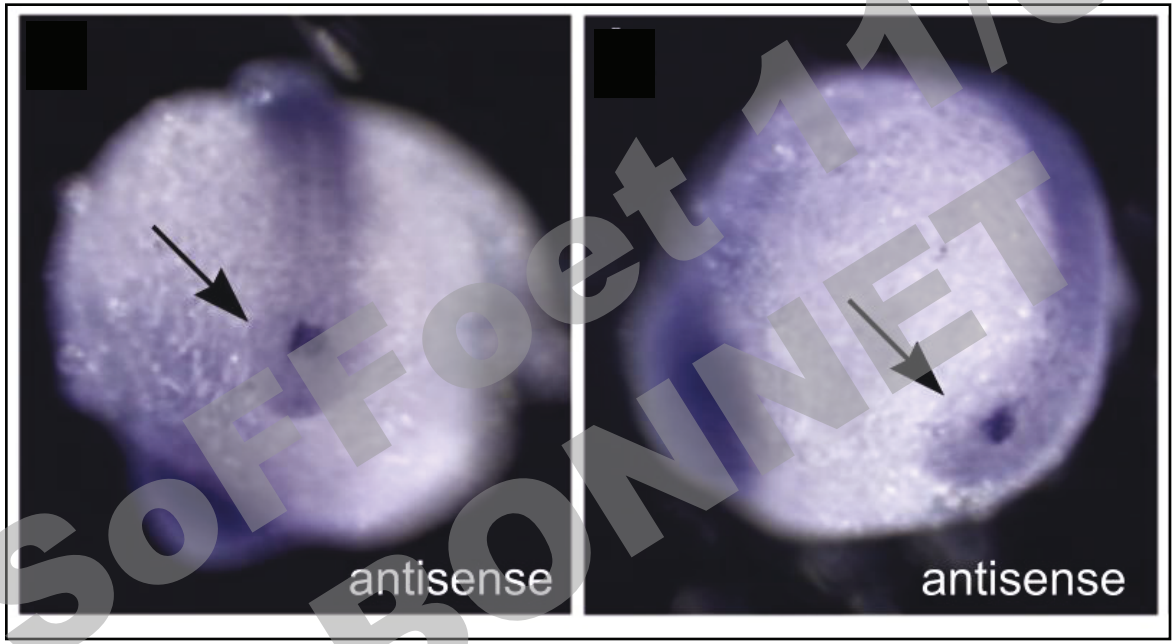
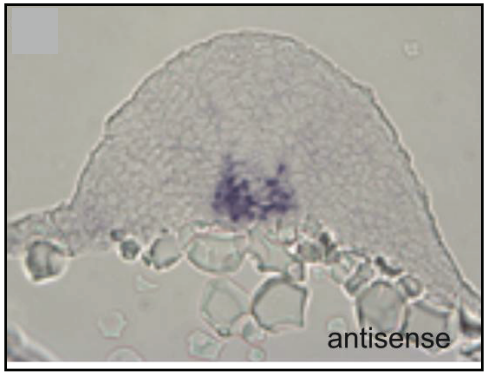
mmp21 expression in zebrafish



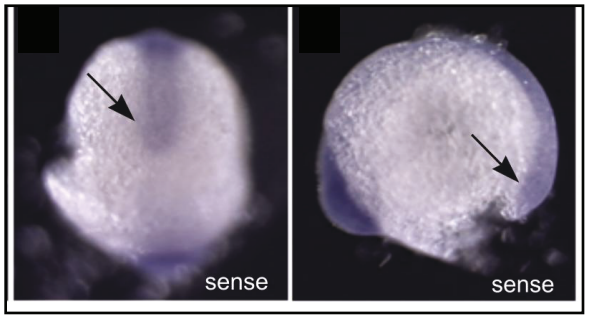
Whole-mount *in situ* hybridization



Embryos at 12hpf



Transverse section



mmp21 is expressed only in the Kupffer's vesicle at 10-20 hpf

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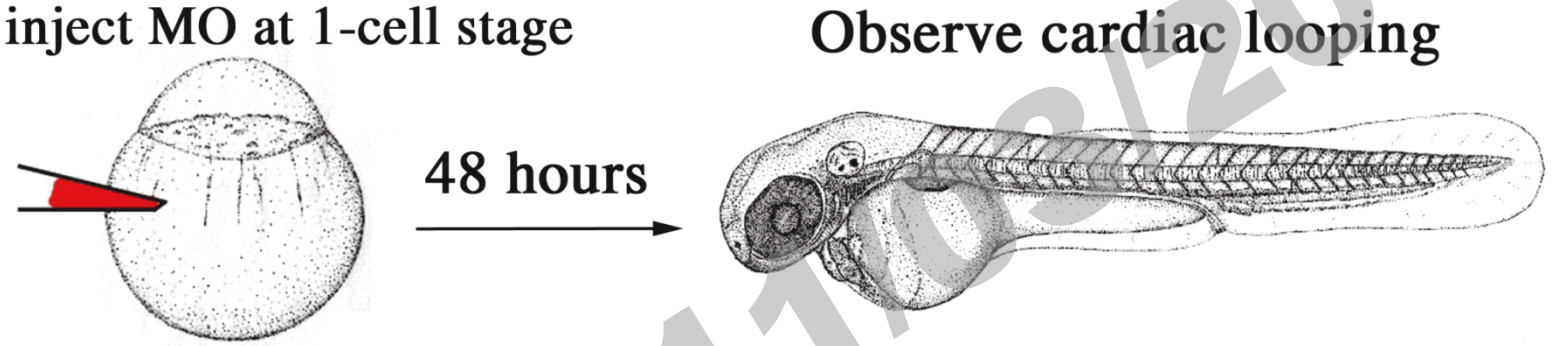
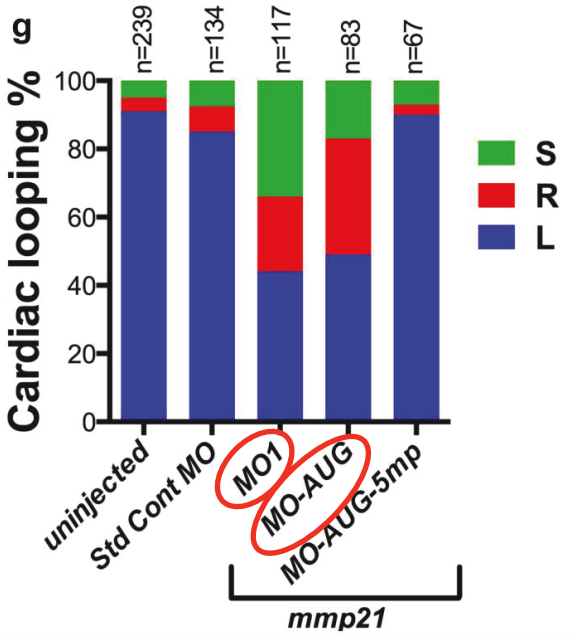
mmp21 knock-down in zebrafish



transgenic cardiac Cmlc2:EGFP reporter line



Morphant zebrafish display a randomized heart looping

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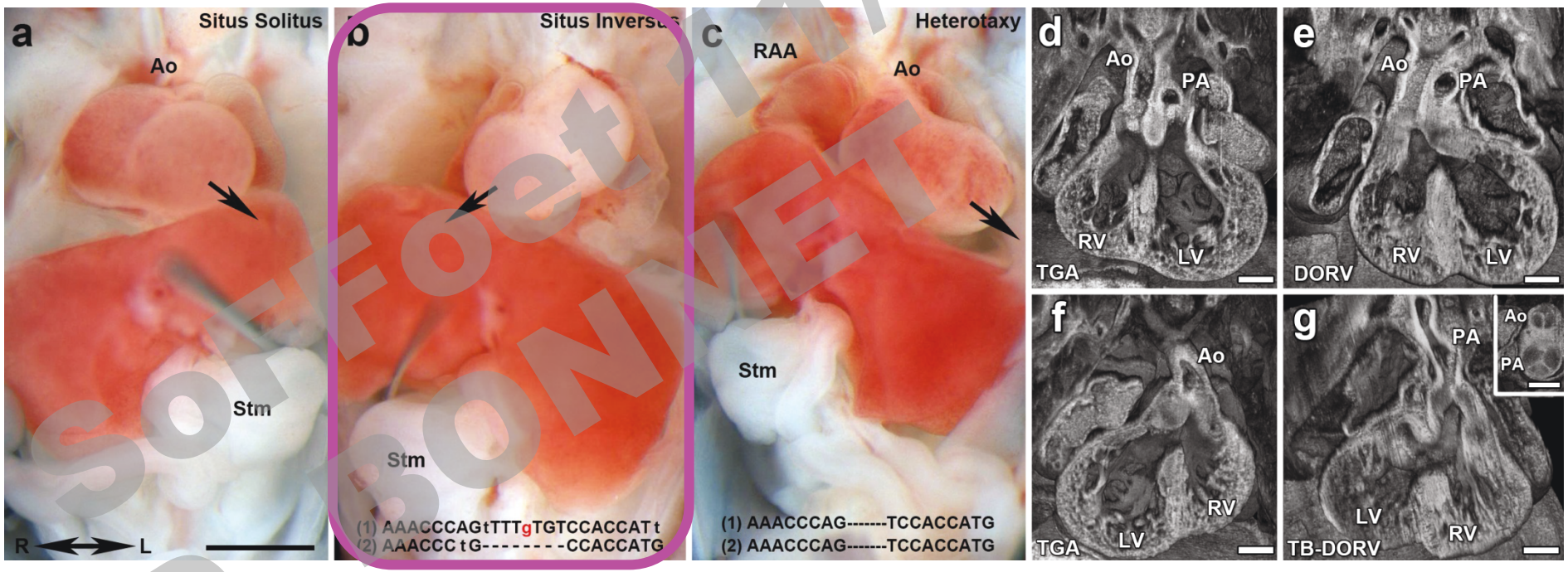
Modeling MMP21 mutations in mice using CRISPR/cas9 genome editing

C. Lo (Pittsburgh University) and the Jackson Laboratory

knock-in the p.Ile226Thr missense mutation (Family 1)

Fs/Fs (12/15)
I226T/Fs (3/15)

CHDs and other laterality phenotypes



Supporting pathogenicity of the mutation

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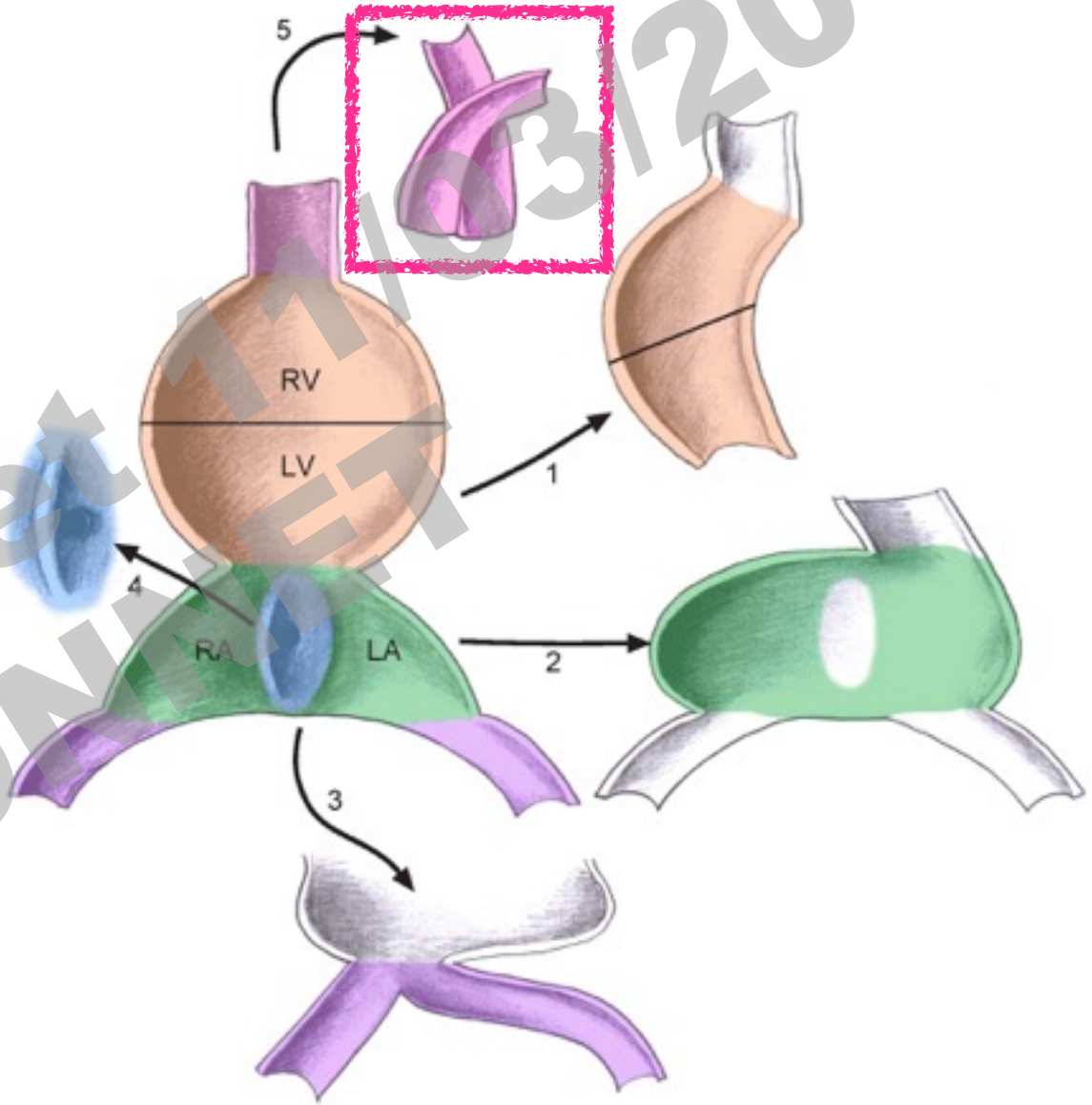
***Papuina pulcherrima*, with a dextral shell**

5 levels of asymmetry in the developing heart



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TGA is a laterality defect

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

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.

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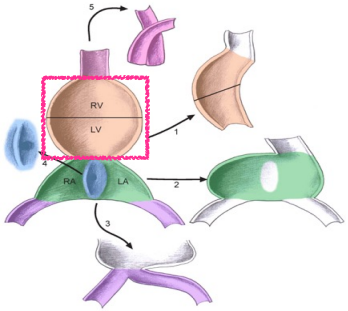
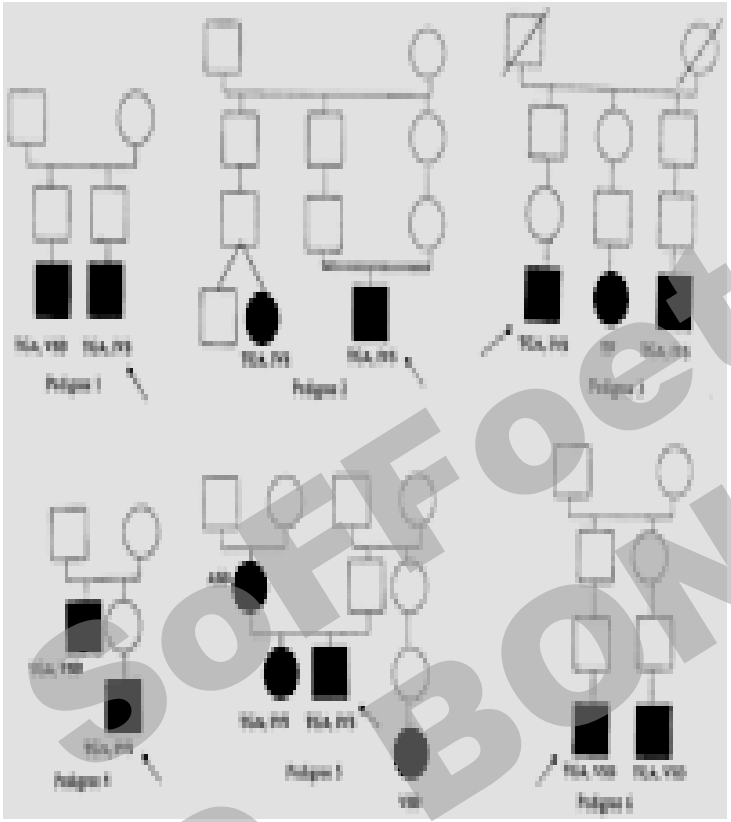
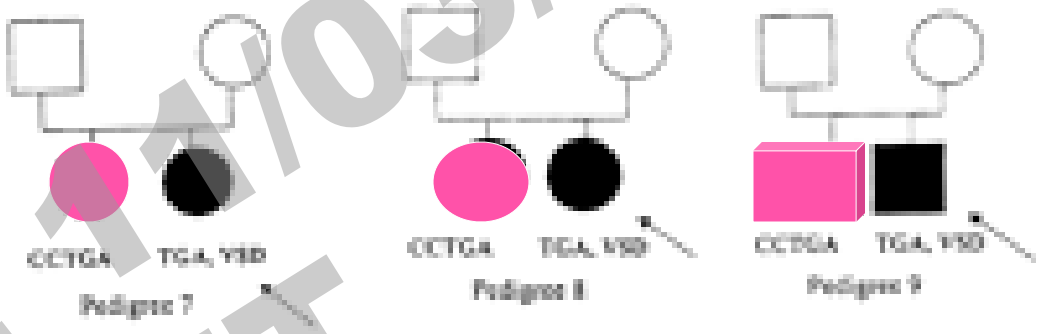
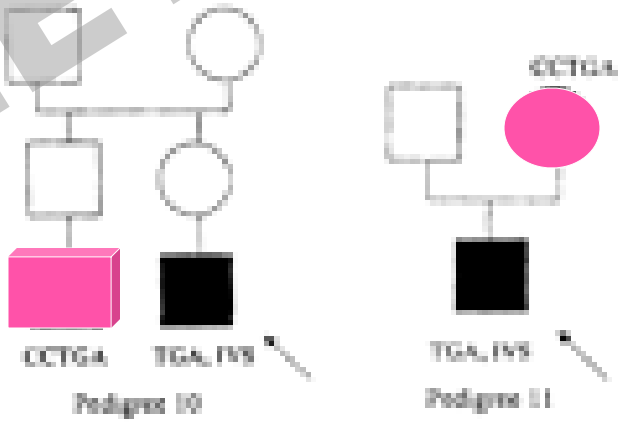


Families TGA & CC-TGA







Families CCTGA + TGA

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Laterality defects

Global randomisation

- Heterotaxy syndromes

Partial randomisation: segmental defects

- AV discordance
- Double discordance
- TGA and other malpositions of the great arteries with no spiraling of great vessels
  - Anatomically corrected TGA
  - Double outlet right ventricles
- Venous pole segmental defects ?
  - pulmonary veins
  - caval veins

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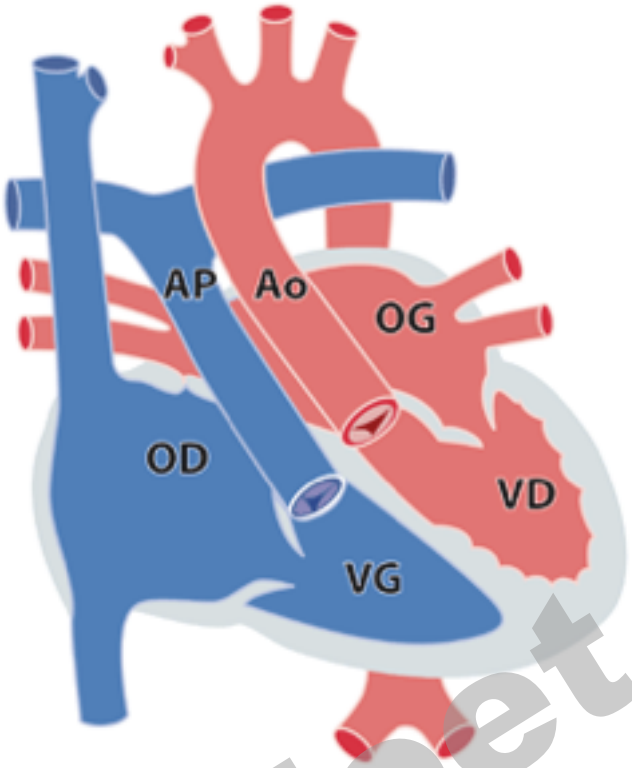

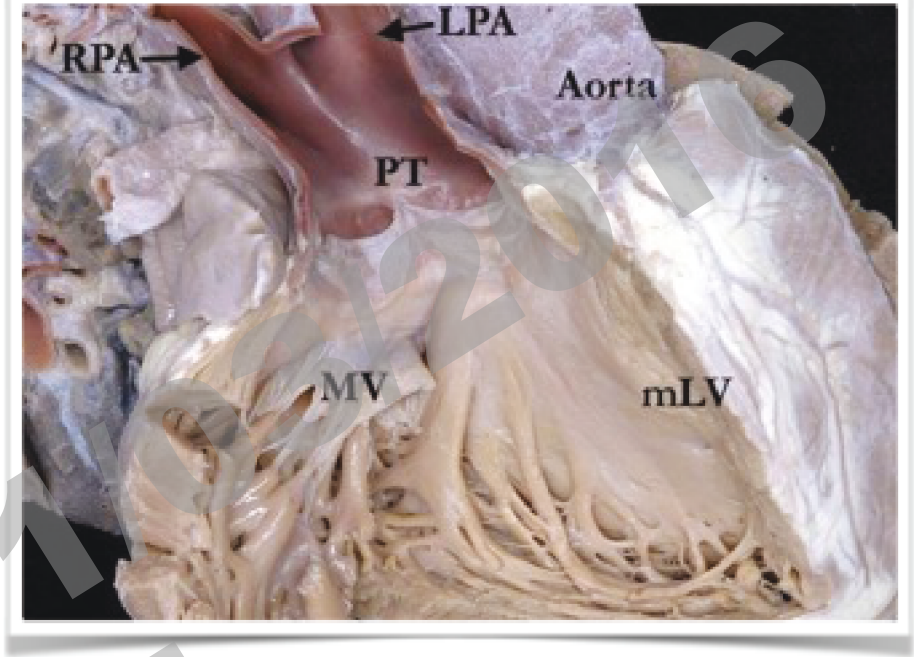

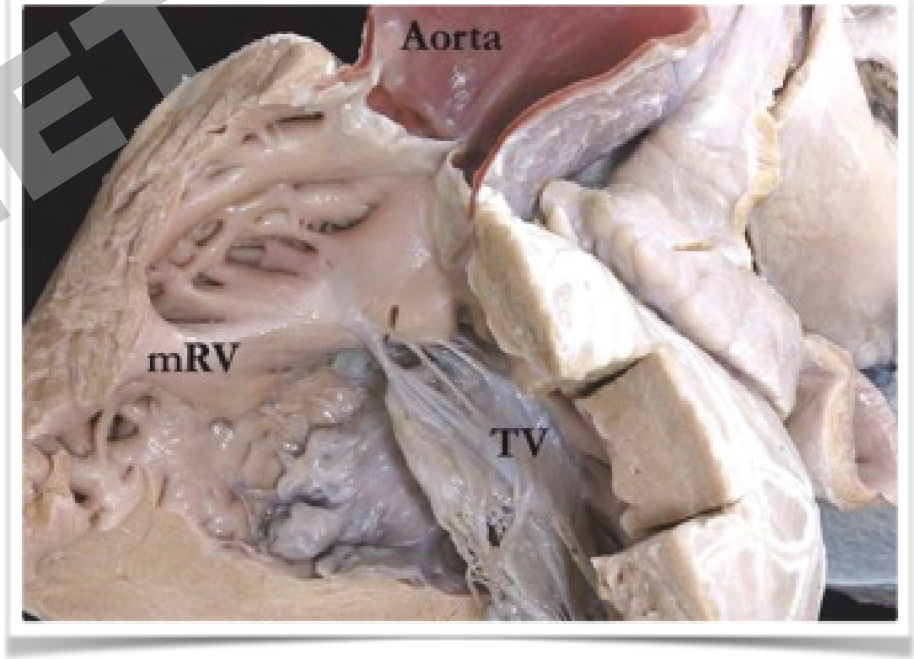

The Double Discordance genome program

Stanilas Lyonnet
Sigolène Meilhac
Damien Bonnet-Fanny Bajolle

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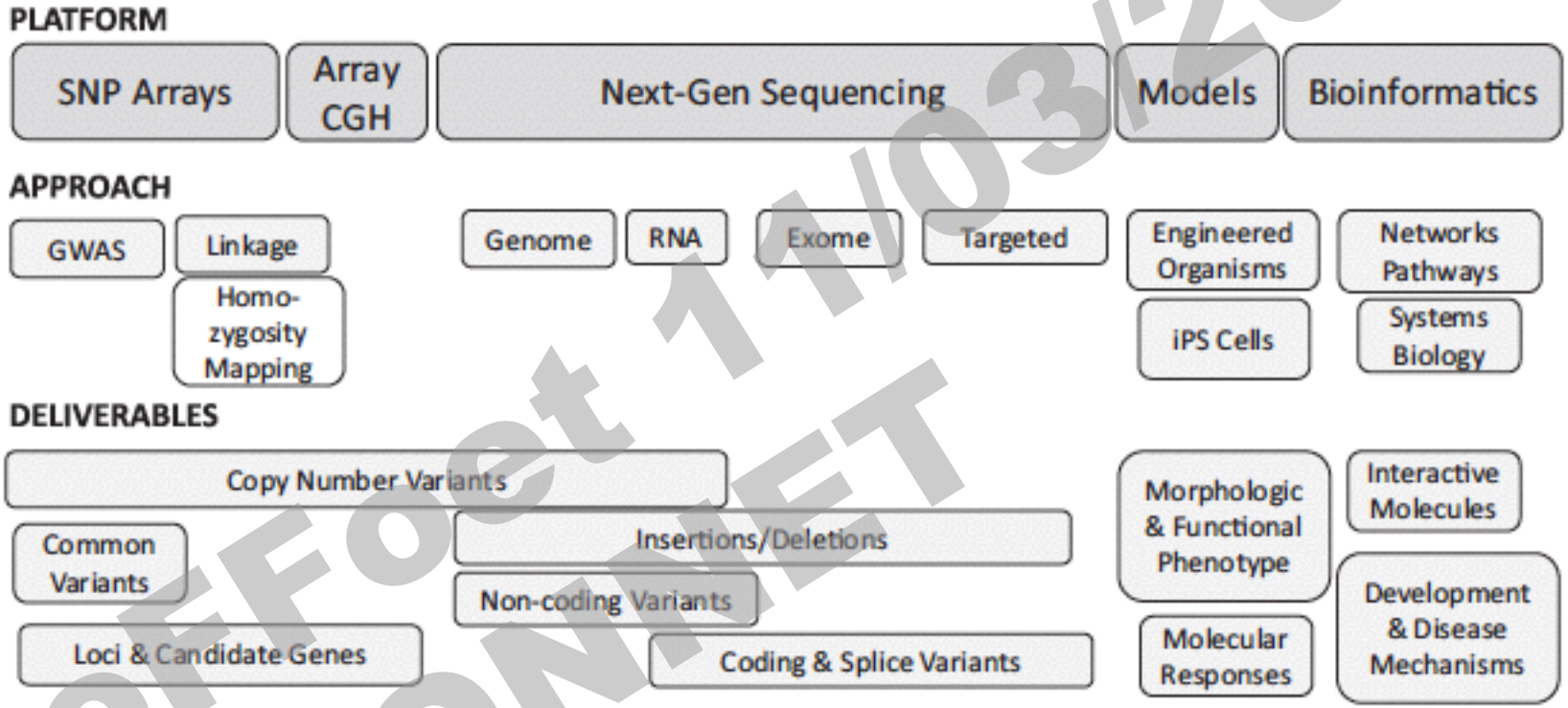
FRANCE GÉNOMIQUE

imagine
INSTITUT DES MALADIES GÉNÉTIQUES



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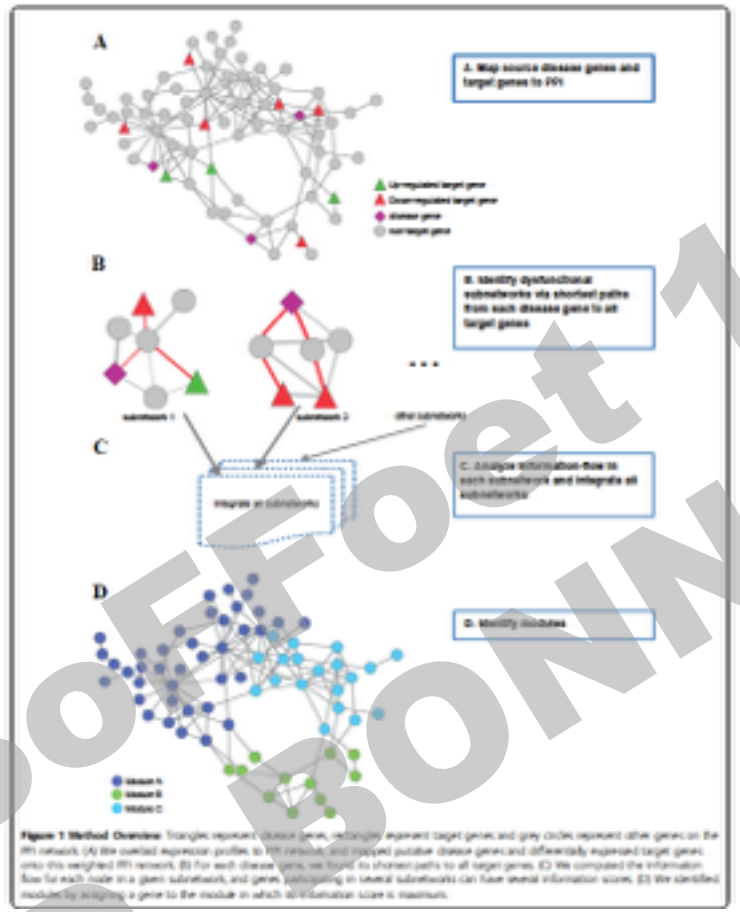
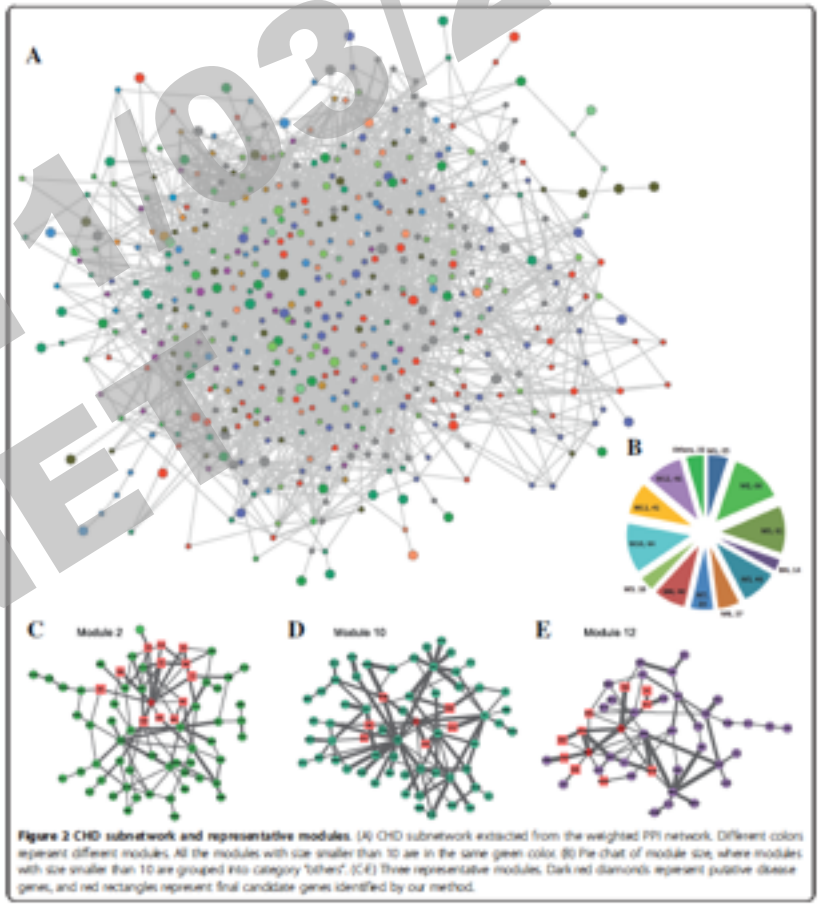
Identification of dysfunctional modules and disease genes in CHD by a network-based approach

Protein-protein interaction, gene co-expression profiles, and causal path from putative CHD genes





Identification of 12 modules and CHD candidate genes

He D et al. BMC Genomics 2011

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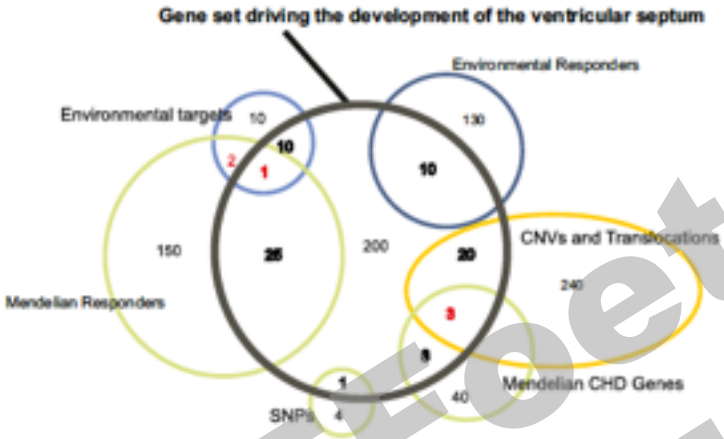
Genetic and environmental risk factors in CHD converge in protein networks driving heart development



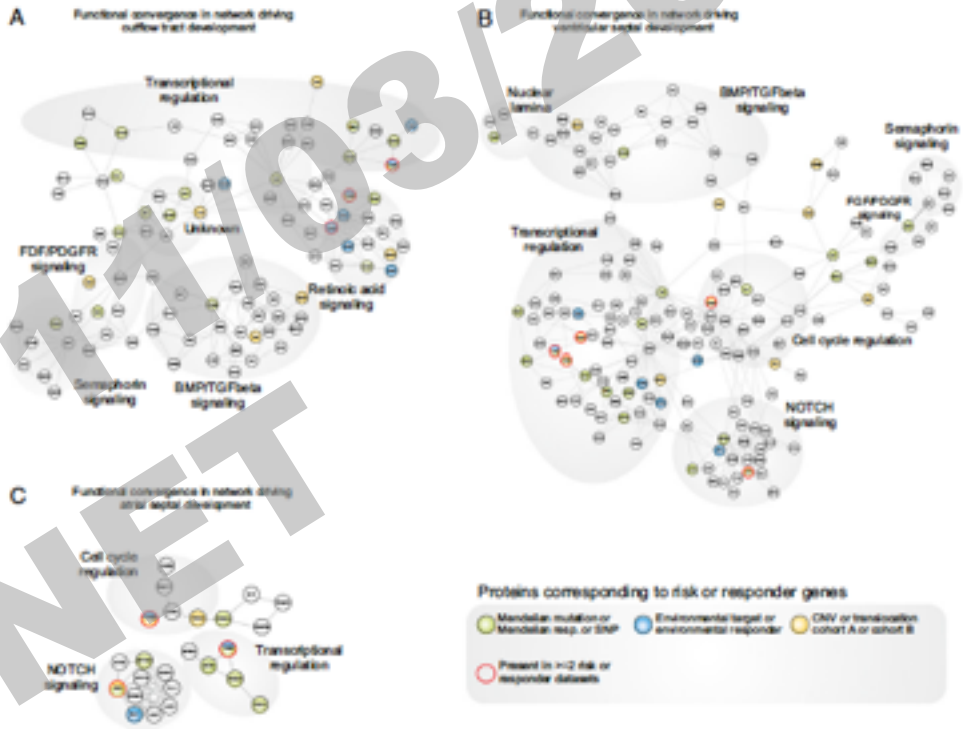


Fig. 2. Functional convergence of CHD risk and responder datasets in cardiac developmental networks. (A–C) Functional convergence was analyzed in predefined developmental programs (11), deduced from protein-protein interaction networks driving the development of the outflow tract (A), ventricular septum (B), and atrial septum (C). Circles represent gene-encoded proteins (gene names viewable with the Adobe zoom tool or at www.cbs.dtu.dk/suppl/ddg/). Lines represent protein-protein interactions. Colors indicate datasets of Mendelian risk genes, SNPs, or Mendelian responder genes (green), CNVs and translocations from cohort A or B (gold), or environmental target or responder genes (blue). Red circles indicate proteins belonging to more than one dataset.

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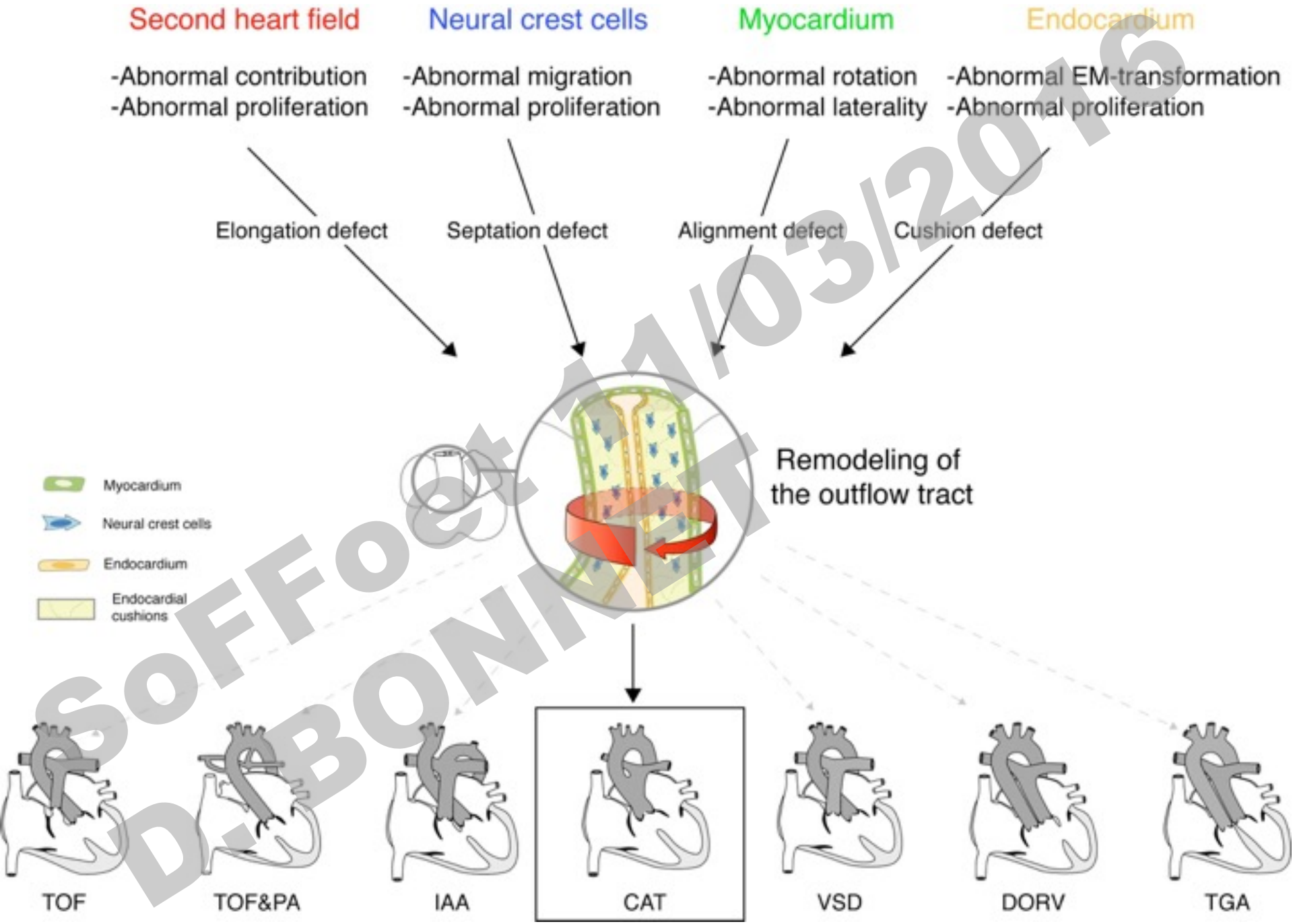
Lage K et al. PNAS 2012



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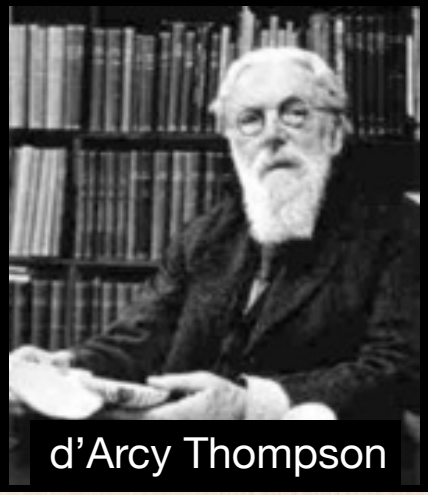
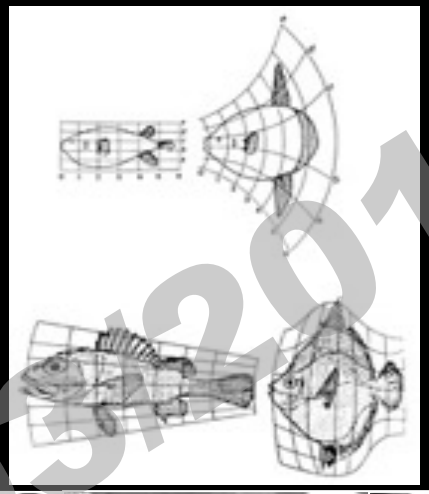
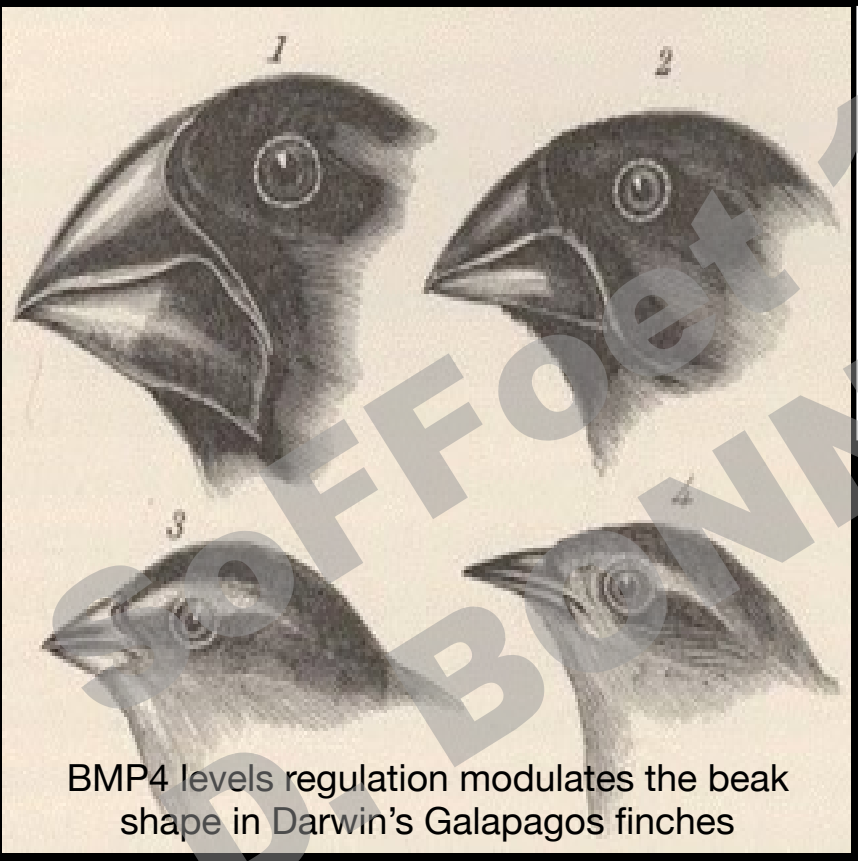
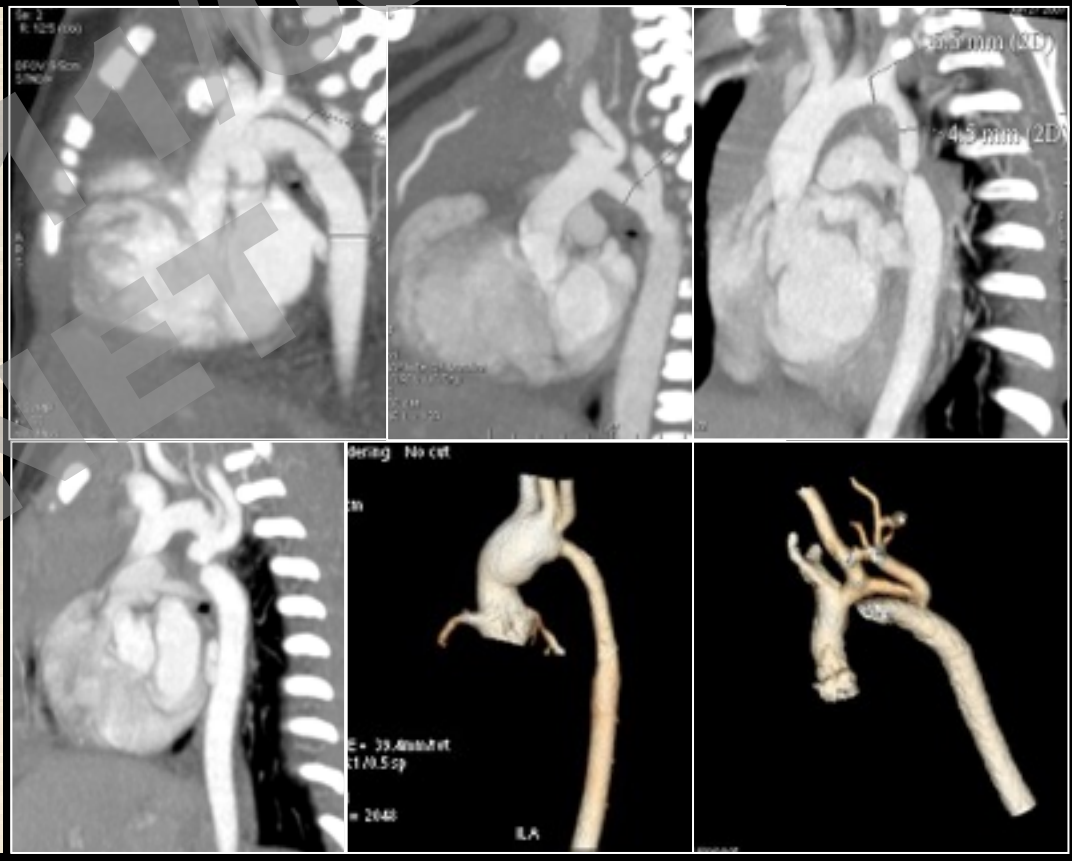


d'Arcy Thompson





BMP4 levels regulation modulates the beak shape in Darwin's Galapagos finches



Abzhanov A et al. Science 2004

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