Génétique cardiovasculaire:
« Concepts & nouvelles approches »

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L'unité de recherche de l'institut du thorax
Inserm UMR 1087 / CNRS UMR 6291
Nantes, France

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MASTER 1 Thorax

Unité d'enseignement « Recherche en Physiopathologies humaines »

23-11-2023

Inserm cnrs UNIVERSITÉ DE NANTES CENTRE HOSPITALIER UNIVERSITAIRE DE NANTES

Translational approach to investigate cardiovascular diseases

Centre for
Clinical Investigations
(CIC 'thorax')

"FROM BEDSIDE TO BENCH TO BEDSIDE"

patients

600 people
85 clinicians

bedside

Improved patient care

Nantes Université

CENTRE HOSPITALIER UNIVERSITAIRE DE NANTES

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2004

Inserm

cnrs

bench

Combining basic research and translational research programs

Inserm 1087/CNRS 6291
72 researchers
4 Teams:

- **Human Genetics**
- Ion channels and cardiopathies
- Vascular & Pulmonary Diseases
- Cardiometabolic diseases

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Research programs

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Approches génétique en 2023: objectifs

1- Concepts, outils & modèles: (Dynamique des génomes)

- **Variants rares (→ ++ maladies mendéliennes)**
  - Approches Liaison génétique – approches en famille **(clonage positionnel)**
  - Séquençage SANGER; séquençage d'exome (WES)
  - Séquençage de génomes complets (WGS) → **accès à l'ensemble**

du spectre de fréquences alléliques des REGIONS CODANTES & NON CODANTES; épigénétique)

- **Variants fréquents**
  - Etude d'association (GWAS); approches en population **(Génotypage)**

→ **augmentation du risque**

2- Applications en Génétique Cardiovasculaire

- Troubles de conduction cardiaque de l'enfance
- Dysfonction sinusale - cardiomyopathie
- **Troubles du rythme ventriculaire: Le** syndrome de Brugada

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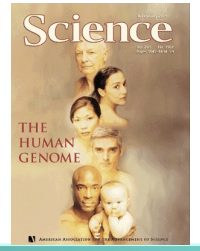
Disease gene identification allowed by a spectacular acceleration of knowledge of the architecture of the human genome

Microsatellites

Genetic Maps



1990 **2001** 2010

SNPs

GWAS

WES

WGS

Rare diseases

(Rare diseases / Rare variants)

Common diseases

(Common diseases / common variants)

- **new sequencing technologies**
- **new databases**
- **… new disease models**

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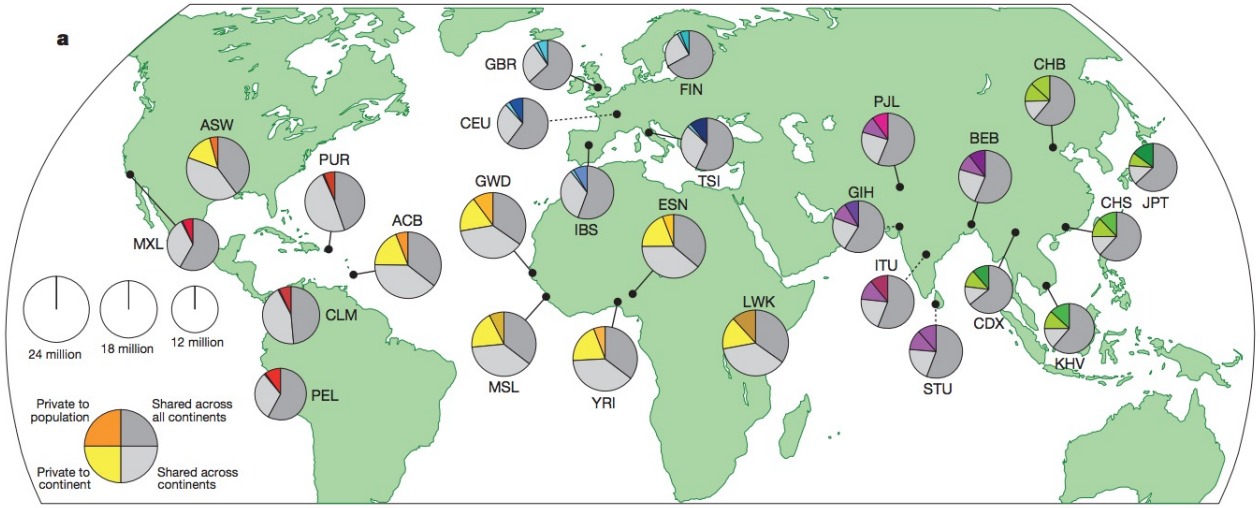
ARTICLE

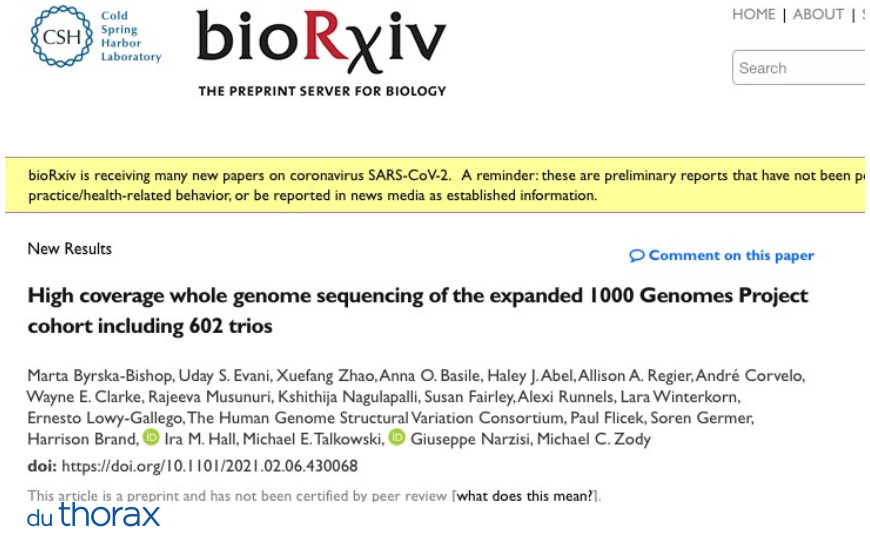
OPEN
doi:10.1038/nature15393

A global reference for human genetic variation

The 1000 Genomes Project Consortium*

- 2504 individuals from 26 different populations
- Broad spectrum of genetic variations:
  - 88 millions variants (MAF >1%)





Cold Spring Harbor Laboratory

bioRxiv

THE PREPRINT SERVER FOR BIOLOGY

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Search

bioRxiv is receiving many new papers on coronavirus SARS-CoV-2. A reminder: these are preliminary reports that have not been p practice/health-related behavior, or be reported in news media as established information.

New Results

Comment on this paper

High coverage whole genome sequencing of the expanded 1000 Genomes Project cohort including 602 trios

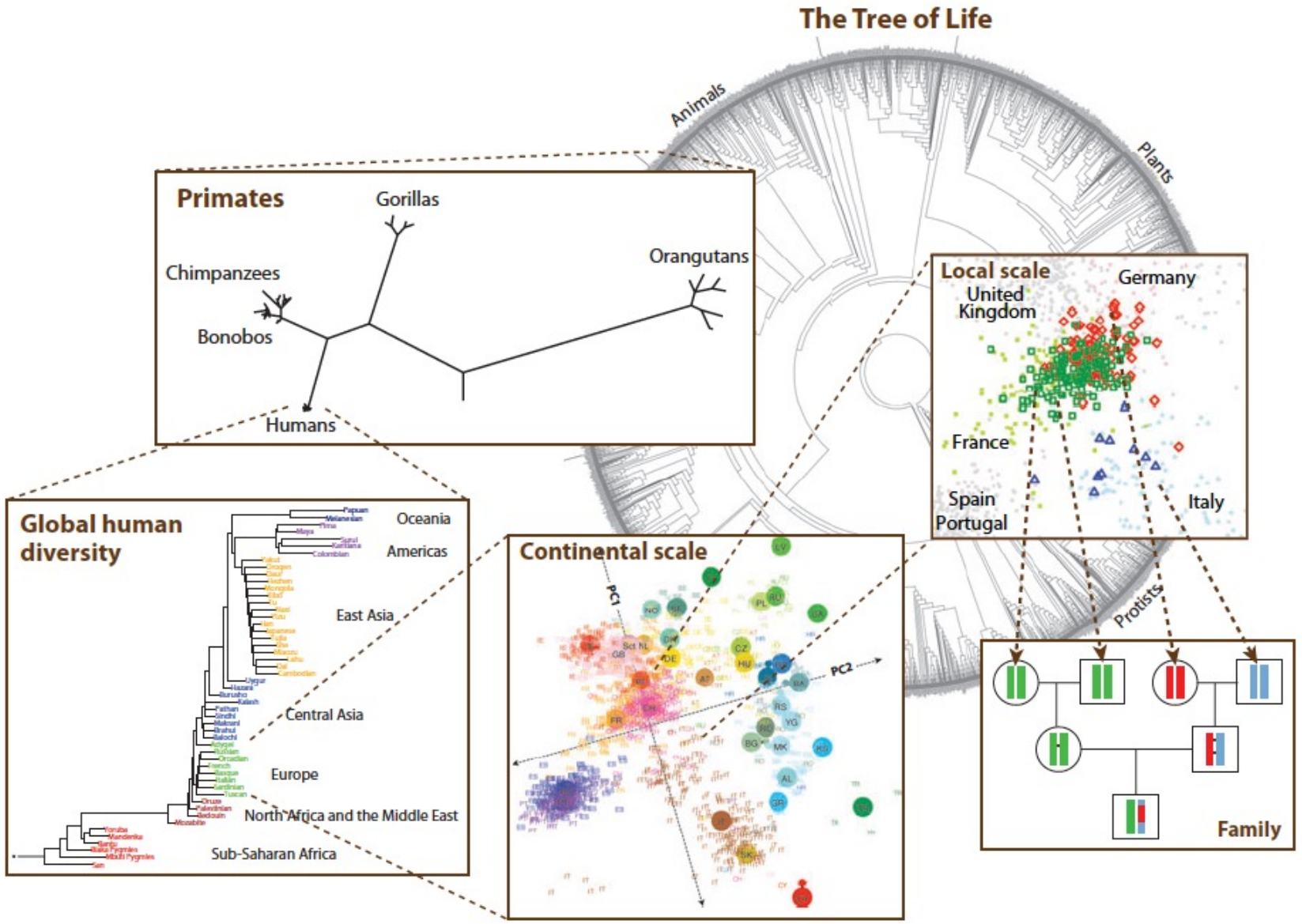
Marta Byrska-Bishop, Uday S. Evani, Xuefang Zhao, Anna O. Basile, Haley J. Abel, Allison A. Regier, André Corvelo, Wayne E. Clarke, Rajeeva Musunuri, Kshithija Nagulapalli, Susan Fairley, Alexi Runnels, Lara Winterkorn, Ernesto Lowy-Gallego, The Human Genome Structural Variation Consortium, Paul Flicek, Soren Germer, Harrison Brand, Ira M. Hall, Michael E. Talkowski, Giuseppe Narzisi, Michael C. Zody

doi: https://doi.org/10.1101/2021.02.06.430068

This article is a preprint and has not been certified by peer review [what does this mean?].

du thorax

Stratégies génétique & dynamique des génomes



Novembre & Ramachandran.
Ann Rev Genomics Hum Genet **12**:245, 2011

- 1000 genome project (population)
- Bases de données : gnomAD, UK biobank (patients)

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Variants rares

MAF (fréquence de l'allèle mineur) < 0,1 %
Variations « récentes
Variants à effet fort

*Approche familiale –Maladies Mendéliennes
Souvent dans les gènes (exons)

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Variants fréquents

MAF < 5 %

→ Variations « anciennes » maintenues durant l'évolution

→ Approche en population – Maladies fréquentes

« Common variant – common disease »

Variants à effet faible
Augmentation du risque de développer une maladie d'environ 1- 5 %
Souvent dans les régions non codantes
Modulation de l'expression d'un gène

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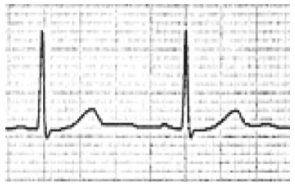
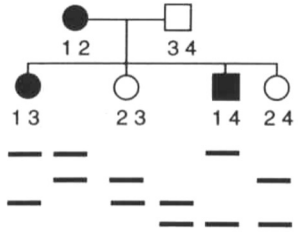
Clonage positionnel : « génétique des grandes familles »

Variants rares



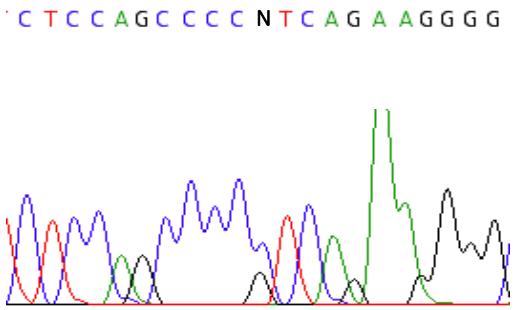
Patient



Analyse de liaison

Locus

Gènes candidats



Mutation

Physiopathologie
Diagnostic génétique

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Modes de transmission

Mode de transmission	Localisation du locus responsable du trait	Principales caractéristiques	Exemples de phénotypes
Autosomique dominant	Chromosome 1-22	-hommes et femmes sont atteints avec une fréquence équivalente -les enfants atteints ont un parent atteint	Huntington, syndrome de Marfan
Autosomique récessif	Chromosome 1-22	-hommes et femmes sont atteints avec une fréquence équivalente -les enfants atteints n'ont pas de parent atteint -peut « sauter » une ou plusieurs générations	Mucoviscidose, drépanocytose
Dominant lié à l'X	Chromosome X	-les femmes atteintes ont 50% d'enfants atteints -les hommes sont plus sévèrement atteints -toutes les filles de père atteints sont atteintes	Syndrome de Rett
Récessif lié à l'X	Chromosome X	-beaucoup plus d'hommes atteints que de femmes -les hommes atteints ont une mère saine (porteuse) -le trait n'est jamais transmis de père à fils -peut « sauter » une ou plusieurs générations	Hémophilie
Lié à l'Y	Chromosome Y	-tous les hommes atteints passent le trait à leur fils -les femmes ne sont pas atteintes	Stérilité
Mitochondrial	Pas chromosomique ADN mitochondrial	-tous les enfants d'une femme atteinte sont atteints -les hommes atteints ne transmettent pas le trait	Neuropathie optique de Leber

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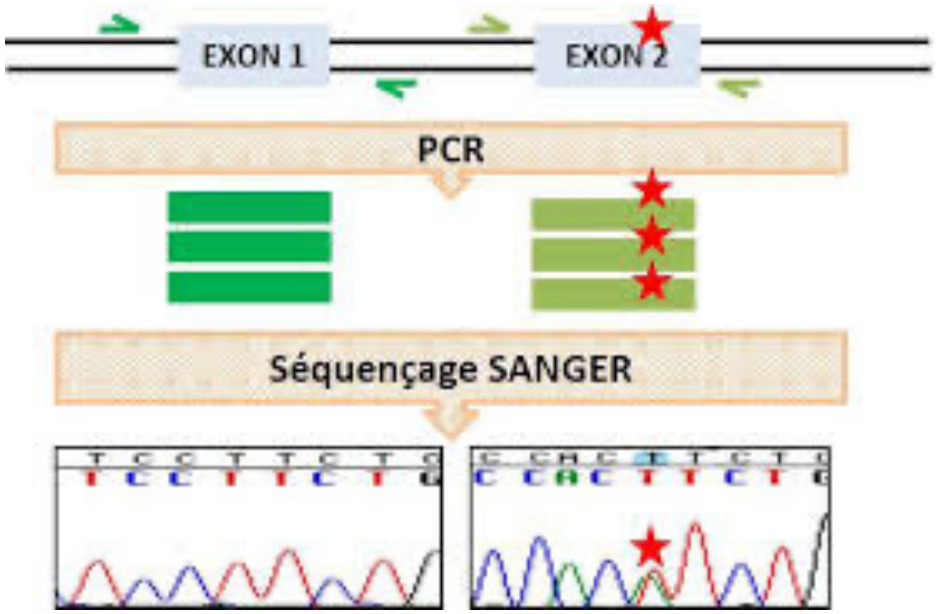
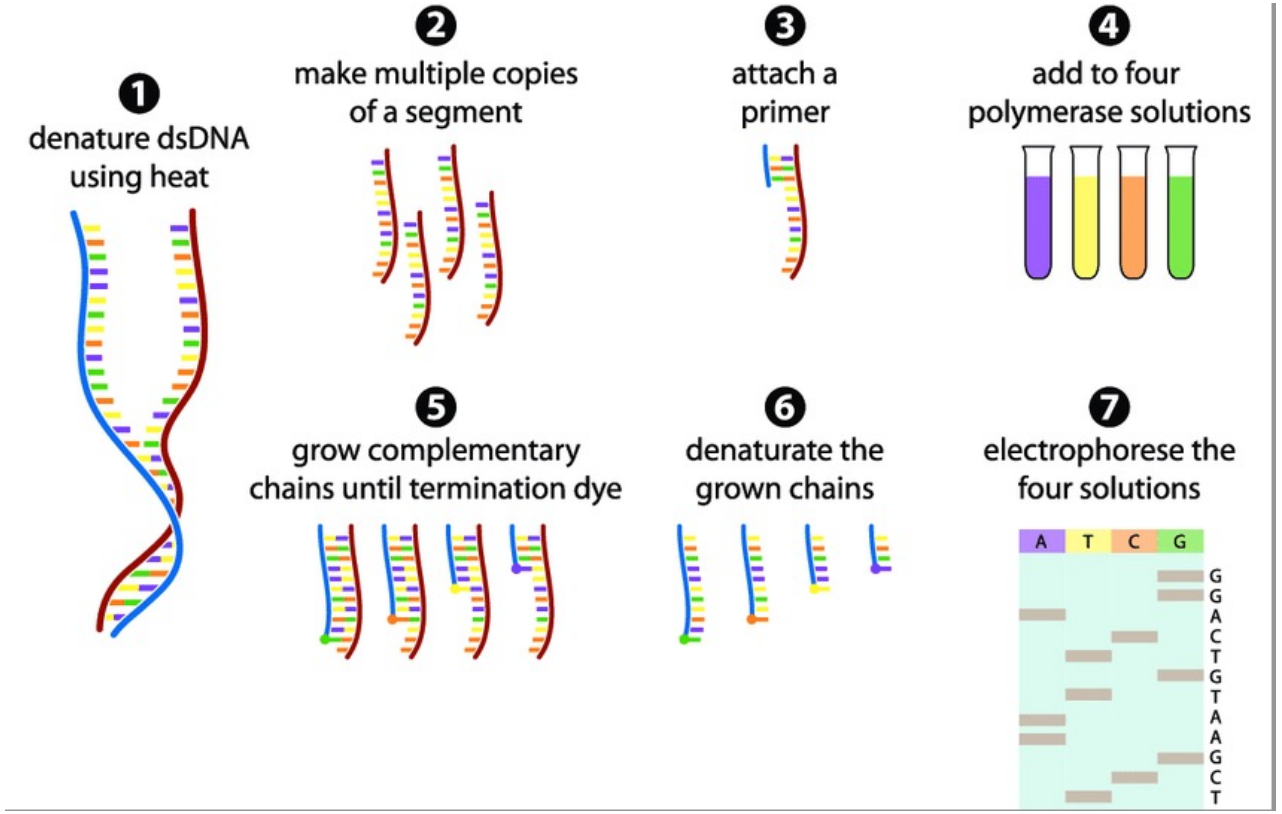
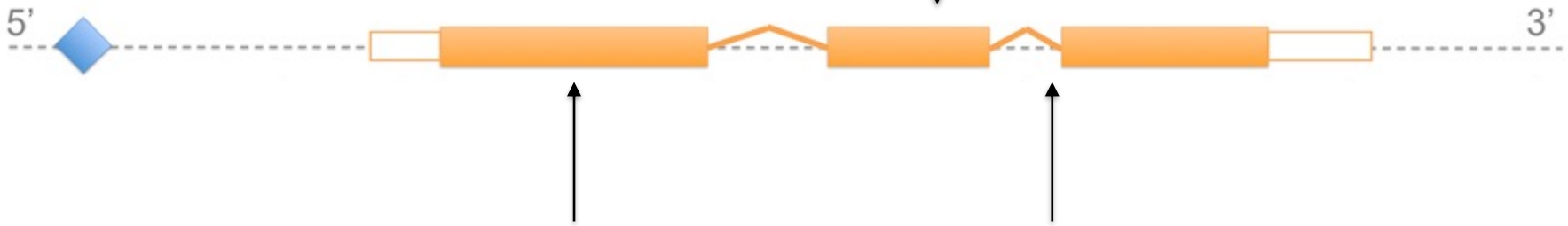
Clonage positionnel : Séquençage SANGER





Effet des variants génétiques

Variant non-sens (stop)
- Changement du cadre de lecture (stop)



Variant faux-sens
Changement d'un acide aminé

Variant intronique (++ épissage)

- Effet de ces mutations variables selon la protéine
  - Haplo insuffisance (50 % de réduction d'activité
  - Gain de fonction (ex. SCN5A /LQT3; fuite de courant sodique)
  - Effet dominant négatif (peptide poison; ex Canaux K^+; structure $\alpha 4$)

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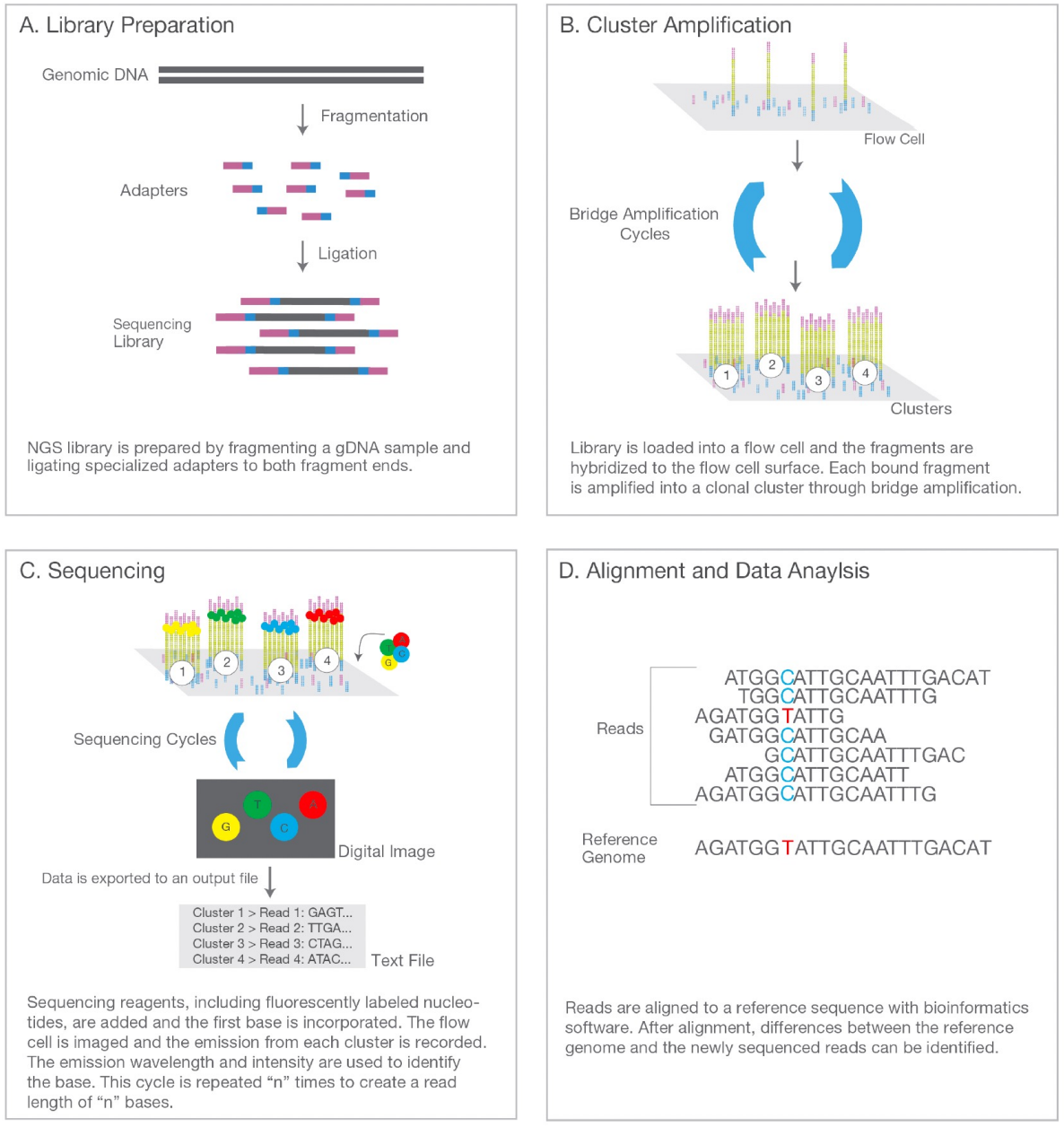
Next generation sequencing – massively parallel sequencing



Next-Generation Sequencing Chemistry Overview—Illumina NGS includes four steps: (A) library preparation, (B) cluster generation, (C) sequencing, and (D) t and data analysis.

- Exome sequencing: capture all coding regions of the genome 50 Mb (< 2% genome)

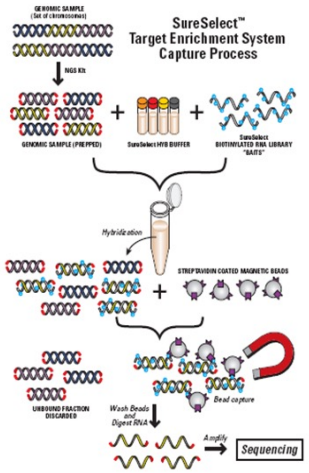
Evolution and Functional Impact of Rare Coding Variation from Deep Sequencing of Human Exomes
Jacob A. Tennessen *et al.*
Science **337**, 64 (2012);
DOI: 10.1126/science.1219240

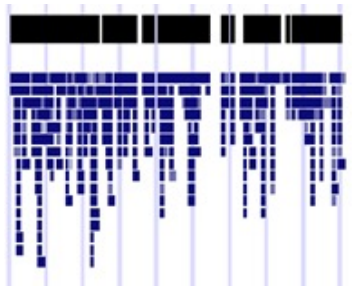
Science
AAAS

- Whole Genome: full spectrum of allelic variability & Copy Number Variations
- RNA-seq
- Epigenetic marks (ATAC-Seq…)

Séquençage d'Exome (NGS)

Sample preparation → Sequencing → Alignment → Variant Calling





```
Reference   ..TTTAGTAATAACAT--GTGCATCTTTAATC..
  Read_01   ..TTTAGGAATAACATGCGTGCA
  Read_02   ..TTTAGGAATAACATGCGTGCATCT
  Read_03   ..TTTAGGAATAACATGCGTCCATCTT
  Read_04   ..TTTAGGAATAACATGCGTGCATCTTTAAT
  Read_05   ..TTTAGGAATAACATGCGTGCATCTTTAATC..
  Read_06   ..TTTAGGAATAACATGCGTGCATCTTTAATC..
  Read_07   ..TTTAGGAATAACATGCGTGCATCTTTAATC..
  Read_08         AATAACATGCGTGCATCTTTAATC..
  Read_09           TAACATGCGTGCATCTGTAATC..
```

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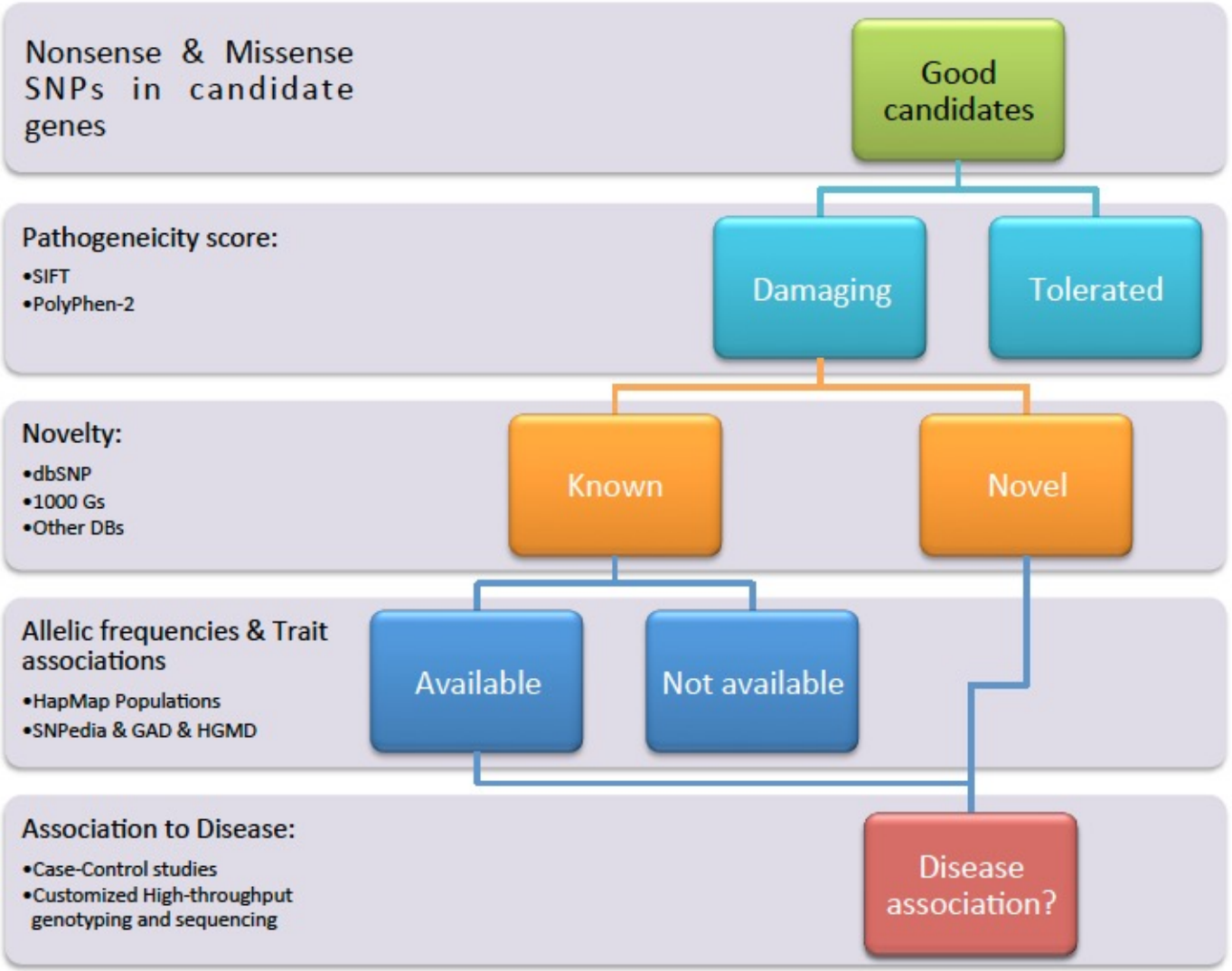
Variant Filtering



Identification de variants rares causaux grâce aux nouvelles technologies de séquençage

CRHOM	POSITION	REF	ALT
5	112175526	G	C
5	125820120	G	C
10	5138672	C	G
22	16449042	T	A
5	56177051	C	G
5	75913610	C	T
6	10894103	C	T
9	102068277	A	C
9	112015730	T	C
1	897829	C	G
1	8415627	T	C
1	17722114	G	A
1	86173964	C	T
1	92765756	A	G
1	113238173	C	T
1	120458982	C	G
1	143378919	T	C
1	146639425	G	A
1	159002410	A	G
1	159021815	T	G
1	177250522	G	A
1	179012995	CAGCG	C
1	242022009	G	A
1	247463963	C	T
1	247492516	C	T
10	17083054	T	C
10	28233186	G	A
10	83635880	A	G
10	83992548	G	T
10	99377056	G	T
10	102265186	C	T
10	102778027	TCTC	T
10	116059031	G	C
10	121347832	C	A
10	127429028	A	G
11	1298431	G	A
11	1463811	G	A
11	1904753	A	G
11	3681258	G	C
11	6636687	G	A
11	27363105	A	C
11	34527245	C	T
11	46401474	G	A
11	55111661	TAA	T
11	55433325	C	T
11	59712732	G	A
11	62287110	C	T
11	63066440	T	G
11	64428387	C	T
11	67265077	C	T
11	73744718	C	A

~ 85.000 variants

1. Variants fonctionnels

Variants non-synonymes et d'épissage

CRHOM	POSITION	REF	ALT
5	112175526	G	C
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10	5138672	C	G
22	16449042	T	A
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1	92765756	A	G
1	113238173	C	T
1	120458982	C	G
1	143378919	T	C
1	146639425	G	A
1	159002410	A	G
1	159021815	T	G
1	177250522	G	A
1	179012995	CAGCG	C
1	242022009	G	A
1	247463963	C	T
1	247492516	C	T

~ 15 000 variants

2. Variants rares

Bases de données externes et internes

CRHOM	POSITION	REF	ALT
5	112175526	G	C
5	125820120	G	C
18	31324584	G	A
1	247492516	C	T

~ 200 variants

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11	34527245	C	T
11	46401474	G	A
11	55111661	TAA	T
11	55433325	C	T
11	59712732	G	A
11	62287110	C	T
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1	113238173	C	T
1	120458982	C	G
1	143378919	T	C
1	146639425	G	A
1	159002410	A	G
1	159021815	T	G
1	177250522	G	A
1	179012995	CAGCG	C
1	242022009	G	A
1	247463963	C	T
1	247492516	C	T

~ 15 000 variants

2. Variants rares

Bases de données externes et internes

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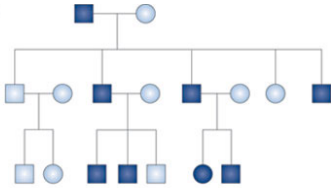
18	31324584	G	A
1	247492516	C	T

~ 200 variants

- Partagés par les individus atteints
  (cas familiaux)



- Liaison génétique

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gnomAD v4.0

gnomAD v4.0

November 01, 2023 in Announcements / Release

Katherine Chao, gnomAD Production Team

Today, we are delighted to announce the release of gnomAD v4, which includes data from 807,162 total individuals. This release is nearly **5x** larger than the combined v2/v3 releases and consists of two callsets: exome sequencing data from 730,947 individuals, including 416,555 individuals from the UK Biobank, and genome sequencing data from 76,215 individuals. Both callsets within v4 were aligned to build **GRCh38** of the human reference genome.

The gnomAD v4 release adds additional global diversity and includes ~**138,000** individuals of non-European genetic ancestry. However, the new inclusion of cohorts such as the UK Biobank means that the proportion of samples with European ancestry is higher than in previous releases. The genetic ancestry group breakdown of gnomAD v4 is:

https://gnomad.broadinstitute.org/news/2023-11-gnomad-v4-0/

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UK Biobank is a large-scale biomedical database and research resource

Researcher log in | Participant log in | Contact us

biobank uk
Enabling scientific discoveries that improve human health

Enable your research | Explore your participation | Learn more about UK Biobank

UK Biobank is a large-scale biomedical database and research resource, containing in-depth genetic and health information from half a million UK participants. The database is regularly augmented with additional data and is globally accessible to approved researchers undertaking vital research into the most common and life-threatening diseases. It is a major contributor to the advancement of modern medicine and treatment and has enabled several scientific discoveries that improve human health.

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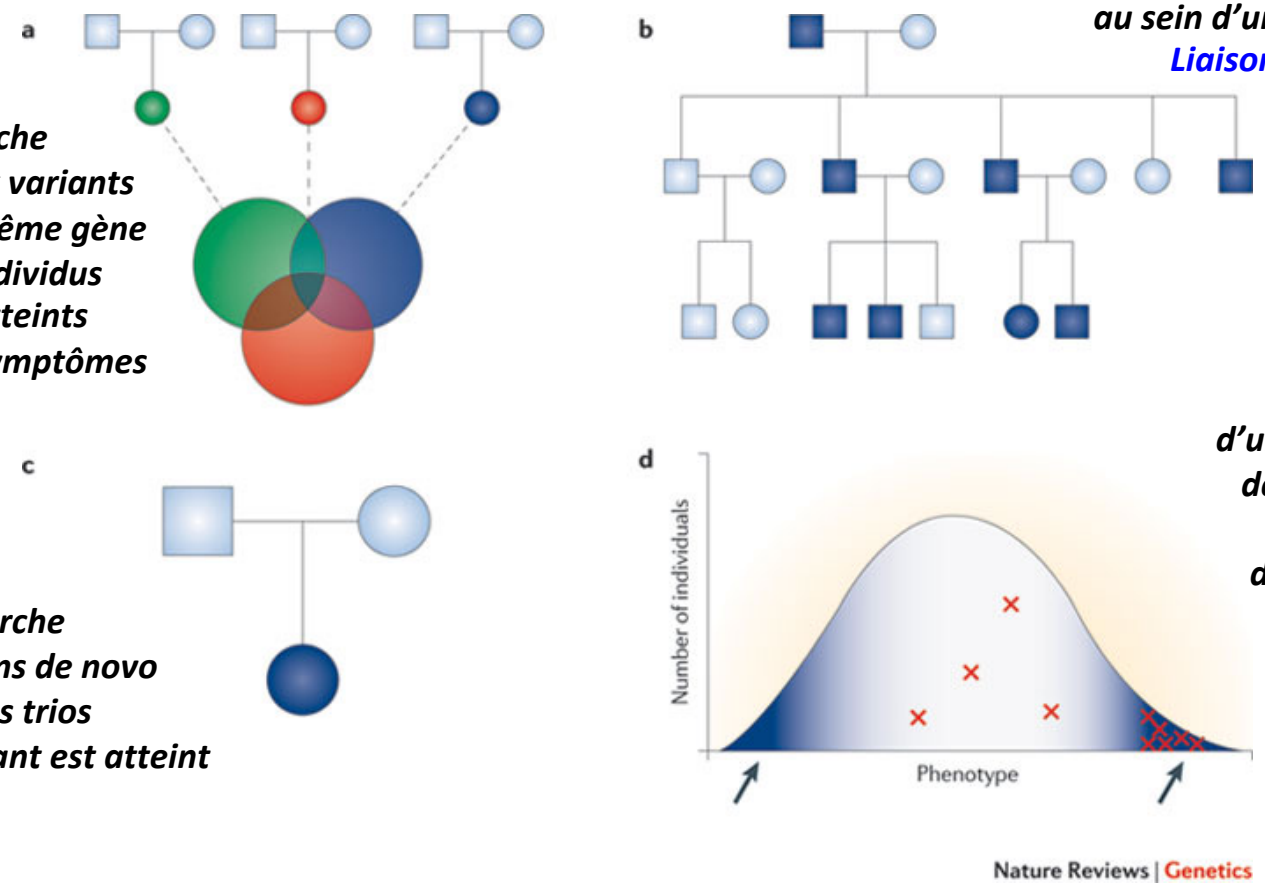
https://www.ukbiobank.ac.uk/

Identification par séquençage d'exomes / génomes de variants rares

Recherche de nouveaux variants partagés entre individus atteints des mêmes symptômes au sein d'une même famille
Liaison génétique

Recherche de nouveaux variants touchant le même gène chez des individus non liés atteints des mêmes symptômes



Recherche de mutations de novo dans des trios dont seul l'enfant est atteint

Recherche d'une concentration de variants rares aux extrêmes d'un phénotype quantitatif

Bamshad et al, *Nat Rev Genet* **12**:745,2011

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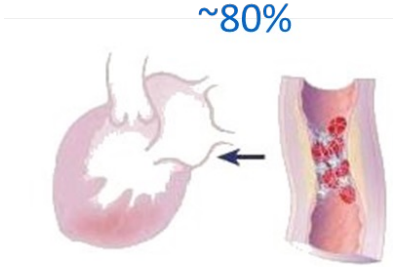
Cardiac arrhythmia

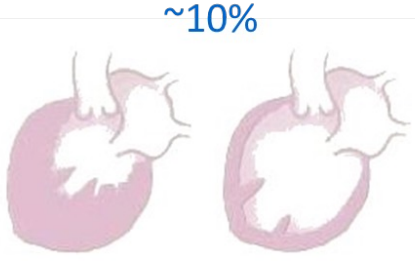
- **Sudden Cardiac Death is a major health burden in industrialized countries**
  - *Ventricular Fibrillation is the most common mechanism*

Myocardial infarction ~80%



Acute plaque destabilization

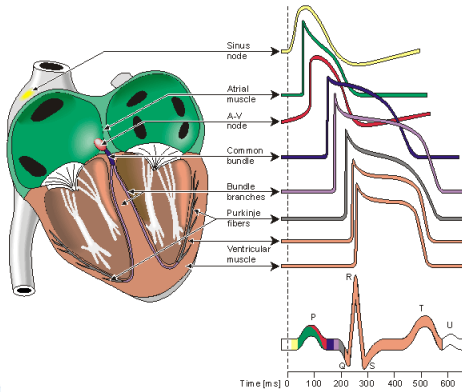
Cardiomyopathy ~10%



Hypertrophic Cardiomyopathy

Dilated Cardiomyopathy

Primary electrical disorders ~10%



⇒ **Brugada syndrome**
⇒ **Early repolarization syndrome**
⇒ **Long QT Syndrome**
⇒ **Catecholaminergic Polymorphic Ventricular Tachycardia**
⇒ **Arrhythmogenic right ventricular cardiomyopathy**
⇒ **Sinus node dysfunction**
⇒ **Cardiac conduction defects**

These electrical disorders provide a homogenous groups of patients with high risk for SCD, which can be considered as a 'sensitized model' for VF

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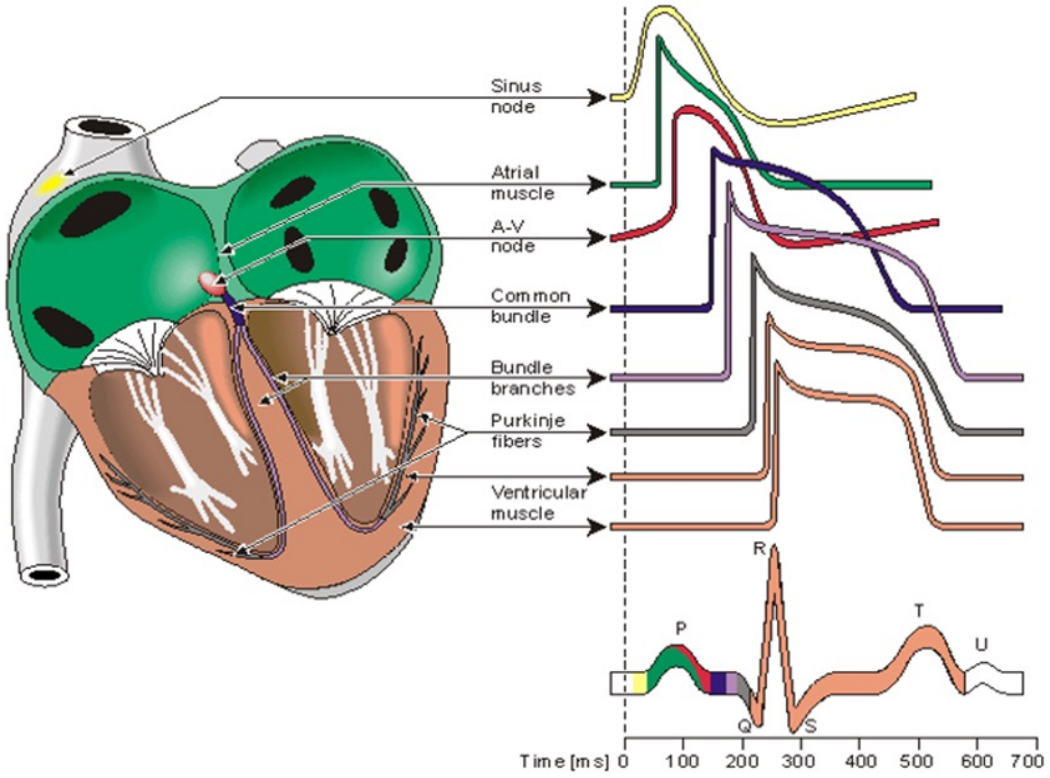
Primary electrical cardiac disorders



Sick sinus syndrome

Atrial arrhythmias

Atrial Fibrillation

Conduction defects

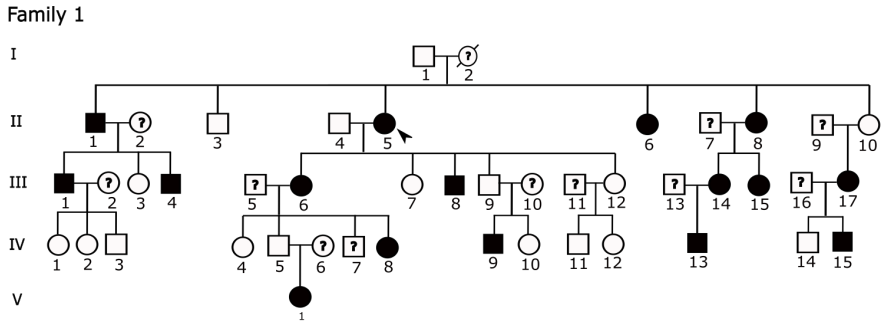
Atrioventricular block

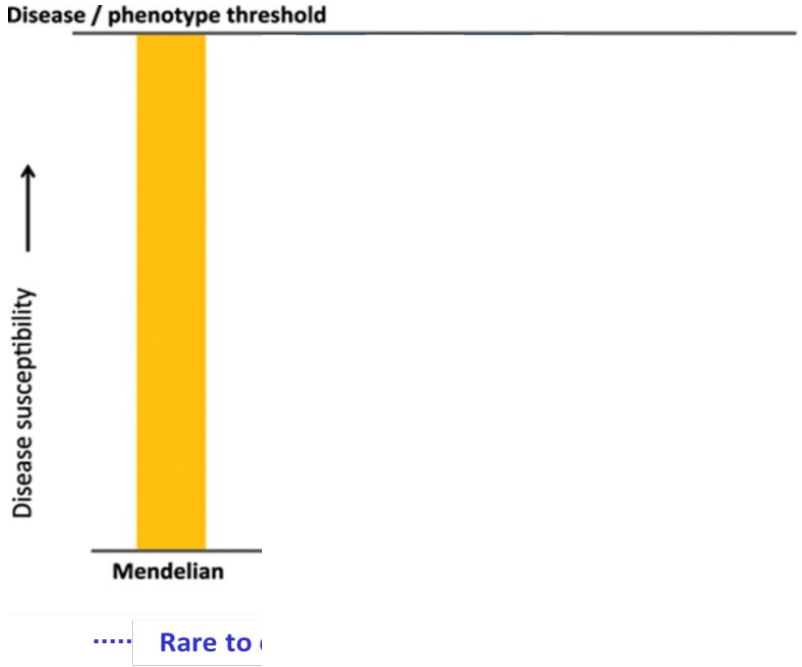
Ventricular arrhythmias

Long QT syndrome

Brugada Syndrome

Early Repolarisation Syndrome





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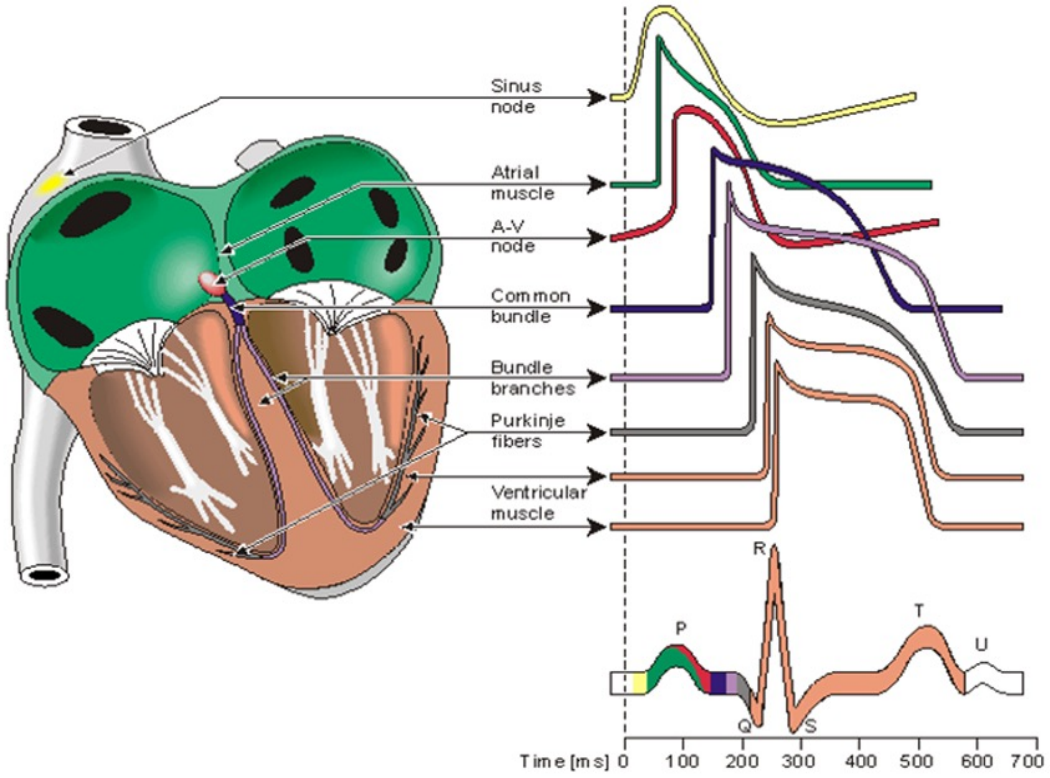
Primary electrical cardiac disorders

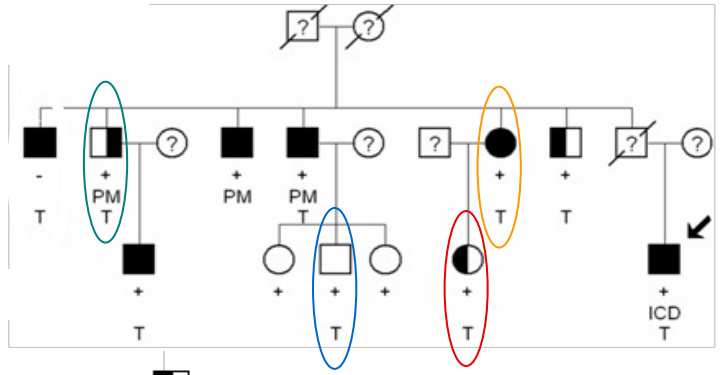


Sick sinus syndrome

Atrial arrhythmias
Atrial Fibrillation

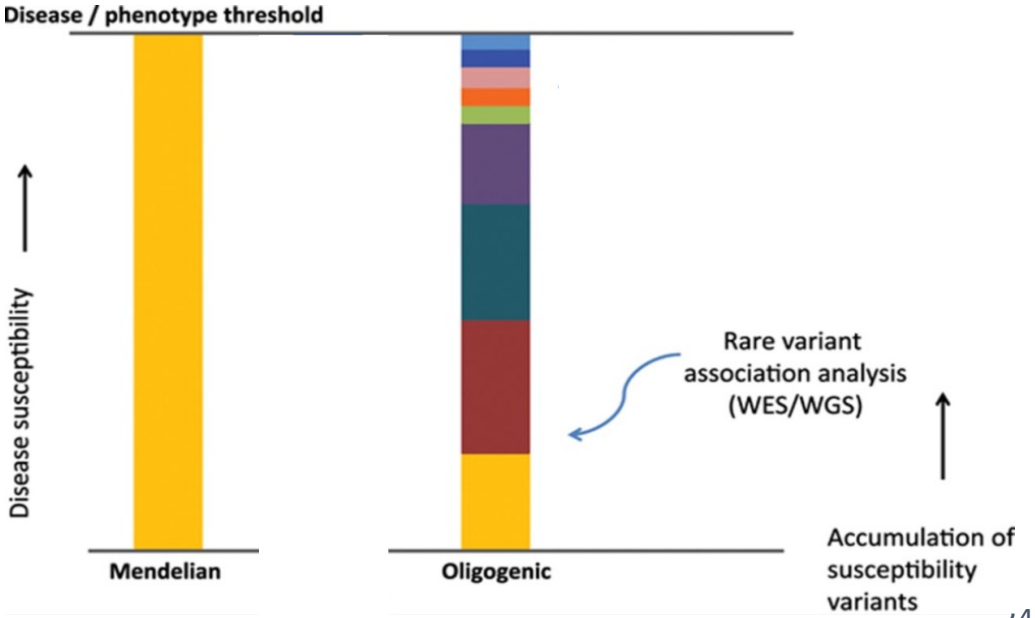
Conduction defects
Atrioventricular bloc

Ventricular arrhythi
Long QT syndrome
Brugada Syndrome
Early Repolarisation



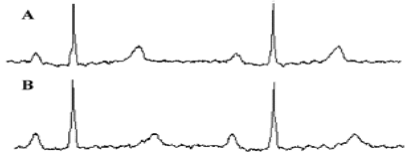
BrS
Conduction disease
Unaffected

Probst *et al.*, 2009



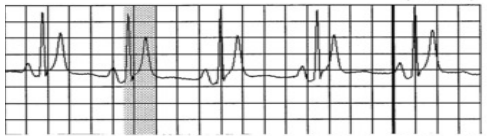
2023 : Cardiac arrhythmias genetics (> 80 genes)

Long QT Syndrome



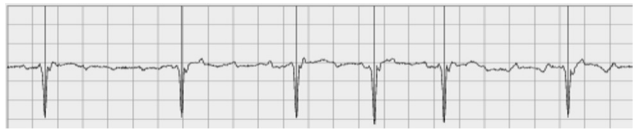
KCNQ1	*KCNJ2*	*KCNJ5*
KCNH2	*CACNA1C*	*SNTA1*
SCN5A	*CAV3*	
ANK2	*SCN4B*	
KCNE1	*CALM1*	
AKAP9	*KCNE2*	

Short QT



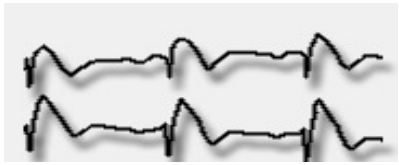
KCNQ1
KCNH2
KCNJ2
CACNA1C
CACN2B
CACNA2D1

Atrial Fibrillation



KCNQ1	*SCN5A*	
SCN3B	*ANK2*	*ABCC9 GJA5*
KCNE1	*KCNE5*	*KCNA5*
KCNE2	*SCN1B*	…
KCNJ2	*SCN2B*	

Brugada Syndrome (27)

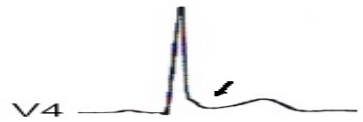


SCN5A	*SCN3B*	*ABCC9*
KCNE5	*KCNE3*	*KCNAB2*
RRAD	*KCND3*	*KCNH2*
GPD1L	*HCN4*	*CACNA1C*
KCNJ8	*SLMAP*	*CACNA2D1*
CACNB2	*TRPM4*	*SCN1B*
RANGRF	*SCN2B*	

Early Repolarization Syndrome (6)

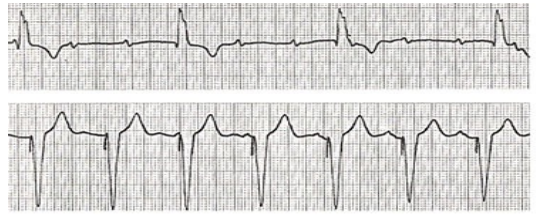
KCNJ8	*CACNB2*
SCN5A	*CACNA2D1*
CACNA1C	*ABCC9*

CPVT



RYR2
CASQ2
TRDN
CALM1

Cardiac conduction block



SCN5A	
SCN1B	*GJA5*
TRPM4	*GJA1*

- Familial rare (linkage/candidate gene)
- Overlap syndrome
- Variable penetrance and expressivity

Arrhythmias gene panel : 109 genes
Centre de référence de Nantes/ Laboratoire de génétique moléculaire
S. Bezieau)

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ACMG Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing

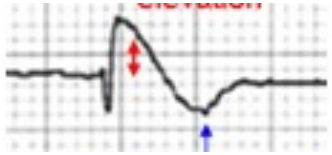
The American College of Medical Genetics and Genomics has published recommendations for reporting incidental findings in the exons of certain genes.

Adenomatous polyposis coli (MIM 175100)	APC (MIM 611731)
Aortic aneurysm, familial thoracic 4 (MIM 132900)	MYH11 (MIM 160745)
Aortic aneurysm, familial thoracic 6 (MIM 611788)	ACTA2 (MIM 102620)
Arrhythmogenic right ventricular cardiomyopathy, type 5 (MIM 604400)	TMEM43 (MIM 612048)
Arrhythmogenic right ventricular cardiomyopathy, type 8 (MIM 607450)	DSP (MIM 125647)
Arrhythmogenic right ventricular cardiomyopathy, type 9 (MIM 609040)	PKP2 (MIM 602861)
Arrhythmogenic right ventricular cardiomyopathy, type 10 (MIM 610193)	DSG2 (MIM 125671)
Arrhythmogenic right ventricular cardiomyopathy, type 11 (MIM 610476)	DSC2 (MIM 125645)
Breast-ovarian cancer, familial 1 (MIM 604370)	BRCA1 (MIM 113705)
Breast-ovarian cancer, familial 2 (MIM 612555)	BRCA2 (MIM 600185)
Brugada syndrome 1 (MIM 601144)	SCN5A (MIM 600163)
Catecholaminergic polymorphic ventricular tachycardia (MIM 604772)	RYR2 (MIM 180902)
Dilated cardiomyopathy 1A (MIM 115200)	LMNA (MIM 150330)
Dilated cardiomyopathy 1A (MIM 115200)	MYBPC3 (MIM 600958)
Ehlers-Danlos syndrome, type 4 (MIM 130050)	COL3A1 (MIM 120180)
Fabry's disease (MIM 301500)	GLA (MIM 300644)
Familial hypercholesterolemia (MIM 143890)	APOB (MIM 107730) LDLR (MIM 606945)
Familial hypertrophic cardiomyopathy 1 (MIM 192600)	MYH7 (MIM 160760)
Familial hypertrophic cardiomyopathy 3 (MIM 115196)	TPM1 (MIM 191010)
Familial hypertrophic cardiomyopathy 4 (MIM 115197)	MYBPC3 (MIM 600958)
Familial hypertrophic cardiomyopathy 6 (MIM 600858)	PRKAG2 (MIM 602743)
Familial hypertrophic cardiomyopathy 7 (MIM 613690)	TNNI3 (MIM 191044)
Familial hypertrophic cardiomyopathy 8 (MIM 608751)	MYL3 (MIM 160790)
Familial hypertrophic cardiomyopathy 10 (MIM 608758)	MYL2 (MIM 160781)
Familial hypertrophic cardiomyopathy 11 (MIM 612098)	ACTC1 (MIM 102540)
Familial medullary thyroid carcinoma (MIM 155240)	RET (MIM 164761)
Hypercholesterolemia, autosomal dominant, 3 (MIM 603776)	PCSK9 (MIM 607786)
Juvenile polyposis syndrome, (MIM 174900)	BMPR1A (MIM 601299)
Juvenile polyposis syndrome, (MIM 174900)	SMAD4 (MIM 600993)
Left ventricular noncompaction 6 (MIM 601494)	TNNT2 (MIM 191045)
Li-Fraumeni syndrome 1 (MIM 151623)	TP53 (MIM 191170)
Loeys-Dietz syndrome type 1A (MIM 609192)	TGFBR1 (MIM 190181)
Loeys-Dietz syndrome type 1B (MIM 610168)	TGFBR2 (MIM 190182)
Loeys-Dietz syndrome type 2A (MIM 608967)	TGFBR1 (MIM 190181)
Loeys-Dietz syndrome type 2B (MIM 610380)	TGFBR2 (MIM 190182)
Loeys-Dietz syndrome type 3 (MIM 613795)	SMAD3 (MIM 603109)
Long QT syndrome 1 (MIM 192500)	KCNQ1 (MIM 607542)
Long QT syndrome 2 (MIM 613688)	KCNH2 (MIM 152427)
Long QT syndrome 3 (MIM 603830)	SCN5A (MIM 600163)

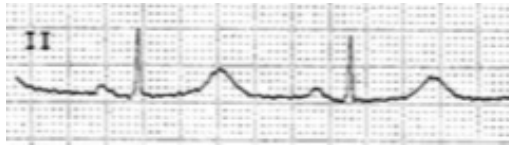
➢ **78 actionable genes**

- 19 cardiac diseases
  - 15 cardiomyopathies (ARVC, DCM, HCM)
  - 4 arrhythmias

- Brugada Syndrome



- Long QT Syndrome (1,2 & 3)



(*SCN5A, KCNQ1, KCNH2*)

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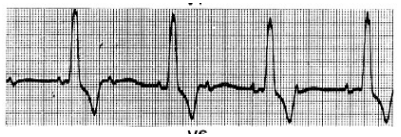
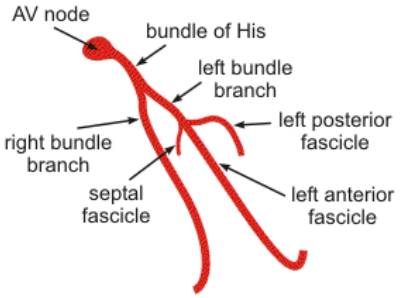
Gene identification by Exome sequencing

Cardiac Conduction Defects



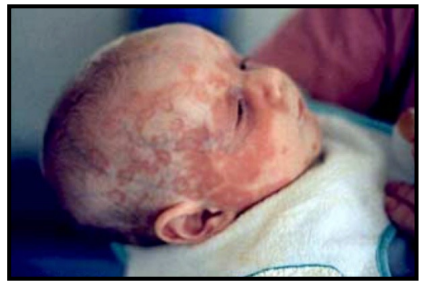
- **Historically isolated Progressive Cardiac Conduction Defects, the most common form of CCD, (Lenègre & Lev disease) was considered as a structural and degenerative disease mostly due to aging, fibrosis in the conduction system**

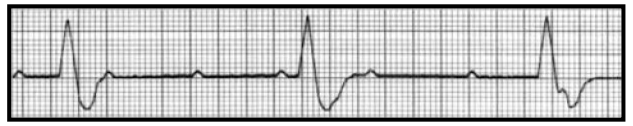


◆ Strong genetic background / genetic heterogeneity:

- **Isolated PCCD: Channelopathy, *SCN5A, SCN1B, TRPM4, GJA5***
- **PCCD & Cardiomyopathies: *NKX2.5, TBX5, PRKAG2, LMNA***

- **Non-immune isolated Congenital AV block is a rare condition**
  - **Prevalence : 1/15 000**
  - **Immunological compound in 80 % congenital AVB**
  - **Mortality : 30 %**





◆ Suspected Strong genetic background

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Search for new Congenital & early childhood AVB index genes: Strategy

- **Patient recruitment:**
  - **141 Index cases**
    - **Diagnosed under age 15**
      (13 centers in France)

European Heart Journal Advance Access published September 14, 2011

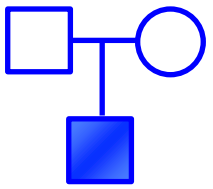
European Heart Journal
doi:10.1093/eurheartj/ehr347

CLINICAL RESEARCH

Characteristics and long-term outcome of non-immune isolated atrioventricular block diagnosed *in utero* or early childhood: a multicentre study

Alban-Elouen Baruteau[1,2,3,4,5,6*], Swanny Fouchard[1], Albin Behaghel[2,3,4,7], Philippe Mabo[2,3,4,7], Elisabeth Villain[6,8], Jean-Benoit Thambo[6,9], François Marçon[6,10], Véronique Gournay[6,11], Francis Rouault[6,12], Alain Chantepie[6,13], Sophie Guillaumont[6,14], François Godart[6,15], Caroline Bonnet[6,16], Alain Fraisse[6,17], Jean-Marc Schleich[2,3,4,6,7], Jean-René Lusson[6,18], Yves Dulac[6,19], Christophe Leclercq[2,3,4,7], Jean-Claude Daubert[2,3,4,7], Jean-Jacques Schott[1], Hervé Le Marec[1,20], and Vincent Probst[1,20]

- **cAVB – trios**



- **15 trios**
  - **No familial history of AV block**
  - **Unaffected parents**

- **Exome sequencing**

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Exome sequencing of 15 trios

➢ 19 *de novo* variants

Trio	Gène	Nucléotide	Acide Aminé	Conséquence
0	*NSUN6*	c.956 C>T	p.R319Q	Faux-sens
1	*GCN1L1*	G>T		Site d'épissage (intronique)
2	*SHBG*	c.74G>A	p.R25H	Faux-sens
2	*CNTROB*	c.137G>A	p.R46Q	Faux-sens
3	*USH2A*	c.82T>A	p.I28L	Faux-sens
3	*SOS2*	c.58G>C	p.R20G	Faux-sens
4	*GPR107*	c.1396C>A	p.L466I	Faux-sens
6	*PTGER3*	C>A		Site d'épissage (intronique)
7	*TTC21B*	c.3641 T>C	p.D1214G	Faux-sens
7	*C7orf61*	c.209 A>G	p.L70S	Faux-sens
8	*TEX29*	G>C		Site d'épissage (5'-UTR)
9	*LPHN2*	c.296 A>G	p.N99S	Faux-sens
9	*RHOBTB1*	c.1259 G>A	p.T420M	Faux-sens
10	*RYR2*	c.10361 G>A	p.R3454H	Faux-sens
11	*ZNF683*	c.1219_1226 delCTGCACTG	p.Q407CfsX 461	Frameshift & protéine tronquée
11	*ZNF22*	c.85 C>T	p.Q29X	Gain d'un codon-stop
11	*GJC1*	c.224 C>T	p.R75H	Faux-sens
11	*ATAD2B*	c.4212 C>G	p.L1404F	Faux-sens
14	*DOPEY2*	c.6697 C>T	p.R2233C	Faux-sens

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Variant selection

	Family A (trio11)	Family B
	French case	Japanese case
Target genes for exon capture	All exons (Trio-exome)	457 genes
Coding variations (*)	13,035	1,040
De novo variants	7	NA
MAF<0.1%	4	8
Sanger validation	4	8
Co-segregation	NA	3
Candidate variations	4	3
Common mutation	*GJC1* -c.G224A, (Cx45-p.R75H)	

*: nonsynonymous, stop-gain, stop-loss, splice site replacement, or indel frameshift
NA: not applicable
¶; found in the proband (II:1) but not in his parents

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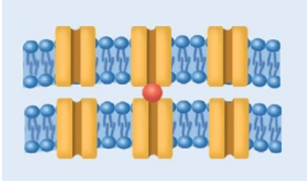
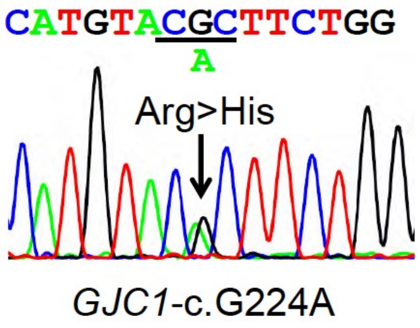
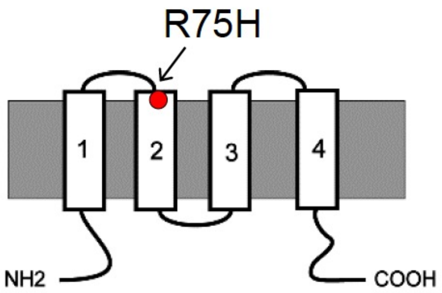
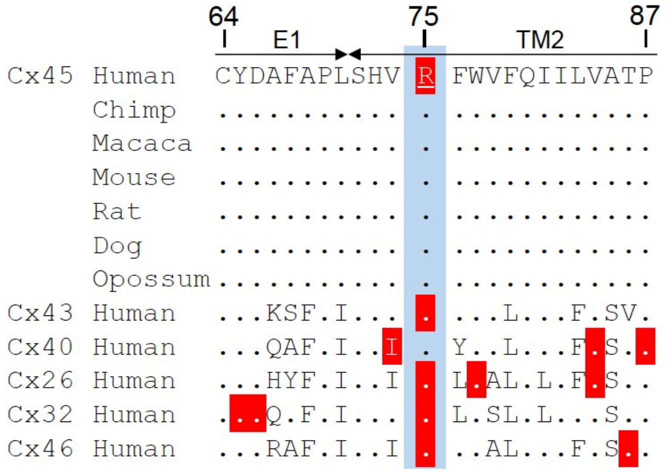
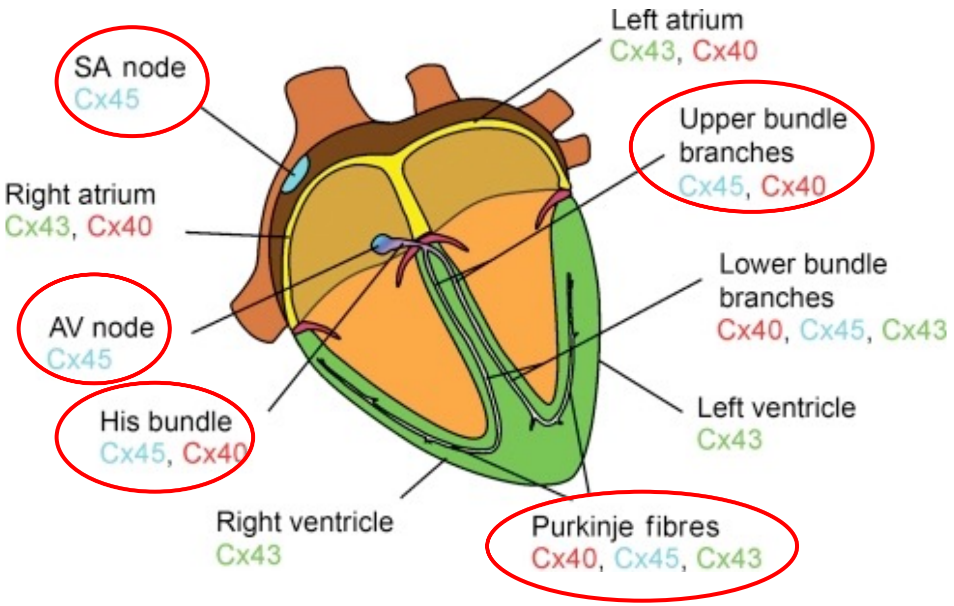
Novel *GJC1* mutation (Cx45-p.R75H)









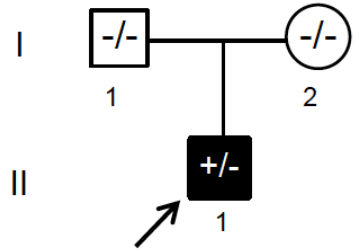


- Connexin family of proteins consists of more than 21 members varying in their biophysical properties

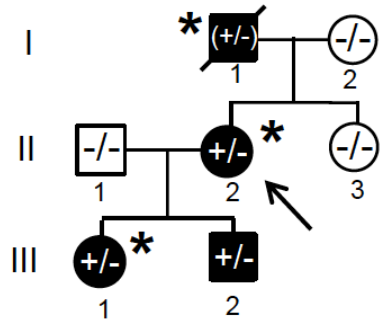
- CX40, 43, **45** heart specific

Sander Verheule et al Front. Pharmacol., 2013

Family A

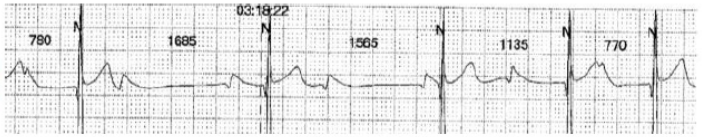


Family B

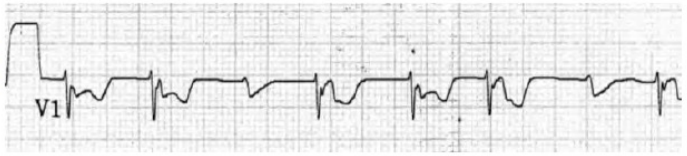


* PM

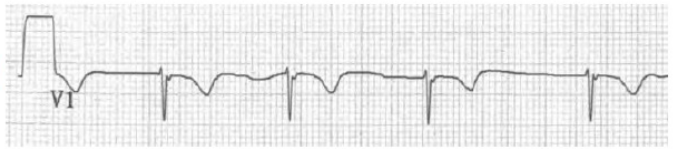
II:1
(2 yo)



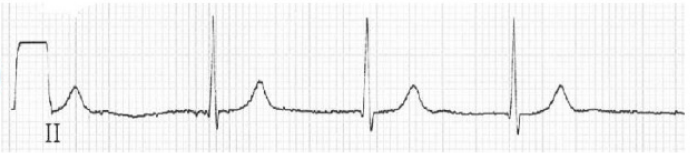
(4 yo)



(5 yo)

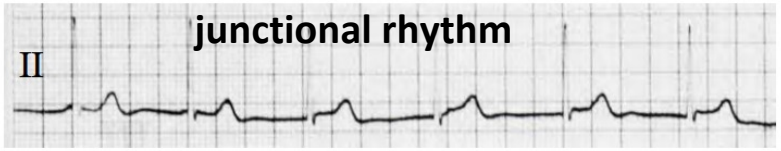


(9 yo)



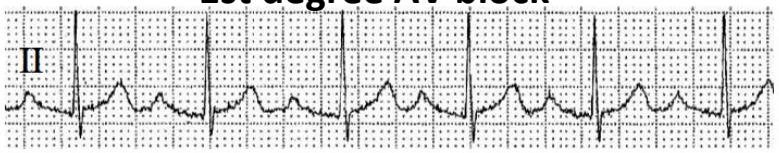
3rd degree AV block & progressive loss of P wave

II:2
(9 yo)

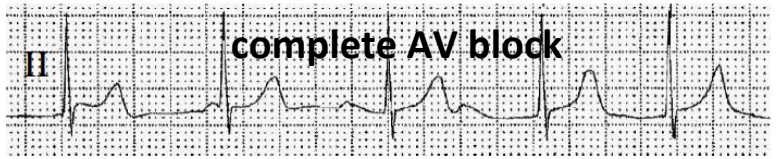


1st degree AV block

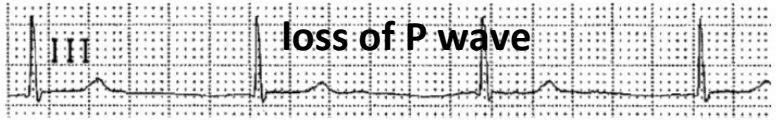
III:1
(8 yo)



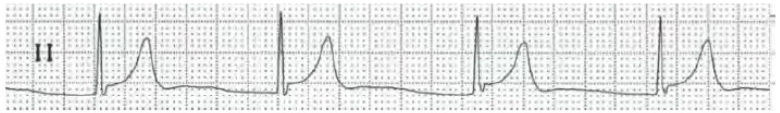
(12 yo)



(14 yo)

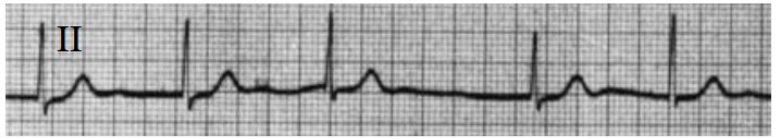


III:2
(14 yo)



junctional rhythm

I:1
(37 yo)



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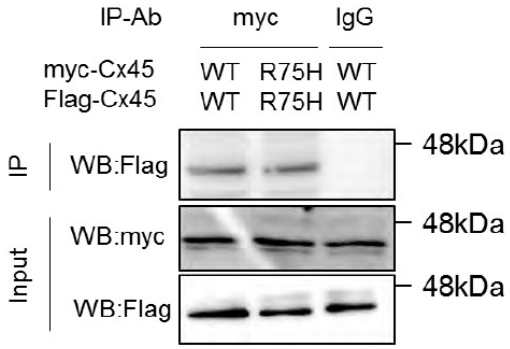
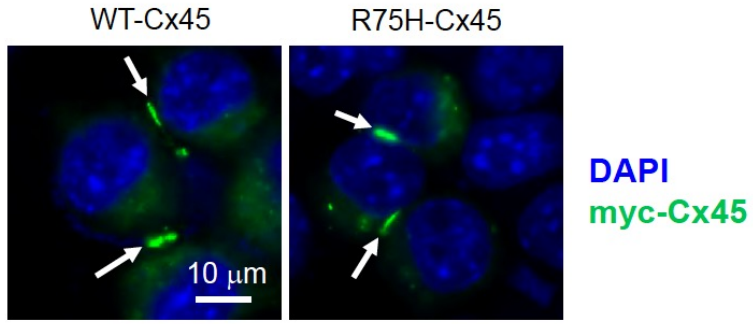
In vitro studies

1- Normal hemichannel assembly and plaque formation

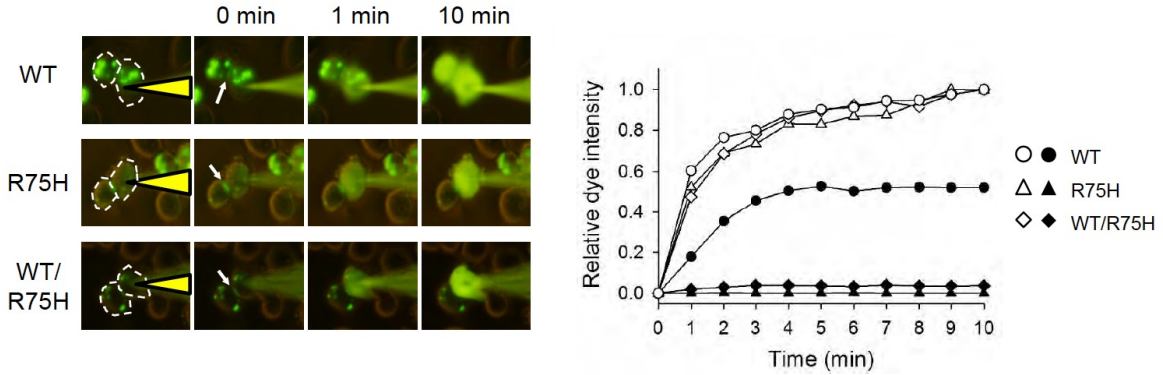
(Co-IP /Immunofluorescent)



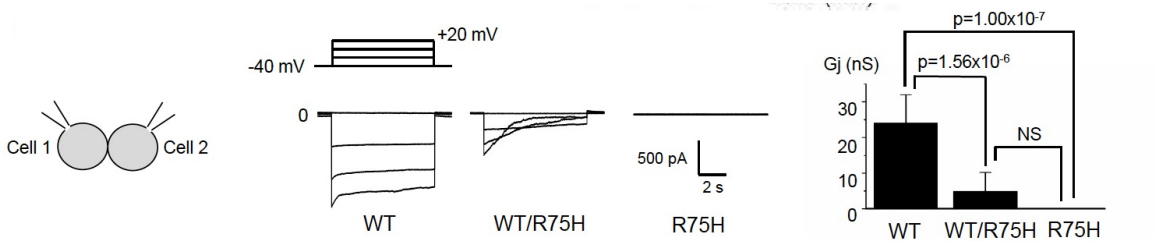
(Immunofluorescent)



2- Severe reduction of permeation properties (Luciferase dye transfer)



3- Macroscopic conductance (Gj) suppressed in hetero & homomeric channels



- Dominant-negative

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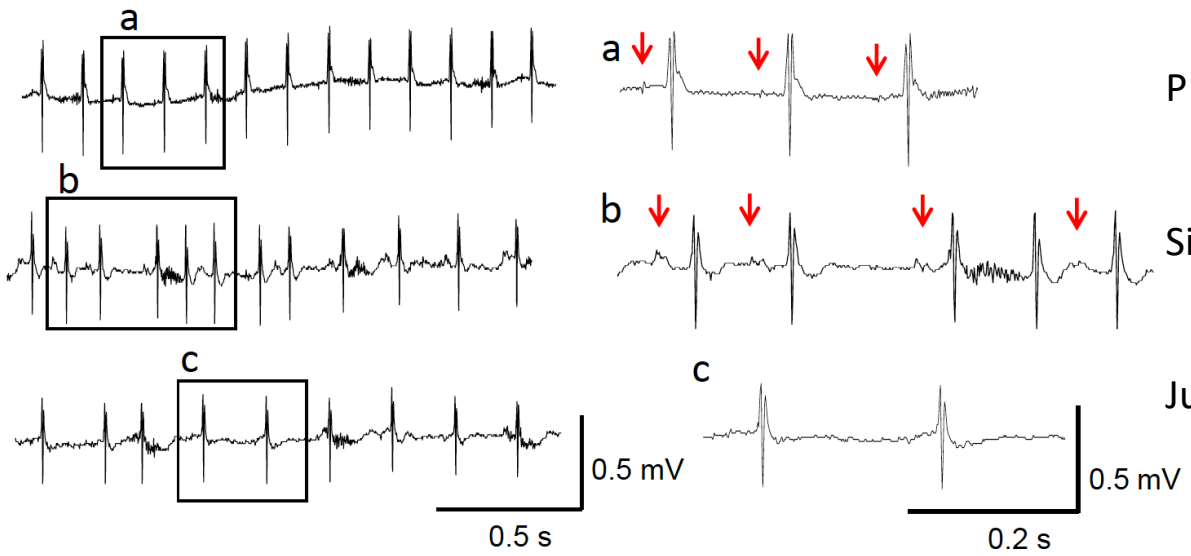
In vivo studies

Electrophysiological properties of conditional *Gjc1* knockout mice

1- Various atrial arrhythmias
(after tamoxifen administration)



- **Pathophysioloy of Cx45-p.R75H in developmental defects?**

Seki et al. J Am Coll Cardiol. 2017

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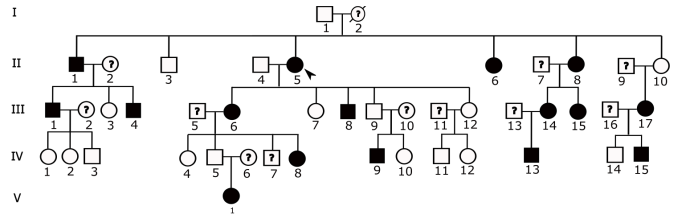
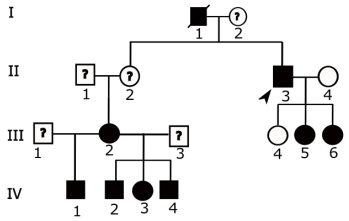
JACC
JOURNAL of the AMERICAN COLLEGE of CARDIOLOGY

Whole Genome Sequencing in unresolved mendelian arrythmias



?



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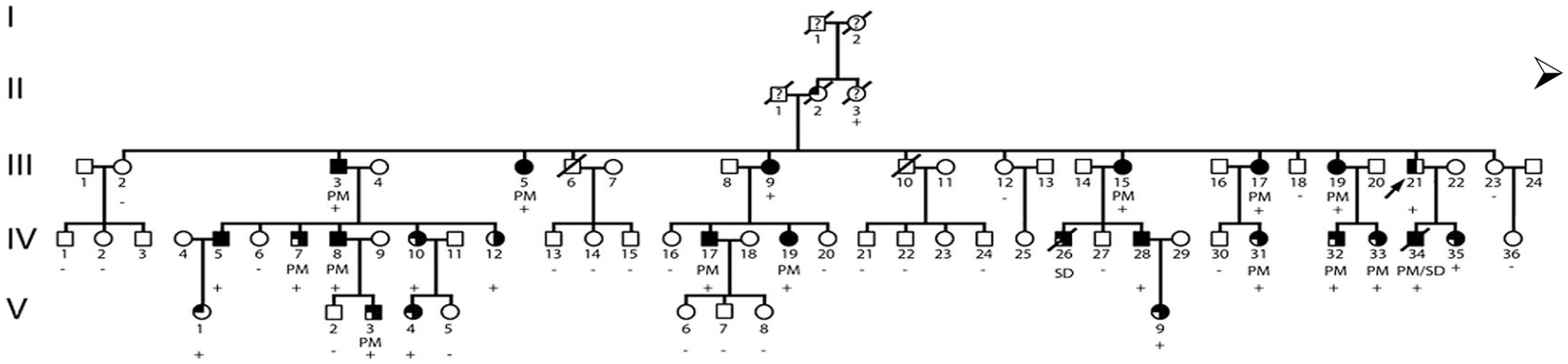
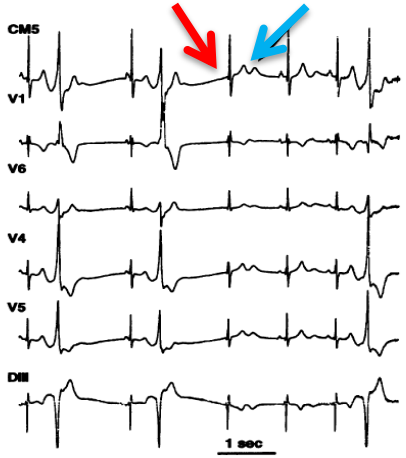
A large family associating sinus node dysfunction & long QT syndrome linked to the *ANK2* gene



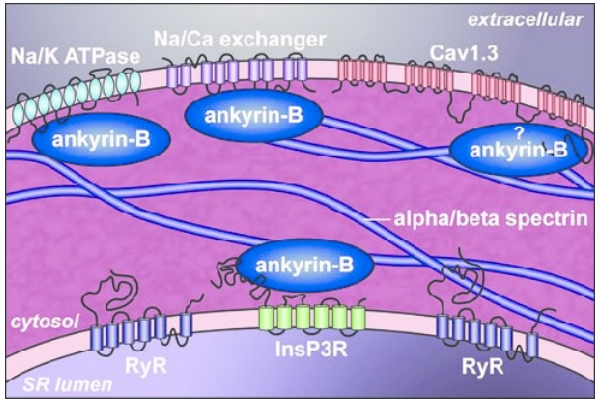
- 24 affected cases
- 2 sudden death (ventricular arrhythmias)



- Long QT syndrome
- Sinus node dysfunction (SND)
- Atrial fibrillation (AF)

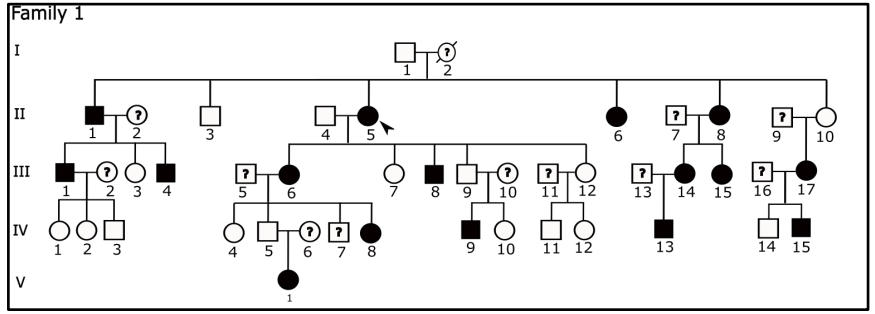
➢ **4q25 linkage**

➢ **Loss of function mutation in *ANK2 (Ankyrin)***



Mohler P; Schott JJ at al. Nature. 2003

➢ **Additional families**
- with positive linkage at 4q25 locus
- *no ANK2* mutation



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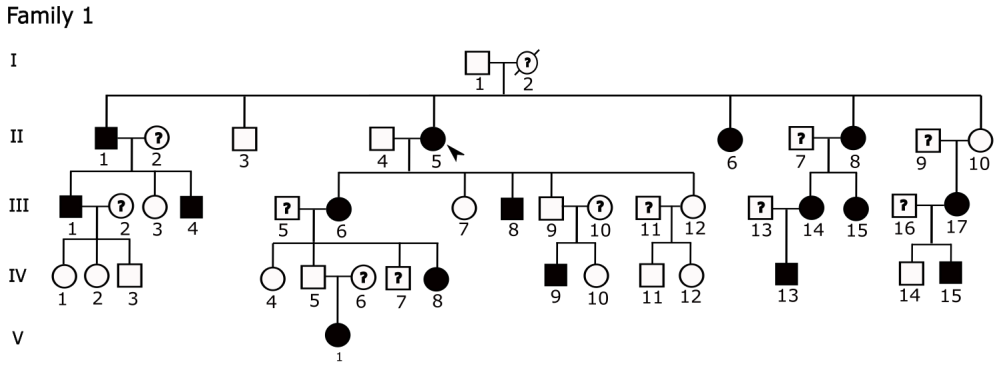
A large *ANK2*-negative family presenting with a complex cardiac syndrome with positive linkage at 4q25 locus



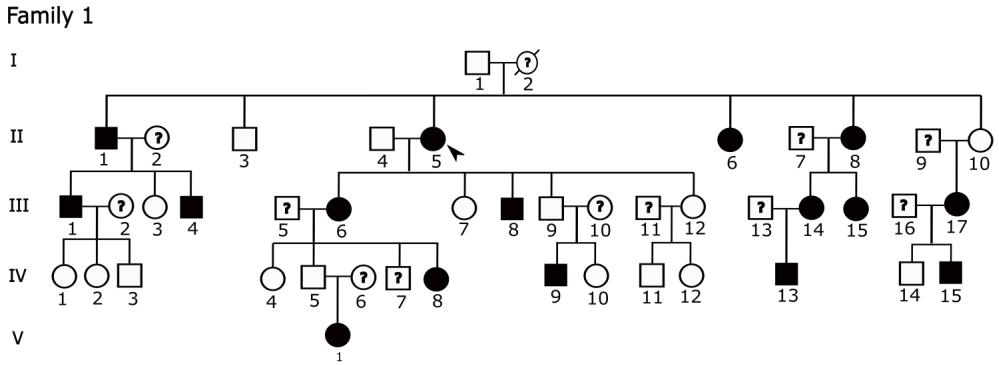
Electrical cardiac disorders:

Sinus node dysfunction
Long QT
Atrial fibrillation

Cardiac developmental defect:

Atrial septal defect
Mitral valve prolapse
LV non-compaction

WGS Identifies a rare 15 kb deletion in a gene desert area



Electrical cardiac disorders:

Sinus node dysfunction
Long QT
Atrial fibrillation

Cardiac developmental defect:

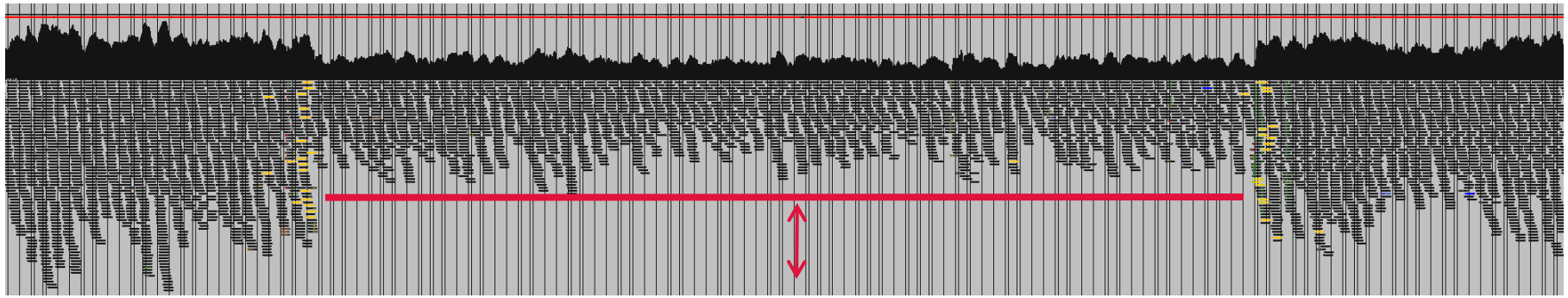
Atrial septal defect
Mitral valve prolapse
LV non-compaction
Vein abnormalities

Linkage analysis
4q25 region associated with Ankyrin B syndrome
PJ.Molher et al 2002

Sanger sequencing
No mutation in *ANK2* gene

Exome sequencing
No mutation in genes located in the 4q25 region

Whole genome sequencing
Identification of a 15kb deletion in a gene desert area



Reduced sequencing depth by 50%

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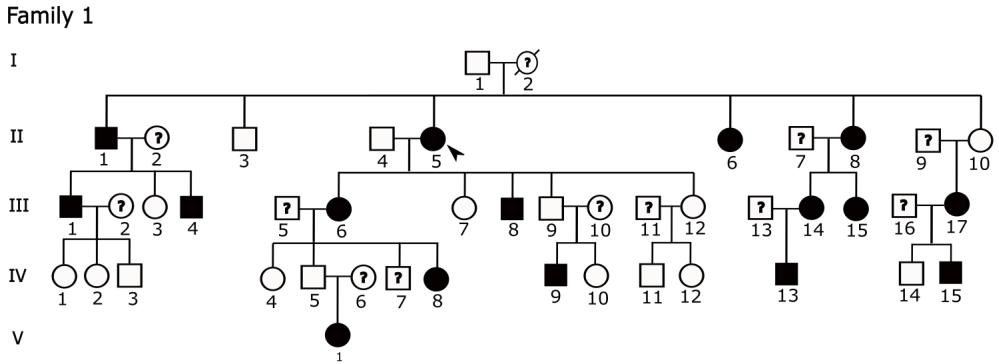
Identification of a large family presenting with a complex cardiac syndrome associated with a 15 kb deletion in a gene desert area



Electrical cardiac disorders:

Sinus node dysfunction
Long QT
Atrial fibrillation

Cardiac developmental defect:

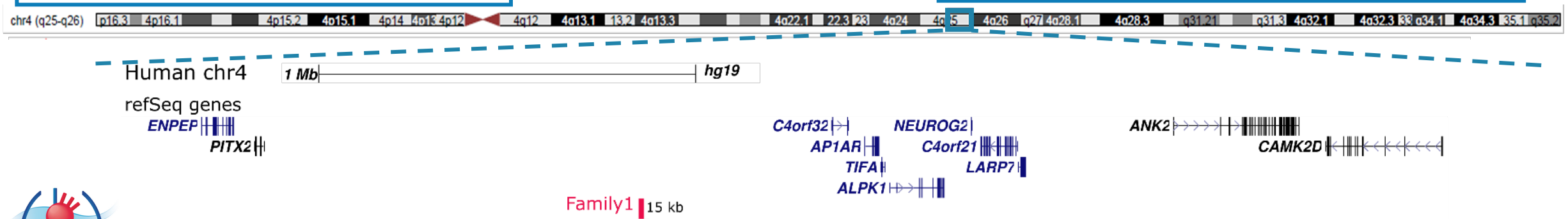
Atrial septal defect
LV non-compaction
Vein abnormalities
Mitral valve prolapse

PITX2

- Cardiac transcription factor essential for the left-right asymmetry of the heart
- Sinoatrial node development
- Risk variants in *PITX2* associated with atrial fibrillation

Ank2

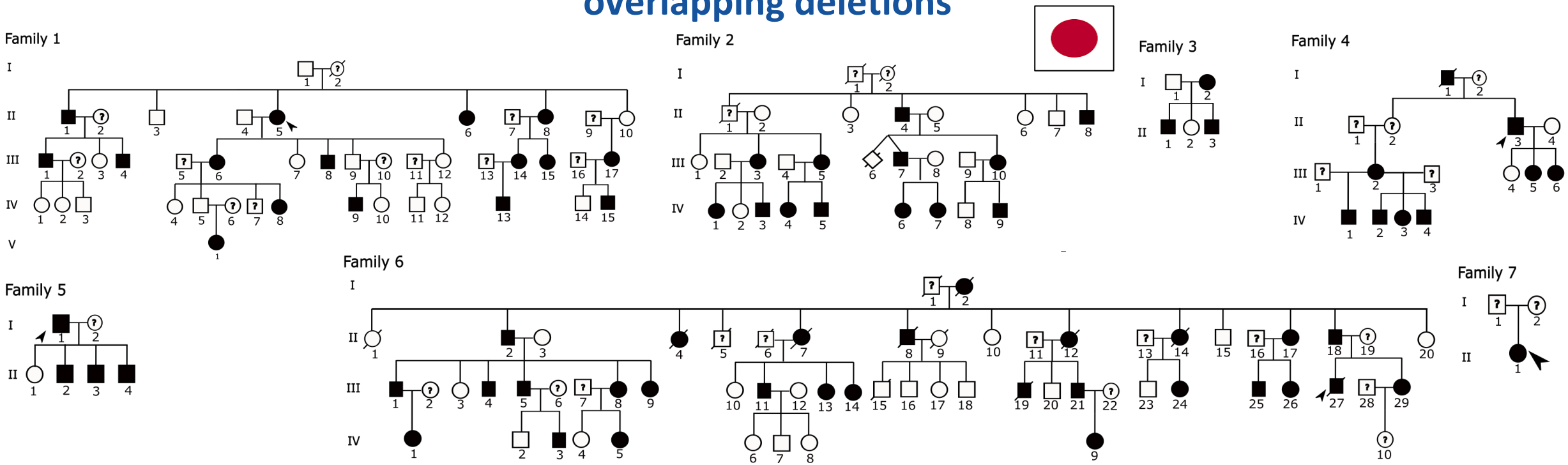
- Localization and membrane stabilization of ion channels
- *ANK2* loss-of-function variants are associated with sinus node disease, atrial fibrillation, ventricular arrhythmia, and risk of sudden cardiac death.

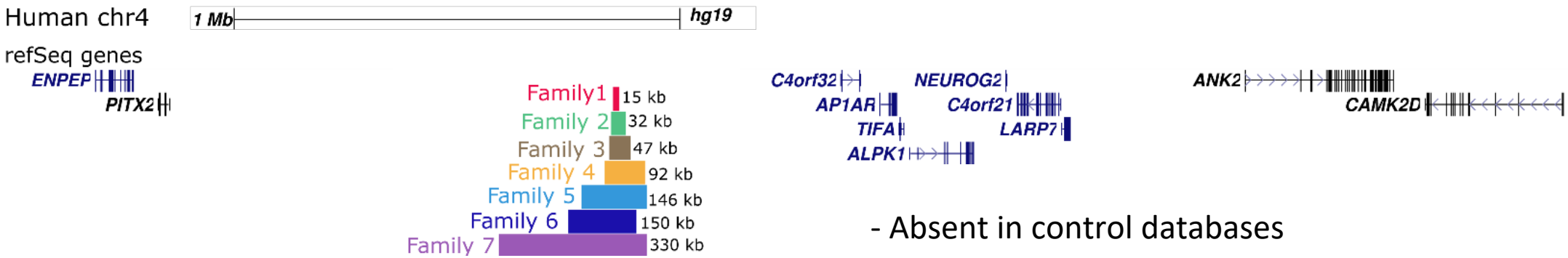


Relevance of this non-coding region to the disease?

Franco et al 2017, Ammirabile et al 2012, Zhang et al 2019, Hill et al 2019, Mohler et al 2003, Le Scouarnec et al 2008, Sucharski et al 2020

Six additional families (4 French and 2 Japanese) presenting with a similar phenotype showing overlapping deletions





- Absent in control databases

Crucial role of this non coding deleted region: regulatory element?

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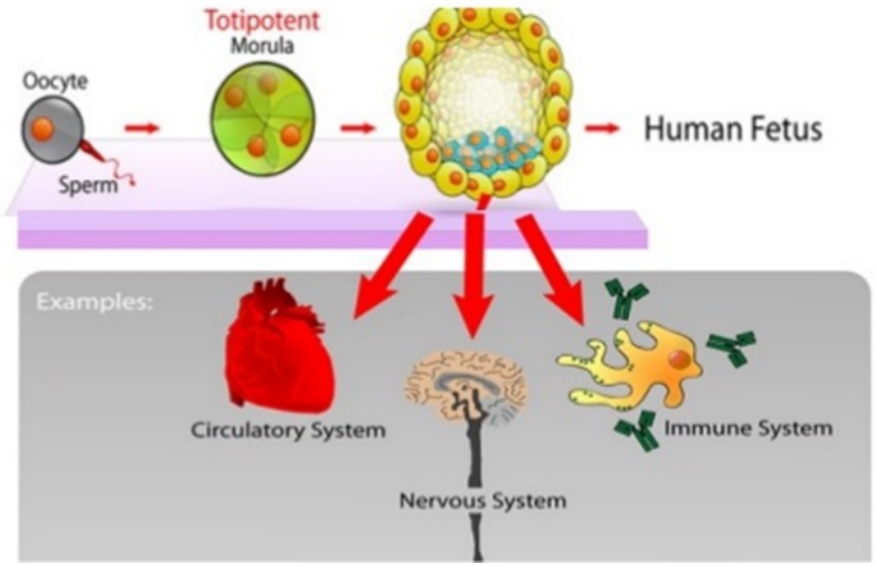
Definition of Epigenetics

Every cell-type in an organism contains the same genetic information but has extremely different shapes and functions.



https://www.slideshare.net/hamivia/definition-of-epigenetics

How cells read the genes

DNA methylation
Chromatin structure and modification
Non-coding RNA (ncRNA)

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Identification of the role of regulatory regions

1- ENCODE databse: Encyclopedia of DNA Elements

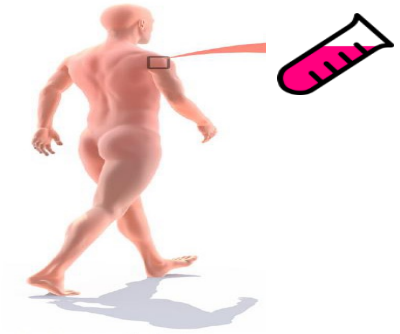
https://www.encodeproject.org/

- Encyclopedia of DNA Elements at UCSC

https://genome.ucsc.edu/encode/

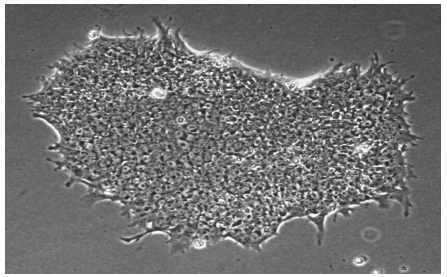
- GENCODE / VISTA …

2- hiPSC (tissue & condition specific)



1. Re-programmation

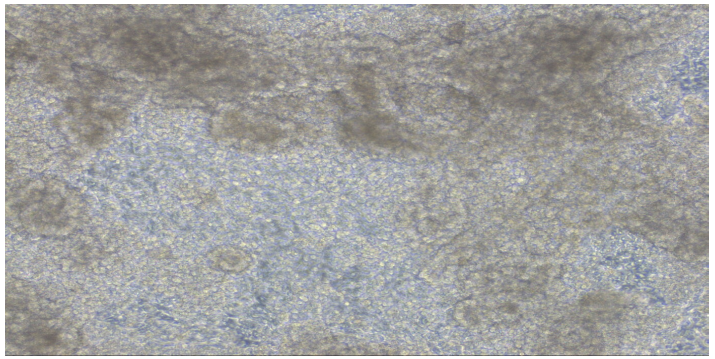
1



Pluripotent cells

2. Differentiation

2



Cardiac human cells

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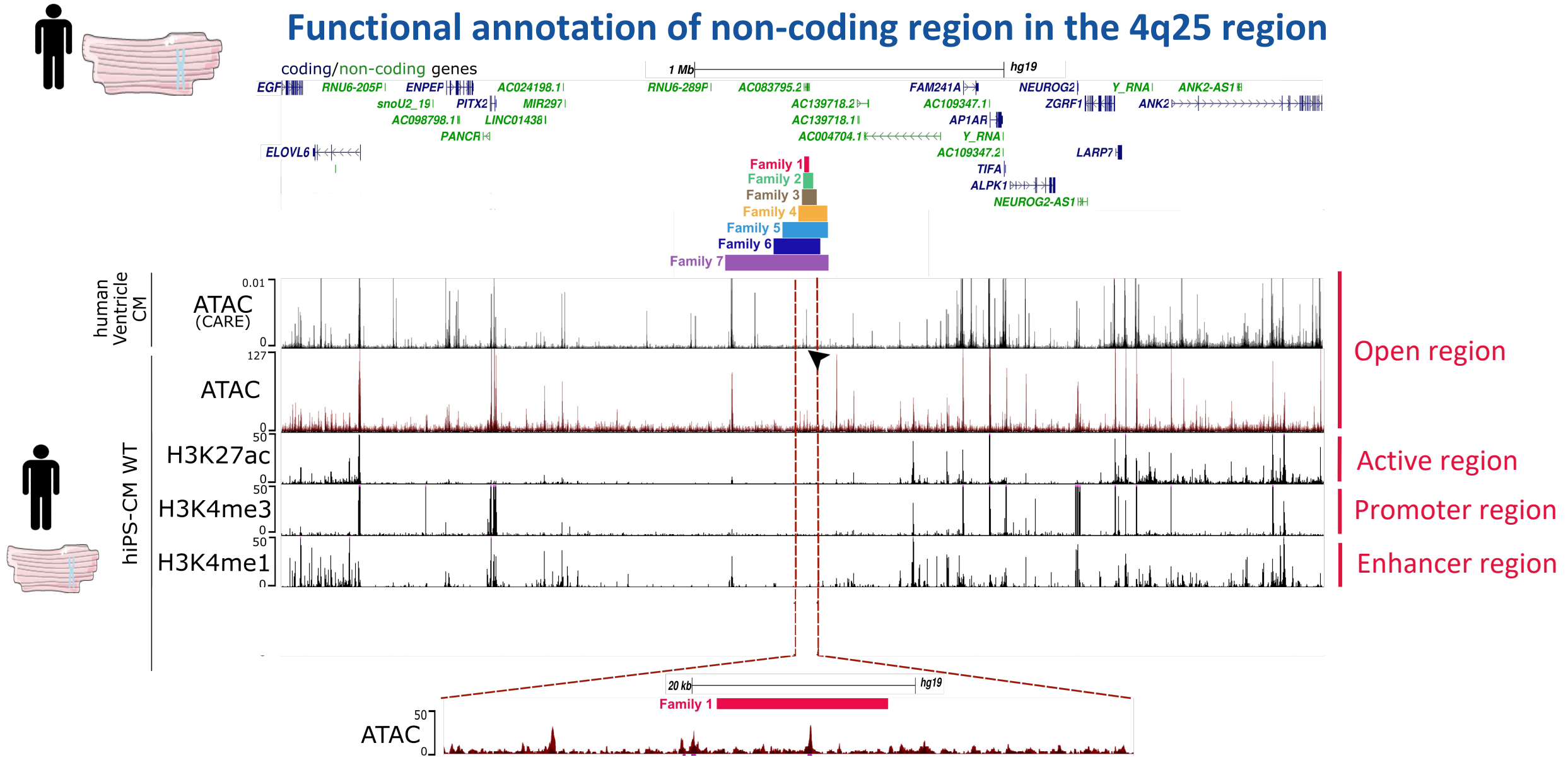
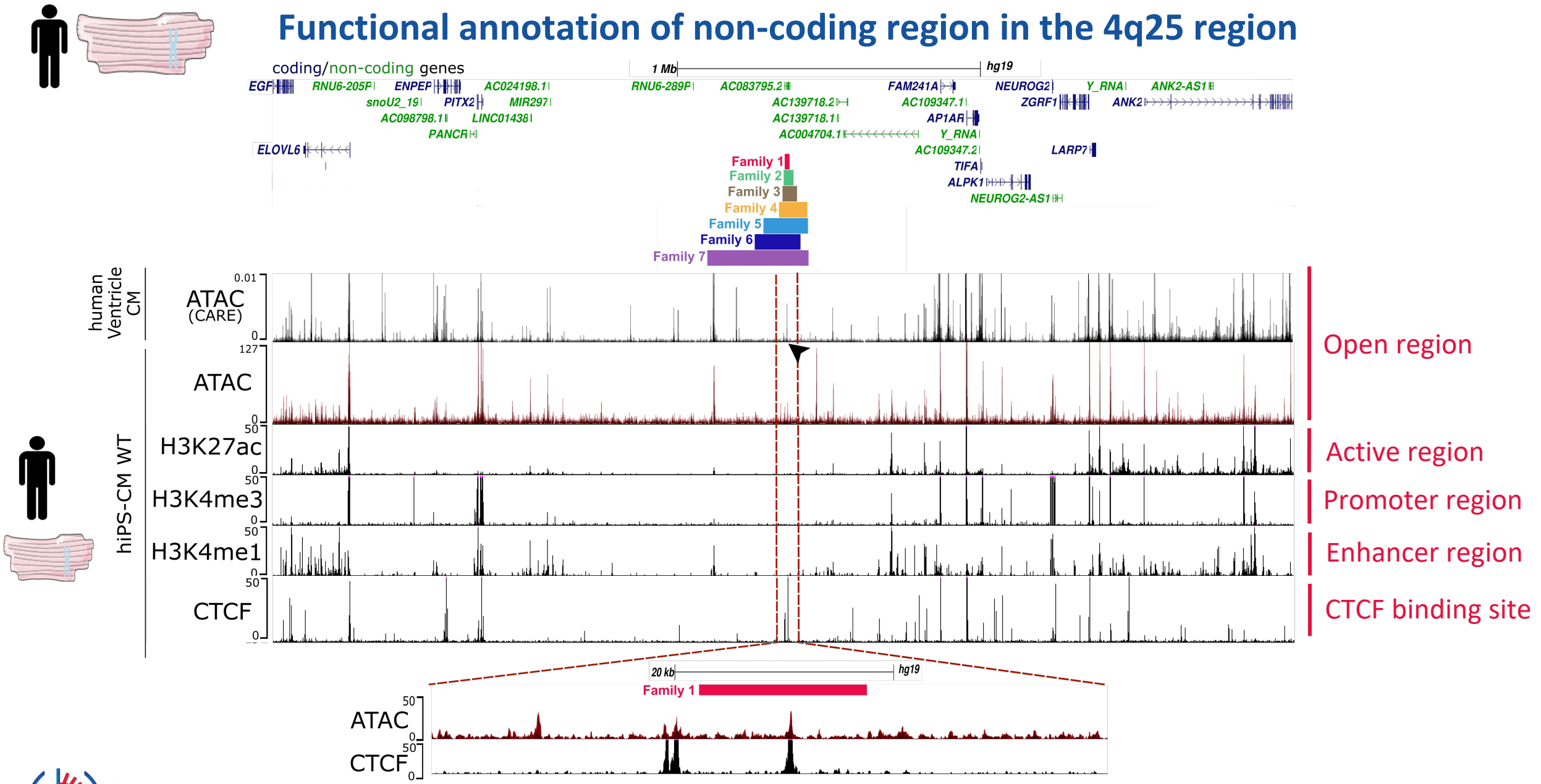
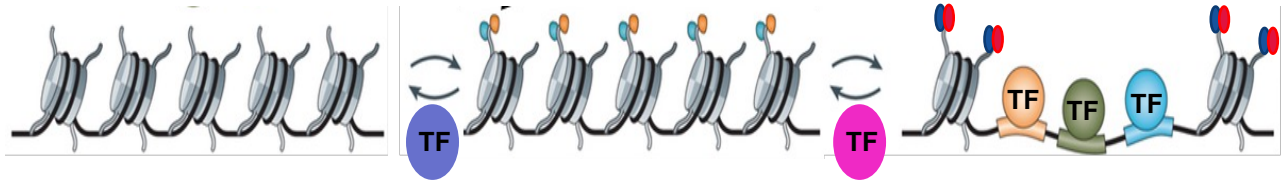
Functional annotation of non-coding region in the 4q25 region



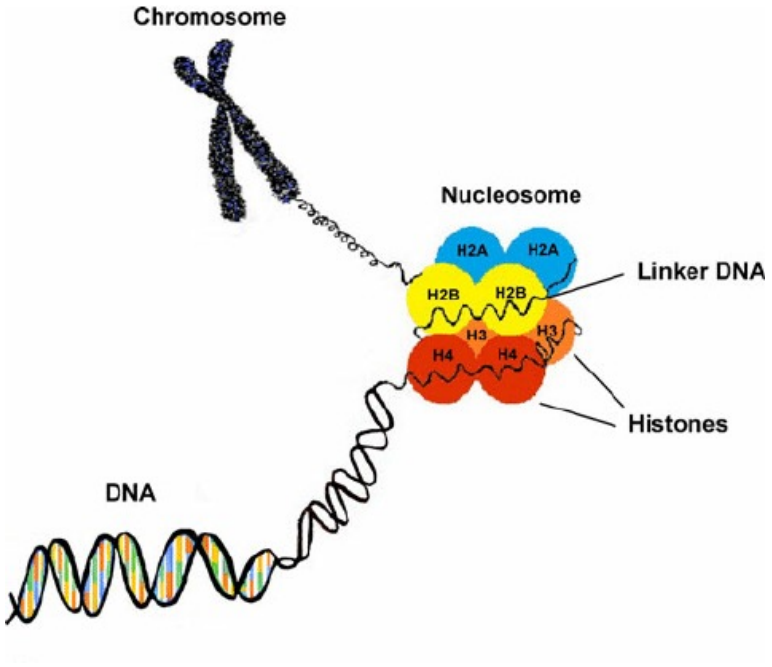
Functional annotation of non-coding region in the 4q25 region



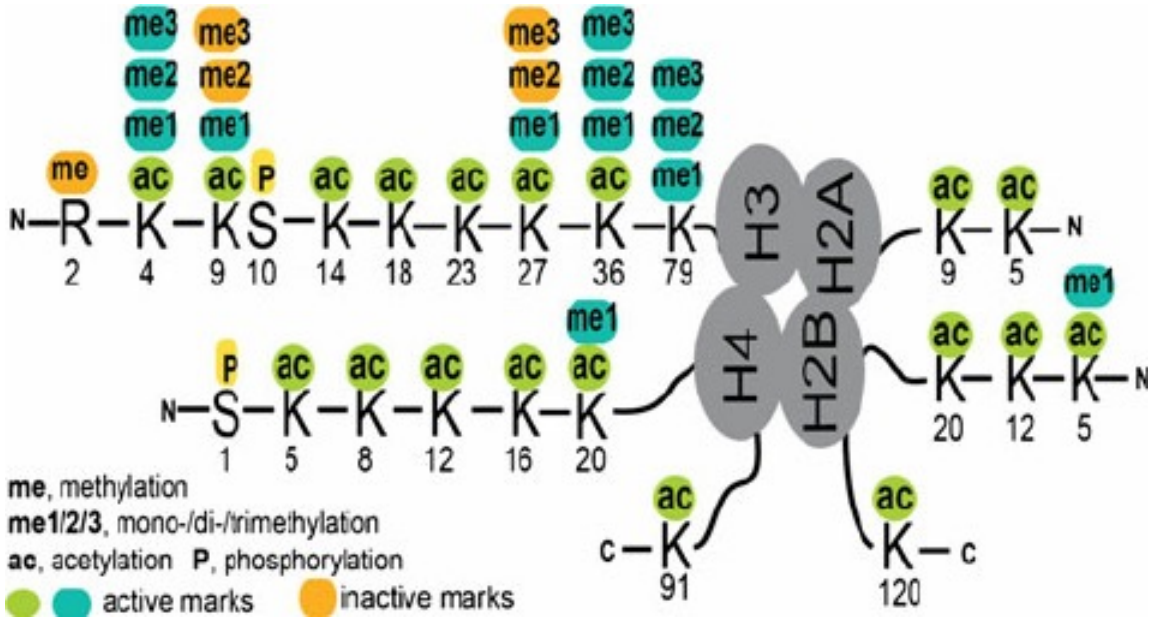
Functional annotation of non-coding region of the genome



Non-active region | Latent region | Open region



Histone modifications



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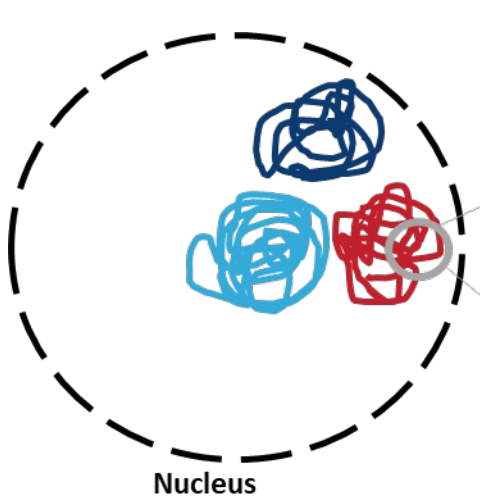
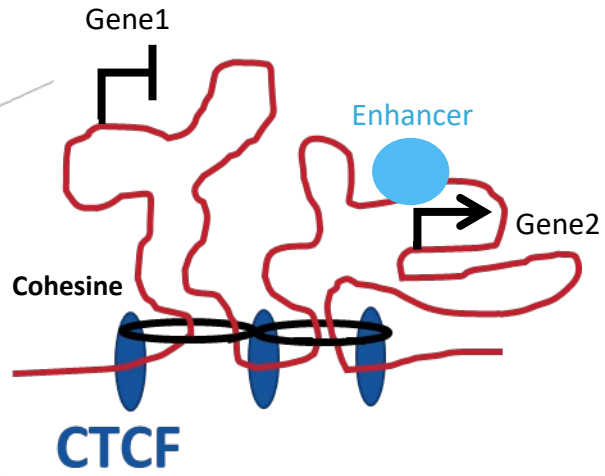
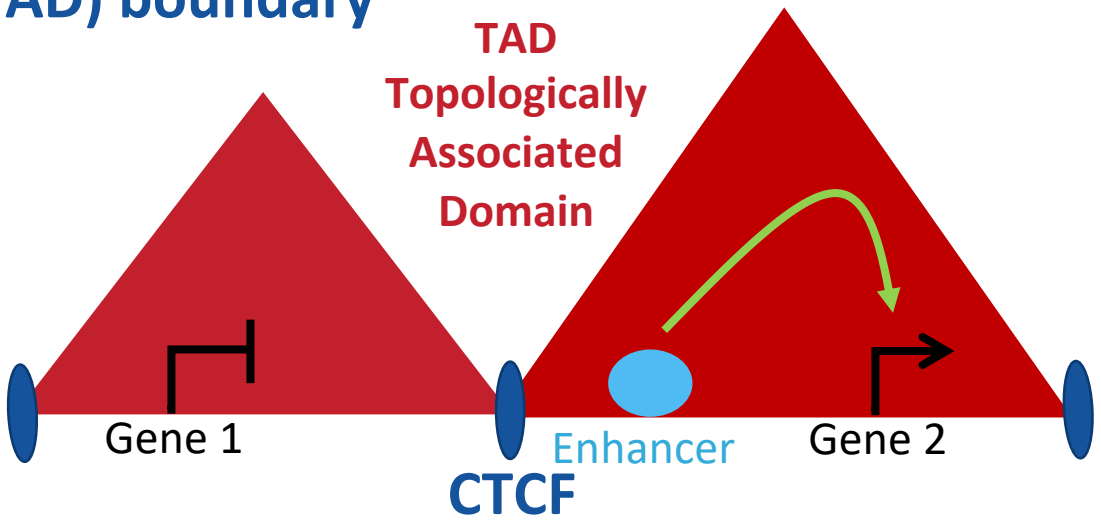
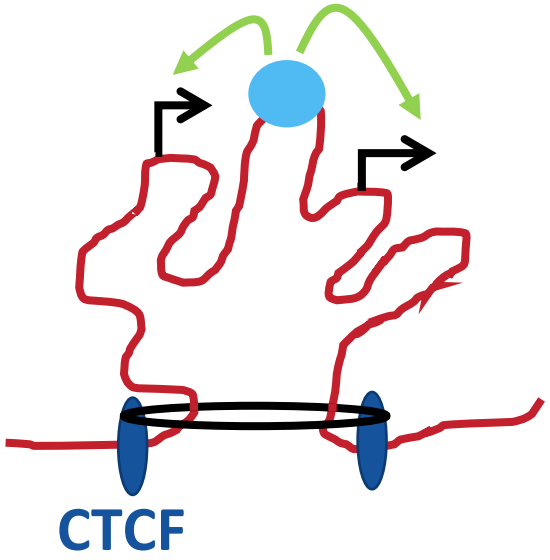
Hypothesis: deletion of the CTCF binding site modifies the Topologically Associated Domain (TAD) boundary

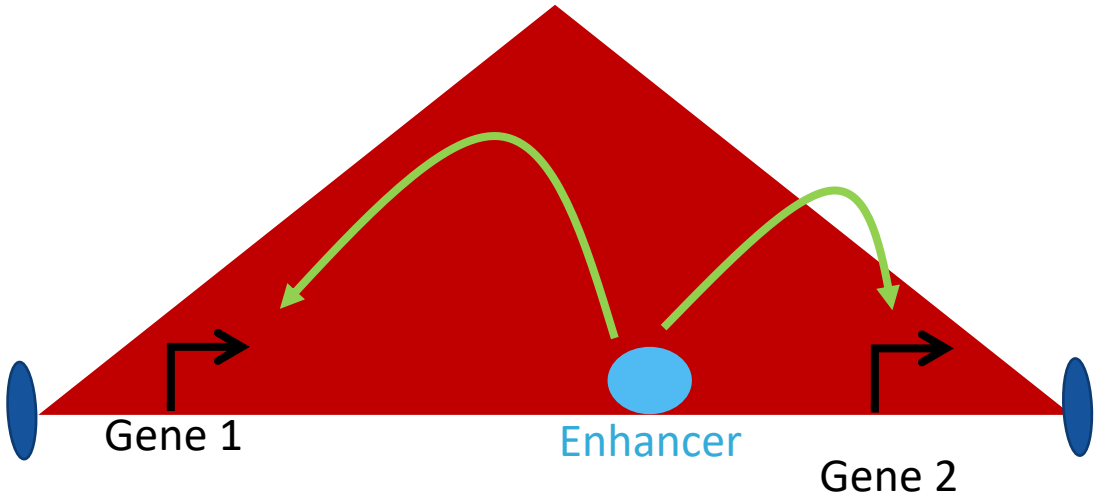






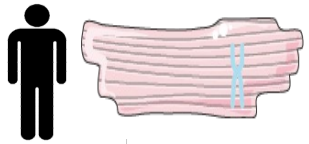
Deletion of CTCF binding site





Lupiañez et al, Cell.2015

Chromatin Conformation Capture HiC: 3D organization of the 4q25 region

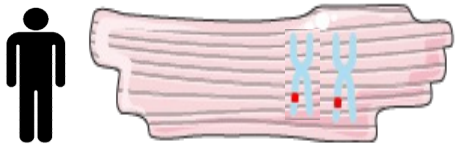


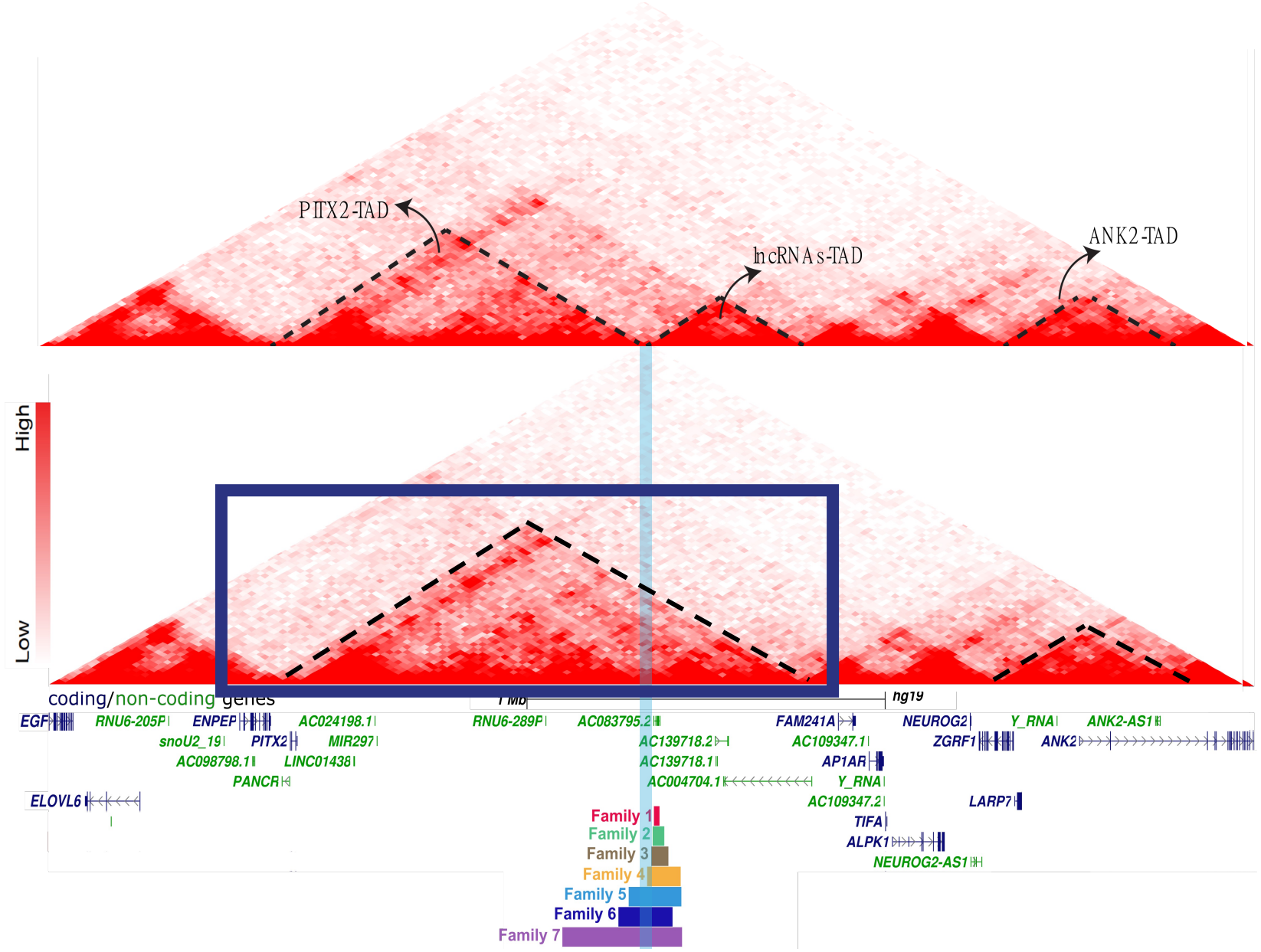
hiPS-CM WT

3D chromatin conformation remodeling with 15kb deletion

hiPS-CM WT

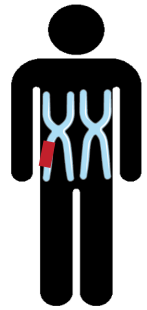
hiPS-CM 15Kb deletion

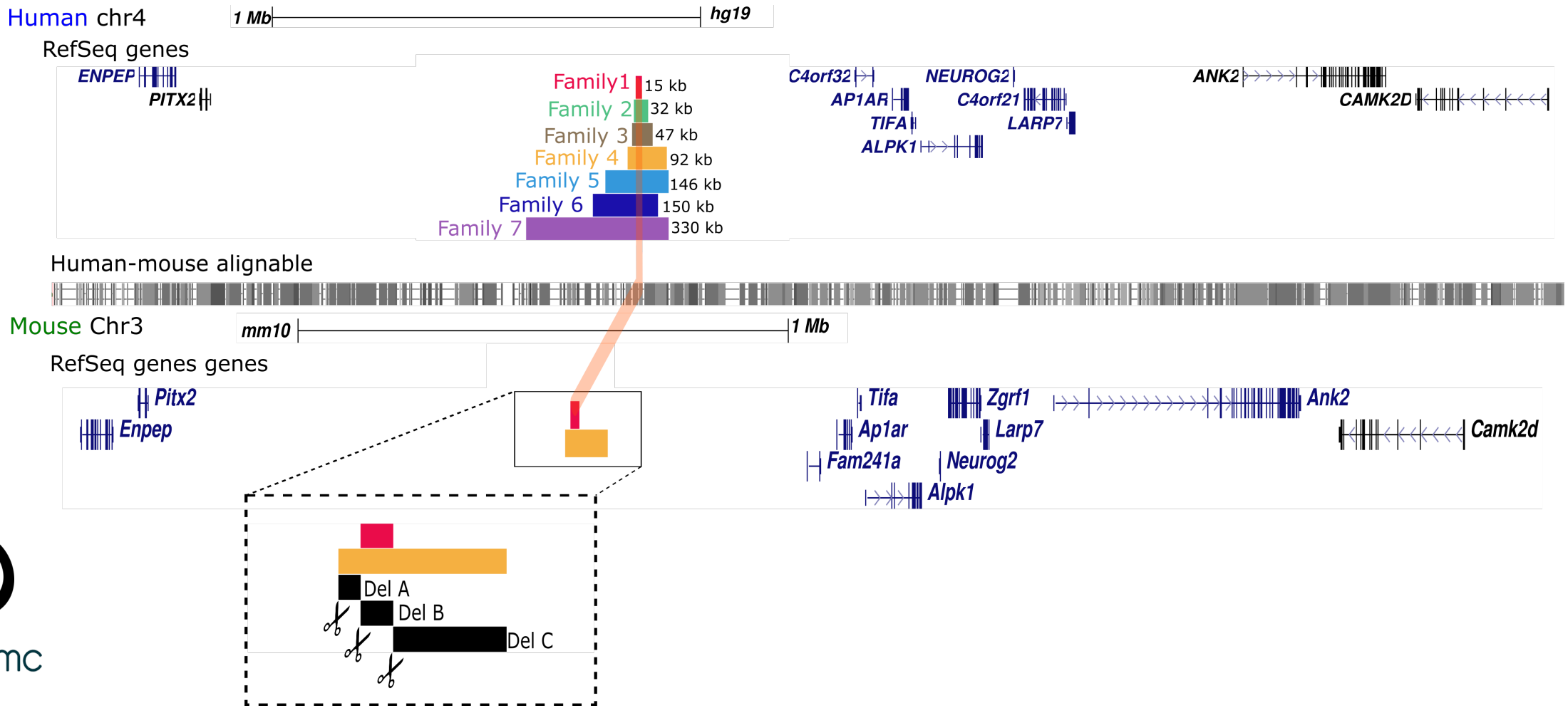




Fusion of the two TADs result in new interactions with *PITX2*

Mouse model to characterize the impact of the deletion on the phenotype





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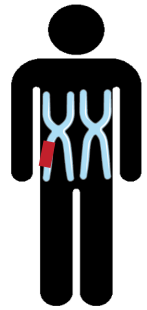
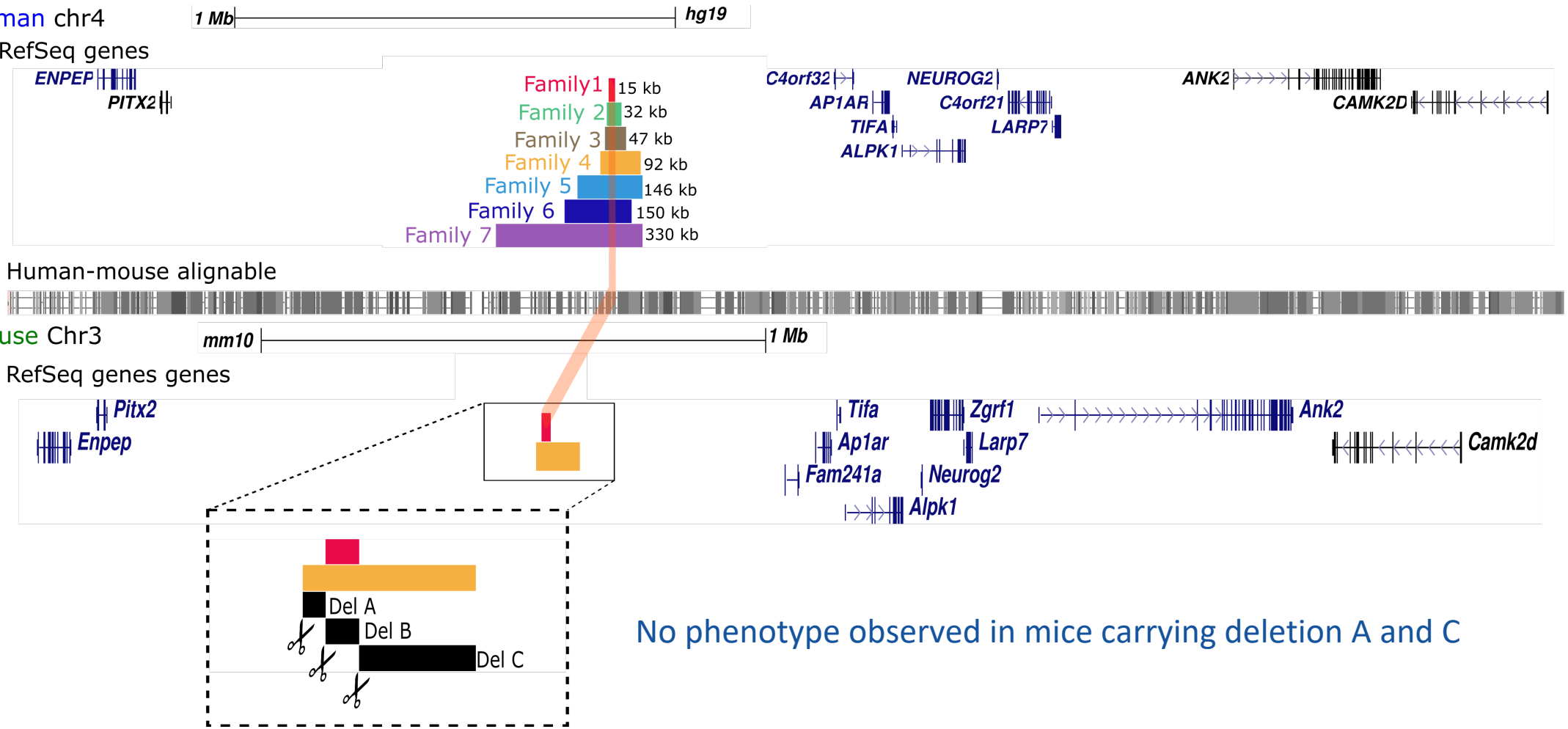
Mouse model to characterize the impact of the deletion on the phenotype





No phenotype observed in mice carrying deletion A and C

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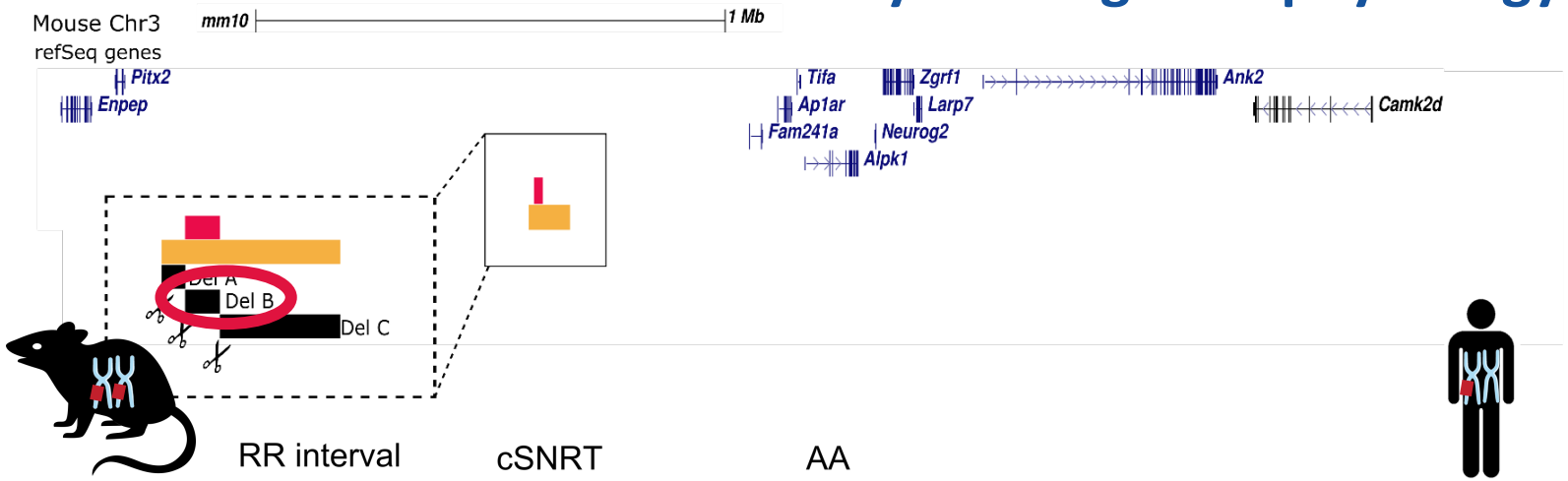
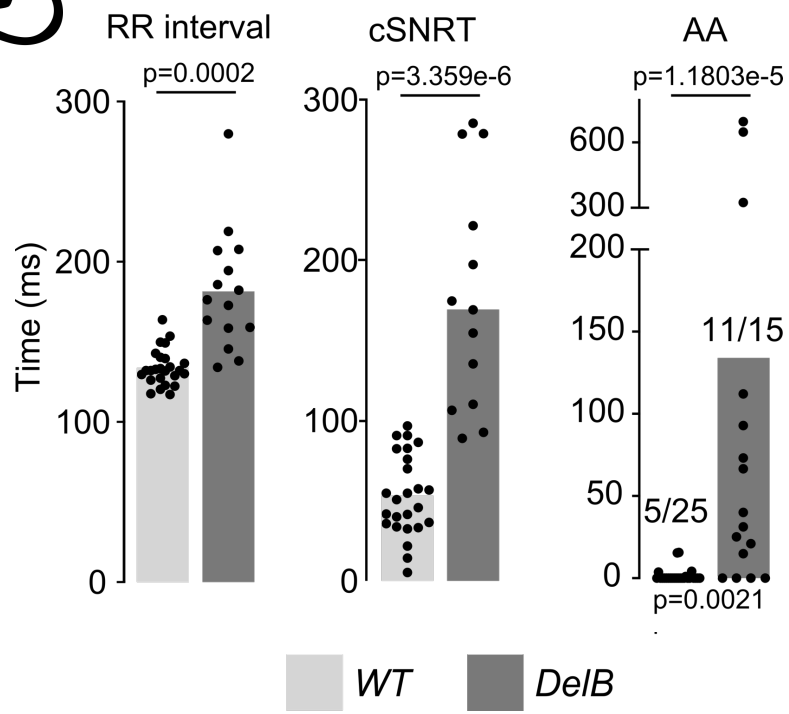
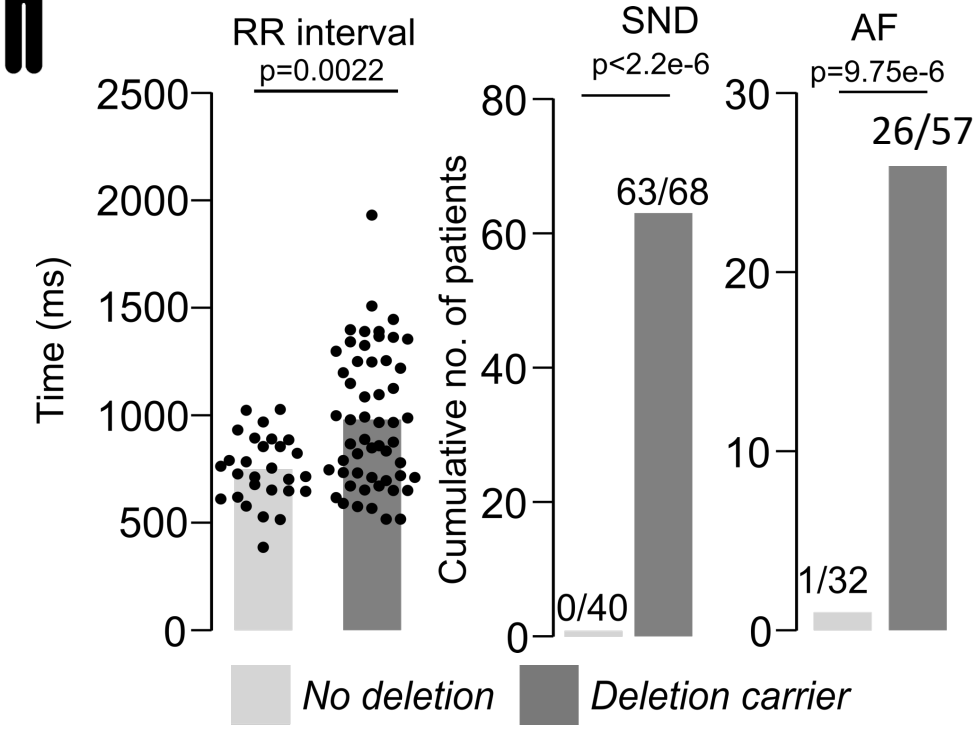
In vivo electrical cardiac activity investigation physiology on mice carrying 15kb deletion



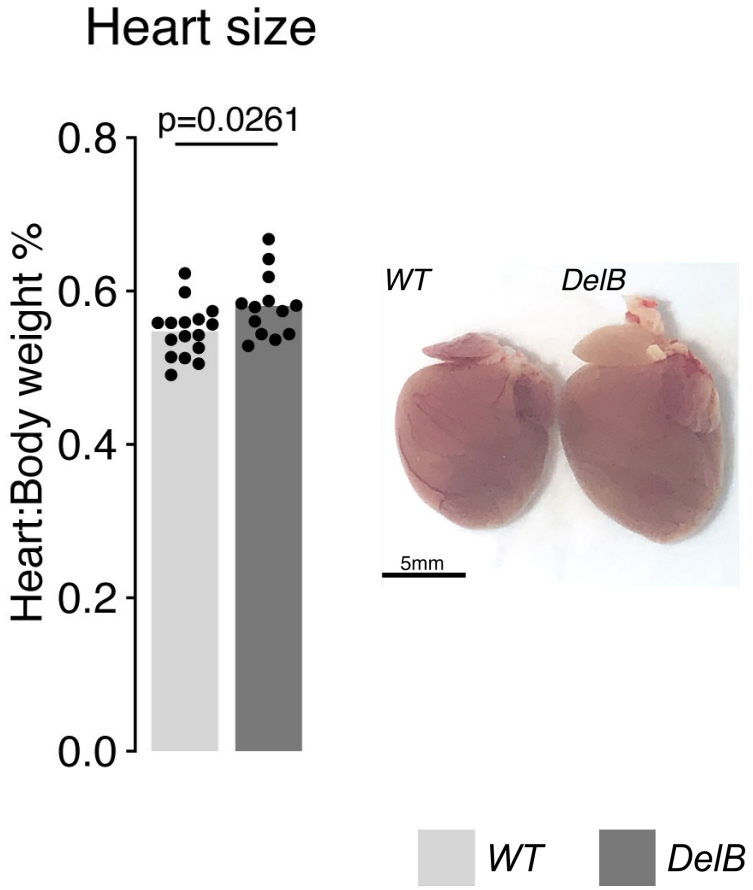


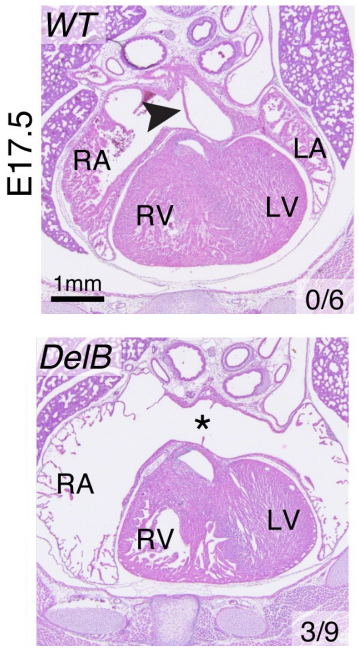


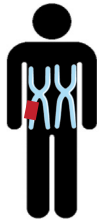
CRISPR Mice show slower heart rates, slower sinus node recovery times, atrial arrythmias

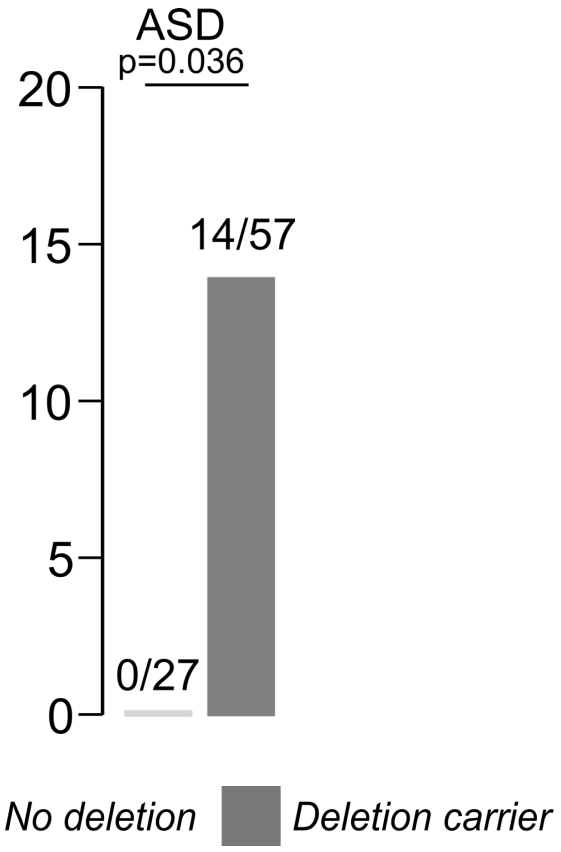
Bradycardia, SND and atrial arrythmia recapitulates electrical phenotype identified in patients

Cardiac morphological characterization of mice carrying 15kb deletion







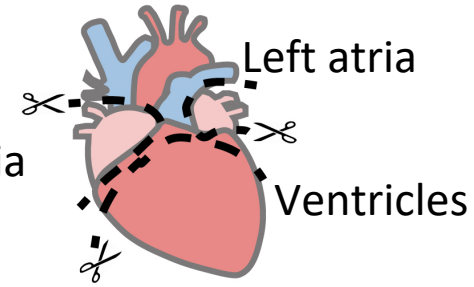


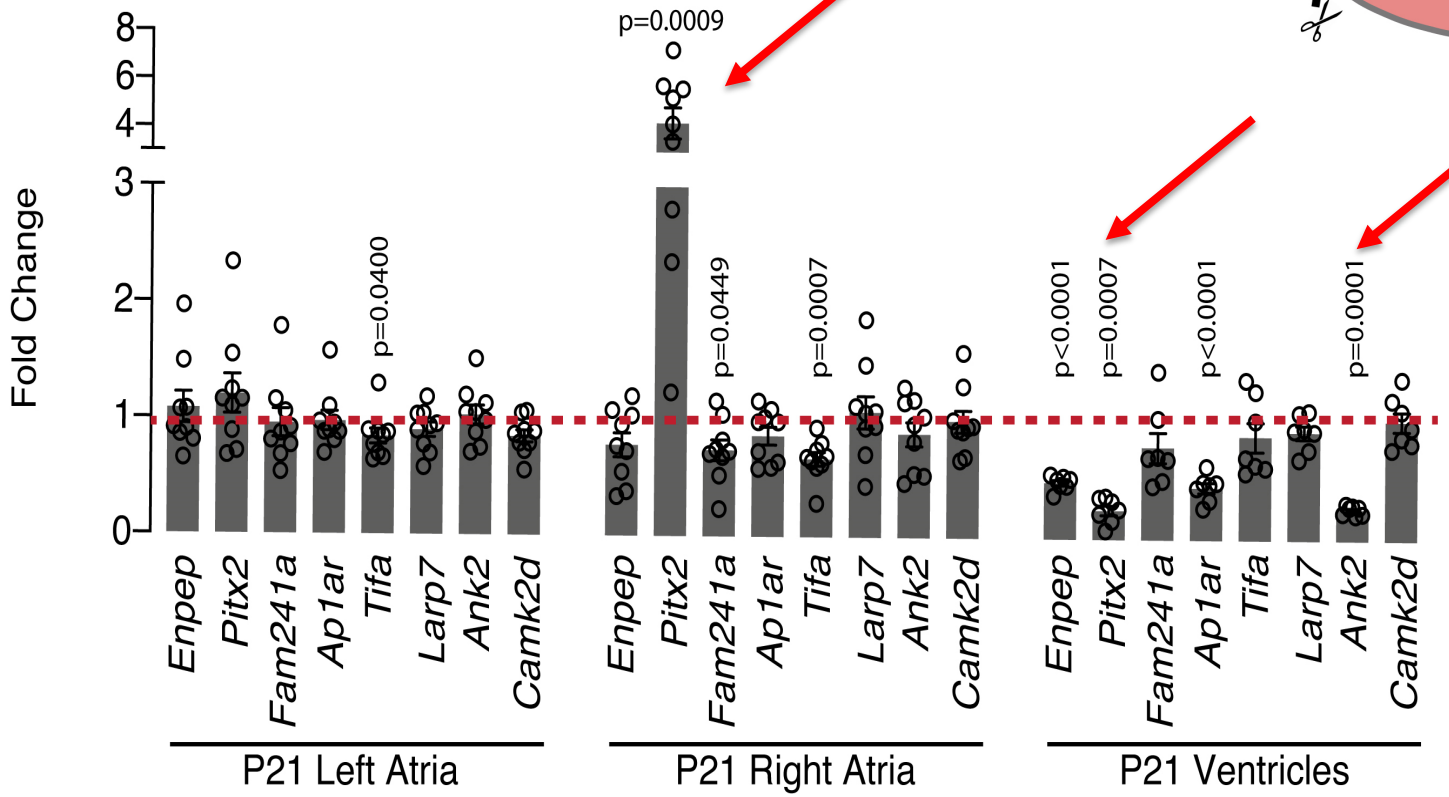
CRISPR Mice show a slightly bigger heart and ASD

Mouse model recapitulates electrical and morphological cardiac phenotype identified in patients

Severe disruption of the SAN-specific genetic program coinciding with the induction of ectopic Pitx2 expression in the SAN

DelB SAN tissue





RNA-Seq:

- -suppressed gene expression encoding:
  - **Tbx3** and **Isl1 (**key pacemaker TFs)
  - **Hcn4 i**on channels that drives pacemaker function

Upregulation of *Pitx2* in the right atria

Downregualtion of *Pitx2* & *Ank2* in the ventricules

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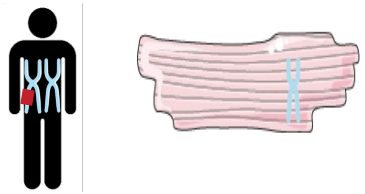
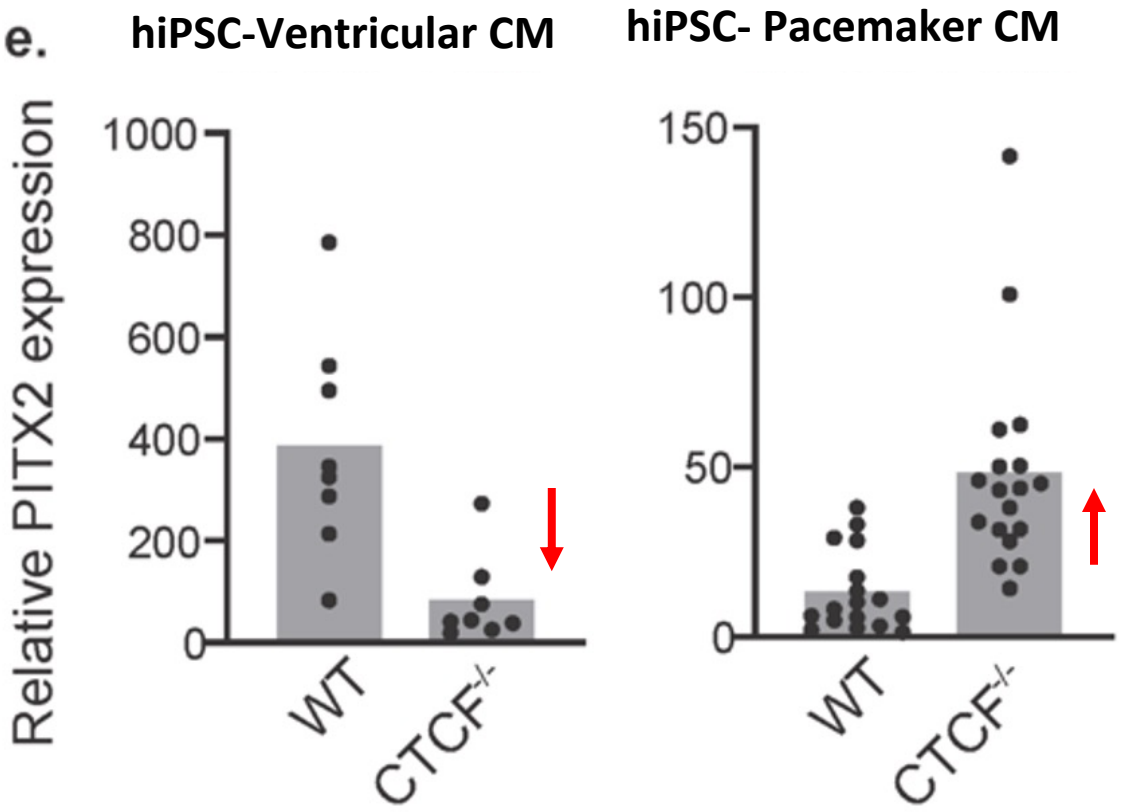
Opposite PITX2 expression pattern in ventricle-like cardiomyocytes and pacemaker cell-like cardiomyocytes





Conclusions

WGS is a powerful approaches to identify new regulatory elements and target genes

- New cardiac entity: CTCF deletion not found in 39 French patients presenting **lone sinus node defects nor in > 10,000 atrial fibrillation cases**
- Cardiac electrical and structural defects are likely mediated by heart compartment-specific dysregulation of *PITX2* expression
  - suppressed gene expression encoding Tbx3 and Isl1 (key pacemaker TFs), Hcn4 ion channels that drives pacemaker function
- Will / can WGS become the new gold standard / genetic testing?

Baudic et al. in revision

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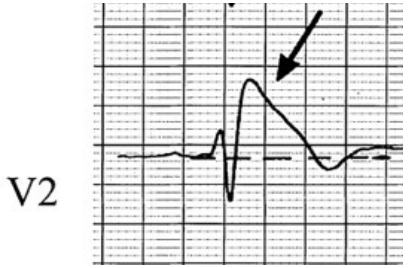
The Brugada syndrome

- Rare disease (1-5/10 000), affecting mainly men (≈ 80%)
- Mean age of diagnosis around age 40
- Ventricular tachycardia, ventricular fibrillation and sudden cardiac death

- Baseline condition or after drug challenge

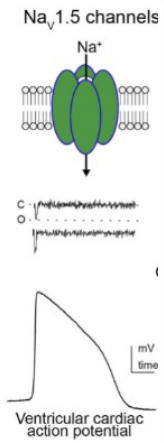
  Typical pattern of ST segment elevation, on the right precordial leads (V1-V3) of the surface ECG

- No cardiac structural defects



Brugada syndrome is a genetic condition:

- Autosomal dominant disease
- ***SCN5A*** loss of function mutations in **~ 20% cases**
  - Decreased upstroke velocity of the cardiac action potential
  - Reduced penetrance and variable expressivity
- 22 additional genes (familial and candidate gene approaches)
- Over all contribution to disease prevalence is still unclear
- **Burden testing**: only rare *SCN5A* variants are significantly associated to BrS



➢ *SCN5A* remains a poor marker for SCD risk stratification (BrS, a polygenic disease?)

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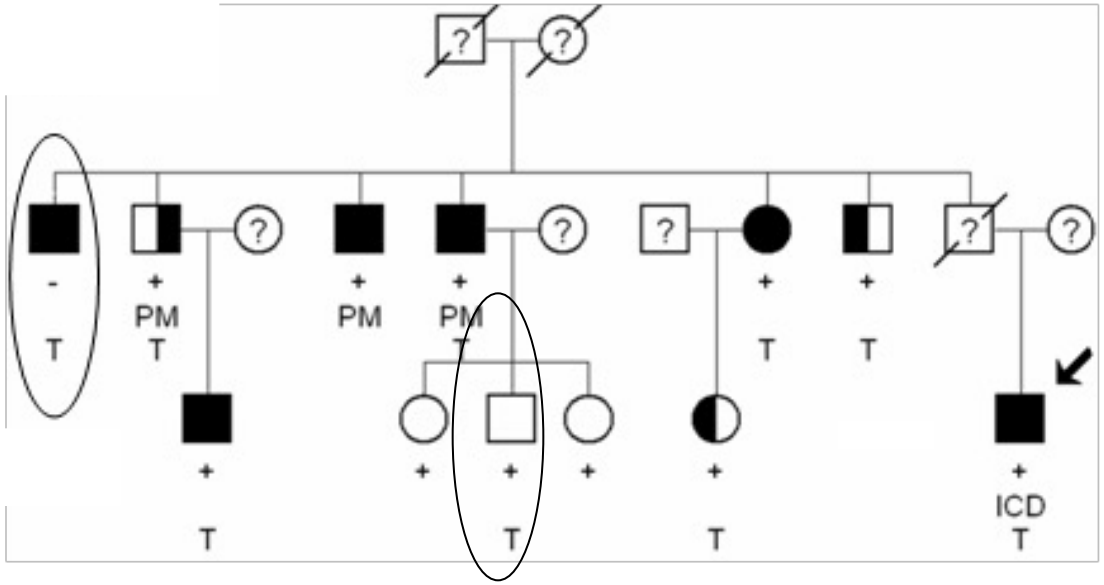
Wilde et al. Circulation 2002
Le Scouarnec et al. Hum Mol Genet 2015

Variable penetrance and expressivity in mendelian diseases

Brugada Syndrome families with non-mendelian segregation



\+ *SCN5A*-p.E161K mutation

BrS

Conduction disease

Unaffected

T Na/channel blocker test

Presence of genetic modulators?

➢ **Role of frequent variants modulating BrS expressivity**

Probst et al., Circ Cardiovasc Genet. 2009

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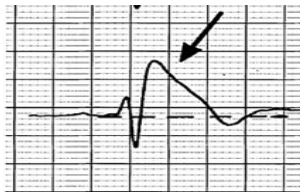
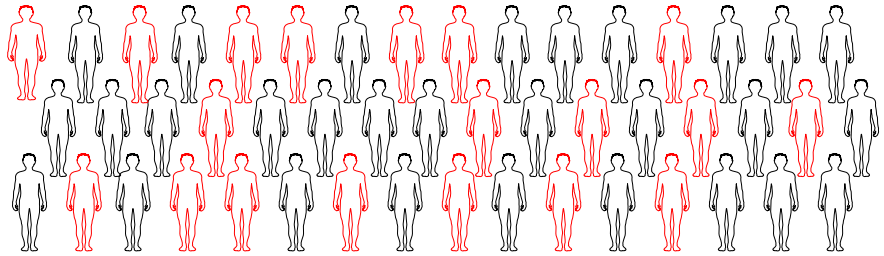
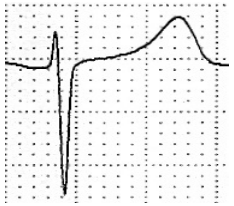
A GWAS on Brugada syndrome: study design

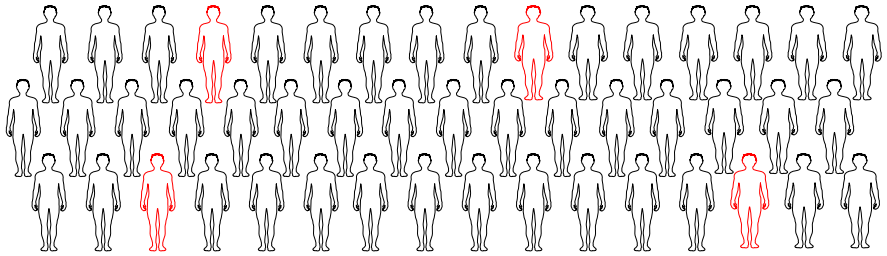


383 index cases with Brugada Type-1 ECG

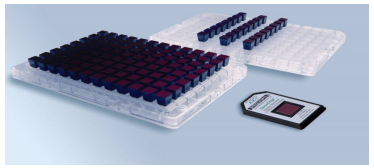




898 control individuals



Affymetrix



Axiom Genome-Wide Human CEU-1 Array Plate

587,352 markers

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Common polymorphisms modulate Brugada syndrome

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in the heart of an international network on Brugada syndrome

Leducq Foundation

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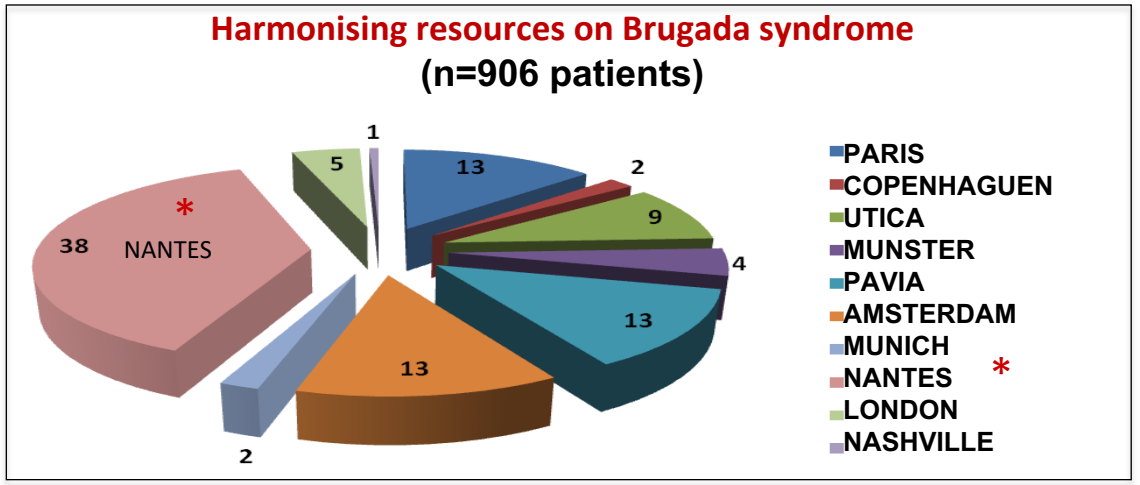
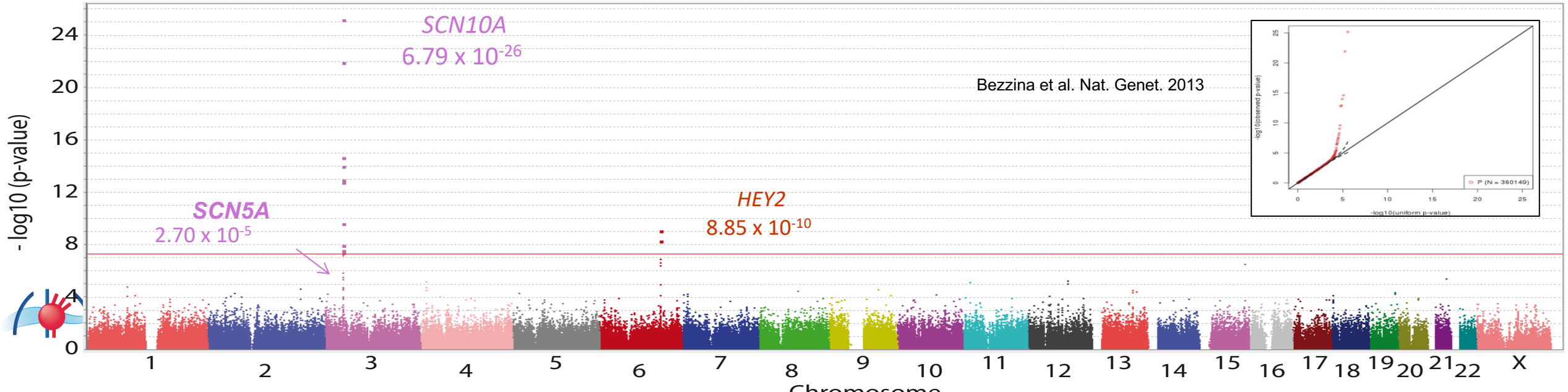
amc

Alliance Against SCD

312 patients
1115 controls

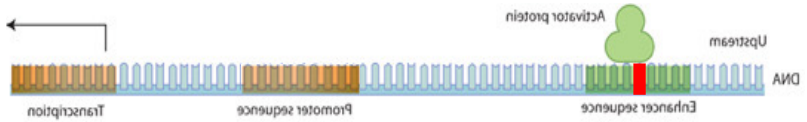


*** National Referral Centre for Inherited Cardiac Arrhythmias**

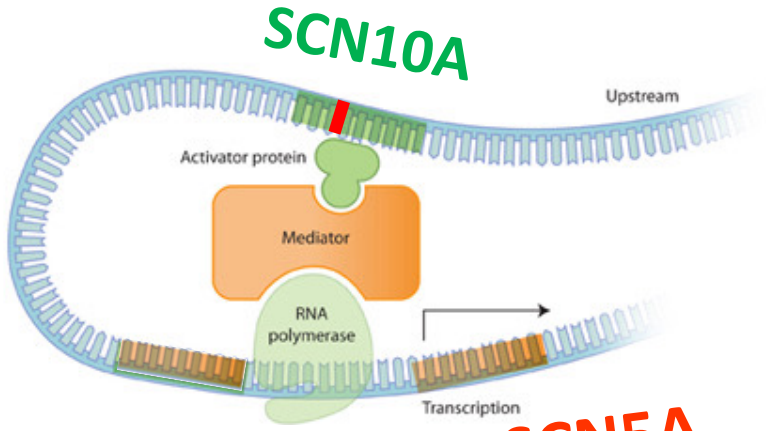


Translate genetic findings to molecular mechanisms

Chromatine conformation



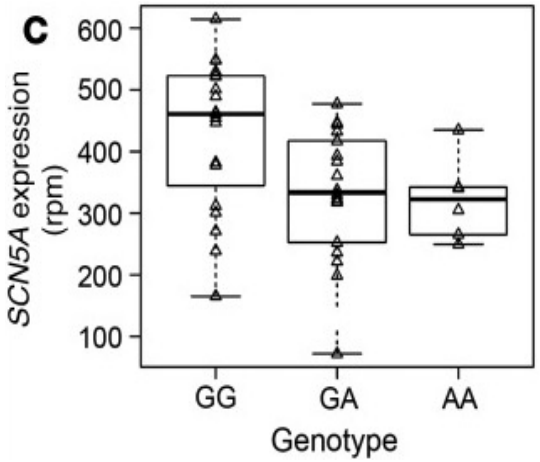
SCN5A SCN10A

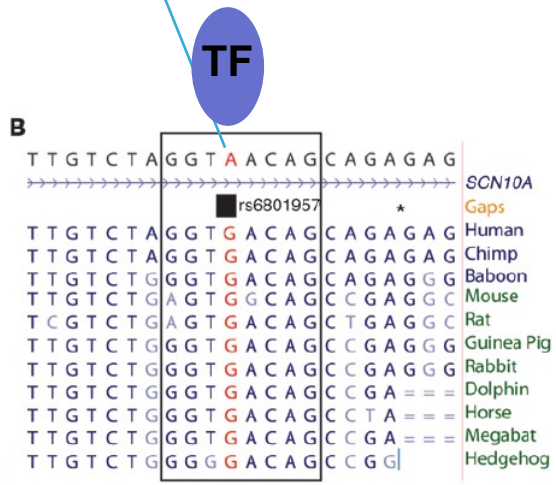


M. van den Boogaard et al. JCI 2012
M. van den Boogaard et al. JCI 2014

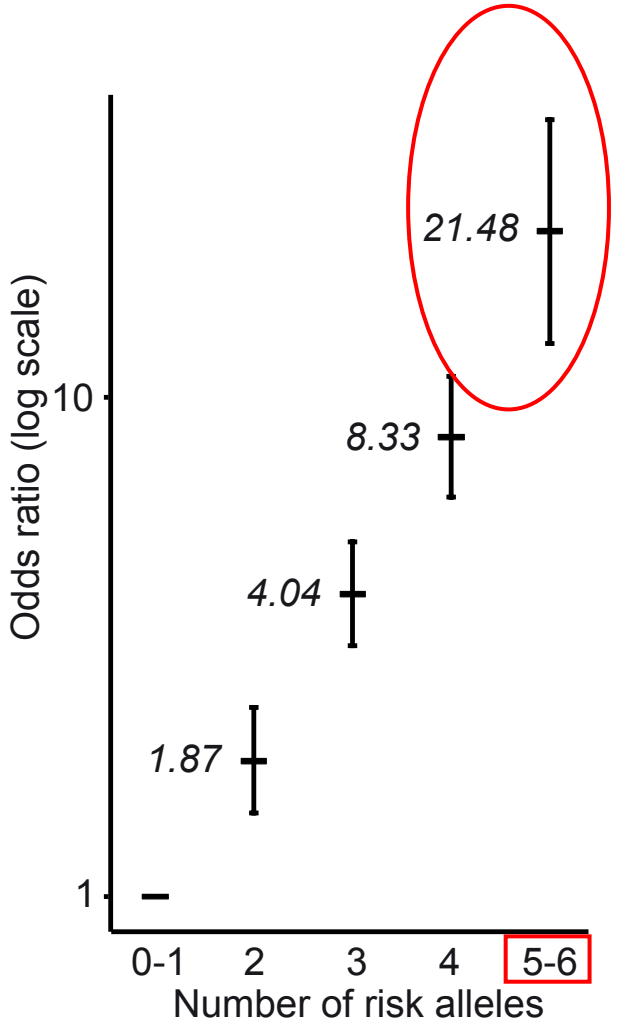
eQTL

- rs6801957 regulates *SCN5A expression in human myocardium*





Cumulative effect of risk alleles at the three loci



Bezzina et al. Nat. genet. 2013

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International Brugada Syndrome Genetics Consortium

Amsterdam UMC
Universitair Medische Centra

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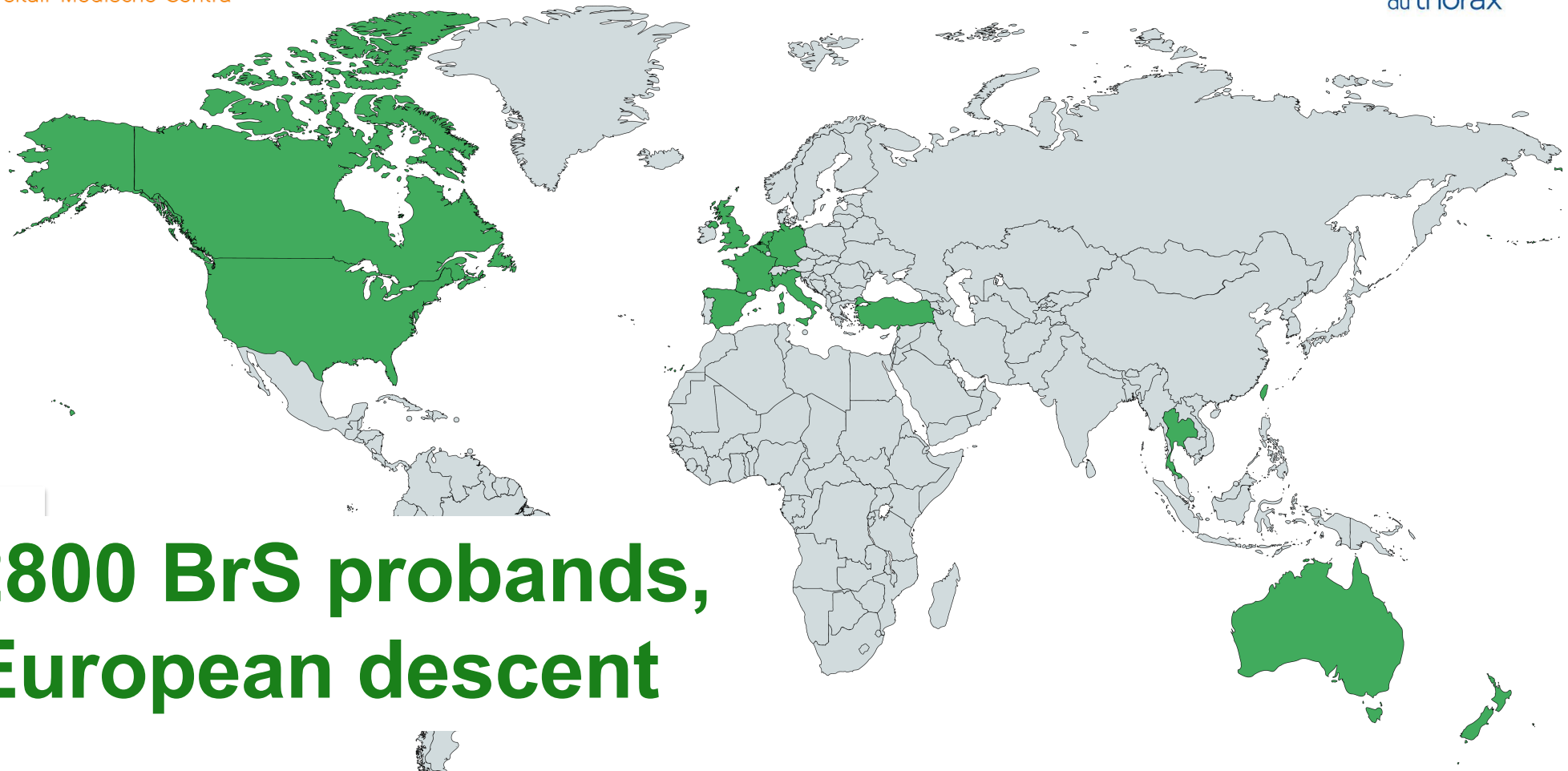

~2800 BrS probands, European descent

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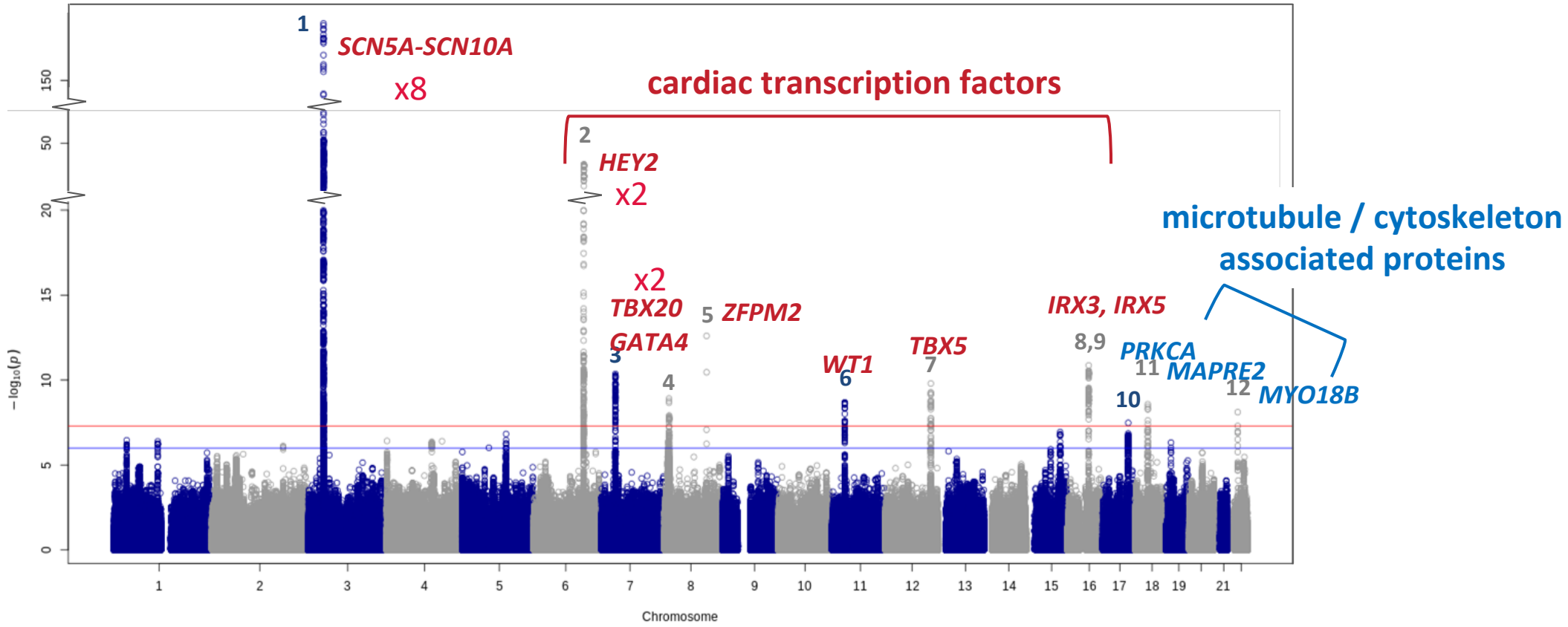
Brugada syndrome Genome-wide association meta-analysis

GWAS: identification of 12 loci associated with Brugada syndrome

21 independent association signals (10 new loci)



New molecular mechanisms

- **BrS heritability (h2):**
  - substantial portion of susceptibility to BrS attributable to common genetic variation
  - 0.17 (LDSC) to 0.34 (GREML)

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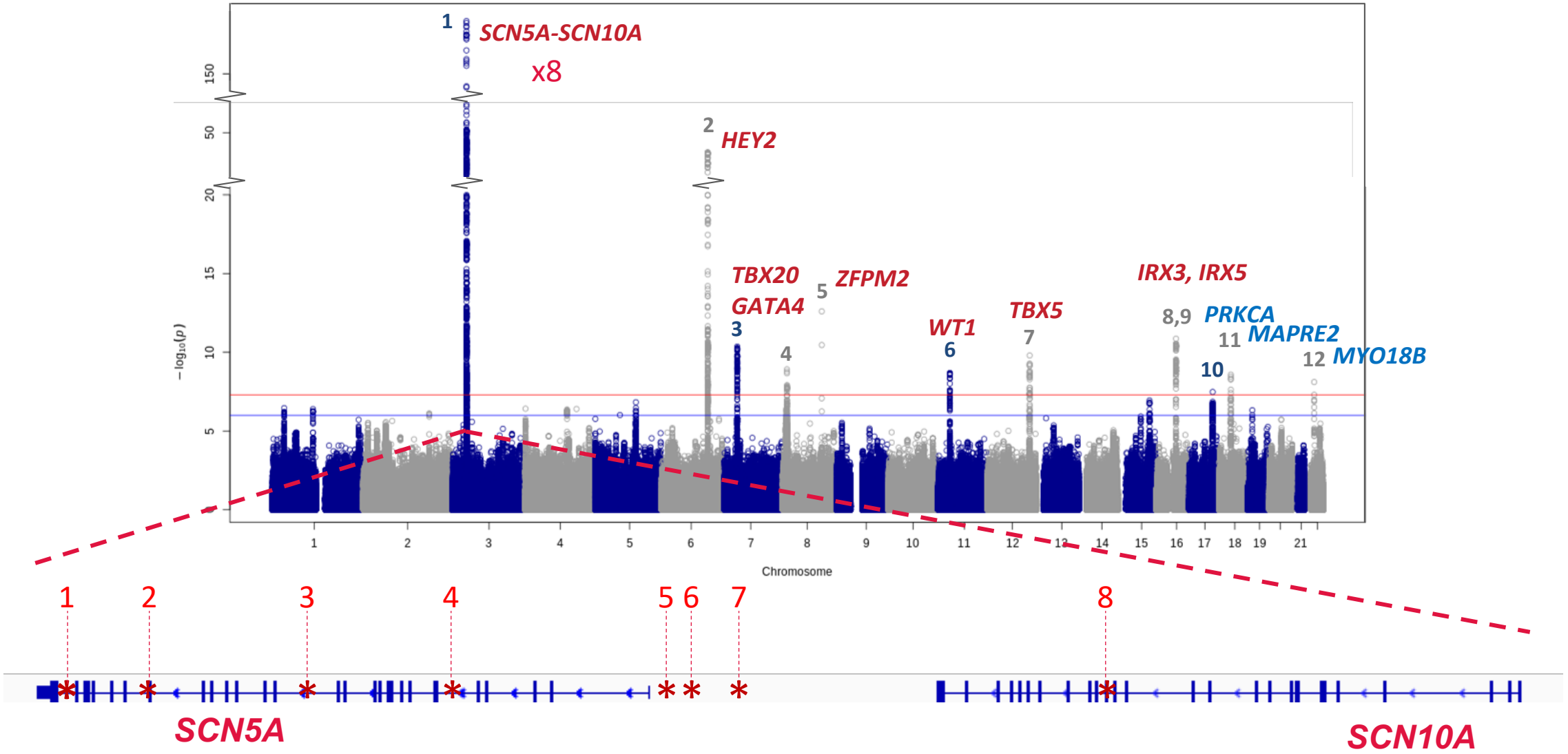
A predominant role of *SCN5A/SCN10A locus risk alleles*



➢ ***SCN5A/SCN10A locus: 8* independent loci**

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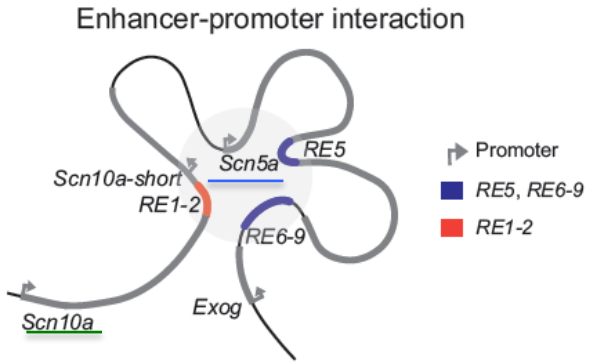
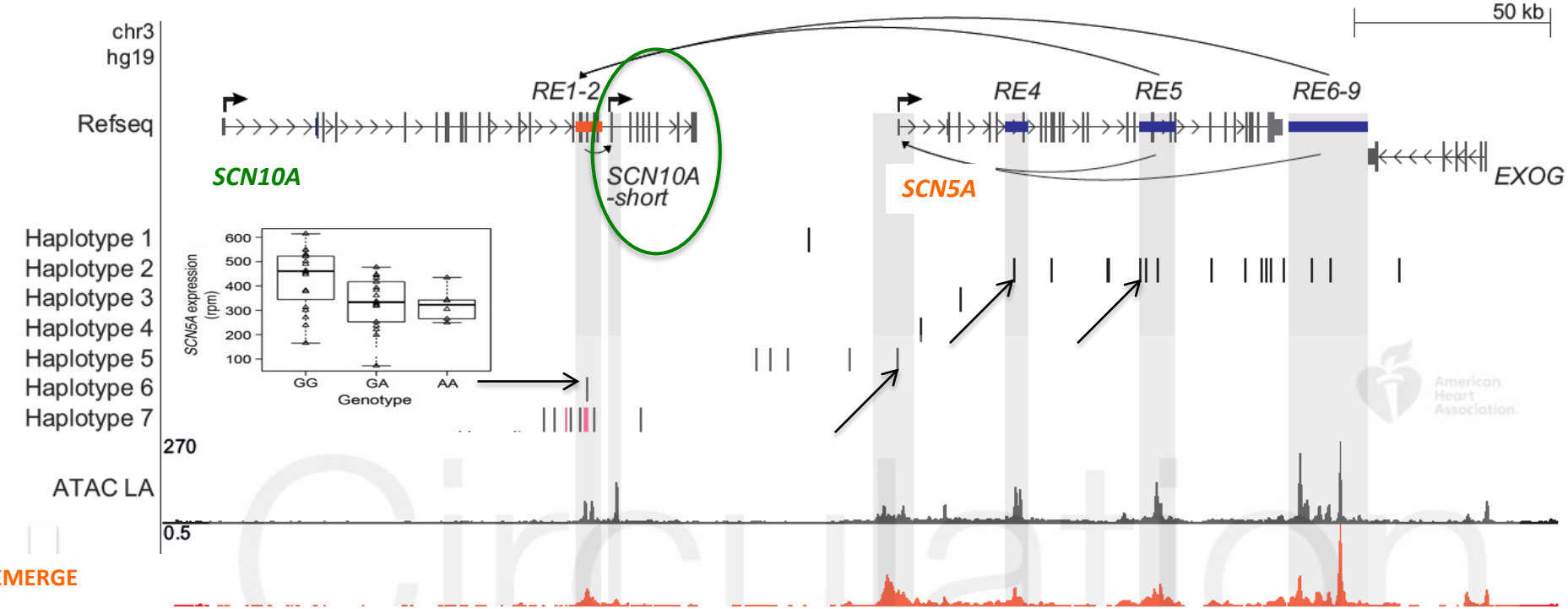
Risk haplotypes co-localize at multiple SCN10A / SCN10A intronic enhancers

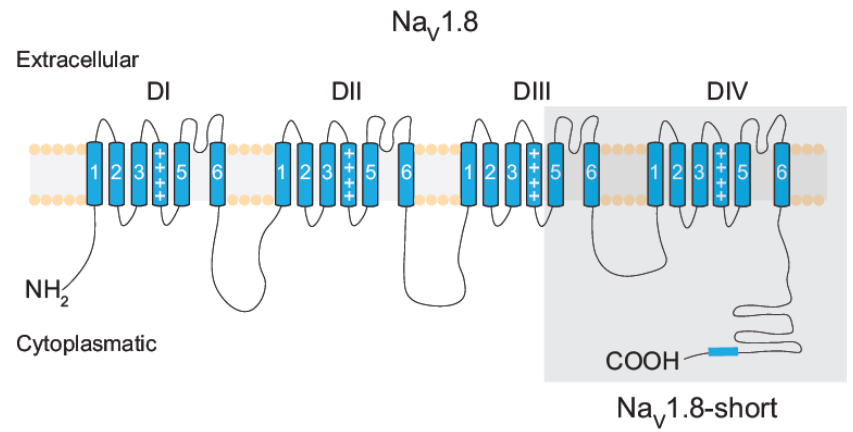


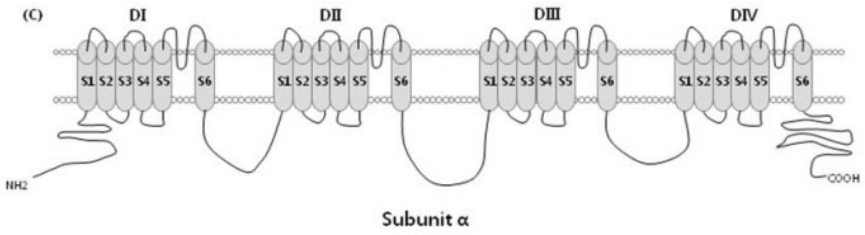


- Epigenetic marks:
- Histone acetylation, methylation
- TF bindings sites
- 3D chromatin conformation

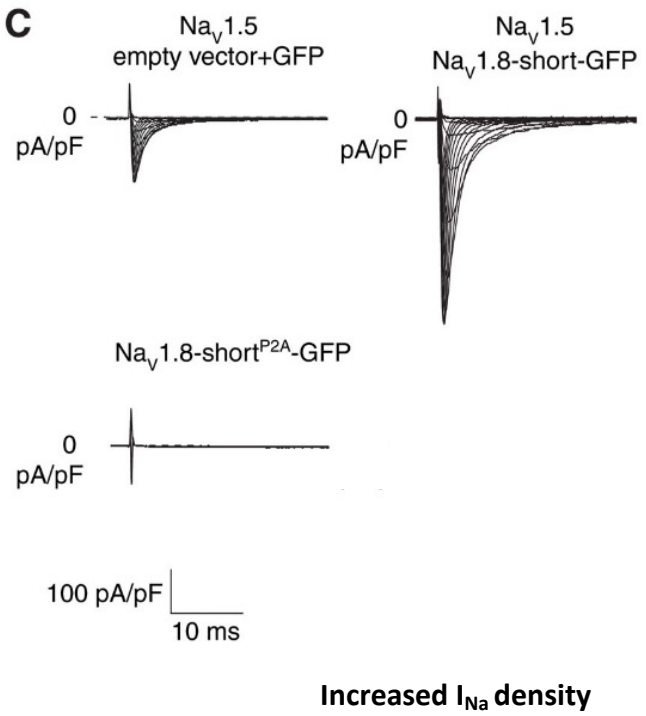
Man et al. Circulation 2021

SCN10A-Short /$Na_V1.8$ modulates sodium currents $Na_V1.5$ in HEK293 cells





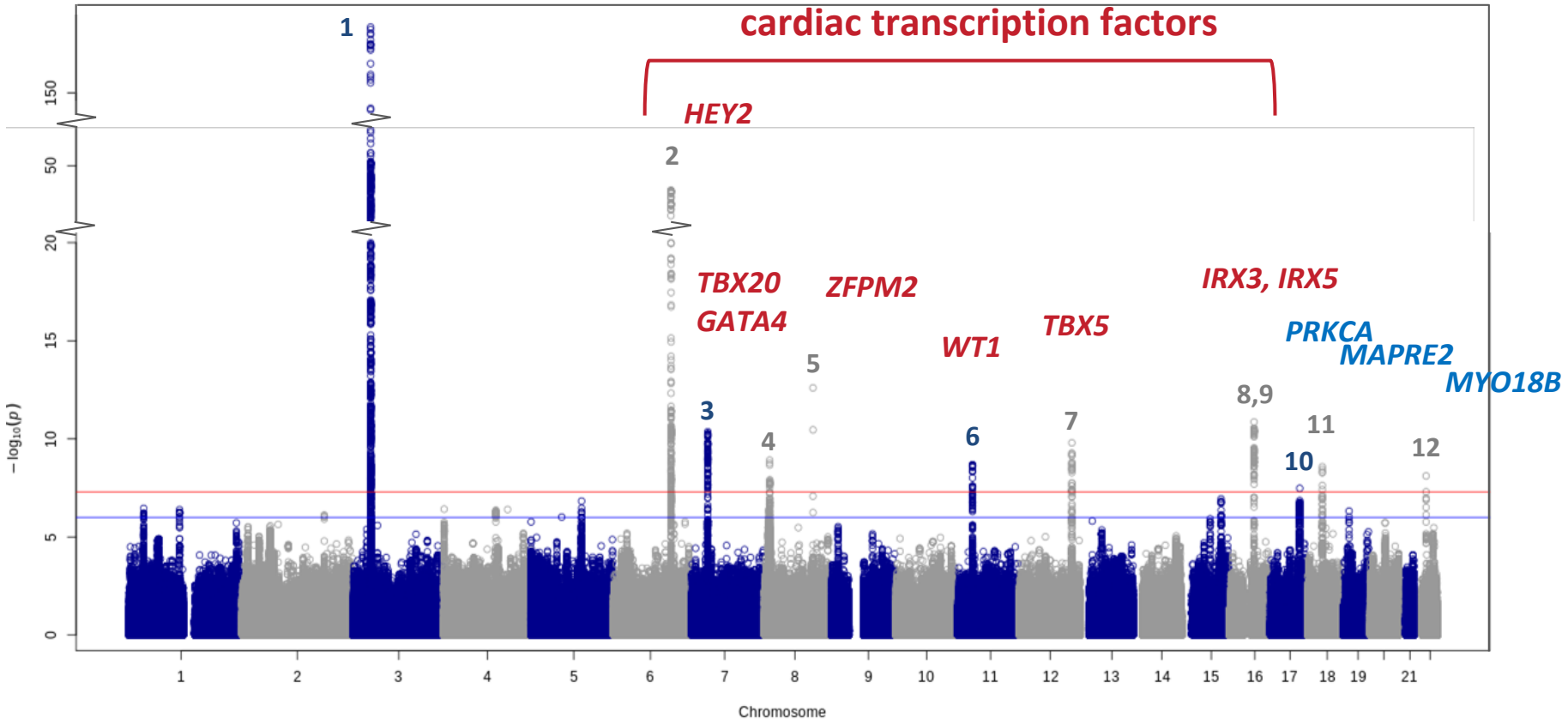
Na_V 1.5



Increased I_{Na} density

Man et al. Circulation 2021

GWAS: identification of 8 loci associated with transcription factors



➢ Implication of TF in regulating ion channels expression in the heart

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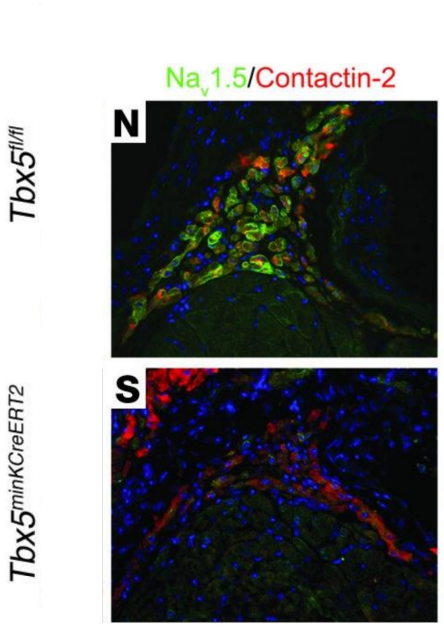
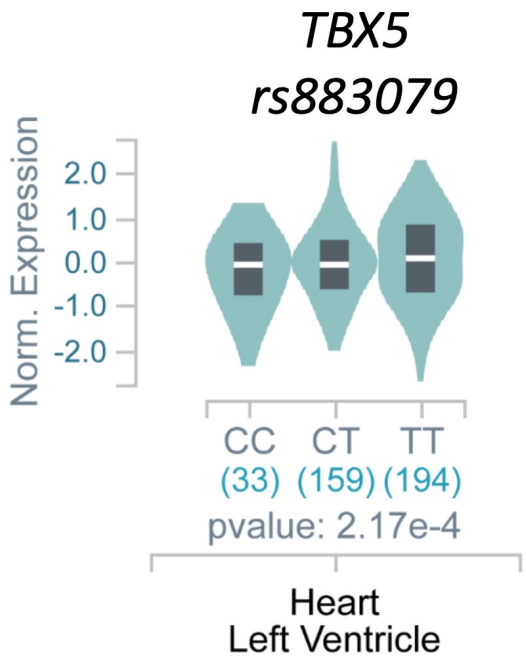
TBX5 drives expression of cardiac conduction system function

TBX5



rs 883079 eQTL



Arnolds et al. J Clin Invest. 2012

- Indirect regulation of Nav1.5 expression by TF

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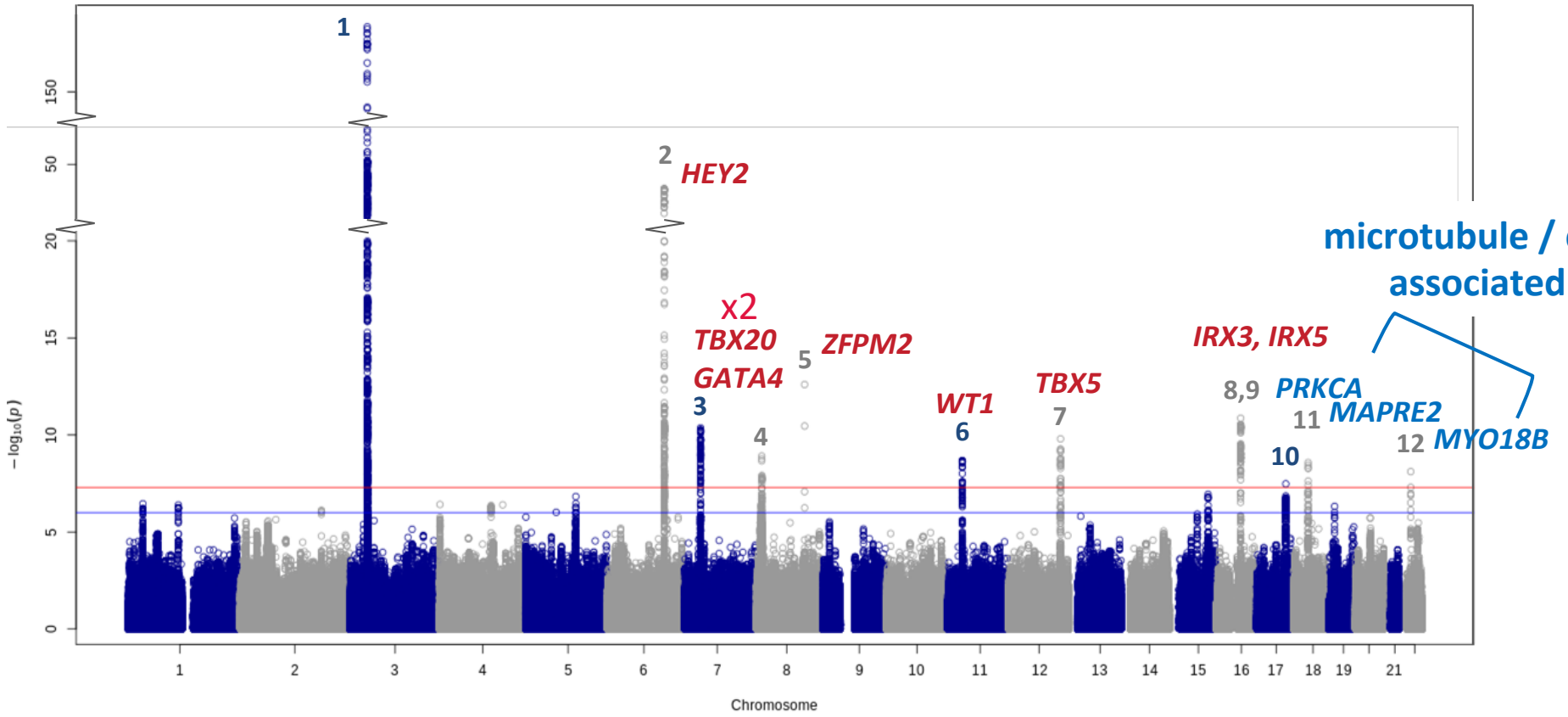
GWAS: identification of 3 loci associated with structural proteins



➢ new molecular mechanisms

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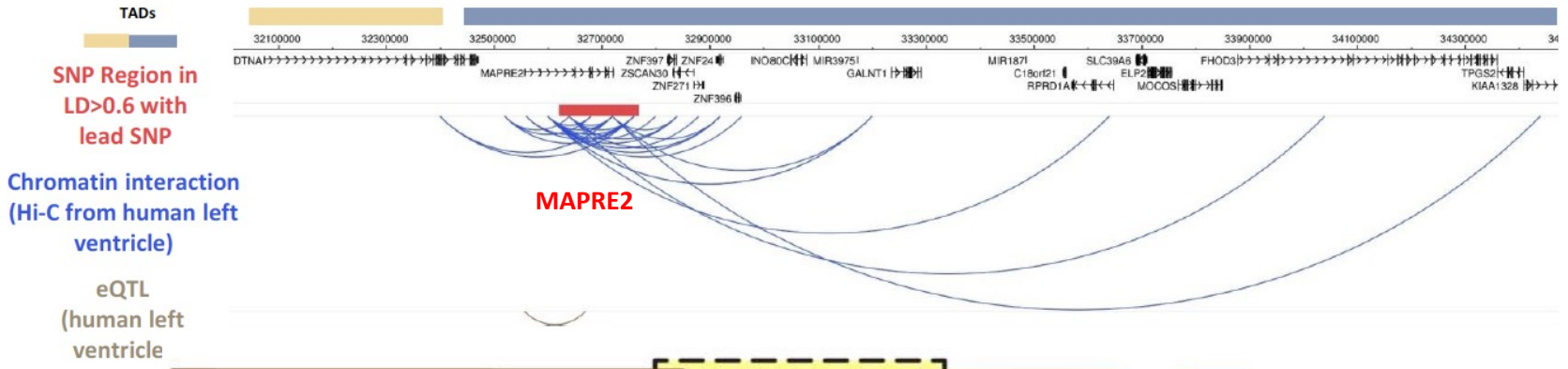
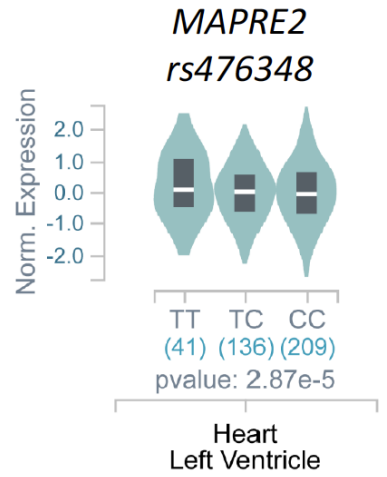
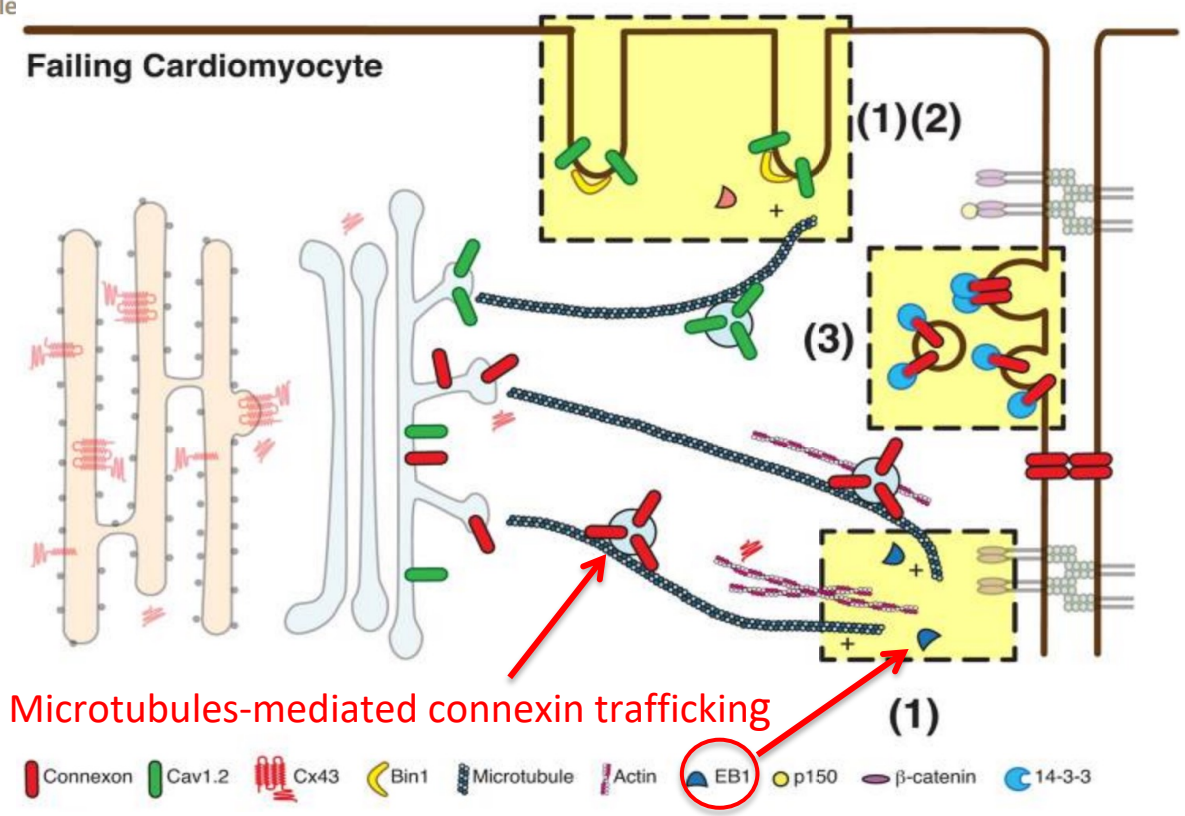
MAPRE2 is a trafficking protein / regulator of microtubules

(microtubule plus-end binding protein (EB2))



eQTL





- EB1 protein, previously described in microtubules-mediated trafficking of connexin in cardiomyocytes

S. Xiao & R M Shaw, Trends Cardiovasc Med. 2015

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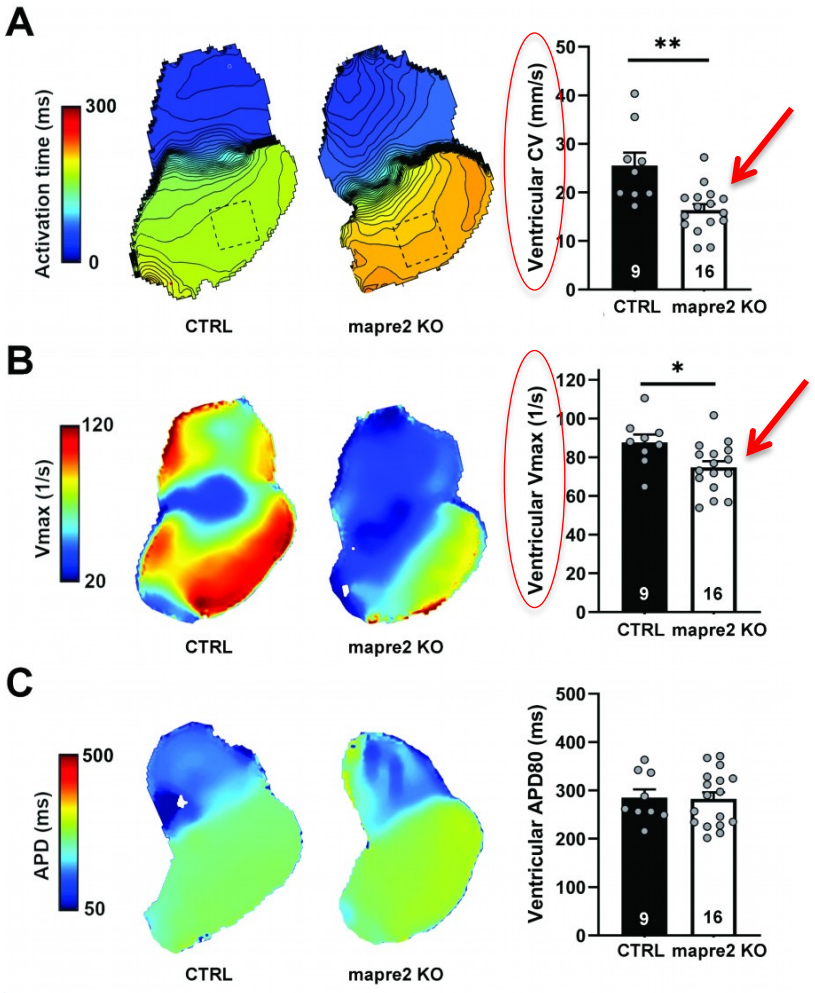
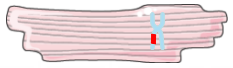
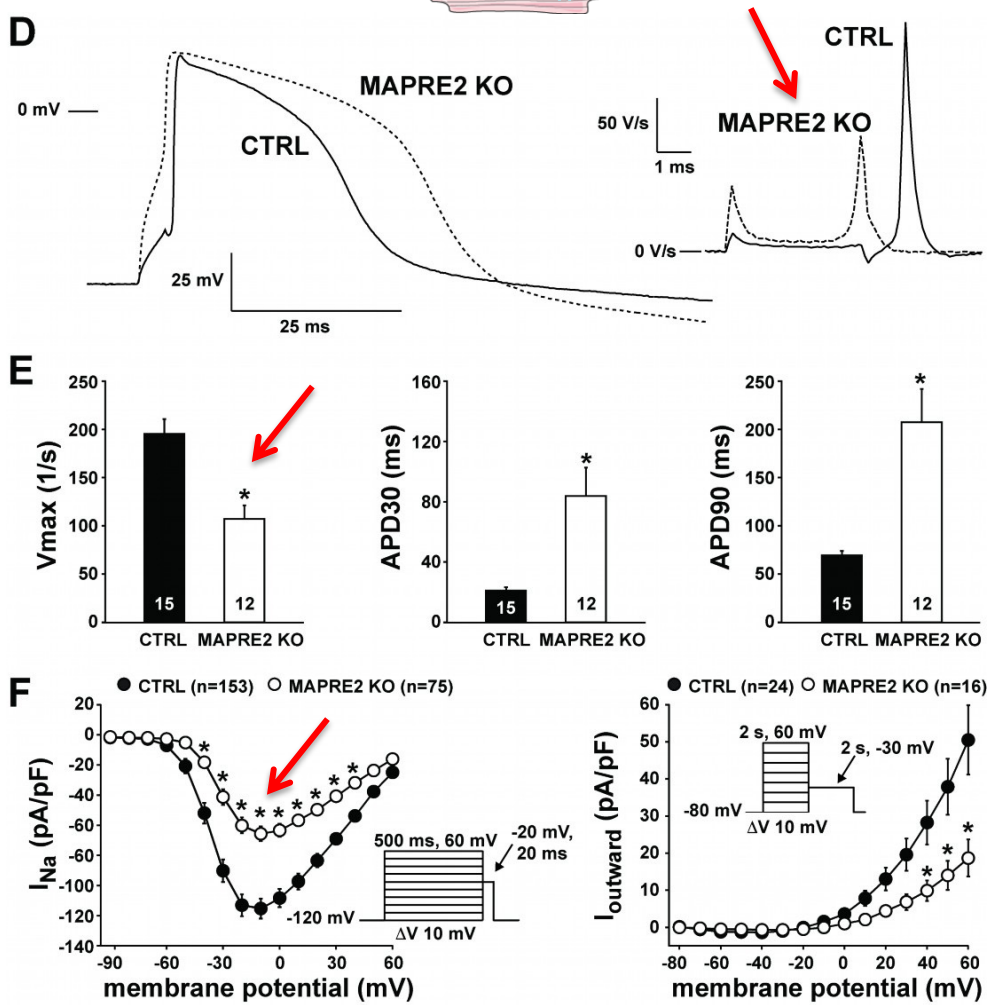
MAPRE2 is a regulator of microtubule organization and impacts on sodium current



iPS-CM





Leducq Foundation

David Chiang
(Boston)

Mariam Jouni
(Chicago)

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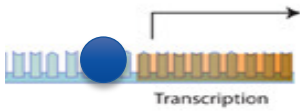
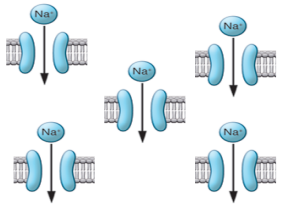
Modulation of microtubule function alters ion-channel trafficking

Multiple levels of sodium current dosage regulation affects in BrS phenotype



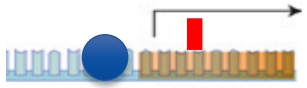
SCN5A



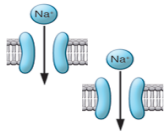
Normal sodium current

Rare coding mutations

(loss of function)



SCN5A



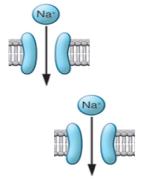
Reduced sodium current

Common non-coding risk alleles

Ion channel control

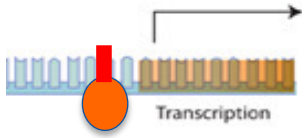
SCN10A

SCN5A

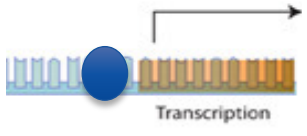


Reduced sodium current

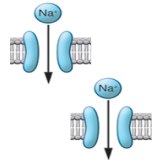
Translational control



TBX5, …

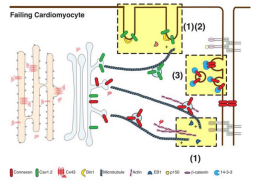


SCN5A

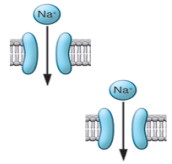


Reduced sodium current

Post-translational control



MAPRE2



Reduced sodium current

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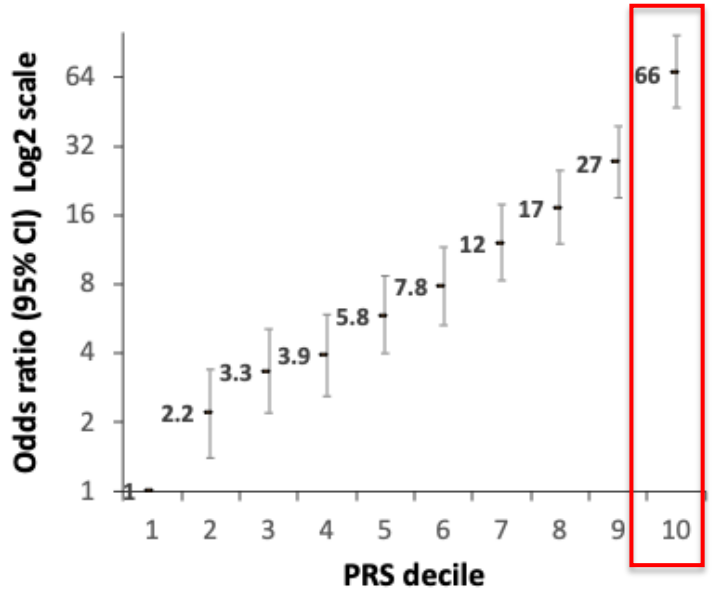
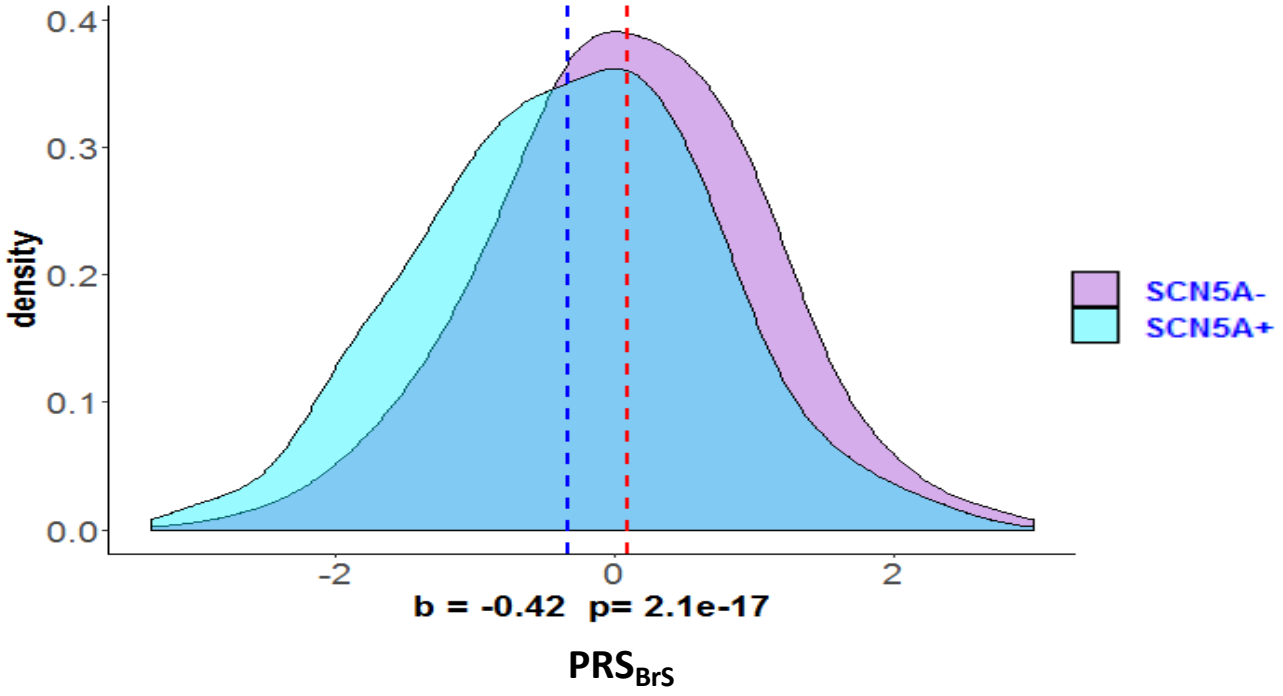
Impact in clinical practice: PRD and risk allele distribution among cases

Cumulative effect of susceptibility variants
(21 loci)

Polygenic risk score



Polygenic Risk Score (PRS) in *SCN5A* $^{+/-}$



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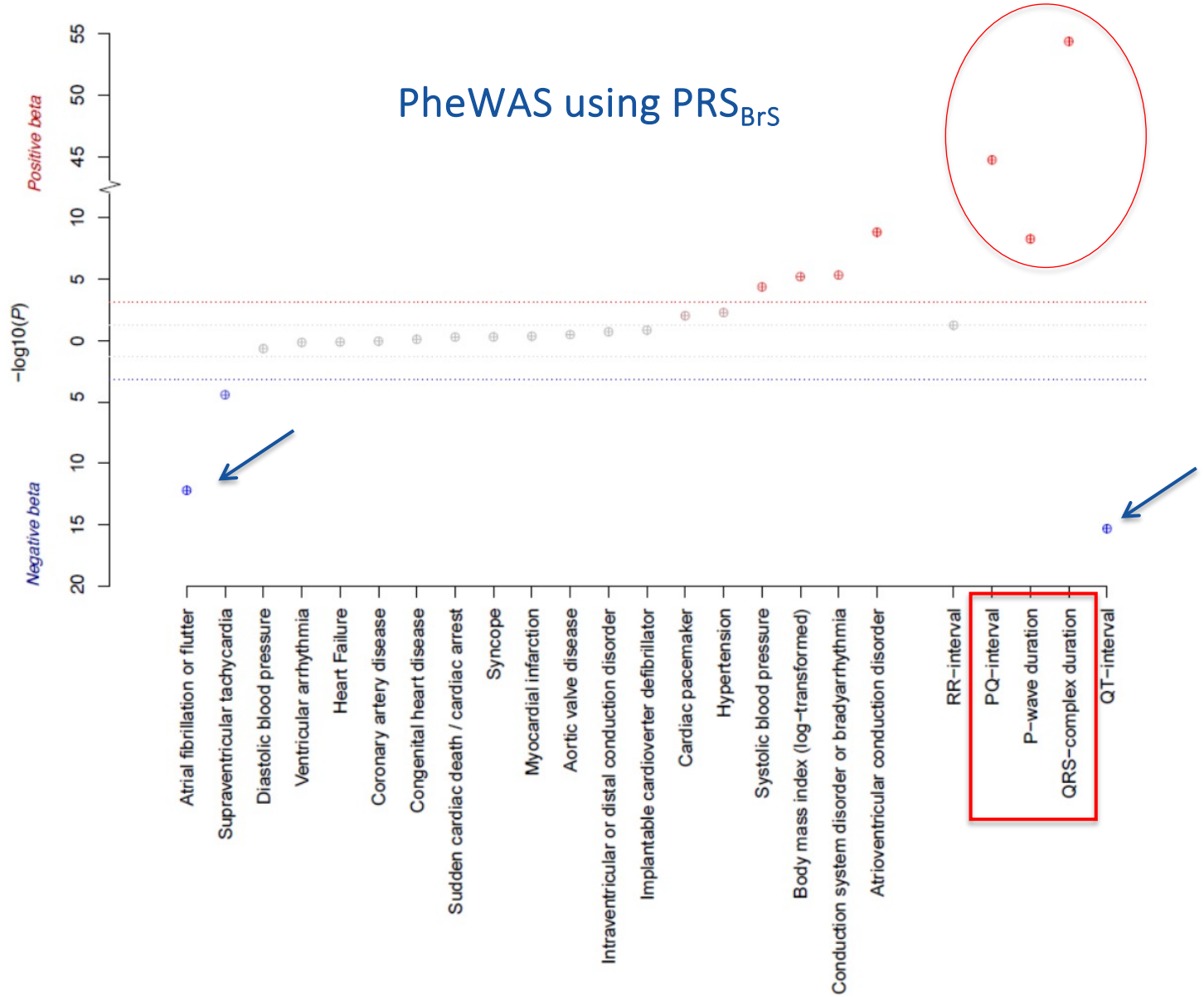
BrS, a relevant model for more complex diseases



Genome-wide association meta-analysis identifies novel Brugada Syndrome susceptibility loci and highlights multiple pathways modulating ion channel dosage



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nature genetics

Barc et al.2022 Mar;54(3):232-239

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Amsterdam UMC
Universitair Medische Centra

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Further perspectives (GWAS)

1- A first Genome-wide association meta-analysis **of Japanese ancestry:** 940 BrS cases and 1,634 controls

2- Genome-wide association meta-analysis combining the Japanese and the European-ancestry datasets: **3,760 BrS cases** and 11,635 controls

→**17 loci** reached the genome-wide significance

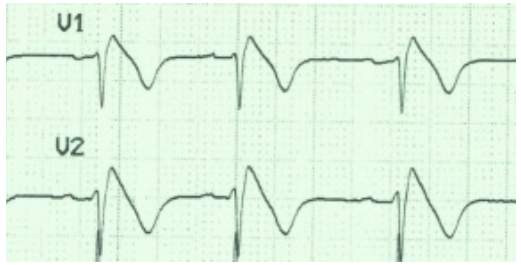
→**5 loci** were newly discovered in the cross-ancestry meta-analysis.

→**Polygenic risk score analysis highlights shared genetic architecture across ancestries**

3- Genome-wide analysis of lethal arrhythmic events among BrS patients identifies 1 VF-associated SNP in Japanese and European BrS

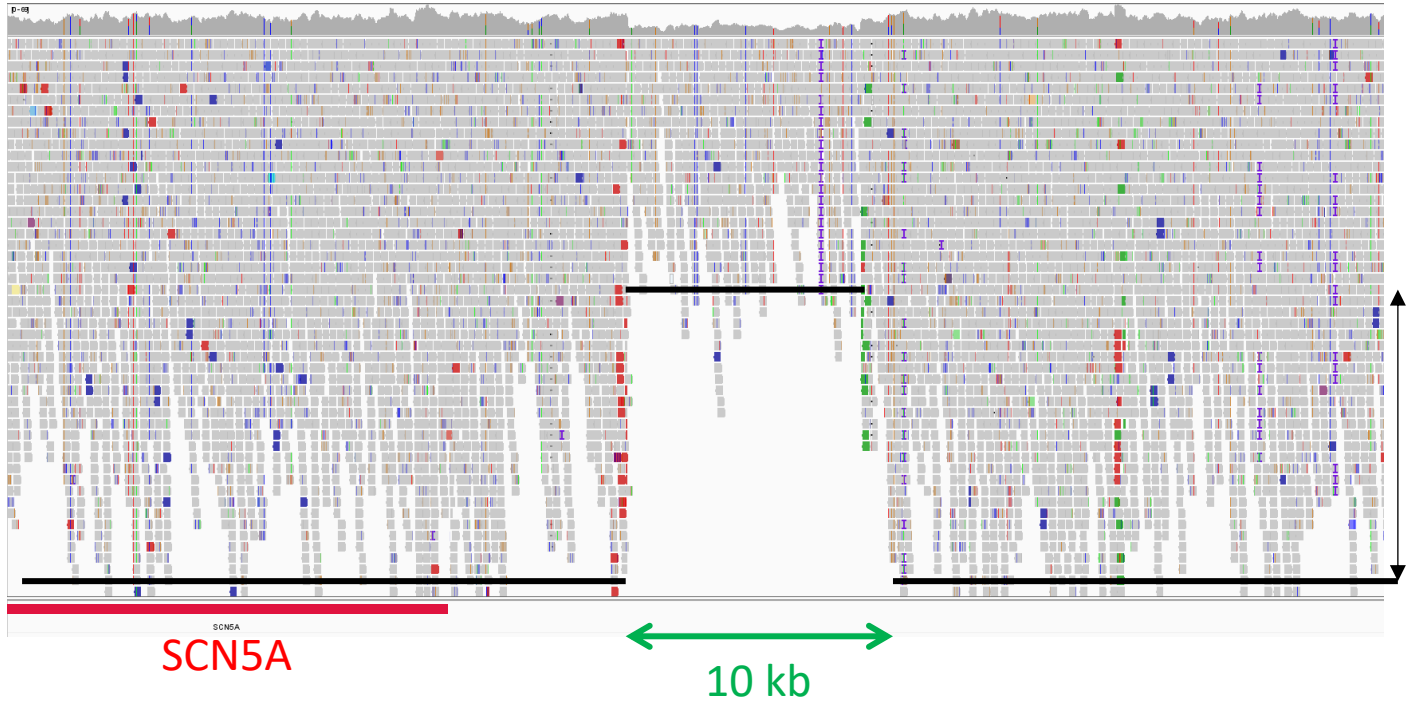
Manuscript in preparation

Whole genome sequencing acccurarly identifies CNVs



→ECG de type I Brugada

3 syncopes → ICD at age 45



50% reduction of covergage
→ hétérozygote deletion

➢ **10 kb deletion in the promoter region of *SCN5A***

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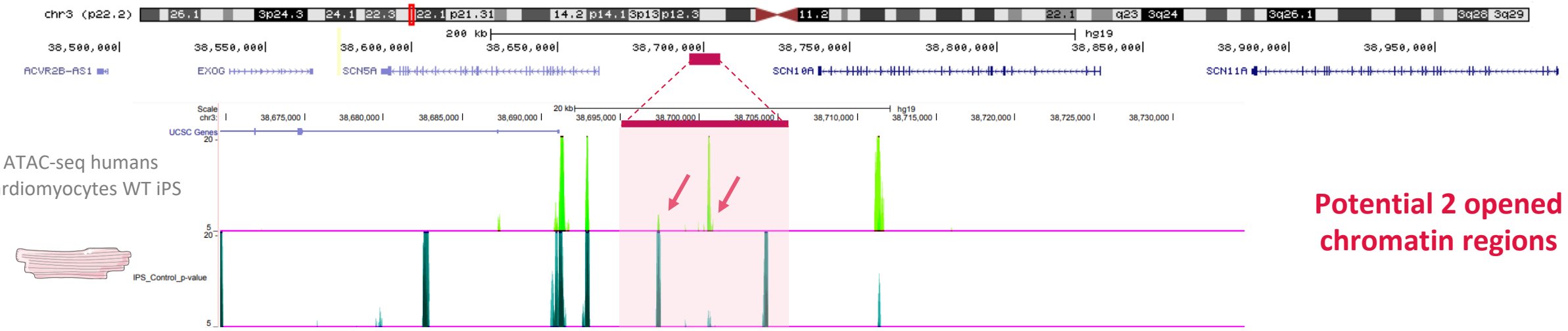
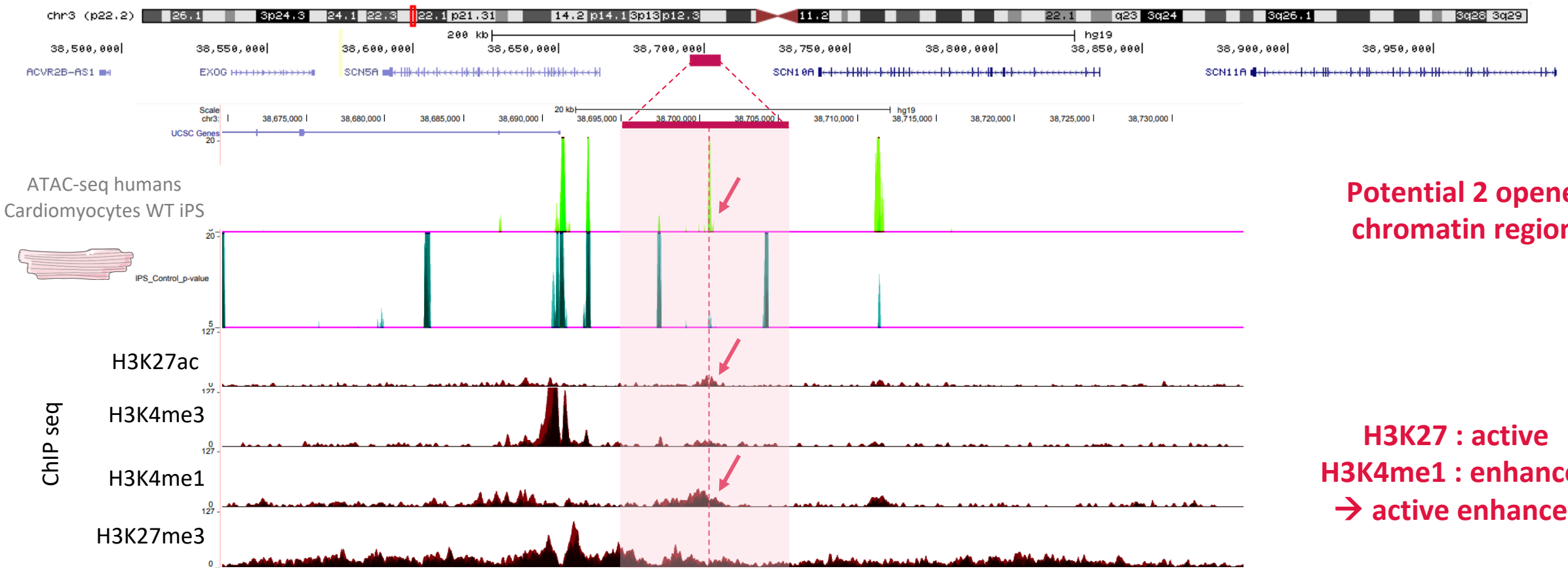
Functioal annotation



ATAC-seq humans
Cardiomyocytes WT iPS

Potential 2 opened chromatin regions

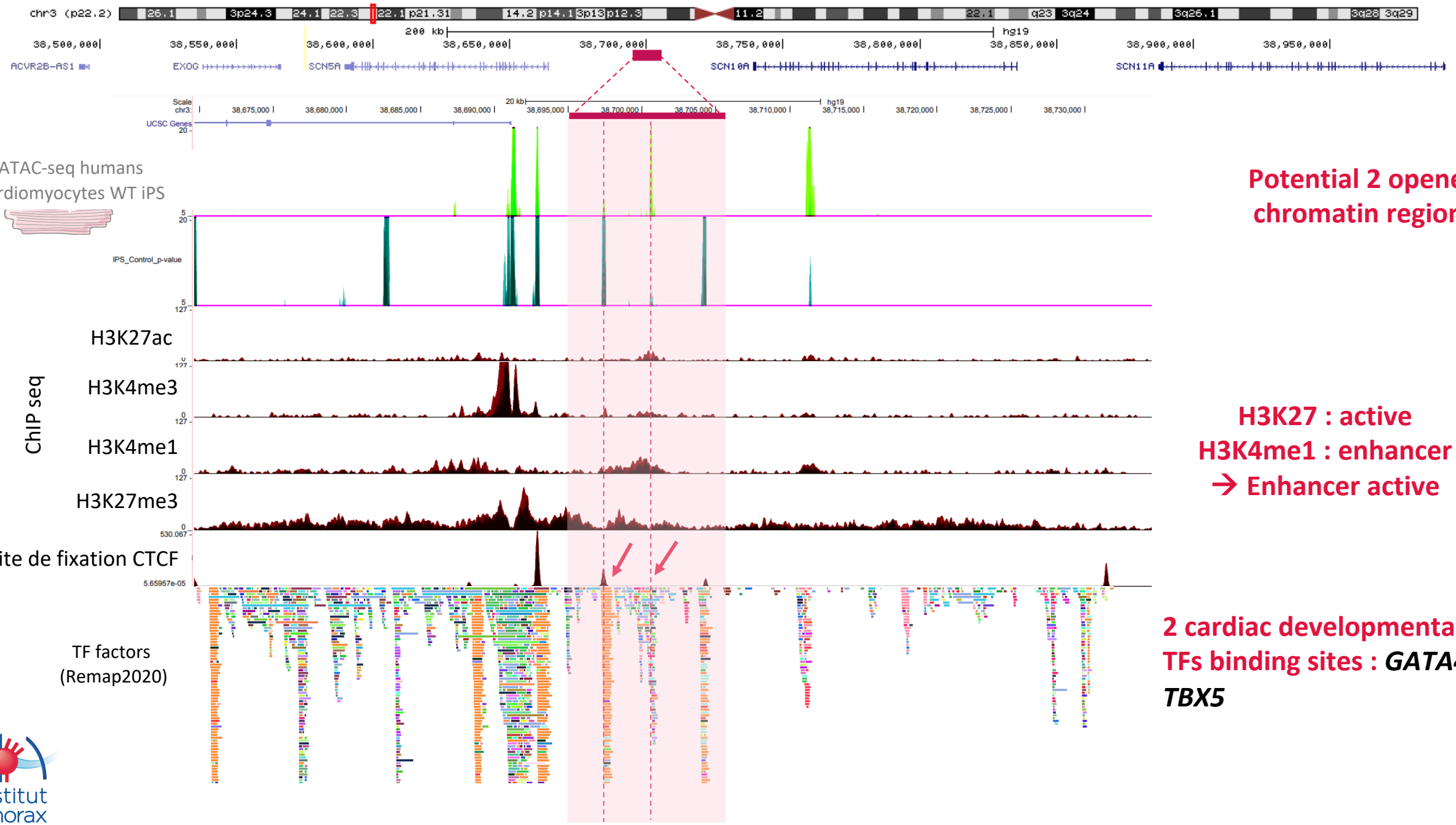
Functional annotation of the deletion region



Potential 2 opened chromatin regions

H3K27 : active
H3K4me1 : enhancer
→ active enhancer

Functional annotation of the deletion region



Potential 2 opened chromatin regions

H3K27 : active
H3K4me1 : enhancer
→ Enhancer active

2 cardiac developmental TFs binding sites : *GATA4, TBX5*

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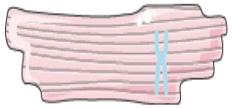
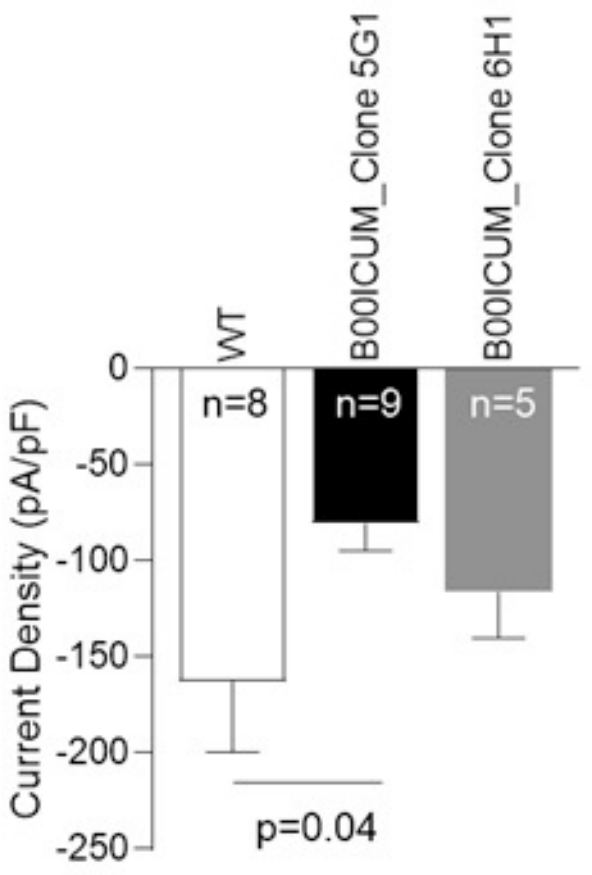
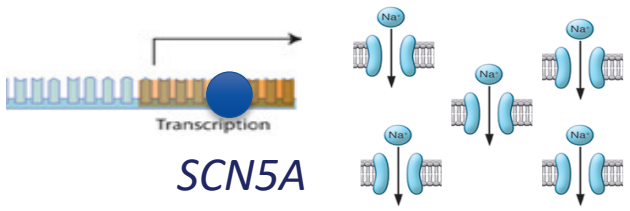
Functional annotation of the 10kb deletion (patch clamping)





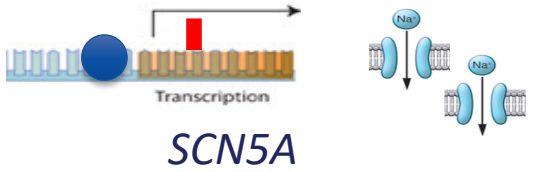
➢ Reduction of I_{Na} density

Multiple levels of sodium current dosage regulation affects in BrS phenotype



Normal sodium current

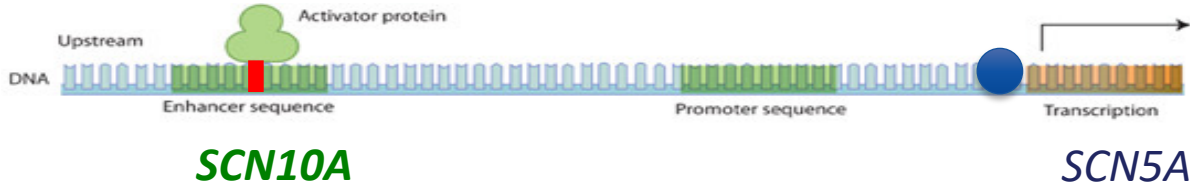
Rare coding mutations



Reduced sodium current

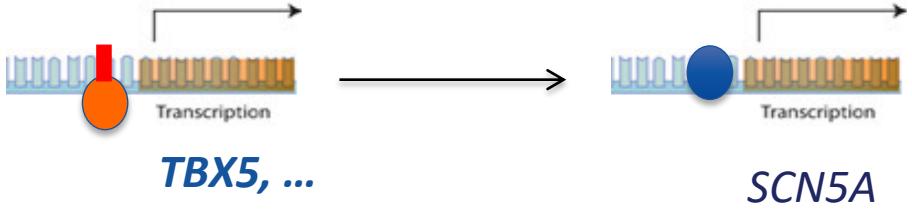
Common non-coding risk alleles

Ion channel control



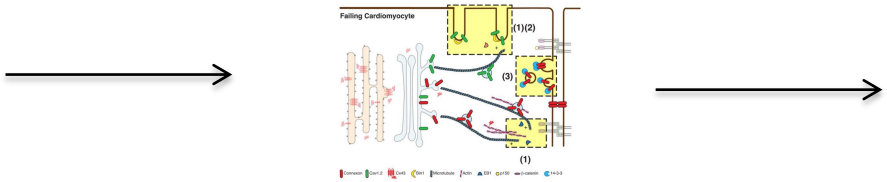
Reduced sodium current

Translational control



Reduced sodium current

Post-translational control



Reduced sodium current

CNVs

Ion channel control

Del

Reduced sodium current

Key take-home messages

- The inheritance model for Brugada syndrome is complex
- Unexpected large effect of common genetic variations on BrS susceptibility
- **The *SCN5A* locus is prominent in disease susceptibility, with strong involvement in both rare and common alleles**
- Strong involvement of transcription regulation impacting ion channels expression, heart development **as well as cardiac structural anomalies**

➢ **THERAPY: From ICD & pharmacology to genetic testing and personalized medicine ?**

- RARE & COMMON VARIANT: GENETIC RISK SCORE
- New pharmacological approaches targeting gene regulatory region?

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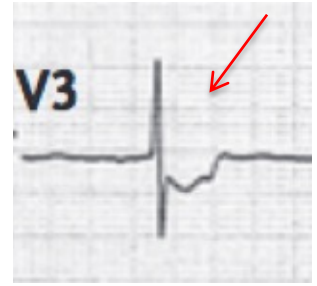
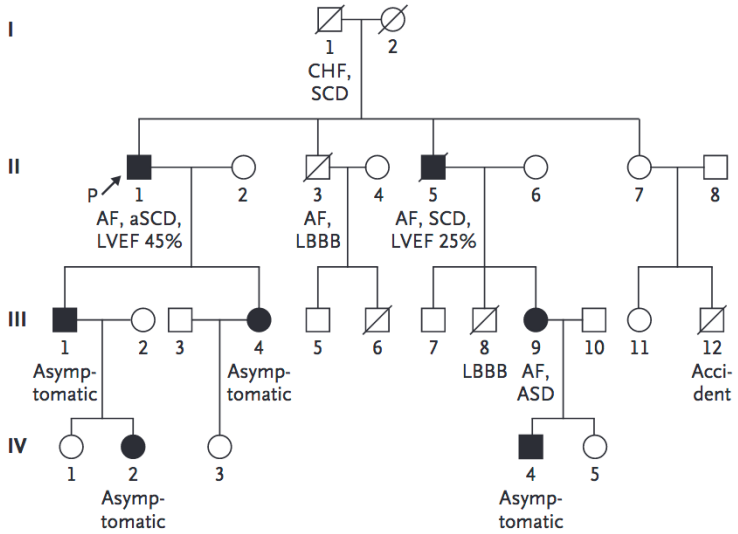
The NEW ENGLAND JOURNAL of MEDICINE

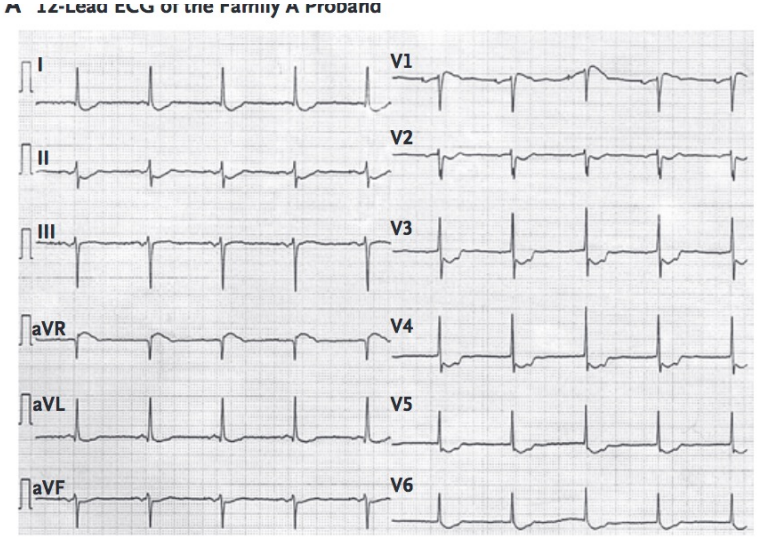
CORRESPONDENCE

(Nov. 2018)



A Novel Familial Cardiac Arrhythmia Syndrome with Widespread ST-Segment Depression





- **The index patient incidental presentation at 36 years of age with an ECG showing deep and persistent, concave-upward ST-segment depression in leads I, II, aVL, aVF, and V2 through V6**
- **Five unrelated families (rare phenotype)**
- **No gene(s) identified (panel, exome, genome…)**

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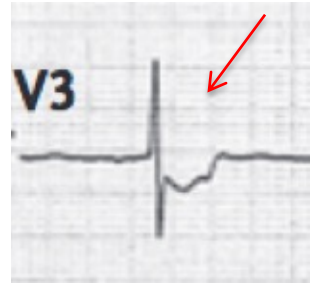
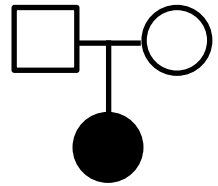
Similar ECG pattern in an isolated case in Nantes

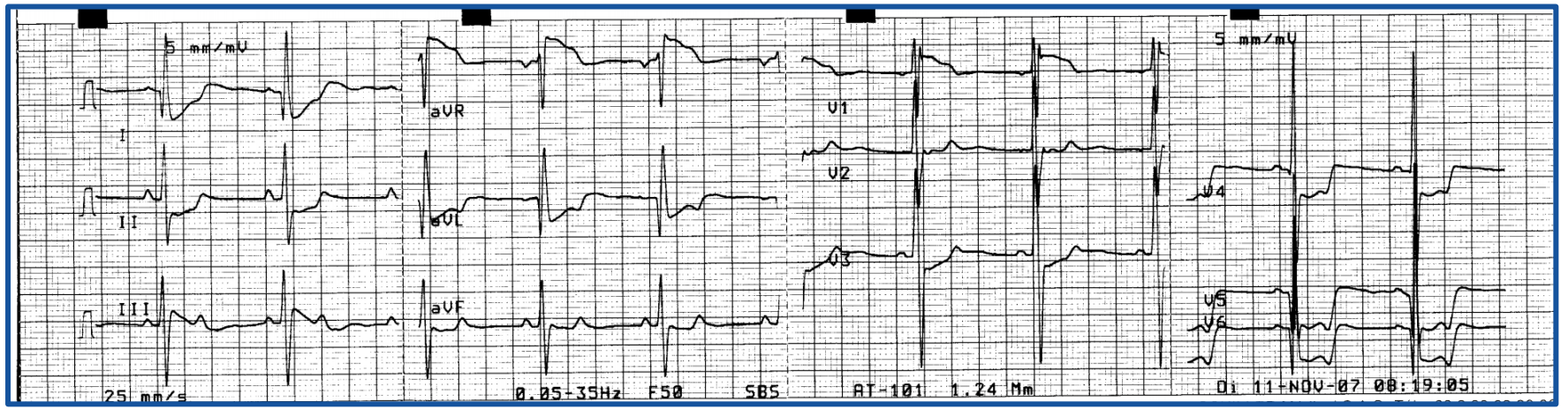


- The 9 y/o index patient (female) presenting cardiac arrest
- Initial diagnostic: Brugada syndrome
- ECG showing deep and persistent, concave-upward ST-segment depression in leads (unrecognized)
- Unresponsive to antiarrhythmic medication
- ICD implantation / multiple shocks
- Unresponsive to ventricular ablations
  - Cardiogenic shock
- Heart transplant



- Both parents are unaffected (normal ECGs)

- Whole genome sequencing (trio)
  - *de novo* mutation ?



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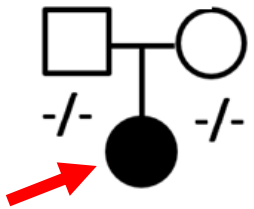
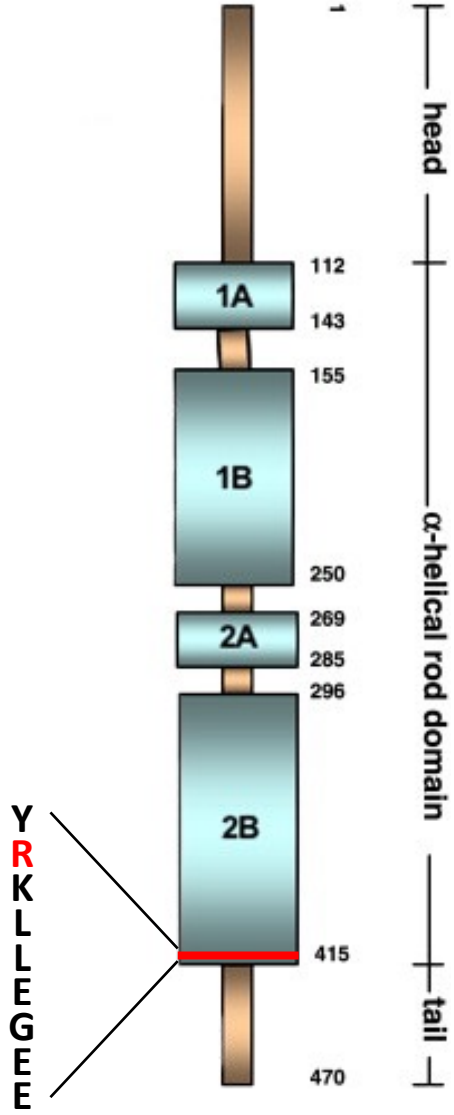
A *de novo* mutation in DES (desmin) gene



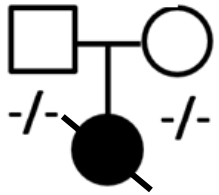
+/-

***DES* p.Arg406Trp**

Ultra-rare (not found in GnomAD)

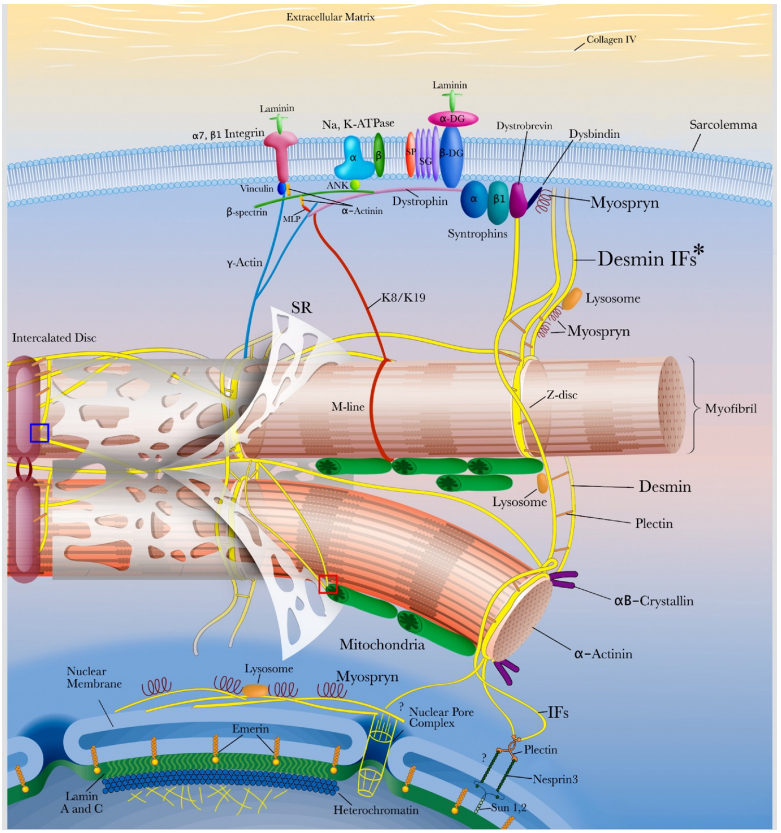


- Same DES p.ARg406Trp mutation with similar ECG

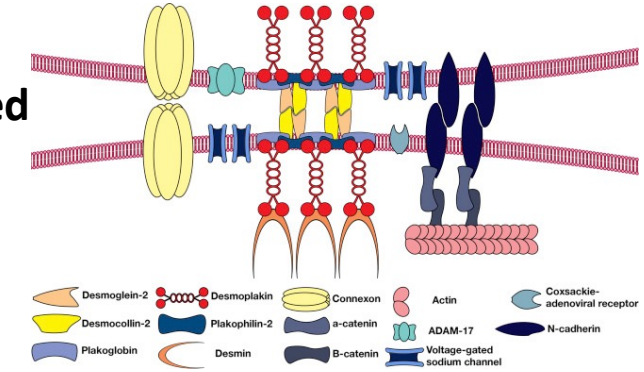


+/-

- Severe rhythm disorder
- Died at 6 months of age in the cardiopediatric emergency care



Intercalated disk



Capetanaki Y *et al*, *Exp Cell Res* 2007; 313(10): 2063-76.

DES p.ARg406Trp mutation is an ultra rare variant but report in the literature

- *de novo*, in 2 other cases (no ECG) in literature & 1 with HCM & complete AV block and ST segment depression
- 1 with familial segregation (father and son; no ECGs documented)

Is DES DES p.ARg406Trp a Desminopathy?

- subgroup of myofibrillar myopathies affecting both skeletal and cardiac muscles
- 50% of mutations carriers have cardiomyopathy: dilated (17%), restrictive (12%), hypertrophic (6%), arrhythmogenic right ventricular (1%) cardiomyopathies
- 60% have cardiac conduction disease or arrhythmias, with atrioventricular block as a hallmark

Index case

- 2009: no structural heart disease (2-D echography) or anomalies of the coronary arteries (tomography)
- Histopathological examination of the explanted heart :
  - increased amounts of epicardial fat, mainly in RV, no fibro-fatty replacement, no dystrophic cardiomyocytes ➔ not an ARVC
- March 2021: no clinical sign of skeletal muscle disease at 23 years old

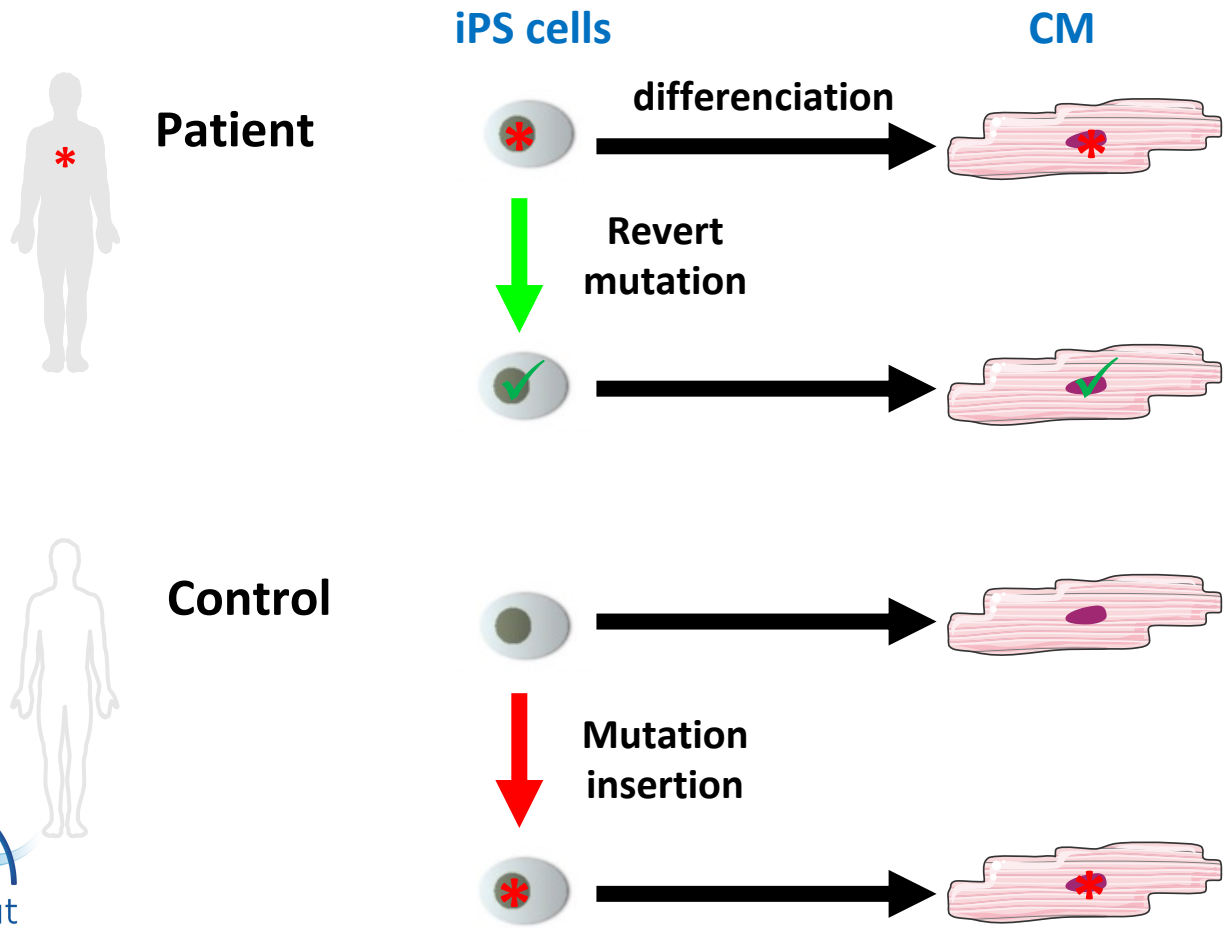
Tsikitis M *et al*, *Biophys Rev 2018*; 10: 1007-31.

Functional analysis

Impact of DESp.R406W on cardiac cells

Michelle Geryk
(F. Charpentier's team)

➔ iPS model (ventricular CM: 2D and Engineered Heart Tissue (EHT)



Next steps

- **Electrophysiological studies**
  - **Patch clamping**
    - **Action potential recording unchanged**
  - **Abnormal mitochondria distribution & structure**
    - **Additional models**
      - **Mouse model (ongoing)**
      - **Purkinje cells (no iPS model**

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Perspectives: from NGS to Digital Health

ENTERING THE BIG DATA ERA

- **High throughput multi-omics approaches**
  - ✓ **Whole genome sequencing**
  - ✓ **Epigenetic**
  - ✓ **Single cell RNA sequencing**

- **Digital enhanced phenotyping**
  - ✓ **MRI**
  - ✓ **…**

Artificial Intelligence

NEW HYPOTHESIS

New biological insights

Better risk stratification

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