Génétique cardiovasculaire: Concepts & applications

L'unité de recherche de l'institut du thorax
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MASTER 1 Thorax

Unité d'enseignement « Recherche en Physiopathologies humaines »

17-11-2022

Approches génétique en 2022: 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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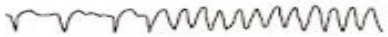
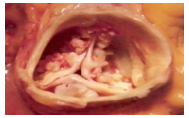
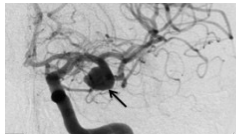
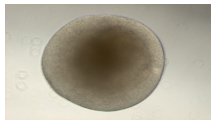
Strategy

Translational approach to investigate the genetic architecture of cardiovascular & developmental diseases

- **Identifying susceptibility genes and molecular mechanisms**


- **Largest collections worldwide**

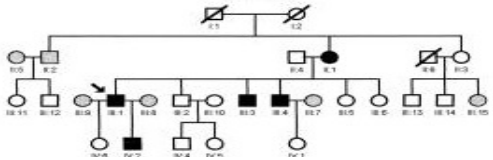




New analytical tools:

Bioinformatics

Biostatistics

Epigenetics



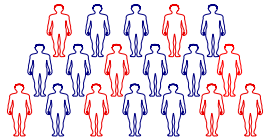
Families

Rare variants

(strong effects)

Exome sequencing

Whole genome sequencing



Population

Common variants

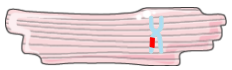
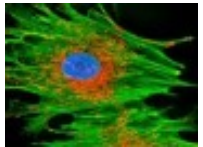
(susceptibility genes)

Genotyping

Whole genome sequencing

NEW GENES

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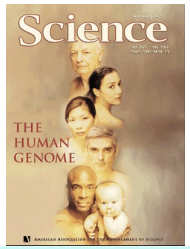
Disease gene identification allowed by a spectacular acceleration of knowledge of the architecture of the human genome:

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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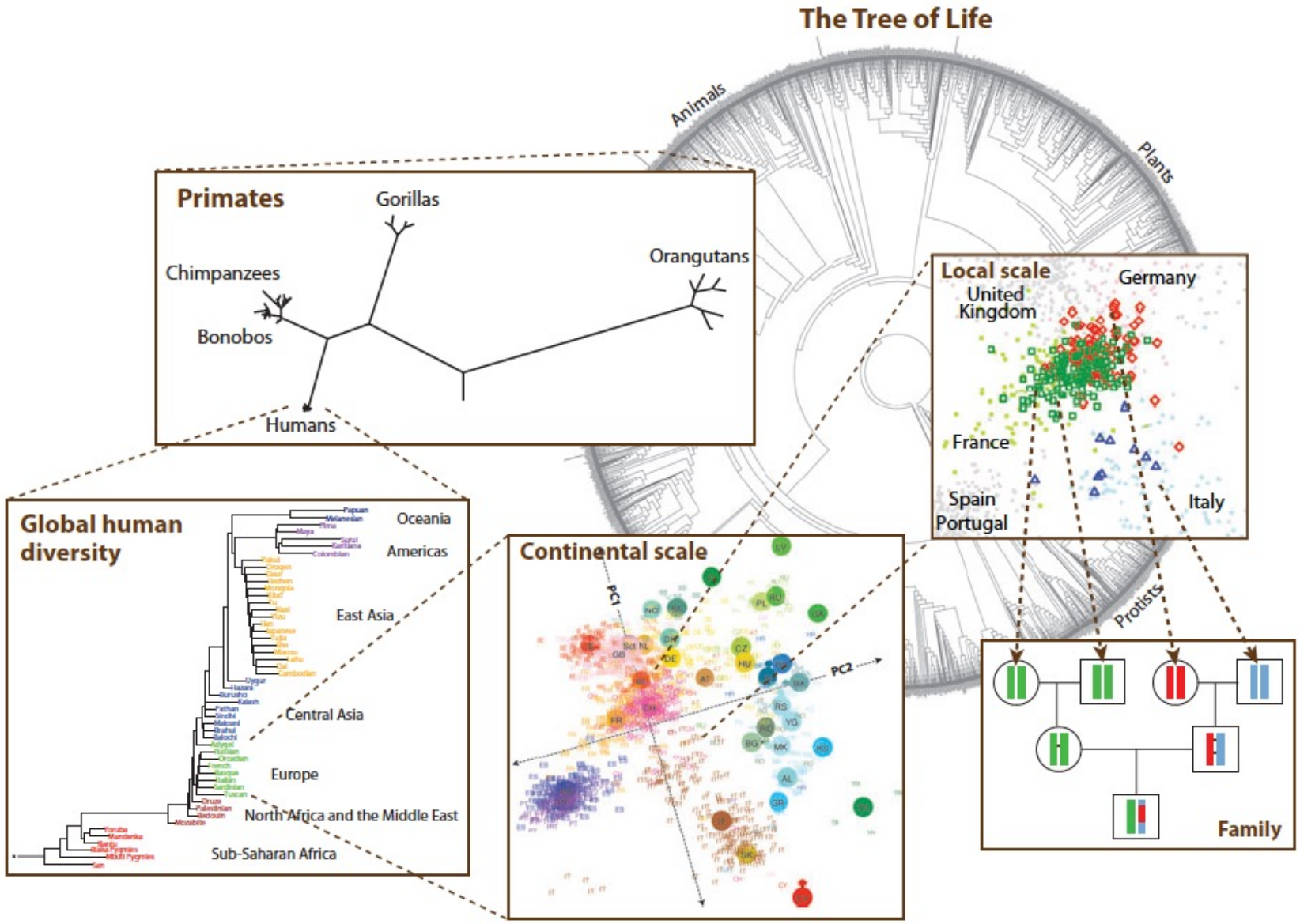
Stratégies génétiques & dynamique des génomes



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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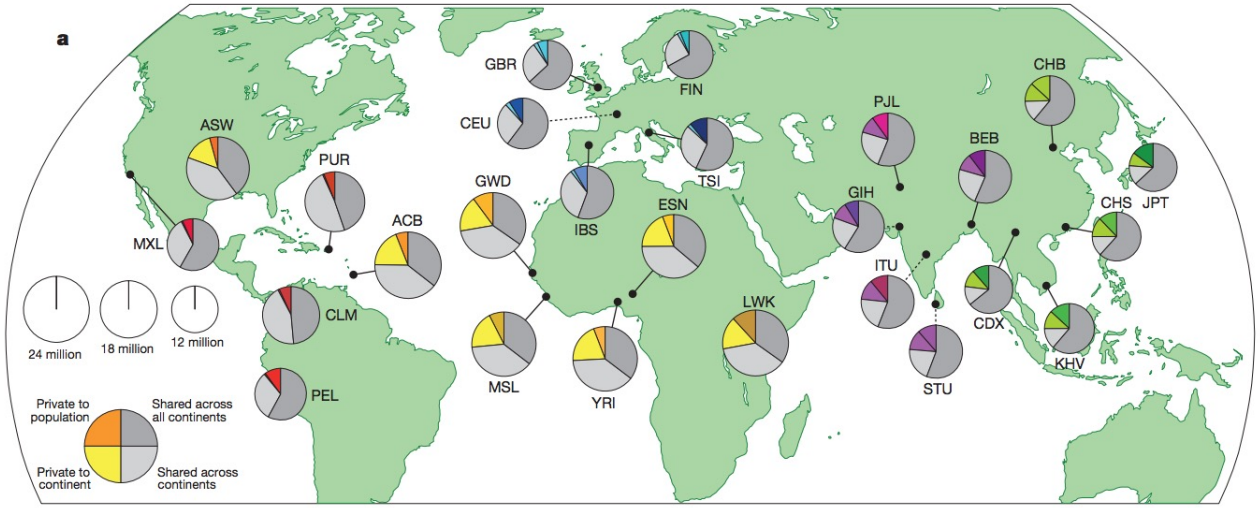
ARTICLE

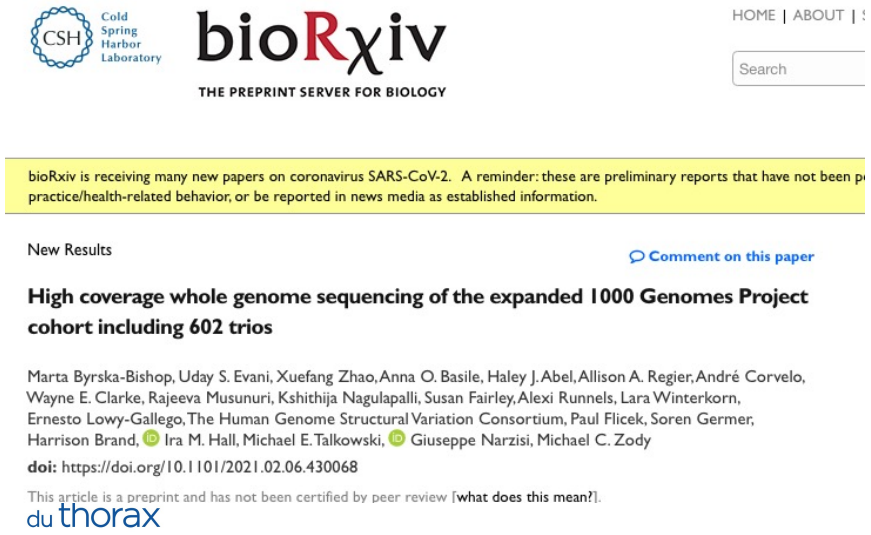
OPEN
doi:10.1038/nature15393

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%)





du thorax

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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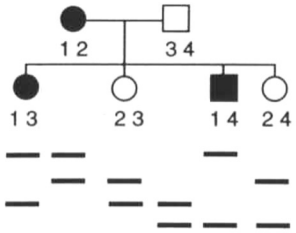
Clonage positionnel :
« génétique des grandes familles »

Variants rares

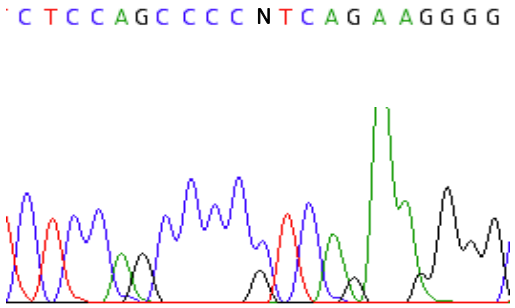
Patient



Analyse de liaison

Locus

Gènes candidats



Mutation

Physiopathologie
Diagnostic génétique

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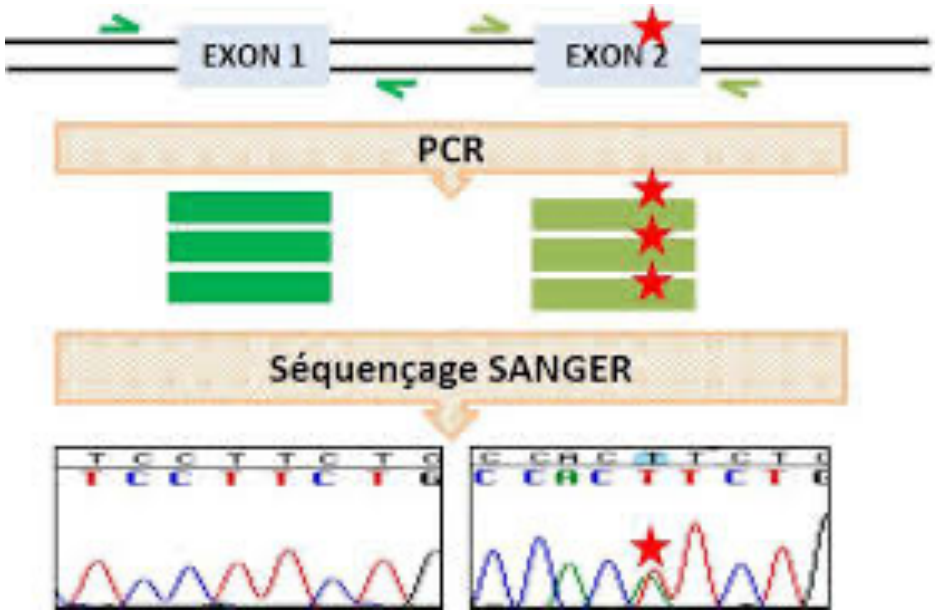
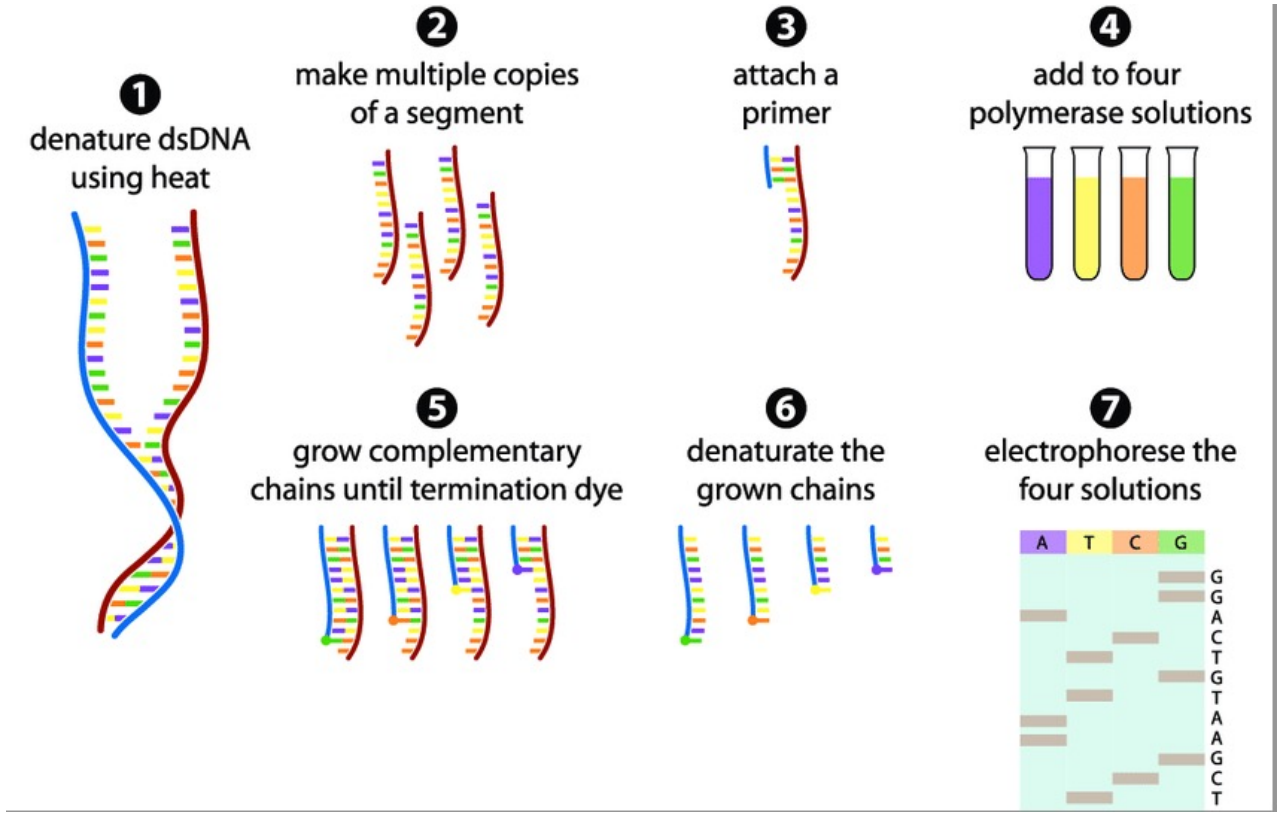
Clonage positionnel : Séquençage SANGER





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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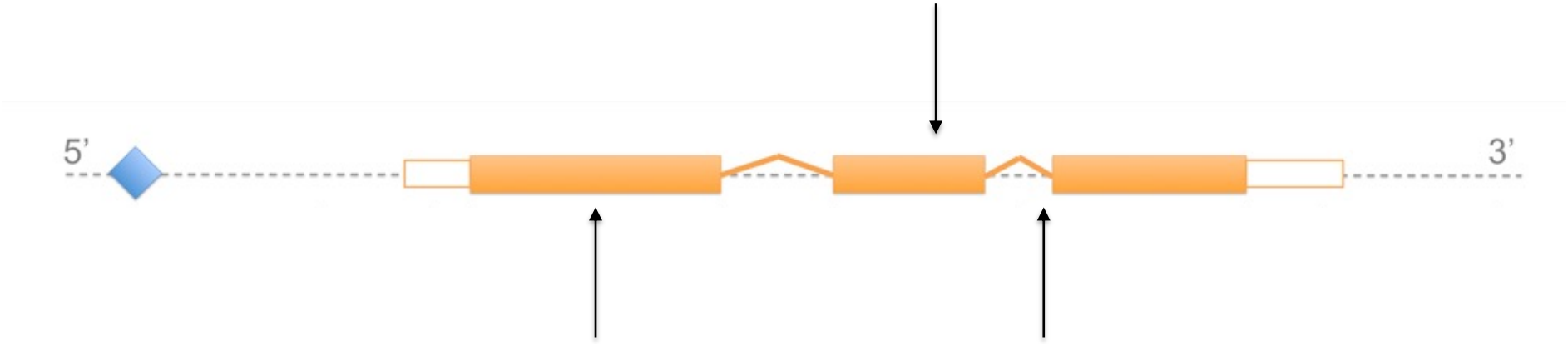
Effet des variants génétiques

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 α4)

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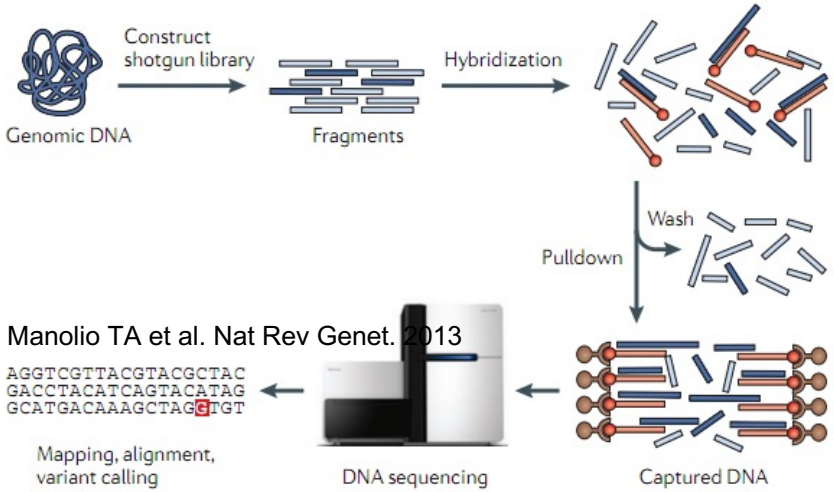
Genetic variation detection improvement by the new technologies

Exome sequencing: capture all coding regions of the genome (1 - 2% soit 50 Mb)

nature



Manolio TA et al. Nat Rev Genet. 2013

Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants

Wenqing Fu[1], Timothy D. O'Connor[1], Goo Jun[2], Hyun Min Kang[2], Goncalo Abecasis[2], Suzanne M
Mark J. Rieder[1], David Altshuler[4], Jay Shendure[1], Deborah A. Nickerson[1], Michael J. Bamshad[1,5], N
& Joshua M. Akey[1]

Science AAAS

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

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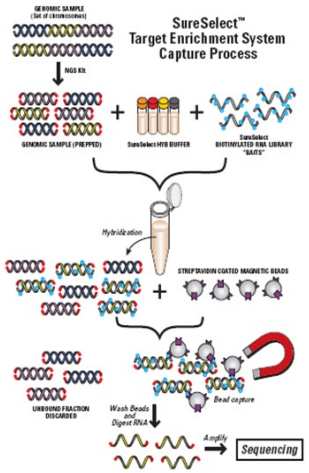
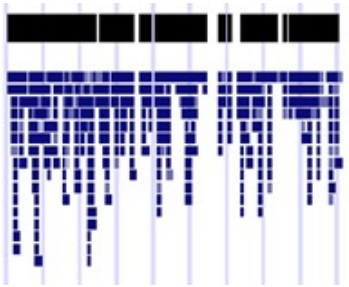
Séquençage d'Exome (NGS)

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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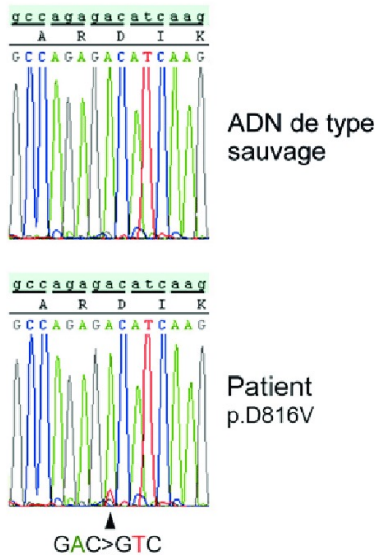
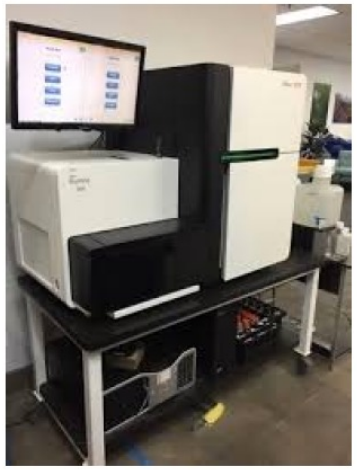
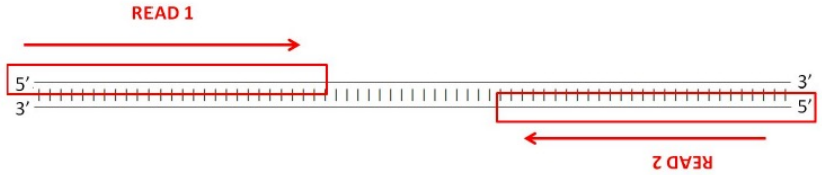
Séquengage « Sanger »

Sample → FASTQ








```
@SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
CAACGAGTTCACACCTTGGCCGACAGGCCCGGGTAA
+SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
BA@7>B=>:>>7@7@>>9=BAA?;>52;>:9=8.=A
@SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
CCAATGATTTTTTTCCGTGTTTCAGAATACGGTTAA
+SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
BCCBA@BB@BBBBAB@B9B@=BABA@A:@693:@B=
@SRR038845.53 HWI-EAS038:6:1:1:360 length=36
GTTCAAAAAGAACTAAATTGTGTCAATAGAAAACTC
+SRR038845.53 HWI-EAS038:6:1:1:360 length=36
BBCBBBBBB@@BAB?BBBBCBC>BBBAA8>BBBAA@
```

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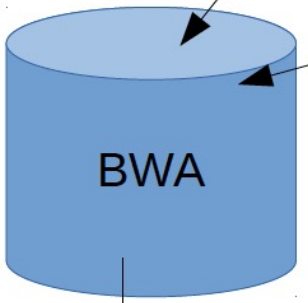
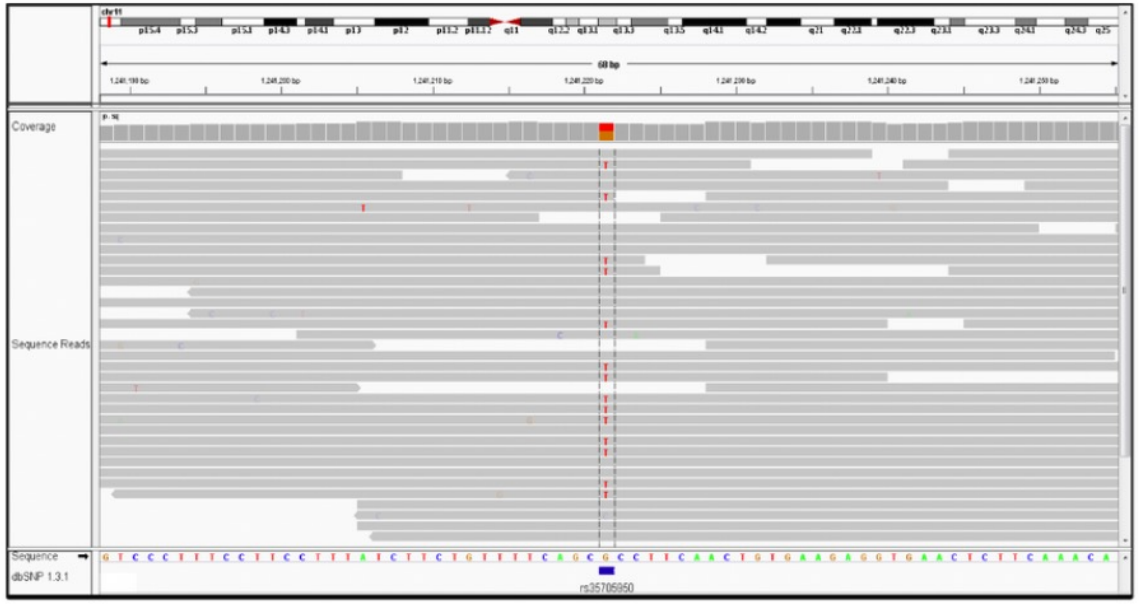
FASTQ → BAM

```
@SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
CAACGAGTTCACACCTTGGCCGACAGGCCCGGGTAA
+SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
BA@7>B=>:>>7@7@>>9=BAA?;>52;>:9=8.=A
@SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
CCAATGATTTTTTTCCGTGTTTCAGAATACGGTTAA
+SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
BCCBA@BB@BBBBAB@B9B@=BABA@A:@693:@B=
@SRR038845.53 HWI-EAS038:6:1:1:360 length=36
GTTCAAAAAGAACTAAATTGTGTCAATAGAAAACTC
+SRR038845.53 HWI-EAS038:6:1:1:360 length=36
BBCBBBBBB@@BAB?BBBBCBC>BBBAA8>BBBAA@
```



BAM



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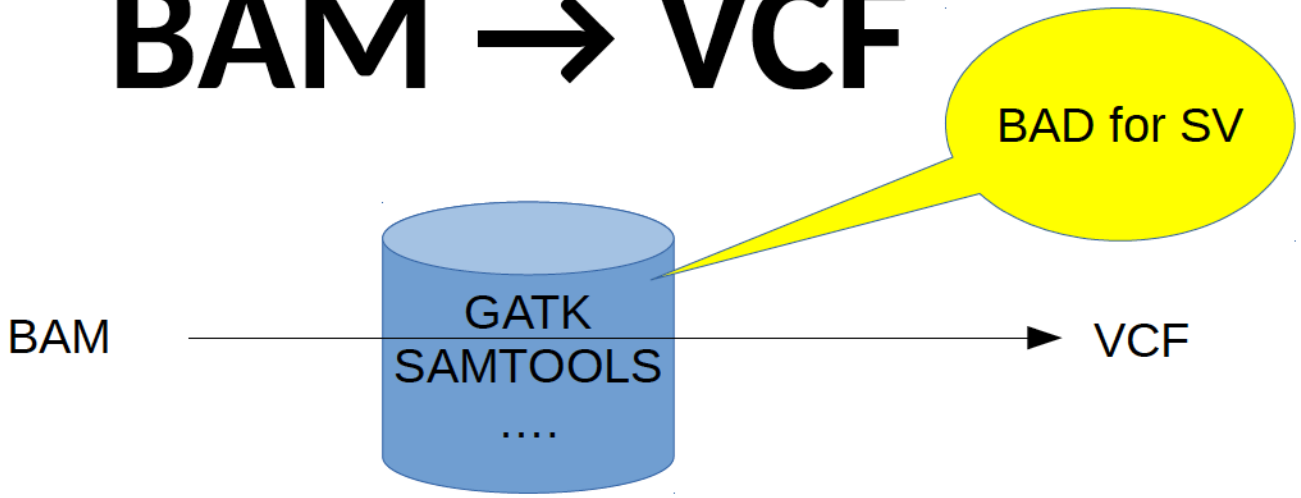
BAM → VCF



CHROM	POS	REF	S1	S2
1	1	A	A/A	A/A
12	234	T	T/A	T/T
20	34	G	G/A	G/G
X	344	C	G/A	C/C

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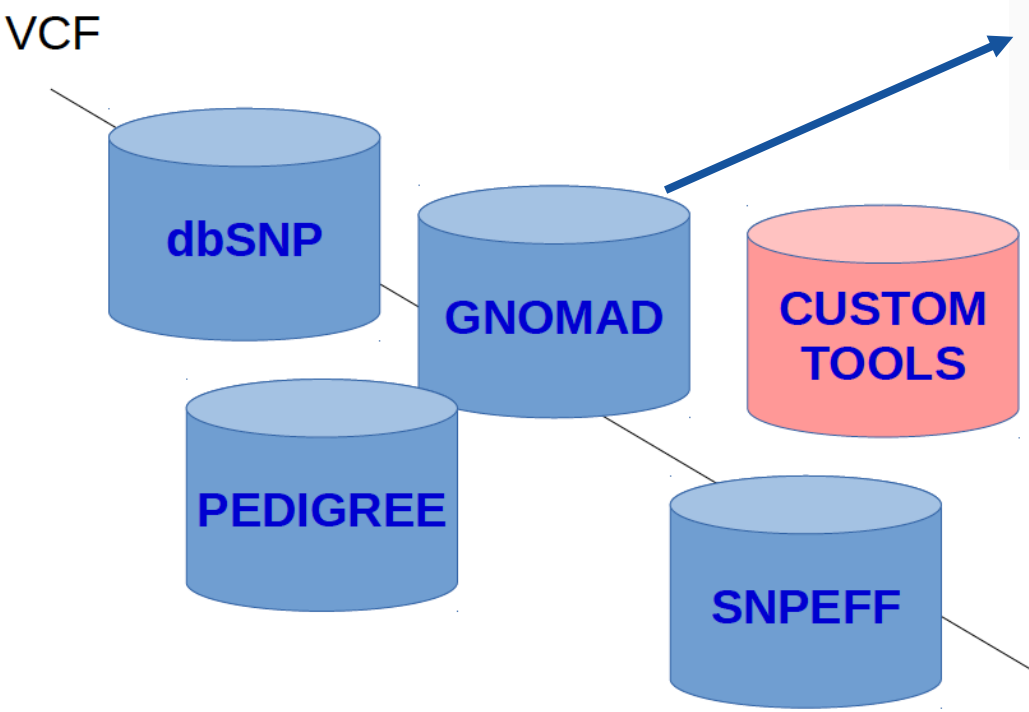
VCF+Annotations



gnomAD

Genome Aggregation Database

https://gnomad.broadinstitute.org/

- v2.1.1 data set (GRCh37/hg19) provides 125,748 exome sequences and 15,708 WGS from unrelated individuals sequenced as part of various disease-specific and population genetic studies.

- v3.1.2 data set (GRCh38) spans 76,156 WGS of diverse ancestries

VCF+annotations

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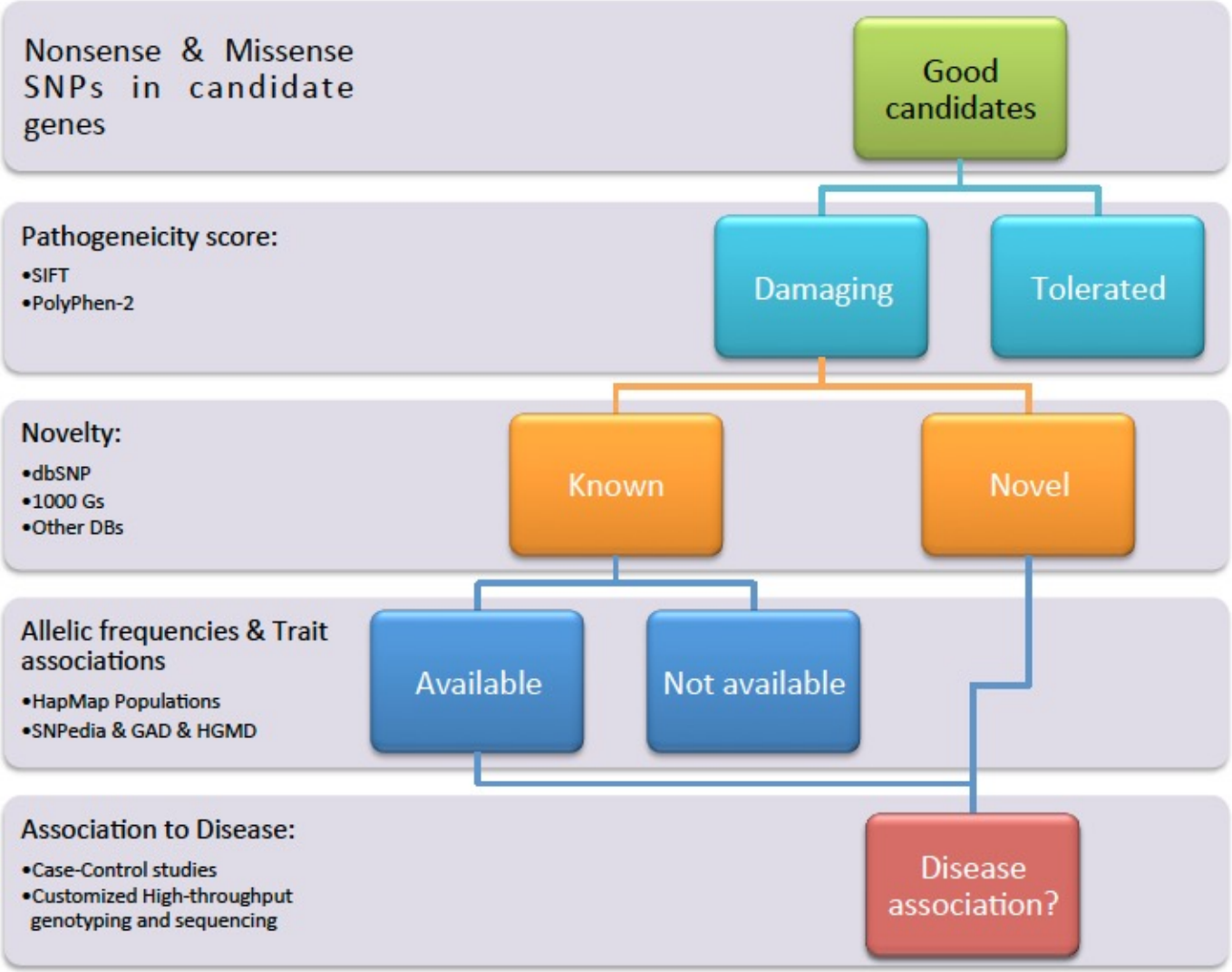
Variant Filtering



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gnomAD browser beta | genome Aggregation Database

Search for a gene or variant or region

Example - Gene: PCSK9, Variant: 1-55516888-G-GA

About gnomAD

The Genome Aggregation Database (gnomAD) is a resource developed by an international coalition of investigators, with the goal of aggregating and harmonizing both exome and genome sequencing data from a wide variety of large-scale sequencing projects, and making summary data available for the wider scientific community.

The data set provided on this website spans 123,136 exome sequences and 15,496 whole-genome sequences from unrelated individuals sequenced as part of various disease-specific and population genetic studies. The gnomAD Principal Investigators and groups that have contributed data to the current release are listed here.

All data here are released for the benefit of the wider biomedical community, without restriction on use - see the terms of use here.

Recent News

February 27, 2017

Official gnomAD release (version 2.0) with browser updates and data available for download.

October 19, 2016

Public release of gnomAD Browser (beta) at ASHG!

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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
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

~ 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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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
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

~ 15 000 variants

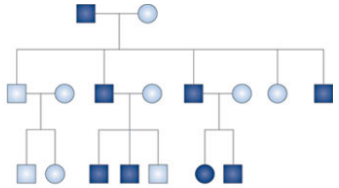
2. Variants rares

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

- Partagés par les individus atteints
  (cas familiaux)



- Liaison génétique

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Classification du niveau de pathogénicité

1- Méthodes « *in silico* » de classification de variants génétiques

✓ Variants localisés dans une séquence codante

SIFT, Polyphen-2, Crescendo, Bongo, SDM, MutPred, PoPMusic, PHD-SNP, Mu-Pro...

✓ Variants localisés dans une séquence non-codante

Eponine, McPromoter, PromoterScan, Match/Transfac, TFSEARCH, ESEFinder, RESCUE-ESE...
+ données ENCODE (Septembre 2012)

2- Méthodes « in vitro »

- tests fonctionnels (bas-débit → Patch clamp (canaux ioniques); souris transgéniques (CRISPR/Cas9)
- évolution technologique: - patch haut-débit
  - modèles cellulaires & animaux : cellules souches pluripotentes induites (IPS); poisson zèbre)

Classification ACMG (American College of Medical Genetics)

- Classe 1 -5: Score de niveau de confiance de pathogénicité:
  pathogenic, likely pathogenic, uncertain significance, likely benign, and benign
- VSI: Variants de signification inconnue

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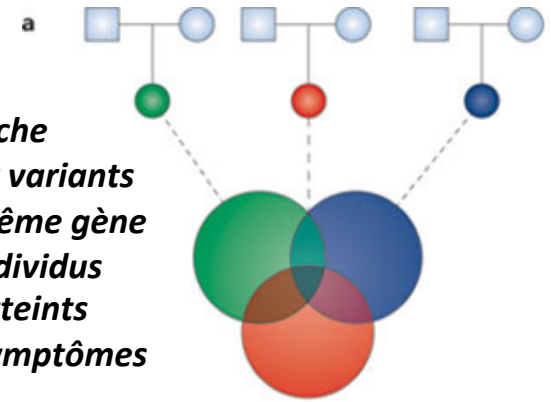
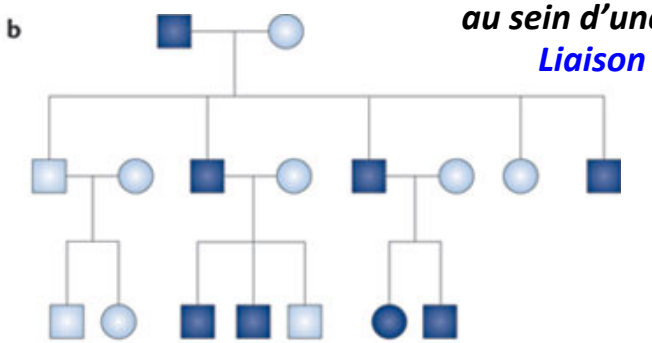
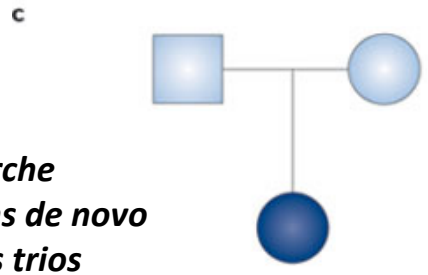
Identification par séquençage d'exomes / génomes de variants rares

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

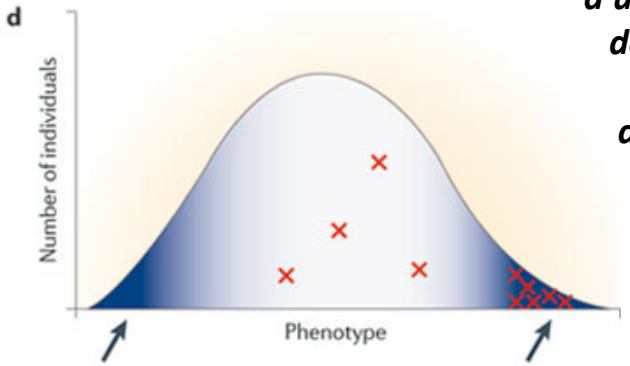






Recherche de mutations de novo dans des trios dont seul l'enfant est atteint

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



Nature Reviews | Genetics

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

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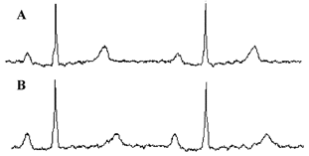
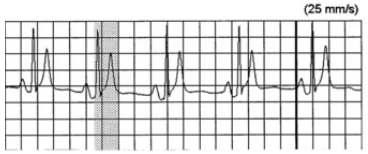
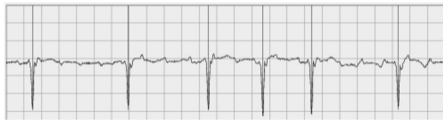
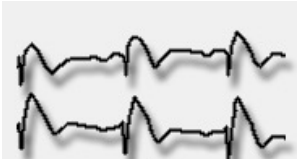
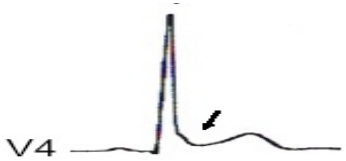
Genetics of primary electrical cardiac disorders



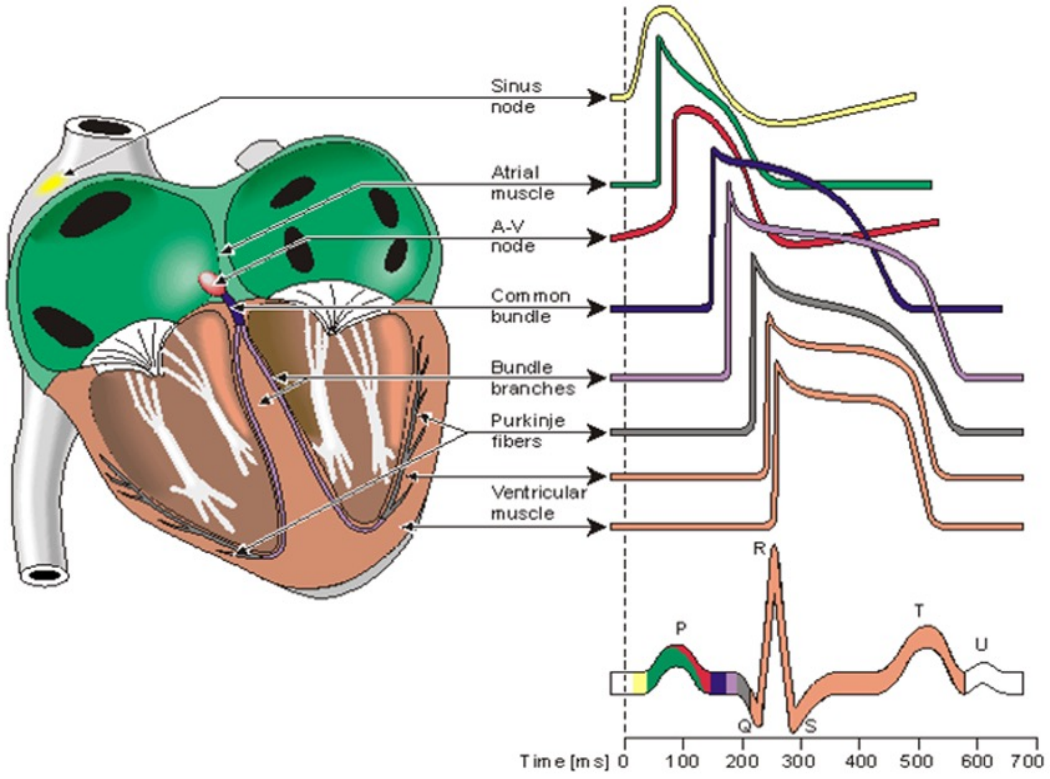








Primary electrical cardiac disorders

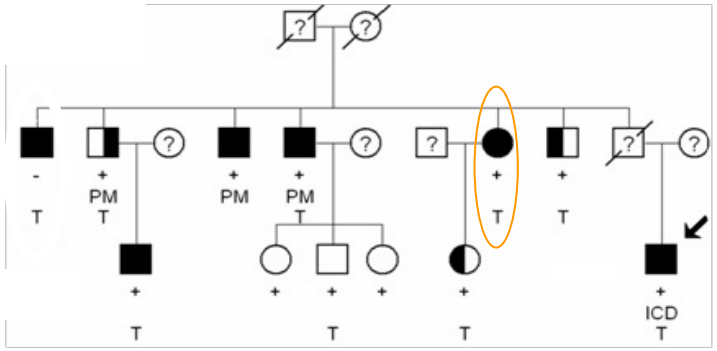


Sick sinus syndrome

Atrial arrhythmias
Atrial Fibrillation

Conduction defects
Atrioventricular bloc

Ventricular arrhythı
Long QT syndrome
Brugada Syndrome
Early Repolarisation

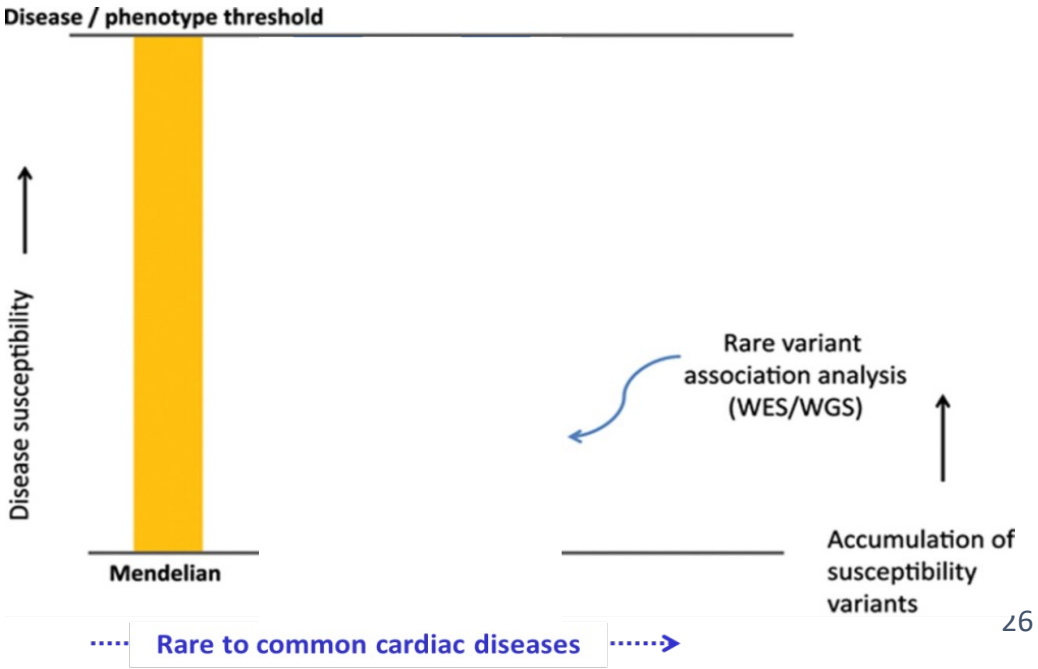


BrS

Conduction disease

Unaffected

Probst *et al.*, 2009



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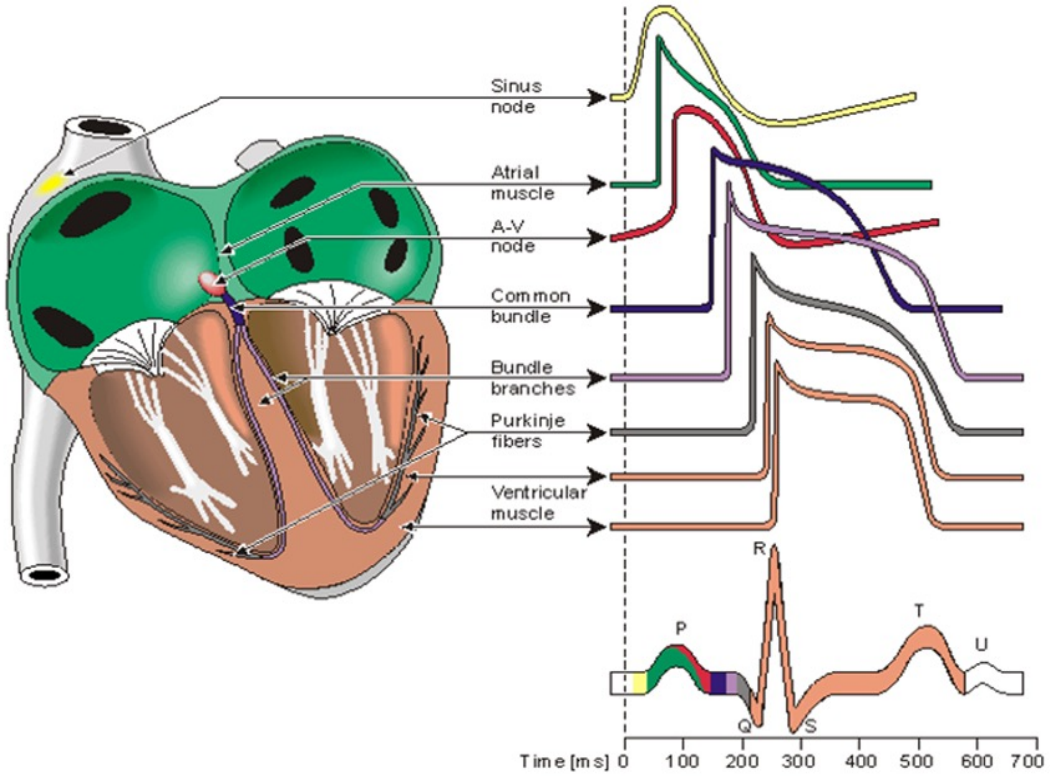
Primary electrical cardiac disorders



Sick sinus syndrome

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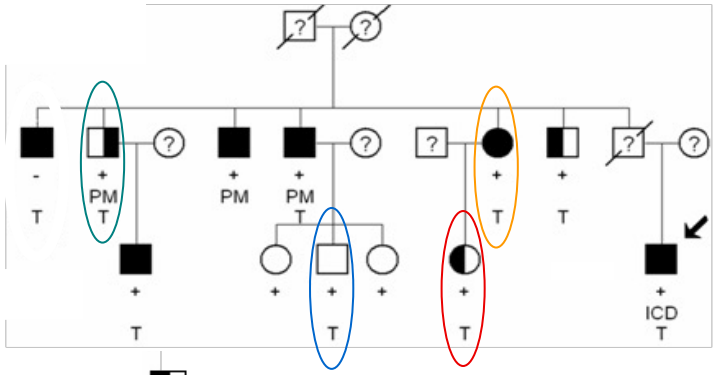
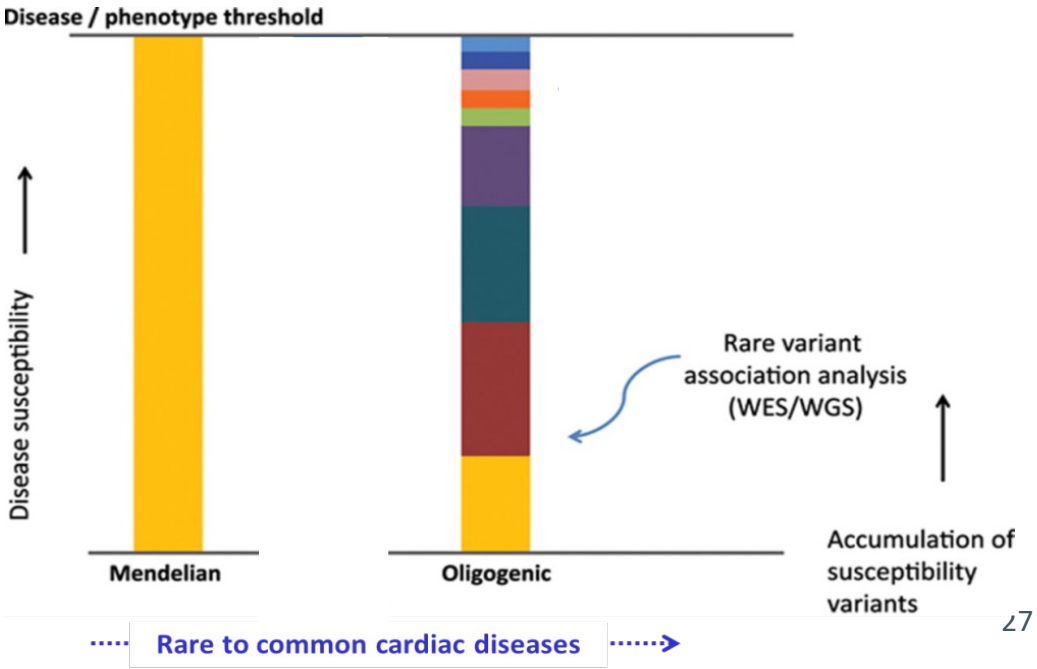


BrS

Conduction disease

Unaffected

Probst *et al.*, 2009



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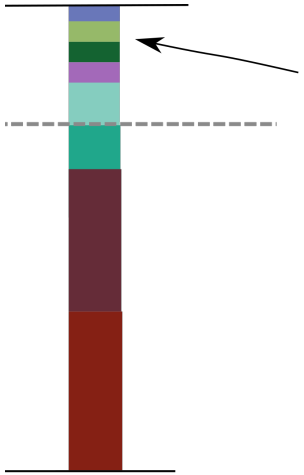
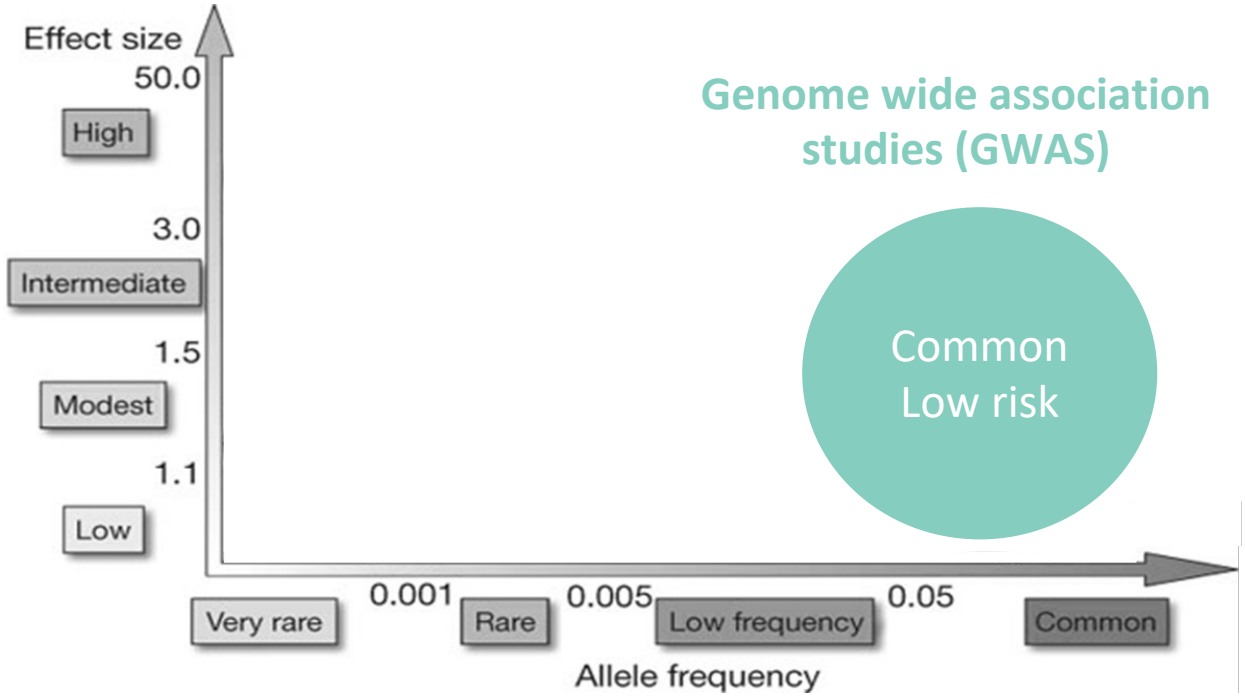
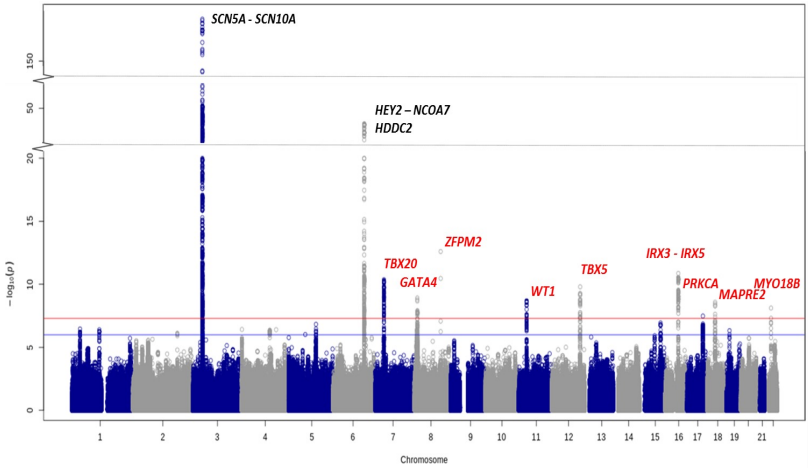
Rare and Common variants associated with cardiac arrhytmia present in coding and non-coding region





Genome wide association studies (GWAS)

Common
Low risk



Genome-wide association analyses identify new Brugada syndrome risk loci and highlight a new mechanism of sodium channel regulation in disease susceptibility

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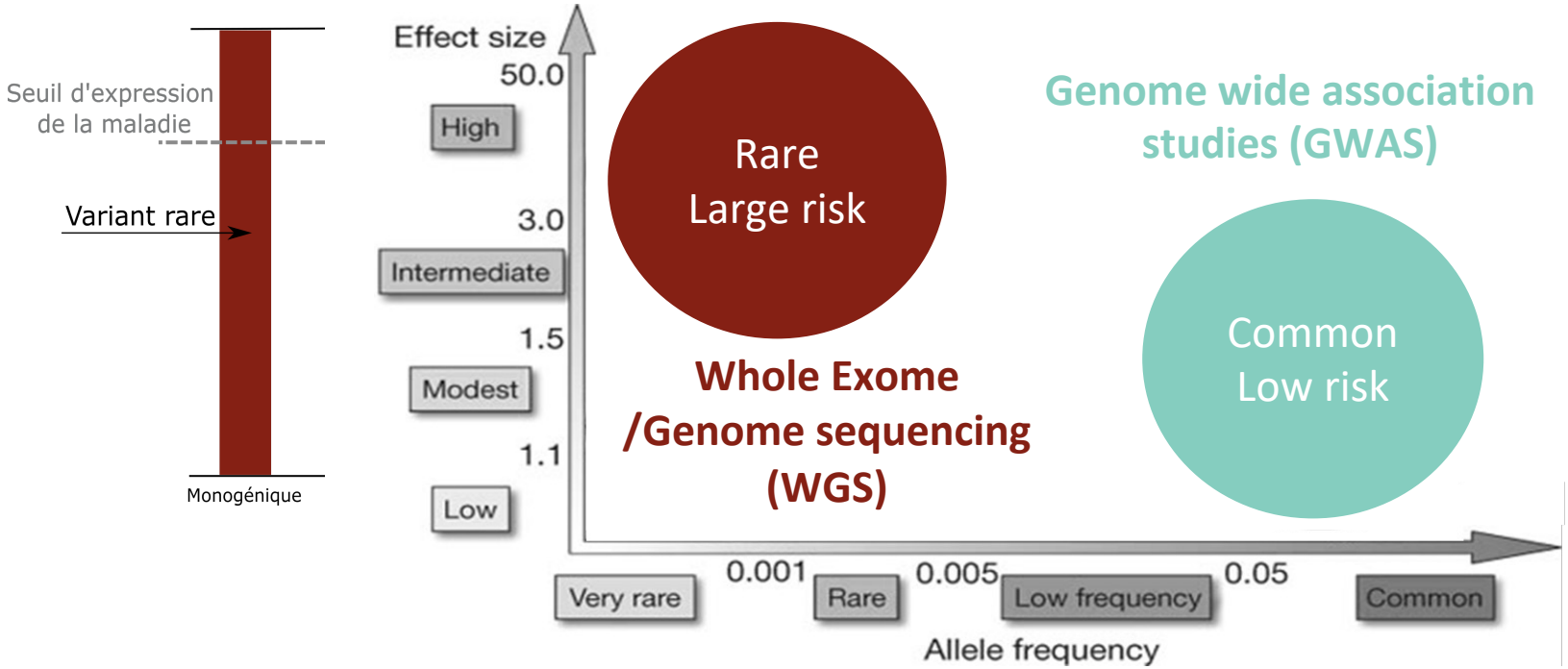
Rare and Common variants associated with cardiac arrhytmia present in coding and non-coding region



Genome-wide association analyses identify new Brugada syndrome risk loci and highlight a new mechanism of sodium channel regulation in disease susceptibility

Common non-coding variant

CTCF-mediated chromatin looping disruption causes PITX2-related novel cardiac syndrome

Rare non-coding variant

Identification of a Deletion Upstream SCN5A associated with Brugada syndrome

De novo mutations in congenital cardiac conduction defects

Rare coding variant

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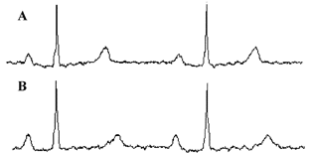
2023 : Cardiac arrhythmias genetics (> 60 genes)

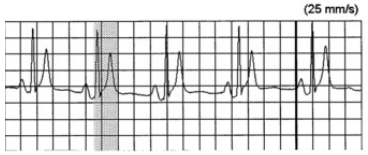
Long QT Syndrome (15)

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



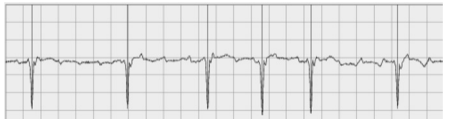
Short QT

KCNQ1
KCNH2 *KCNJ2*
CACNA1C
CACN2B
CACNA2D1



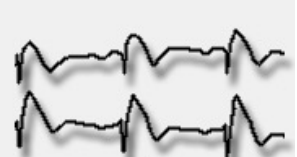
Atrial Fibrillation

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



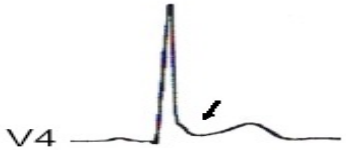
Brugada Syndrome (25)

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



Early repolarization Syndrome

KCNJ8
SCN5A
CACNA1C
CACNB2
CACNA2D1
ABCC9



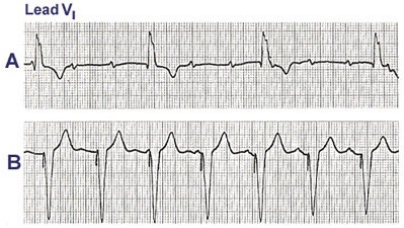
CPVT

RYR2
CASQ2
TRDN
CALM1

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

Progressive conduction (Lenègre)

SCN5A
SCN1B
TRPM4
GJA5 *GJA1*



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

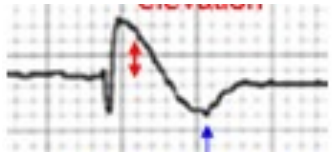
The American College of Medical Genetics and Genomics has published recommendations for reporting incidental findings in the exons of certain genes.

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)

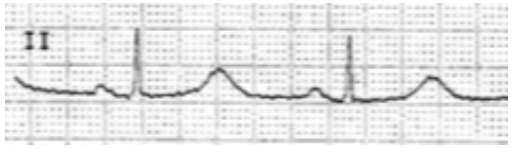
➢ **78 actionable genes**

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

- Brugada Syndrome



- Long QT Syndrome



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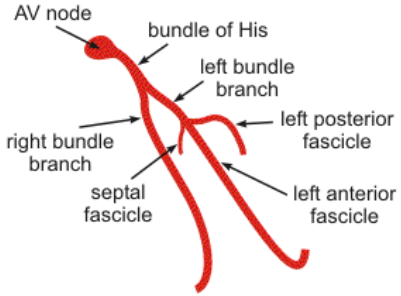
Cardiac Conduction Defects

- **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

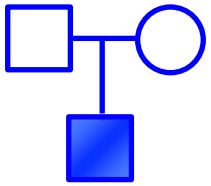
European Heart Journal
doi:10.1093/eurheartj/ehr347

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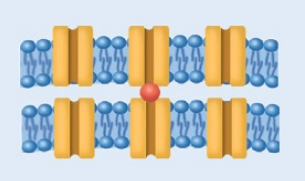
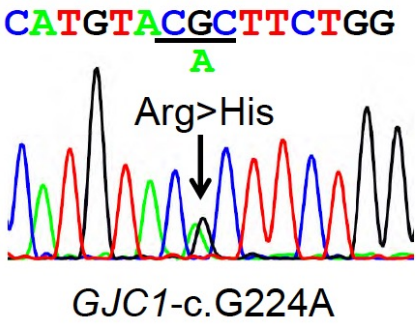
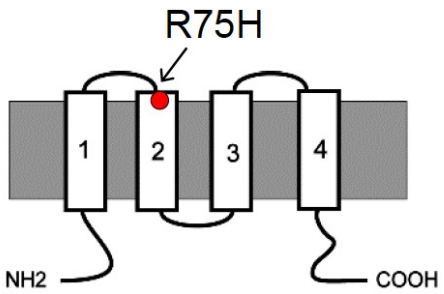
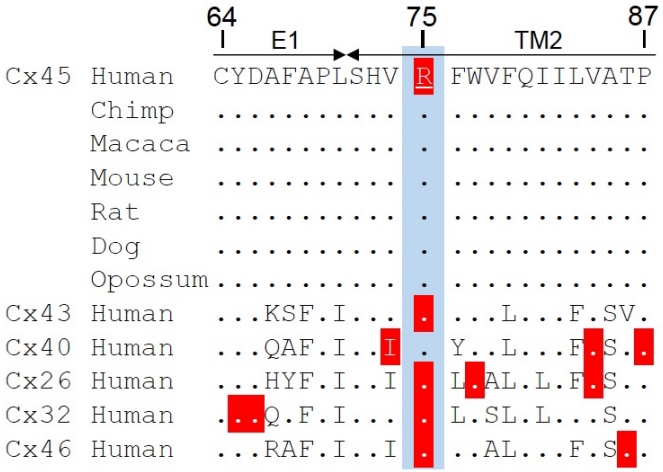
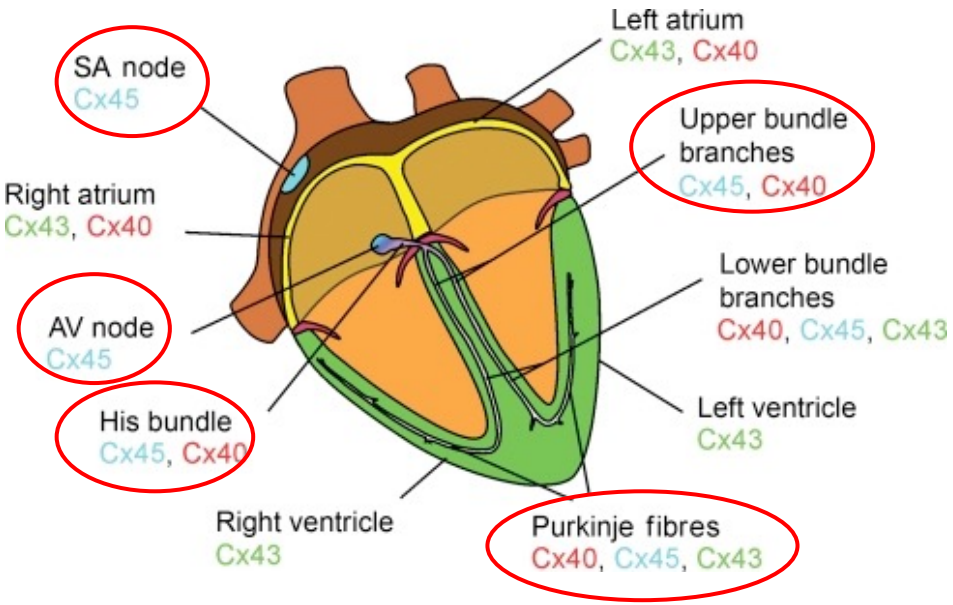
Novel *GJC1* mutation (Cx45-p.R75H)











- 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

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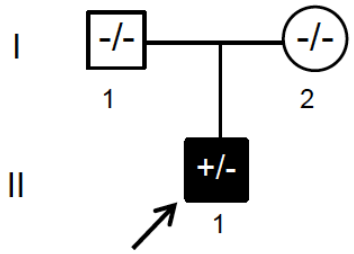
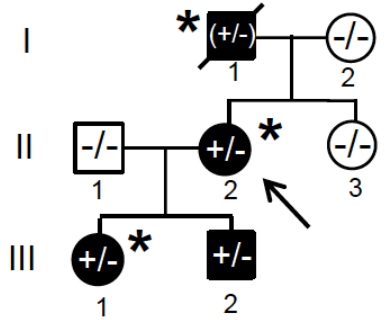
Family A

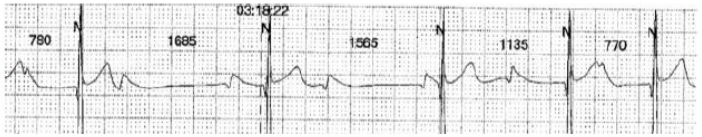


Family B

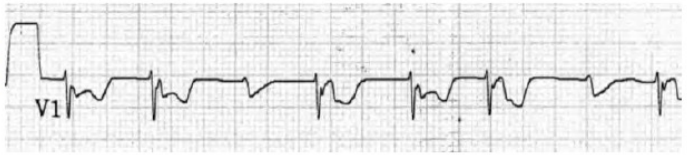


* PM

II:1
(2 yo)



(4 yo)

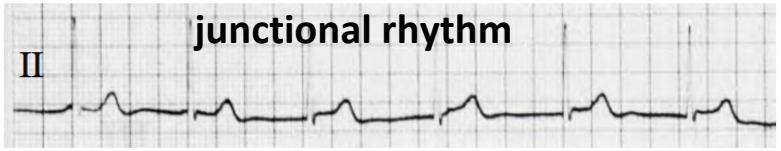


(5 yo)

(9 yo)

3rd degree AV block & progressive loss of P wave

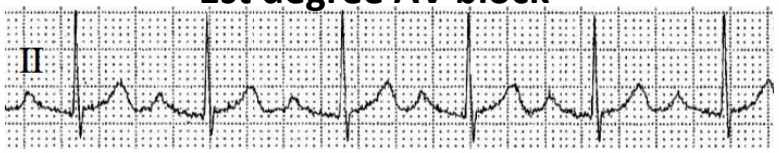
II:2
(9 yo)



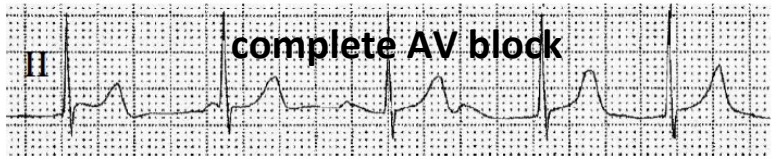
junctional rhythm

1st degree AV block

III:1
(8 yo)

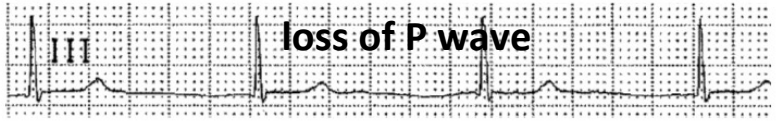


(12 yo)



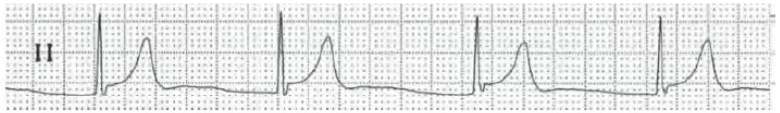
complete AV block

(14 yo)



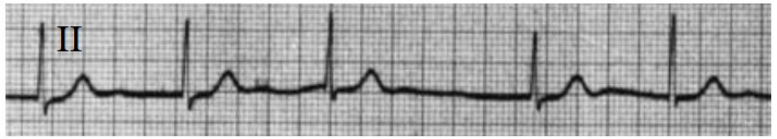
loss of P wave

III:2
(14 yo)



junctional rhythm

I:1
(37 yo)



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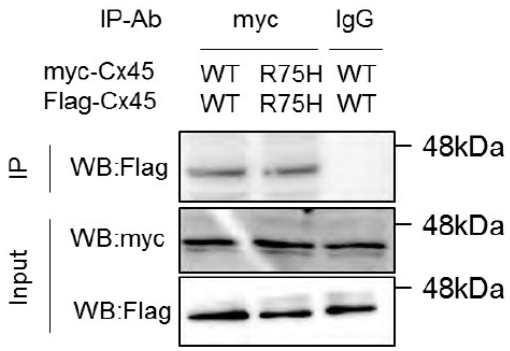
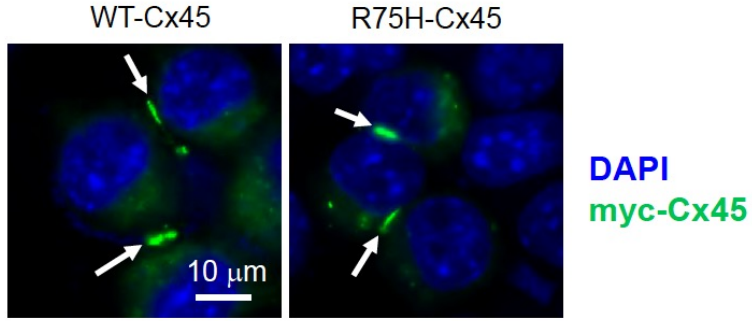
In vitro studies

1- Normal hemichannel assembly and plaque formation

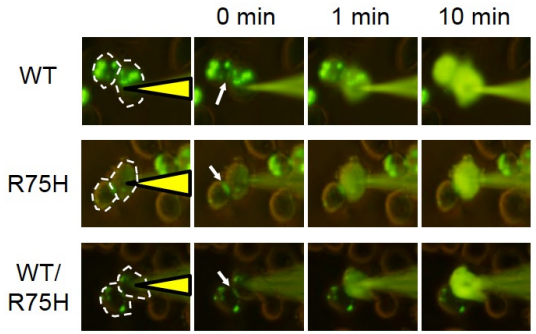
(Co-IP /Immunofluorescent)

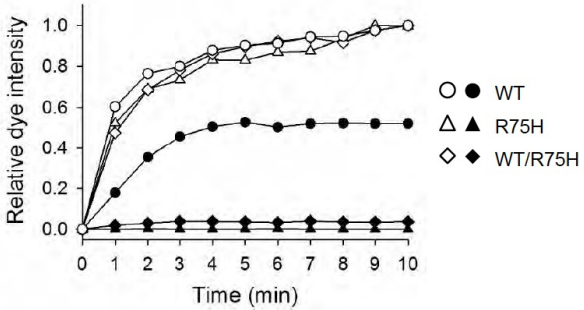


(Immunofluorescent)

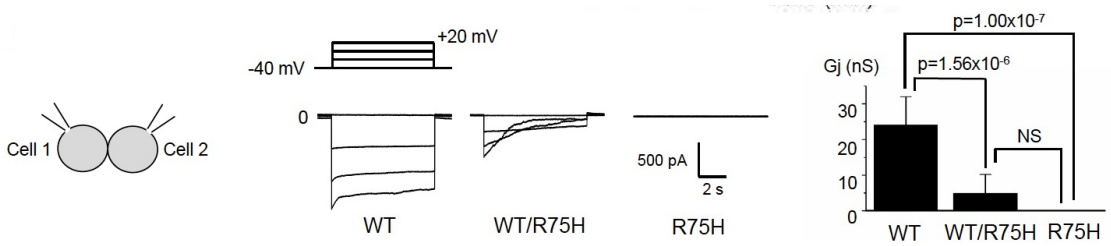


2- Severe reduction of permeation properties (Luciferase dye transfer)





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



➢ Dominant-negative

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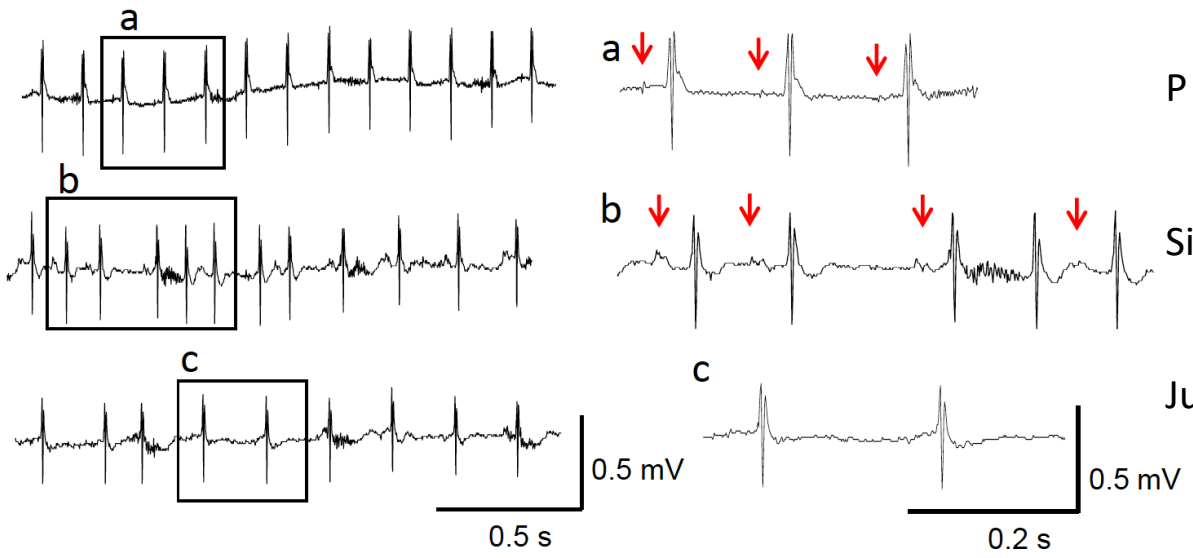
In vivo studies

Electrophysiological properties of conditional *Gjc1* knockout mice

1- Various atrial arrhythmias
(after tamoxifen administration)



P wave wondering

Sinus arrhythmia

Junctional rhythm

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

Seki et al. J Am Coll Cardiol. 2017

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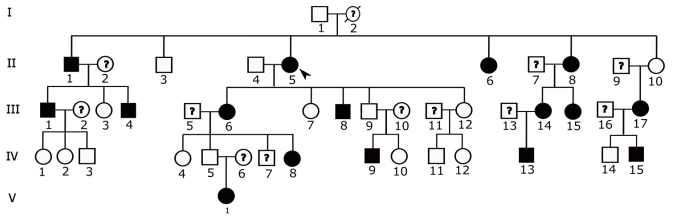
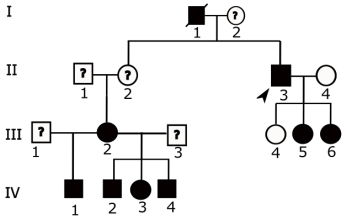
JACC
JOURNAL of the AMERICAN COLLEGE of CARDIOLOGY

Whole Genome Sequencing in unresolved mendelian arrythmias



?



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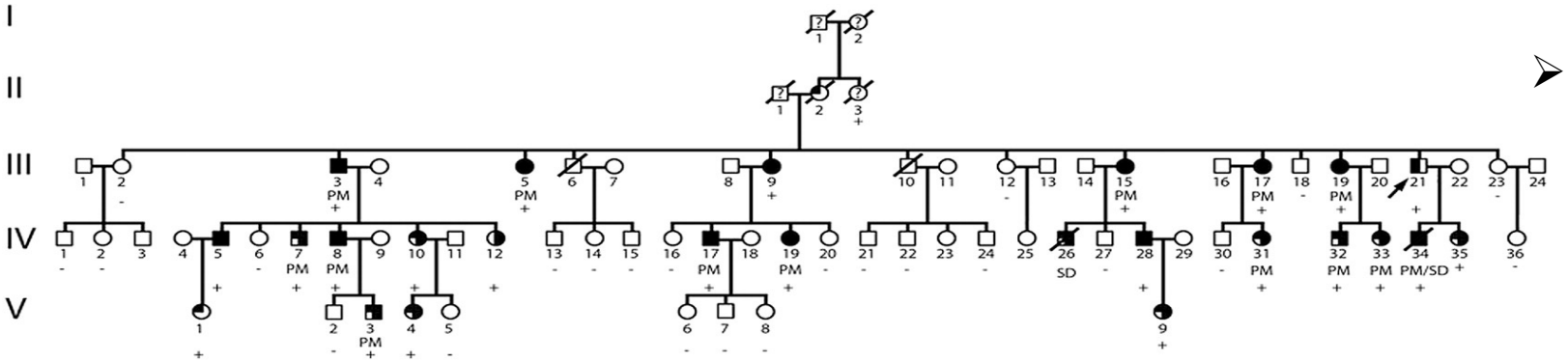
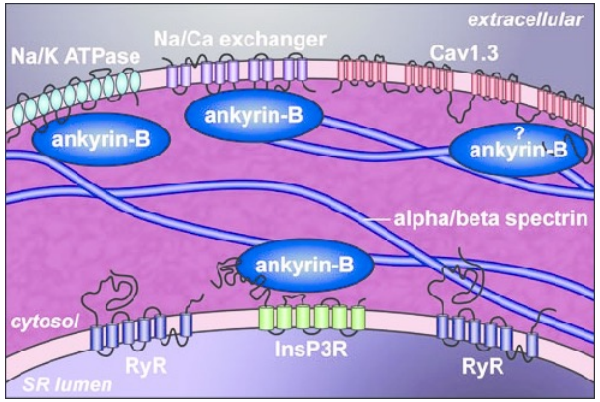
A large family associating long QT syndrome and sinus dysfunction linked to the ANK2 gene (LQT4)

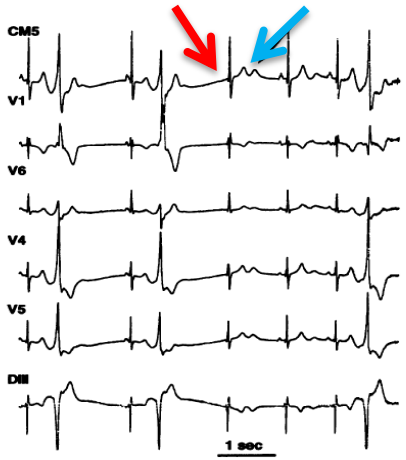


- 24 affected cases
- 2 sudden death (ventricular arrhythmias)

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

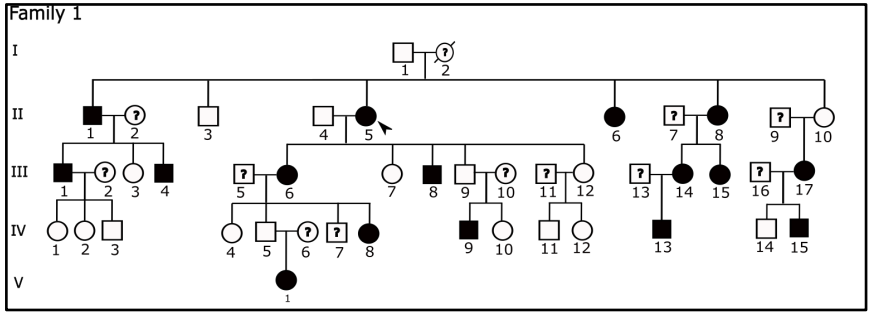


Mohler P; Schott JJ at al. Nature. 2003



- Long QT syndrome (LQT4) T/U waves
- Sinus node dysfunction (SND)
- Atrial fibrillation (AF)

➢ **Additional long QT / SND *ANK2*$^{-/-}$ family but with genetic linkage at 4q25 locus**



➢ **4q25 linkage**

Schott et al. Am J Hum Genet. 1995

Le Scouarnec et al. Proc Natl Acad Sci. 2008

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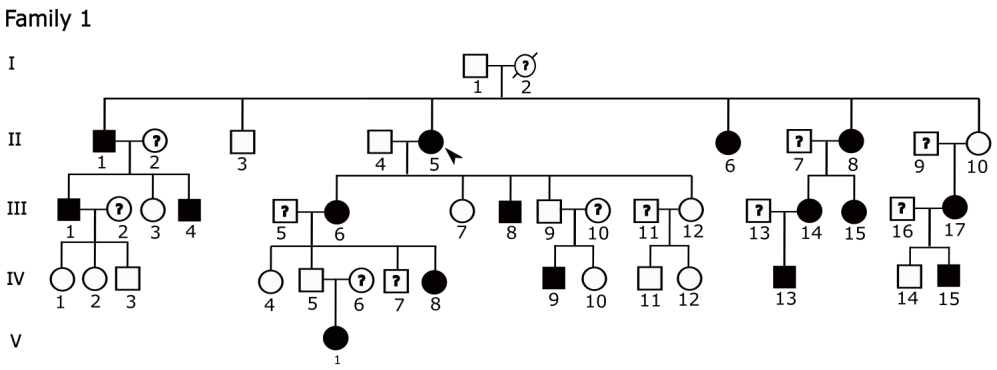
Identification of a large family presenting with a complex cardiac syndrome

17 members present with a complex cardiac syndrome following an autosomal dominant model



Electrical cardiac disorders:

Sinus node dysfunction
Long QT
Atrial fibrillation

Cardiac developmental defect:

Atrial septal defect
LV non-compaction

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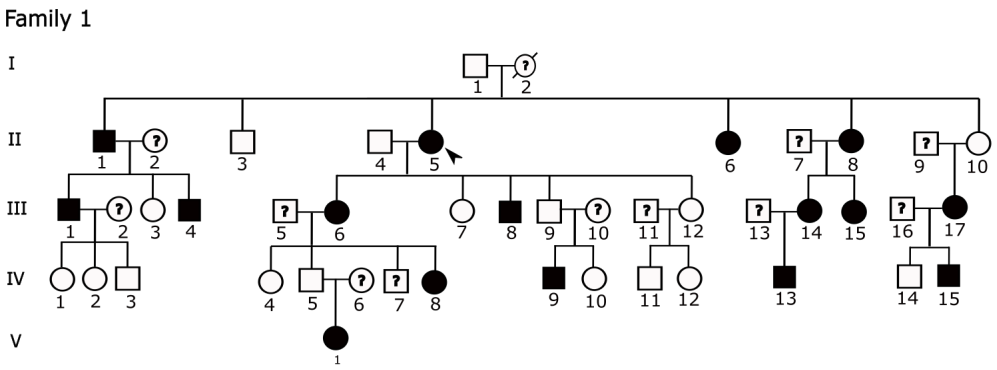
Identification of a large family presenting with a complex cardiac syndrome associated with a 15 kb deletion in a gene desert area



Electrical cardiac disorders:
Sinus node dysfunction
Long QT
Atrial fibrillation

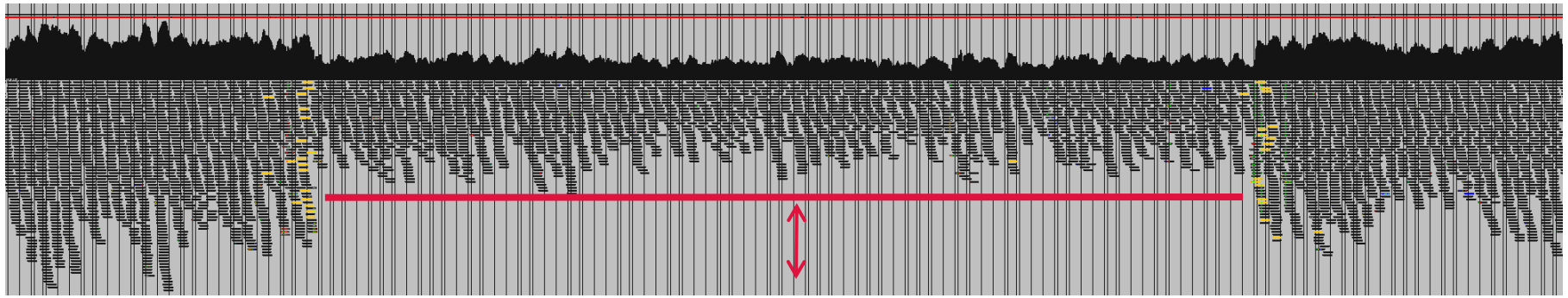
Cardiac developmental defect:
Atrial septal defect
LV non-compaction

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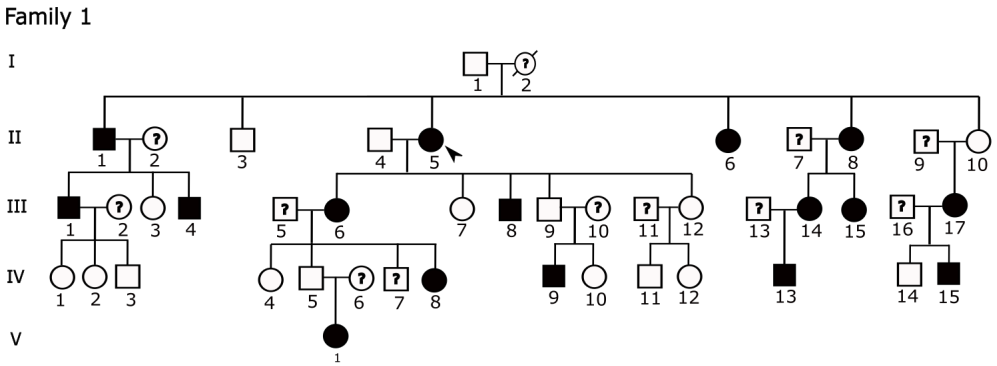
Identification of a large family presenting with a complex cardiac syndrome associated with a 15 kb deletion in a gene desert area



Electrical cardiac disorders:

Sinus node dysfunction
Long QT
Atrial fibrillation

Cardiac developmental defect:

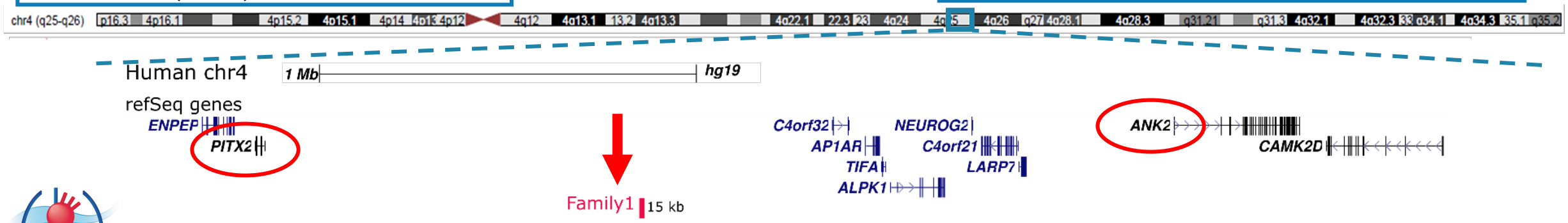
Atrial septal defect
LV non-compaction

PITX2

- Cardiac transcription factor essential for the left-right asymmetry of the heart
- SA development
- Risk variants in *PITX2* associated with atrial fibrillation (GWAS)

Ank2

- Localization and membrane stabilization of ion channels
- *ANK2* loss-of-function variants -> LQT4 syndrome (LQT-SND)



Relevance of this non-coding region in the molecular mechanisms leading to this new syndrome ?

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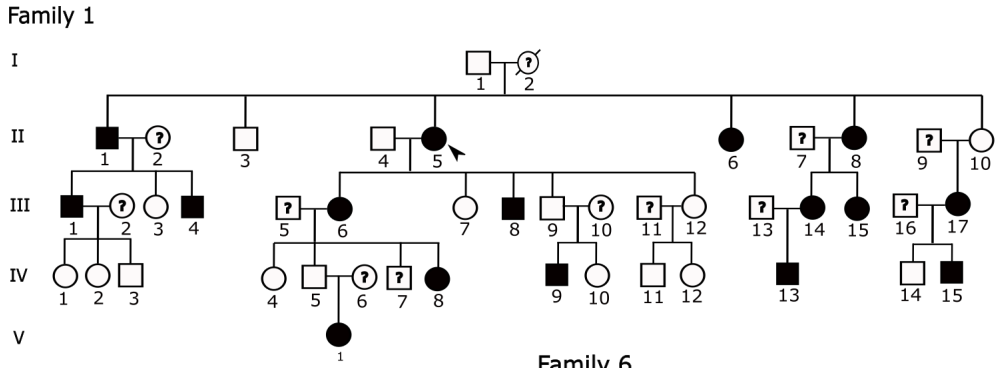
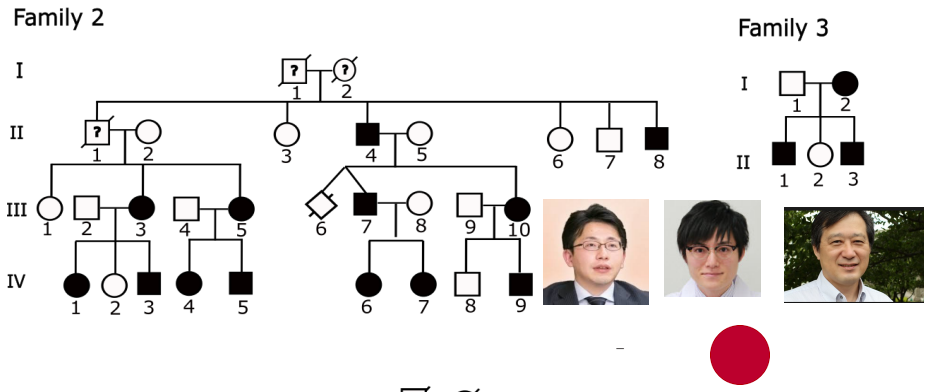
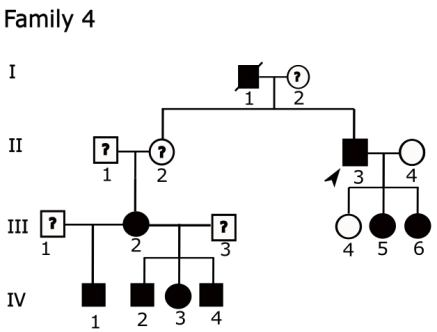
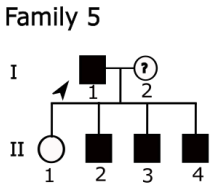
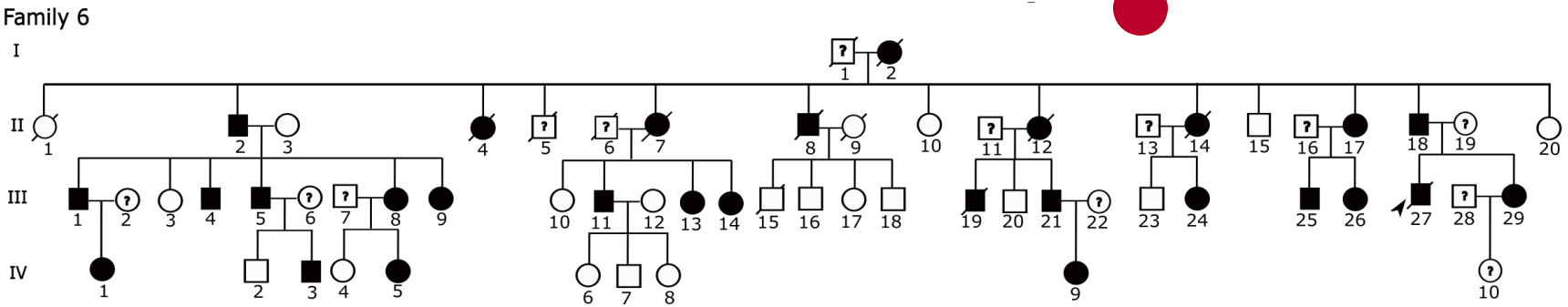
Six additional families (4 French and 2 Japanese) presenting with a similar phenotype showing overlapping deletions

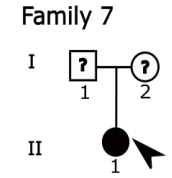


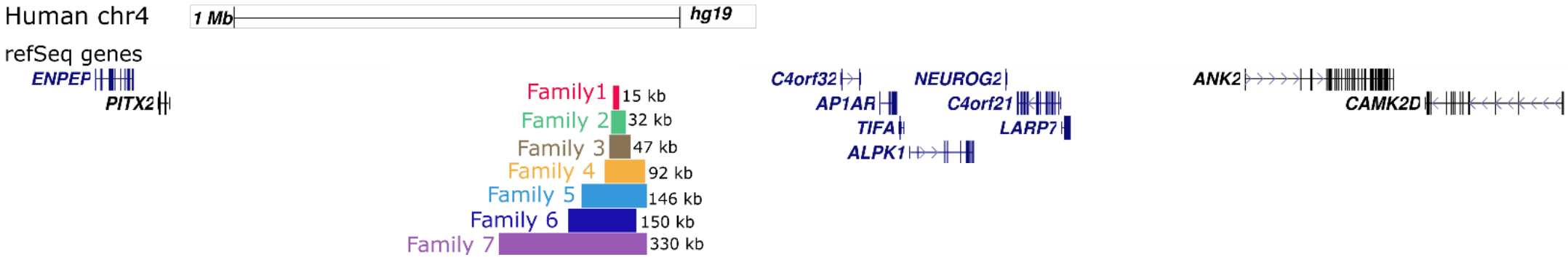












Crucial role of this non coding deleted region

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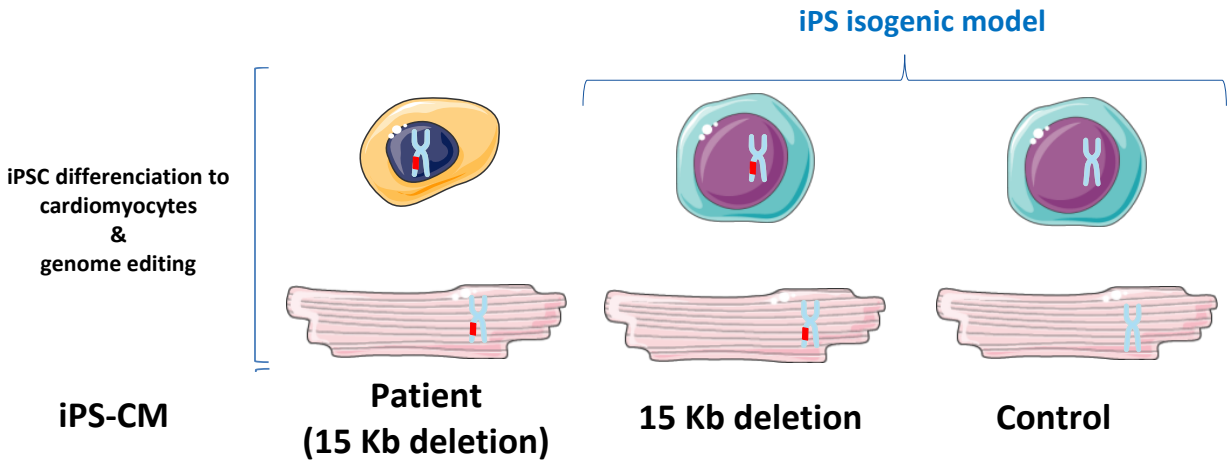
Molecular mechanism

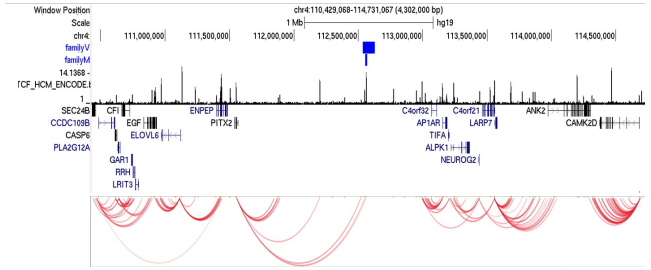
In vitro



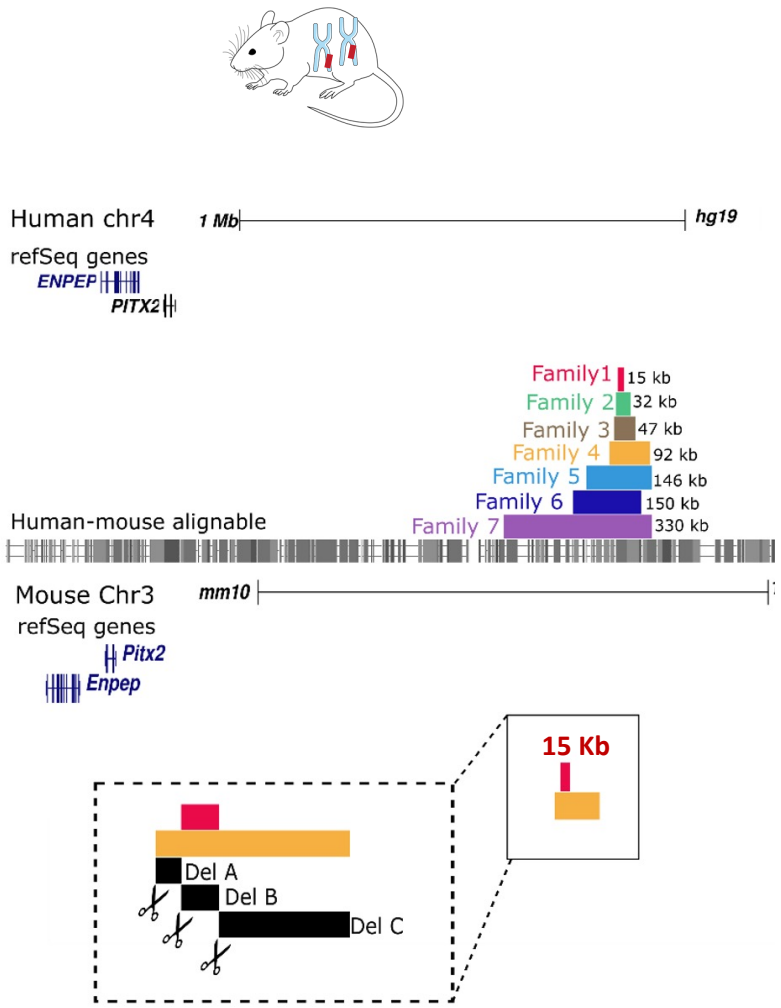
Functional studies

- → Cell morphology
- → Cell beat recording
- → Cardiomyocytes contractility
- → Action potential recording

Chromatin conformation

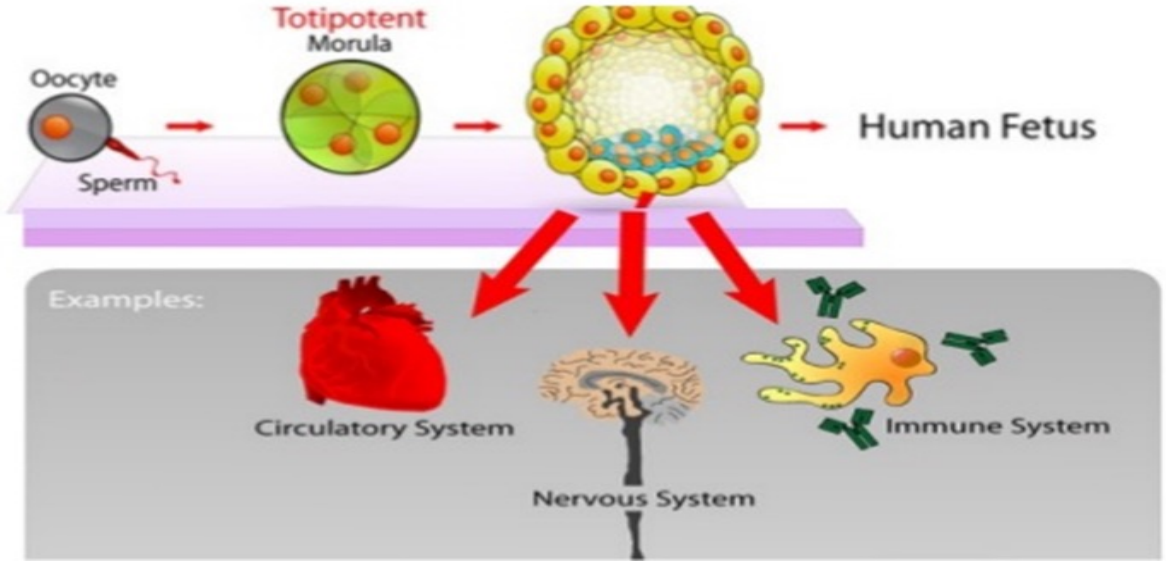


In vivo



Epigenetics: Understanding the non-coding regions of the human genome

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

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Definition of Epigenetics

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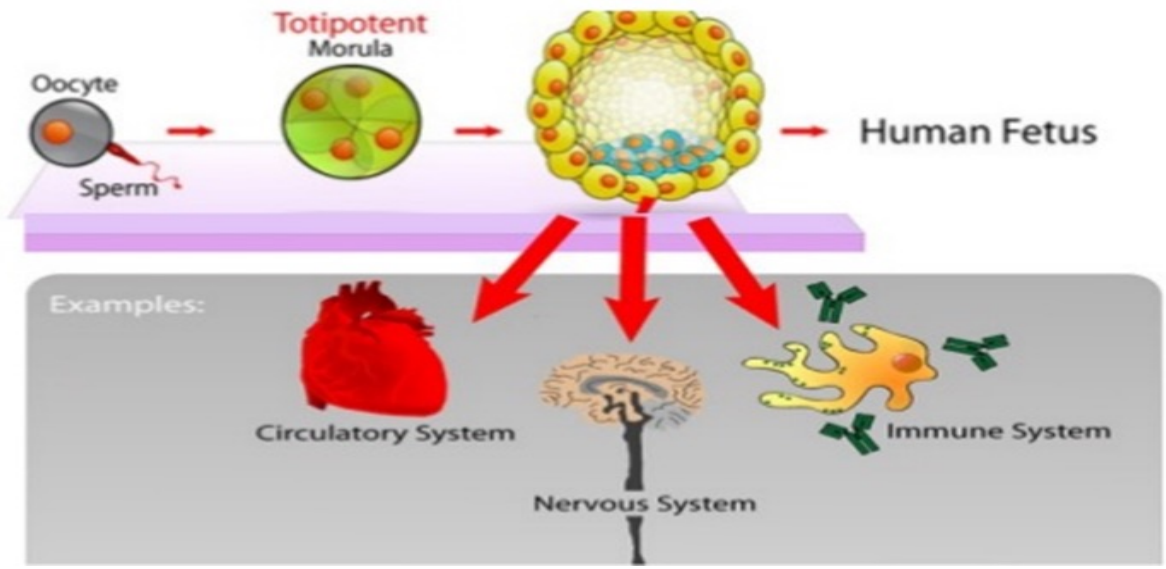

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How cells read the genes

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

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Definition of Epigenetics

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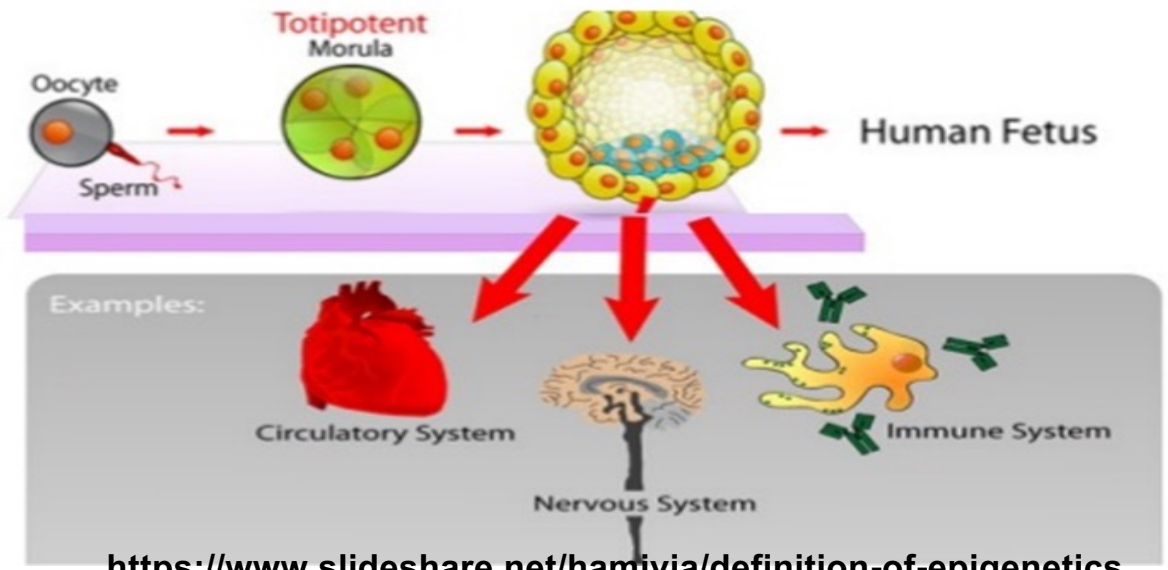

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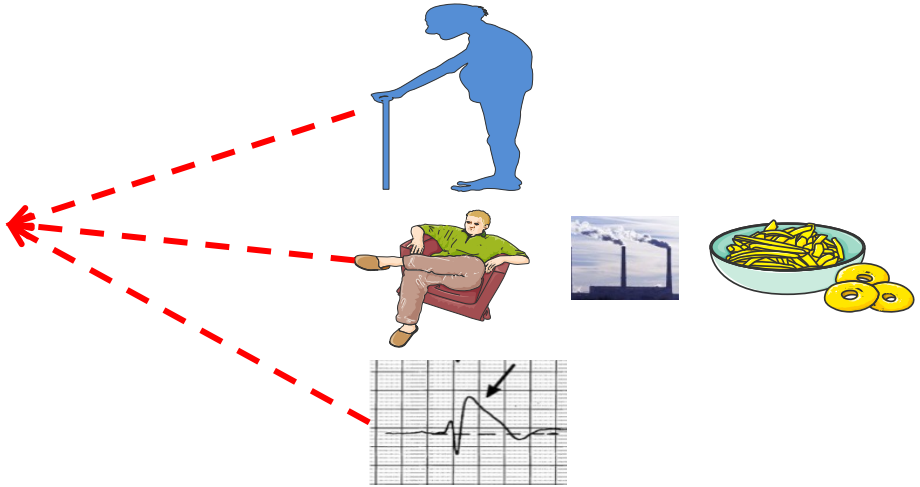
How cells read the genes

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



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Definition of Epigenetics

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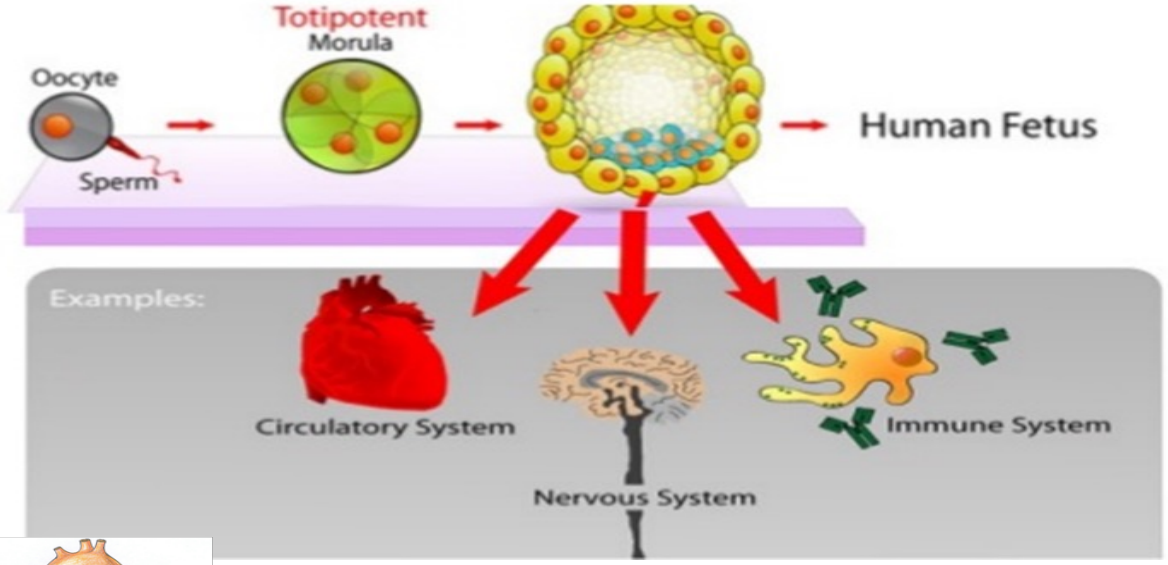
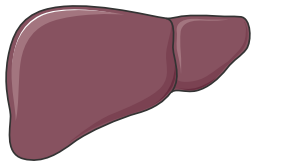
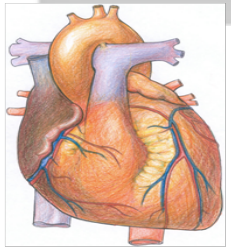
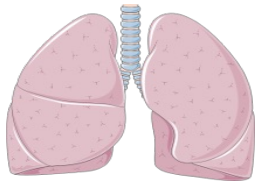
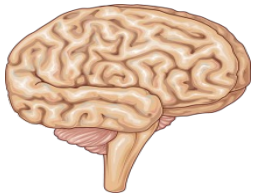


How cells read the genes



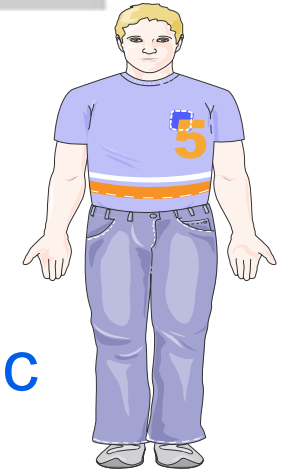






Tissue-specific

Species-specific



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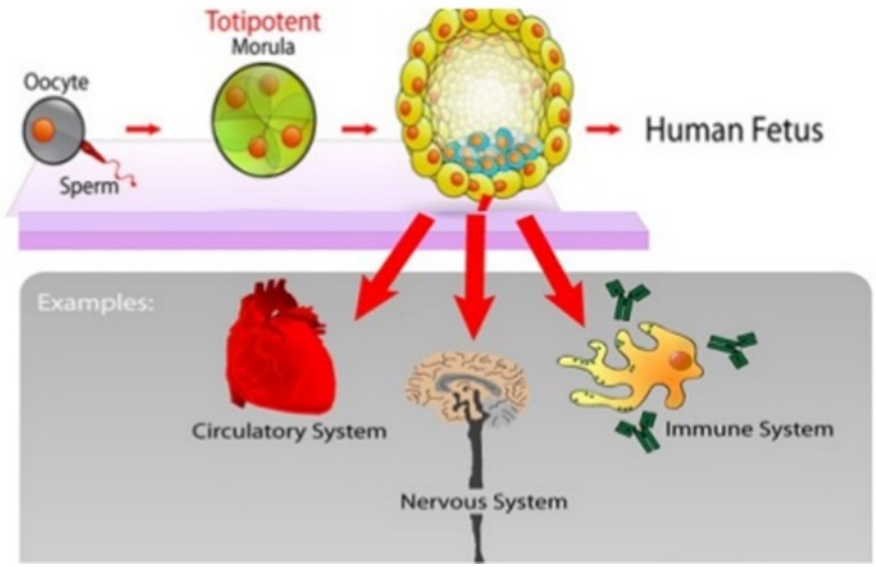
Non-coding region characterization and annotation

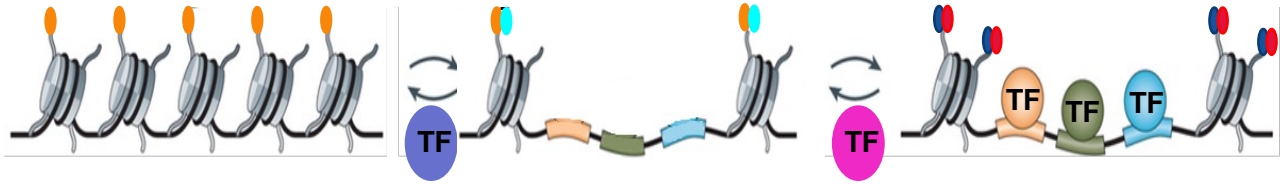


https://www.slideshare.net/hamivia/definition-of-epigenetics

How cells read the genes

ATAC-seq approach: assay for Transposase-Accessible Chromatin

ChIP-seq: chromatin immunoprecipitation –



Non-active region | Latent region | Open region

ChIP-Seq mark	Functional association
H3K4me3	promoters
H3K4me1	enhancers
H3K27ac	Active promoters and enhancers
H3K27me3	Inactive chromatin

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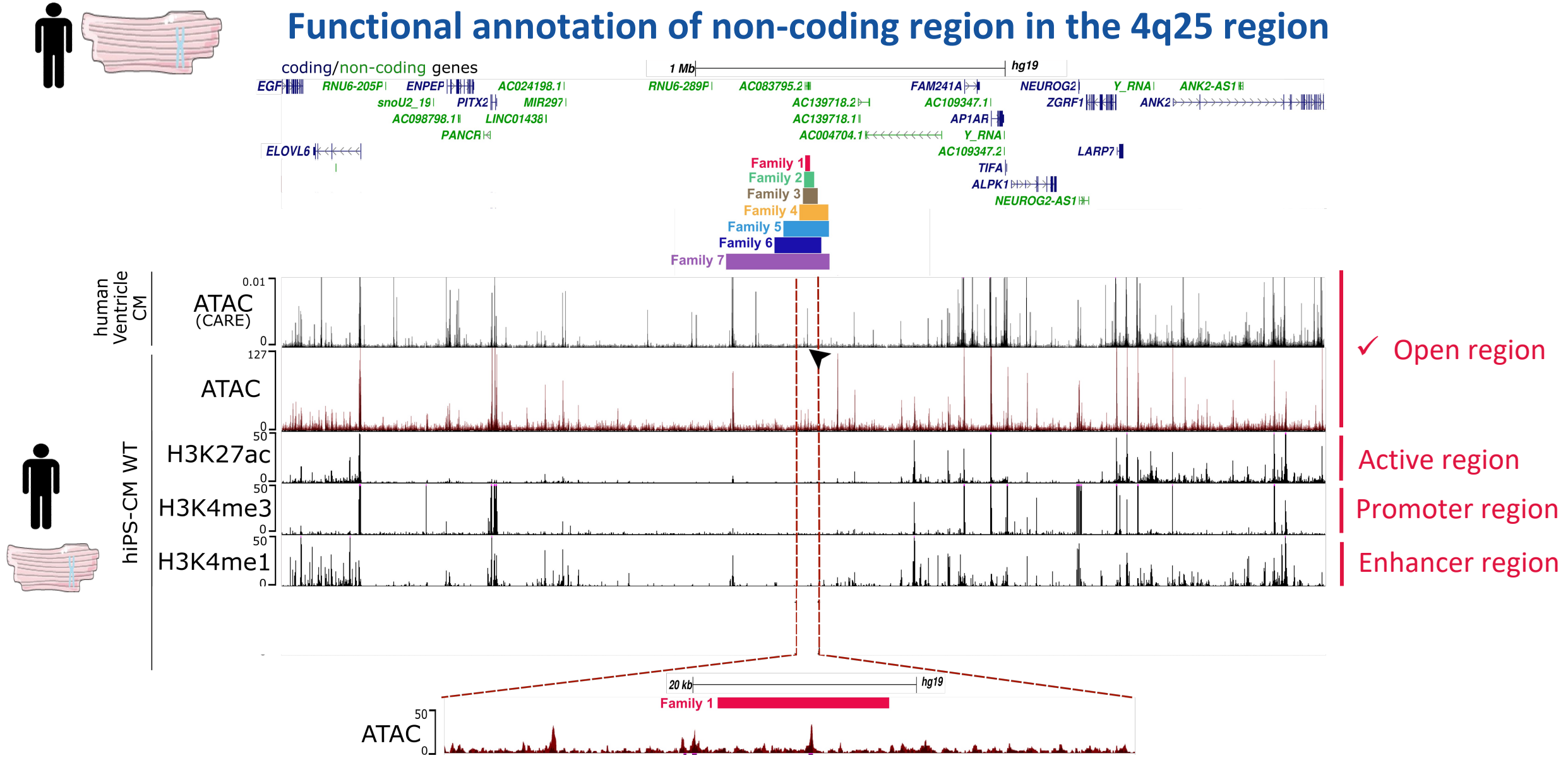
Functional annotation of non-coding region in the 4q25 region



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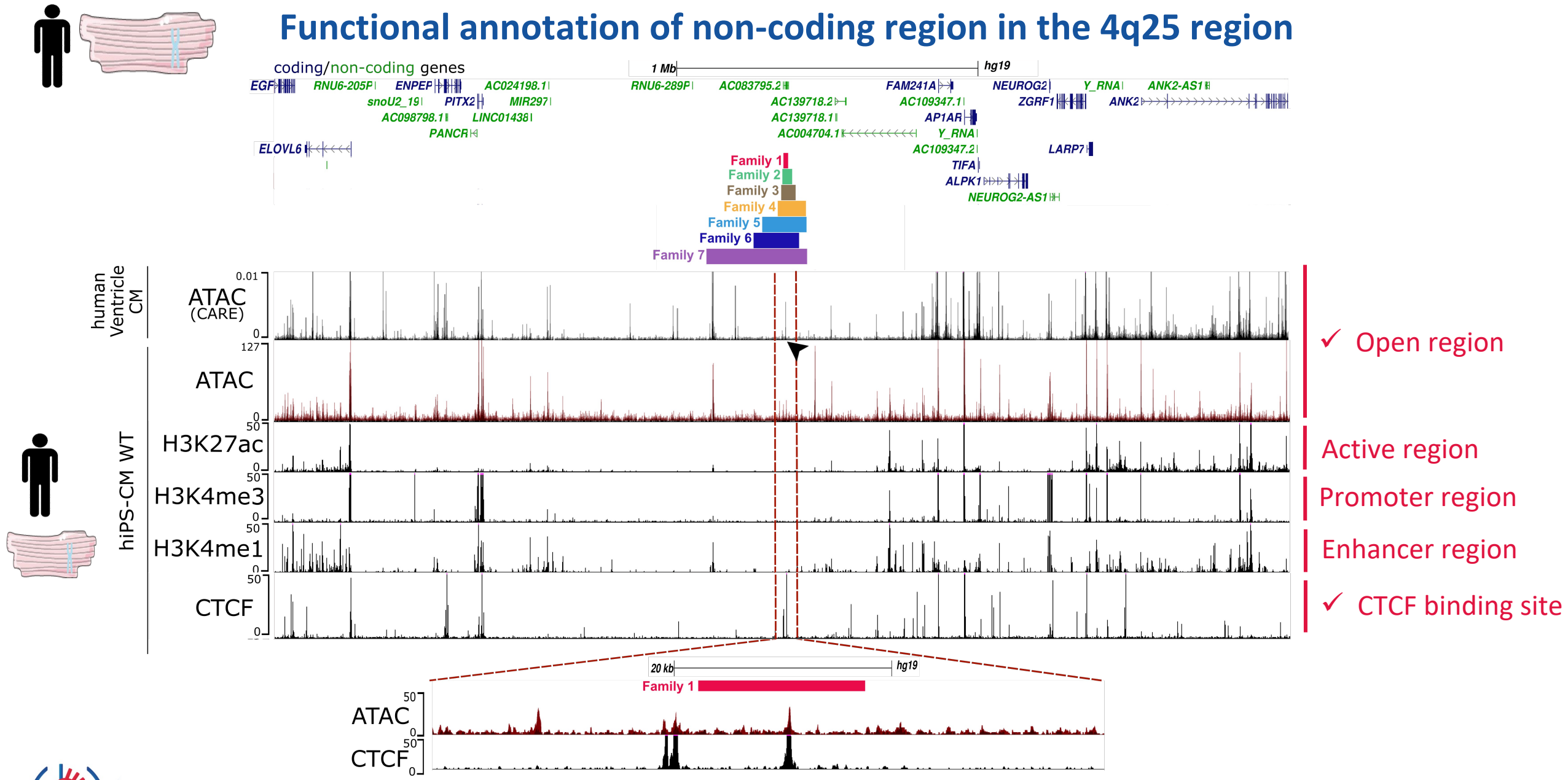
Functional annotation of non-coding region in the 4q25 region



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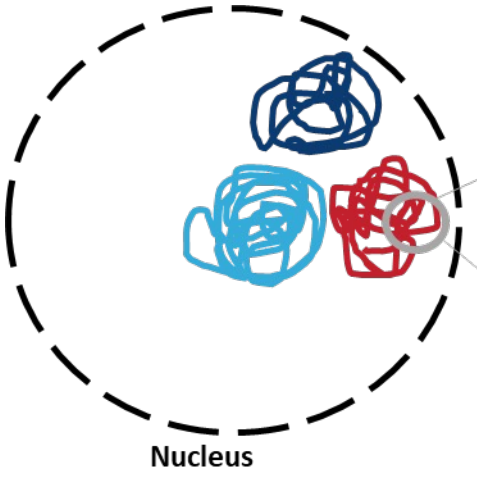
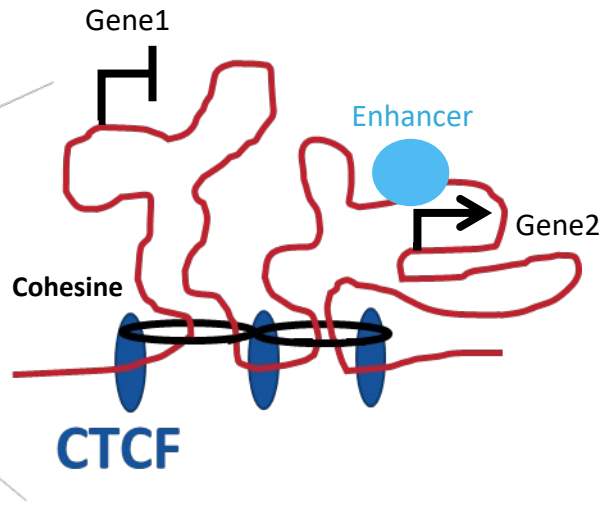
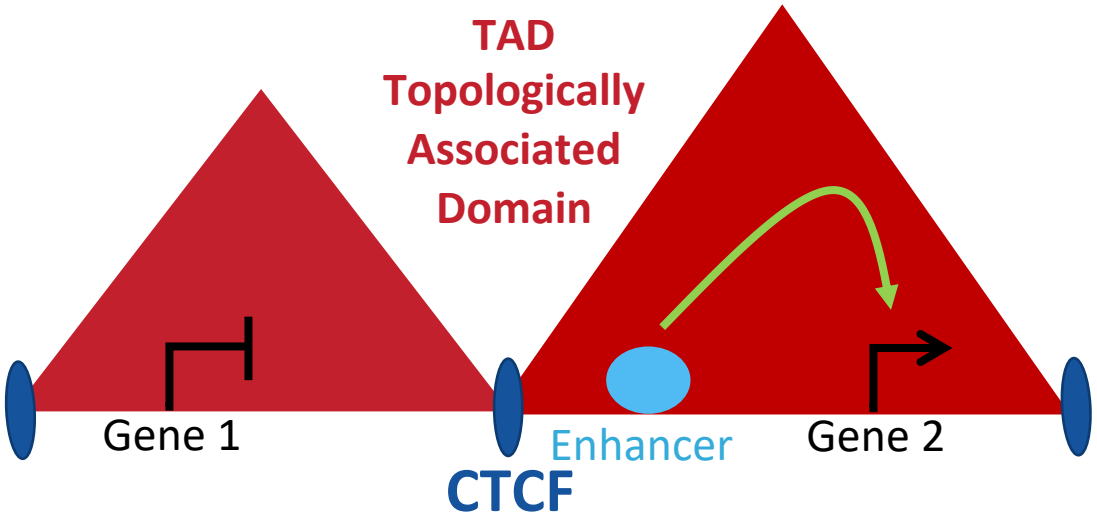
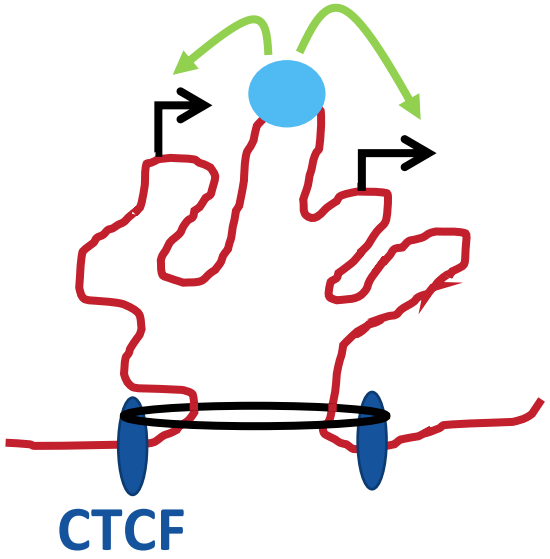
Hypothesis: Deletion of the CTCF binding site modifies the TAD boundary

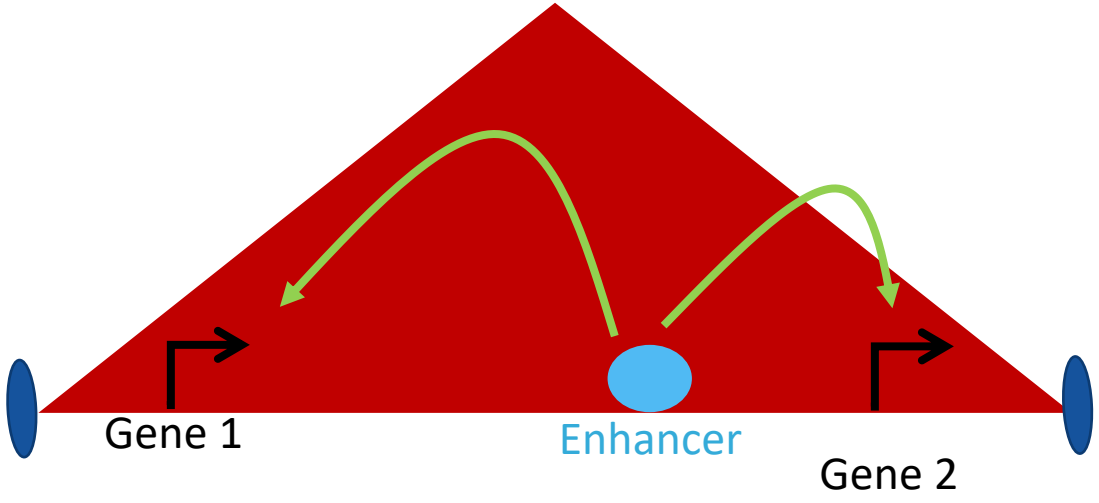






Deletion of CTCF binding site





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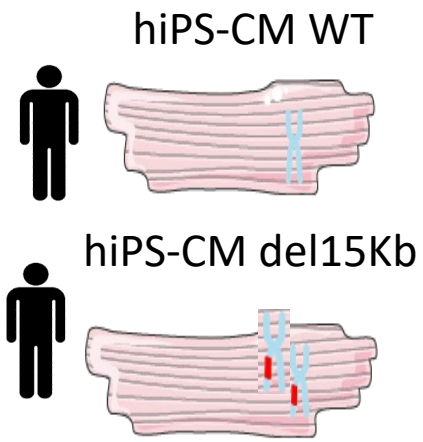
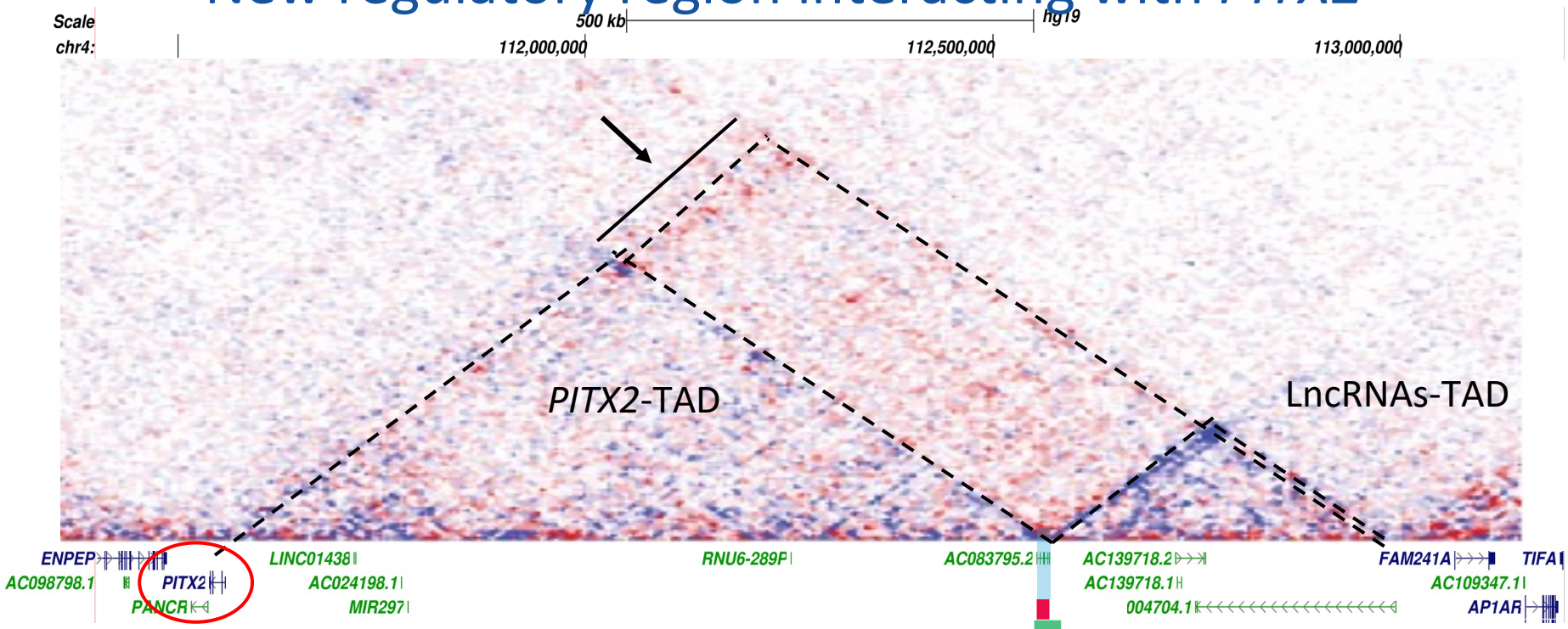
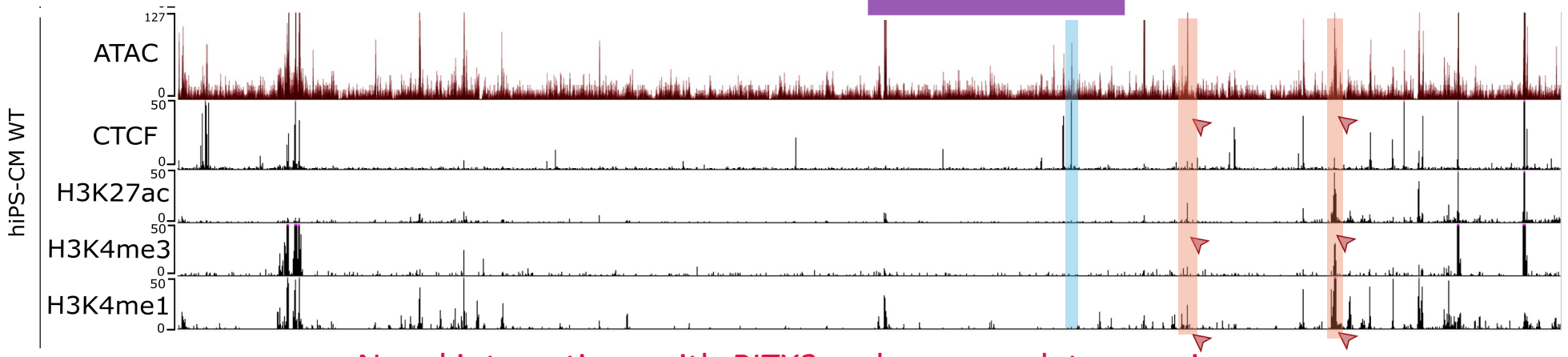
Lupiañez et al, Cell.2015

New regulatory region interacting with *PITX2*







Open region

Active region

Enhancer region

Novel interactions with *PITX2* and new regulatory regions

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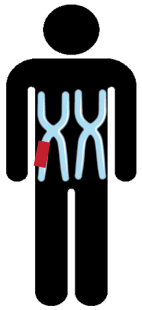
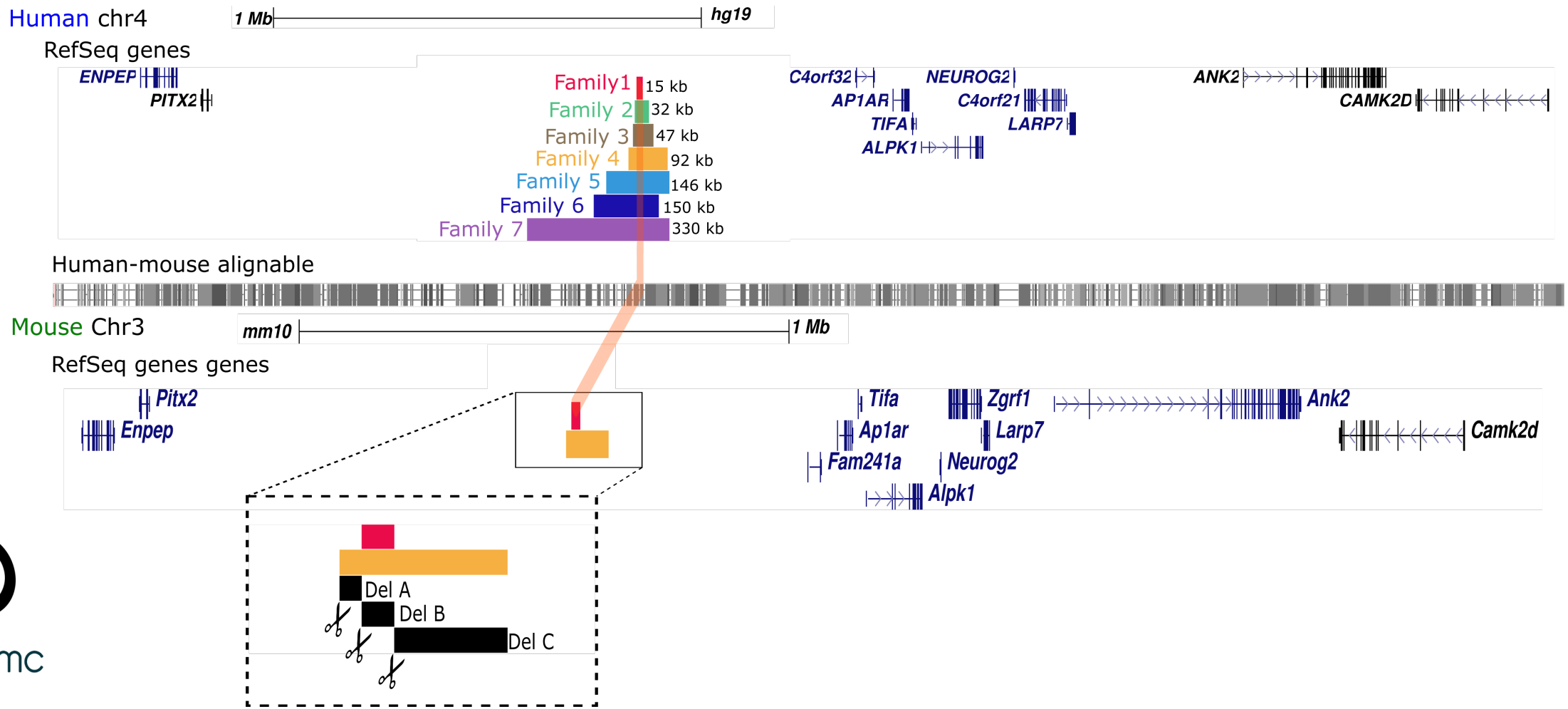
Mouse model to characterize the impact of the deletion on the phenotype





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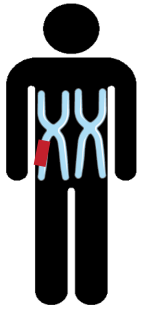
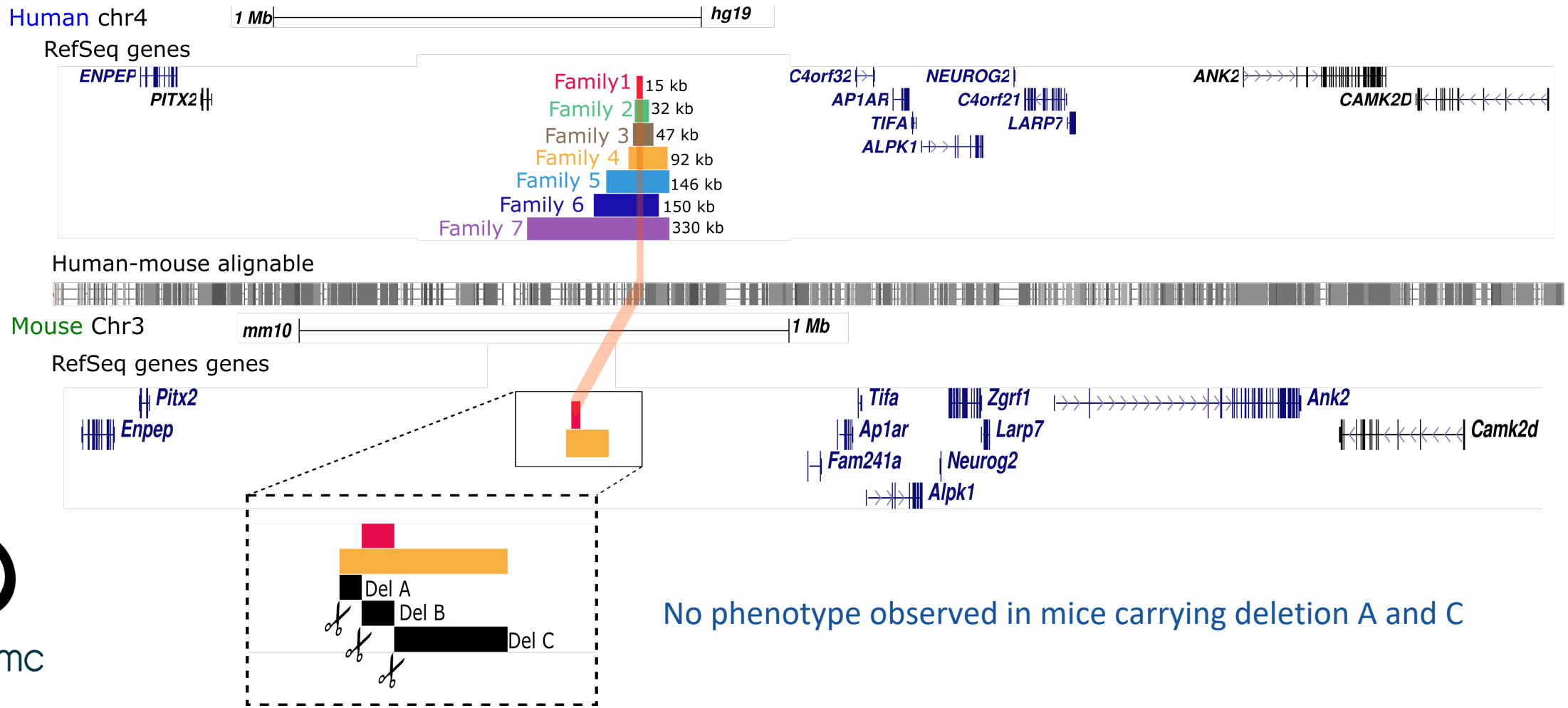
Mouse model to characterize the impact of the deletion on the phenotype





No phenotype observed in mice carrying deletion A and C

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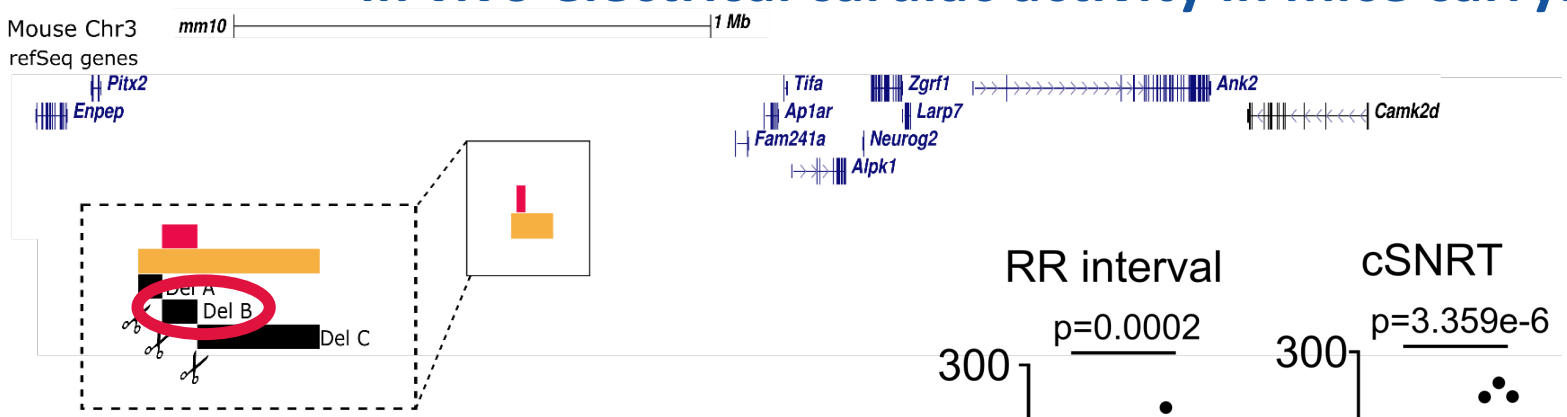
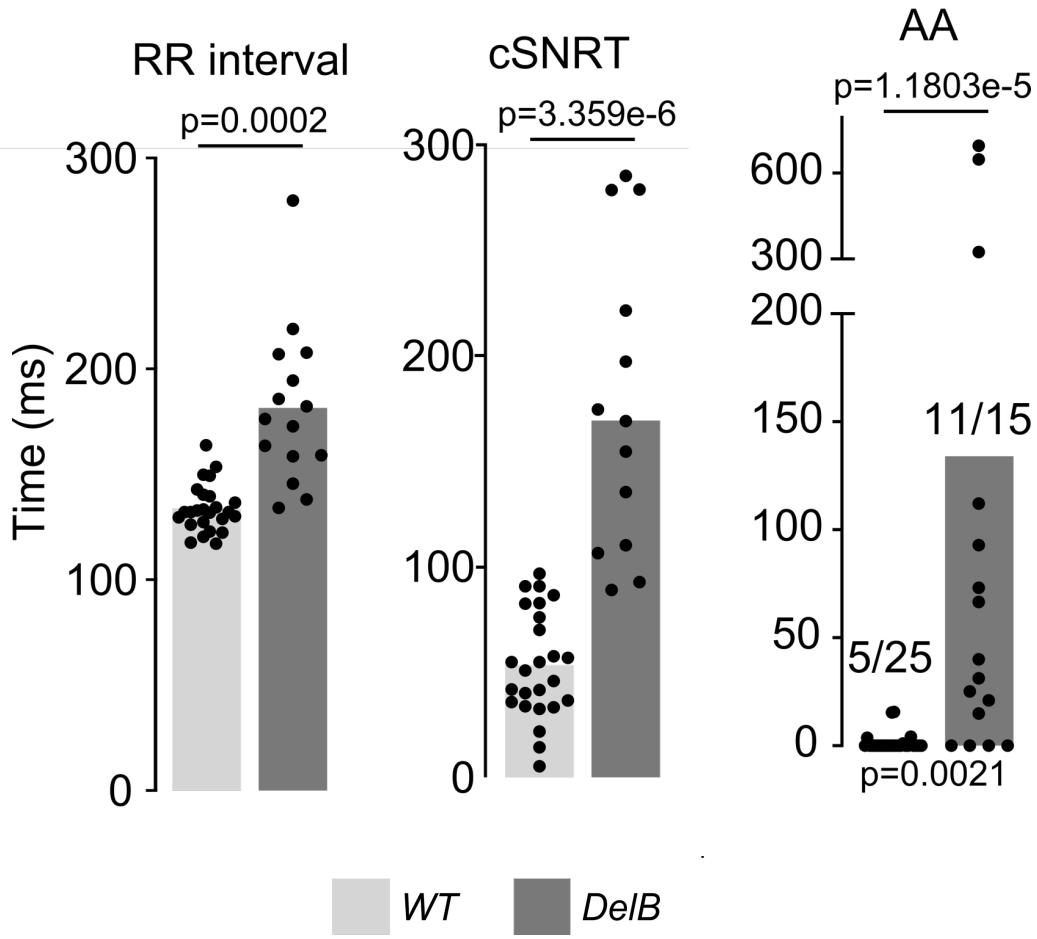
In vivo electrical cardiac activity in mice carrying the 15kb deletion





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

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

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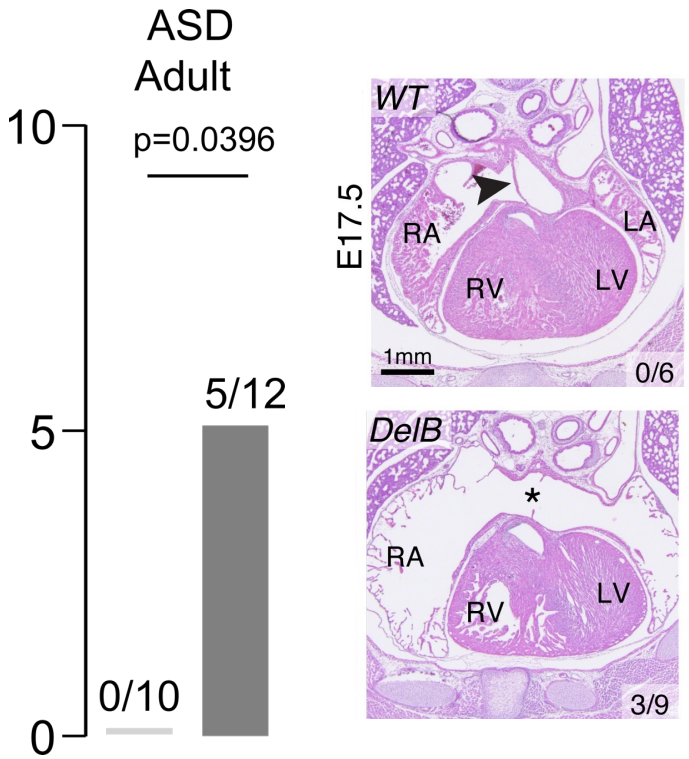
Cardiac morphological characterization in mice carrying the 15kb deletion



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CRISPR mice present ASD

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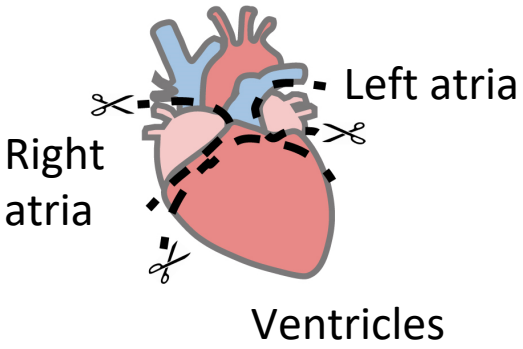
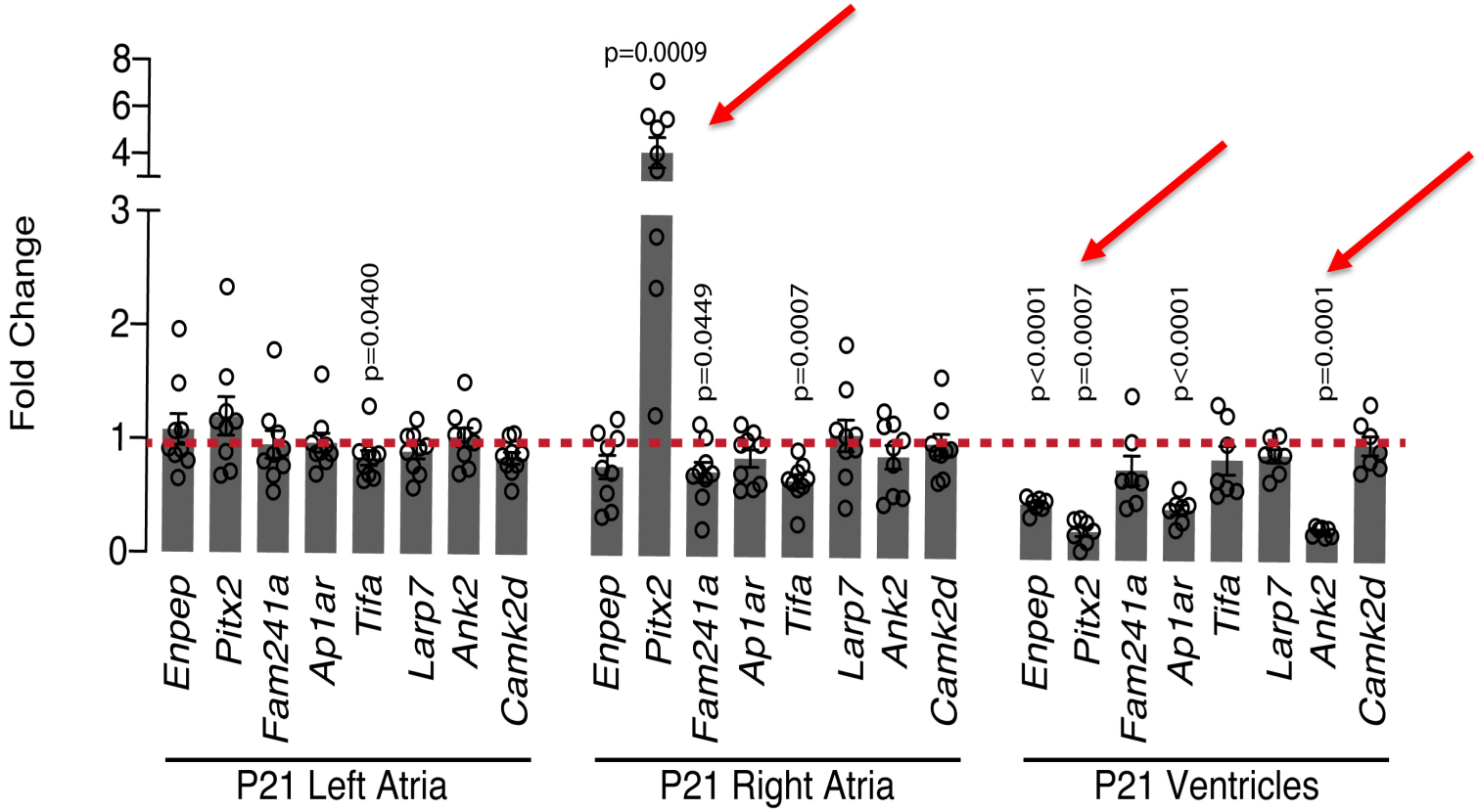
Mouse model recapitulates electrical and morphological cardiac phenotype identified in patients

Transcriptomic analysis





Upregulation of *Pitx2* in the right atria

Downregulation of *Pitx2* & *Ank2* in the ventricles

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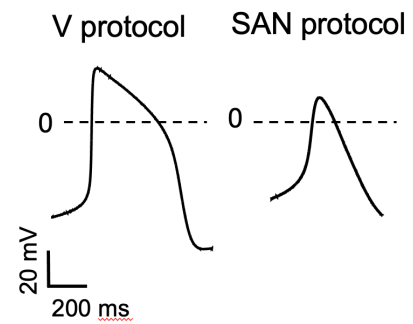
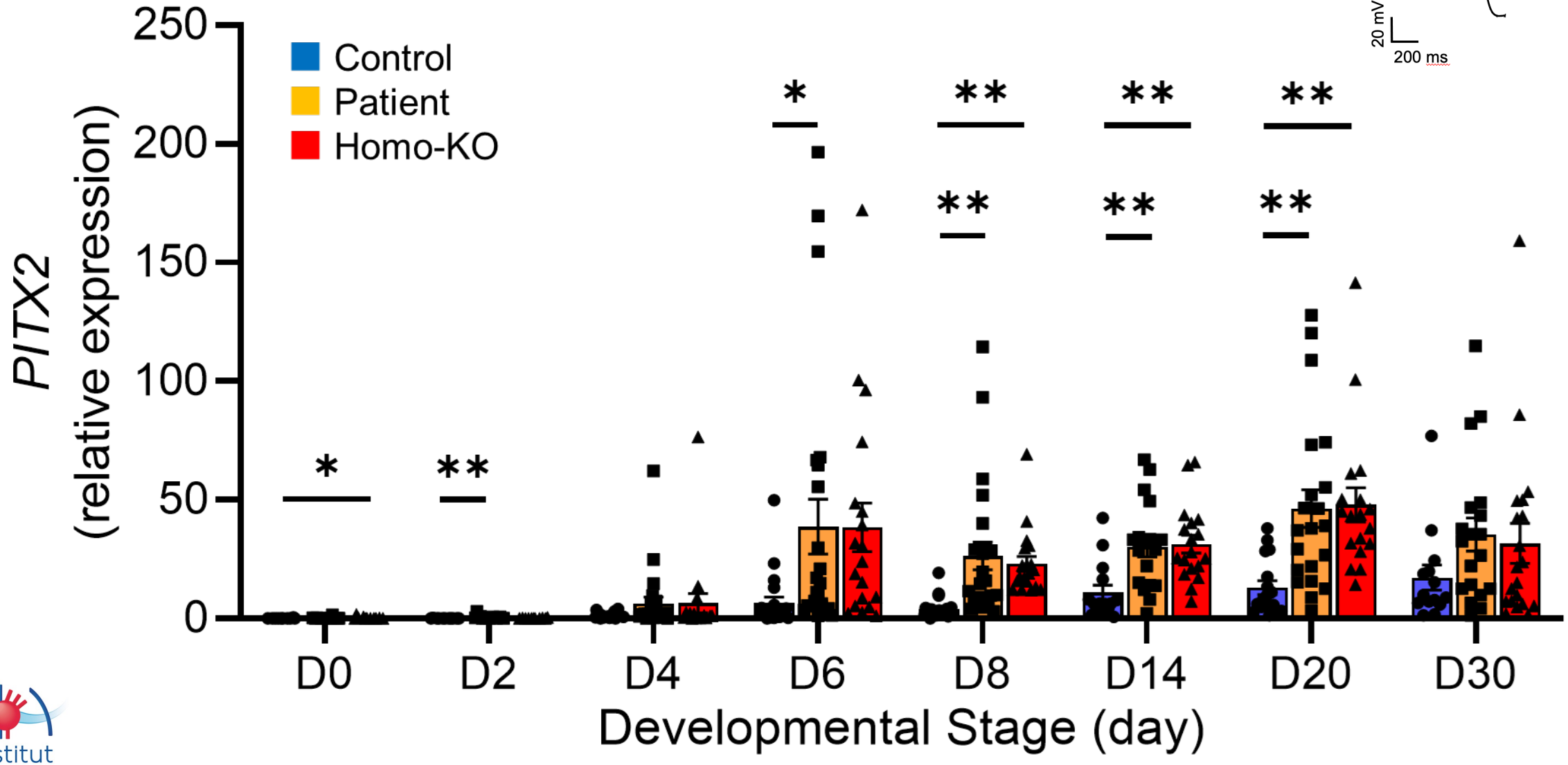
Manuscript in preparation

Overexpression of *PITX2* during differentiation of sinoatrial nodal cells

CTCF- KO (19 bp)





Conclusions & Perspectives

- **WGS & multi-omics are powerful approaches to identify new regulatory elements and target genes in unsolved mendelian diseases**
    - New regulatory mechanism impacting *PITX2* expression
    - New role of *PITX2* in disease: new cardiac syndrome associating atrial, ventricular arrythmias and congenital heart defects (ASD or LVNC)
- **Implications for clinical molecular diagnosis**
    - Search for 4q25-CTCF deletion should firstly be performed in patients presenting a combination of electrical (LQT/SND) and structural heart defects phenotypes
    - Implication of 4q25-CTCF deletions in isolated congenital heart defects is ongoing

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Fibrillation ventriculaire idiopathique

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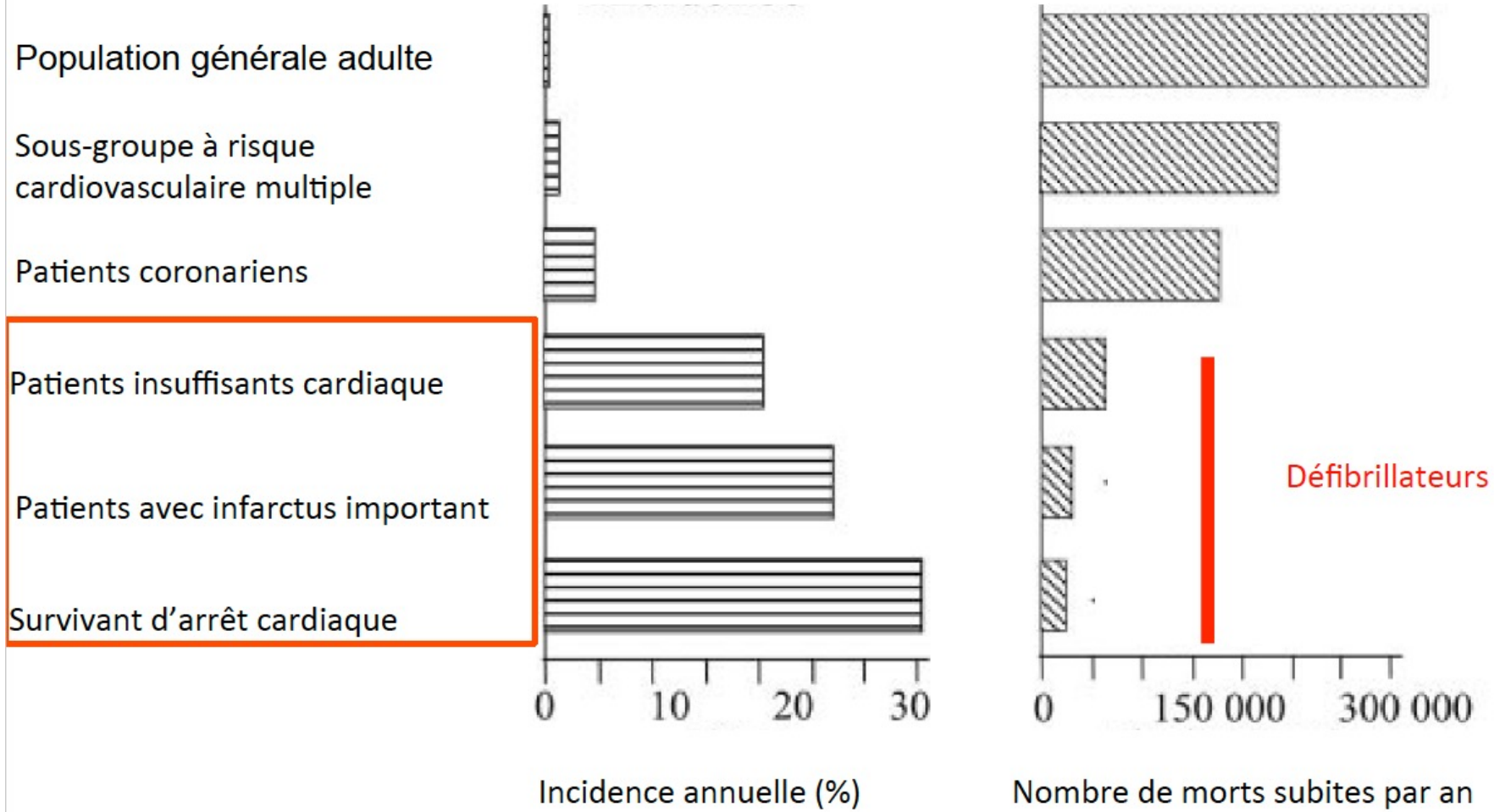
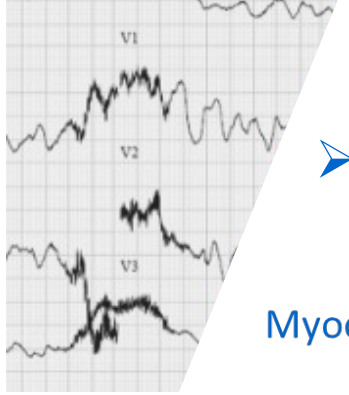
Mort subite cardiaque: population à risque



Myerburg RJ; 2001

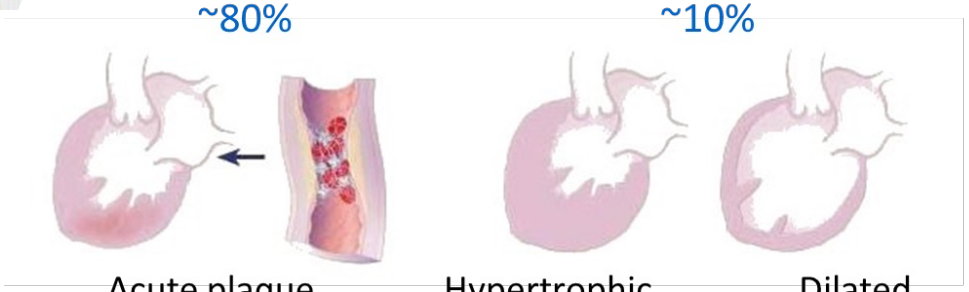


Cardiac arrhythmia

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

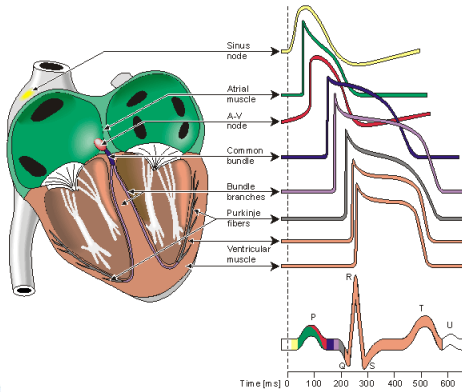
Myocardial infarction ~80%

Cardiomyopathy ~10%



Acute plaque destabilization | 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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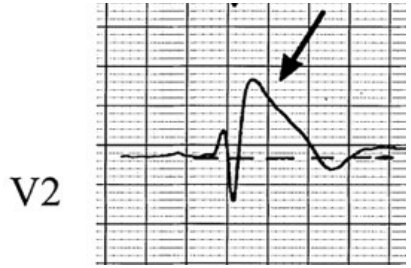
The Brugada syndrome

- 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

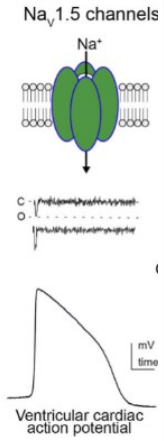
  Typical pattern of ST segment elevation, on the right precordial leads (V1-V3) of the surface ECG

- 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

Human Molecular Genetics, 2015

ORIGINAL ARTICLE

Testing the burden of rare variation in arrhythmia-susceptibility genes provides new insights into molecular diagnosis for Brugada syndrome

Gene	BrS cases ($n = 167$)	Internal controls ($n = 167$)	P-value 1	UK10K controls ($n = 881$)	P-value 2
BrS-susceptibility genes					
SCN5A	20.4% (34)	2.4% (4)	1.4×10^{-7}[a]	2.4% (21)	1.7×10^{-15}[a]
SCN10A	6% (10)	2.4% (4)	0.170	3.5% (31)	0.131
CACNA1C	3% (5)	6.6% (11)	0.199	2% (18)	0.395
PKP2	3% (5)	2.4% (4)	1	1.7% (15)	0.348
CACNB2	1[illegible]% (3)	1.2% (2)	1	0.9% (8)	0.396
KCNH2	1.2% (2)	3.6% (6)	0.283	1.6% (14)	1
TRPM4	1.2% (2)	3% (5)	0.448	1.9% (17)	0.754
KCND3	0.6% (1)	1.2% (2)	1	1.6% (14)	0.488
CACNA2D1	0.6% (1)	0.6% (1)	1	3.3% (29)	0.072
HEY2	0.6% (1)	0.6% (1)	1	0.1% (1)	0.293
SCN2B	0.6% (1)	0.6% (1)	1	0.5% (4)	0.581
SCN3B	0.6% (1)	0.6% (1)	1	0.5% (4)	0.581
ABCC9	—	3% (5)	0.061	1.1% (10)	0.379
SCN1B	—	1.8% (3)	0.248	0.3% (3)	1
RANGRF	—	0.6% (1)	1	0.2% (2)	1
FGF12	—	—	—	0.7% (6)	0.597

Nous ne pouvons pas afficher l'image.

Cases

Controls

Significant enrichment in rare coding variation for *SCN5A* only

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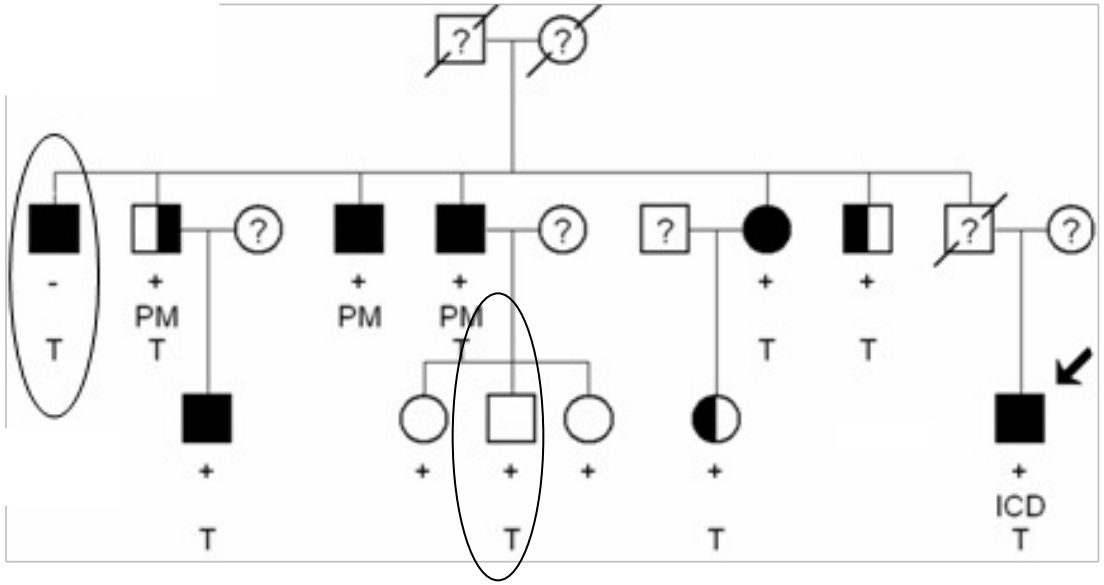
Variable penetrance and expressivity in mendelian diseases

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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Common polymorphisms modulate Brugada syndrome

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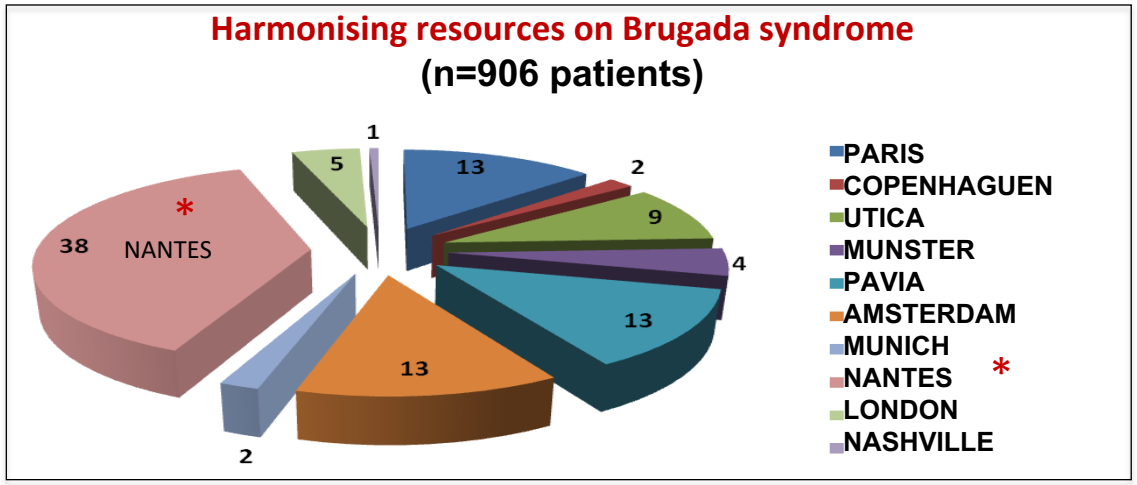
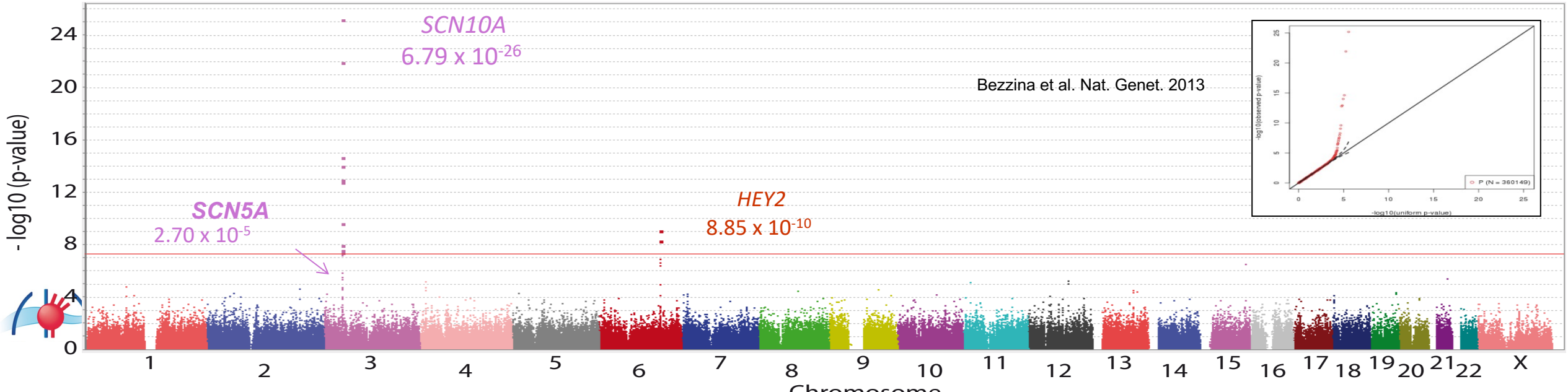
in the heart of an international network on Brugada syndrome

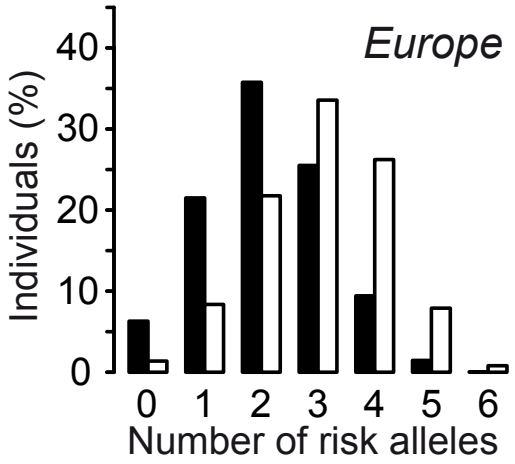


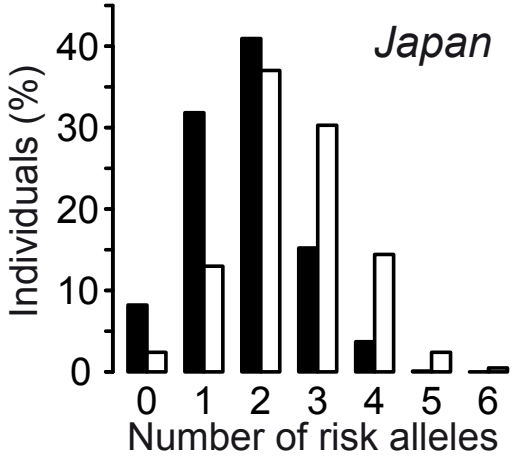
312 patients
1115 controls

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



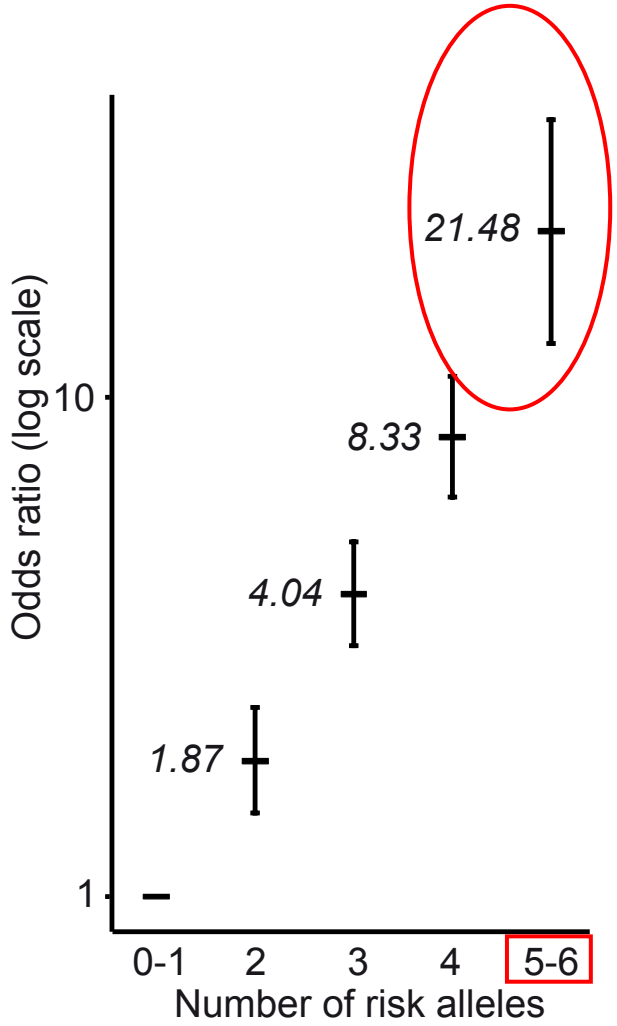
Cumulative effect of risk alleles at the three loci





Ctrls

BrS cases



Bezzina et al. Nat. genet. 2013

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International Brugada Syndrome Genetics Consortium

Amsterdam UMC
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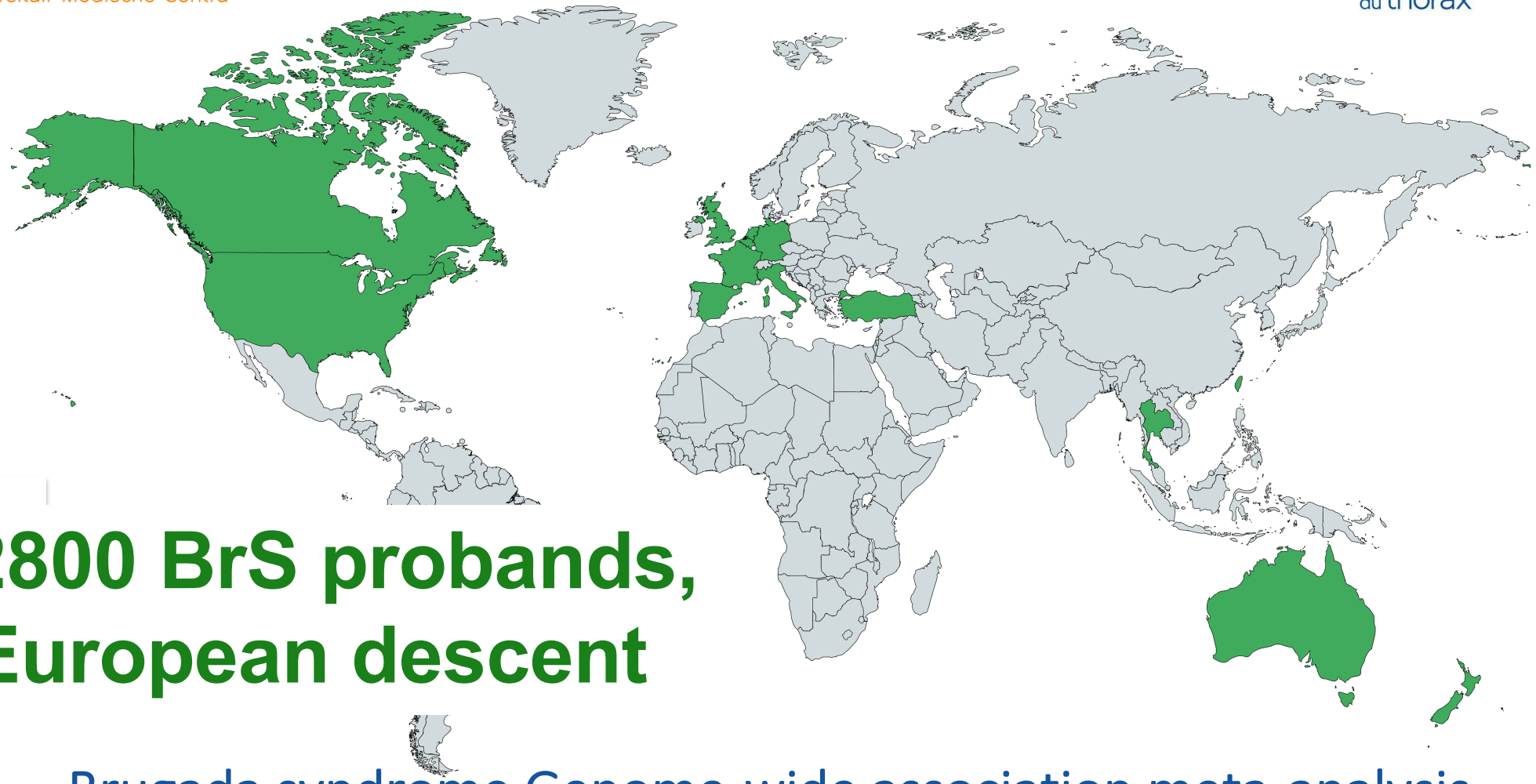

~2800 BrS probands, European descent

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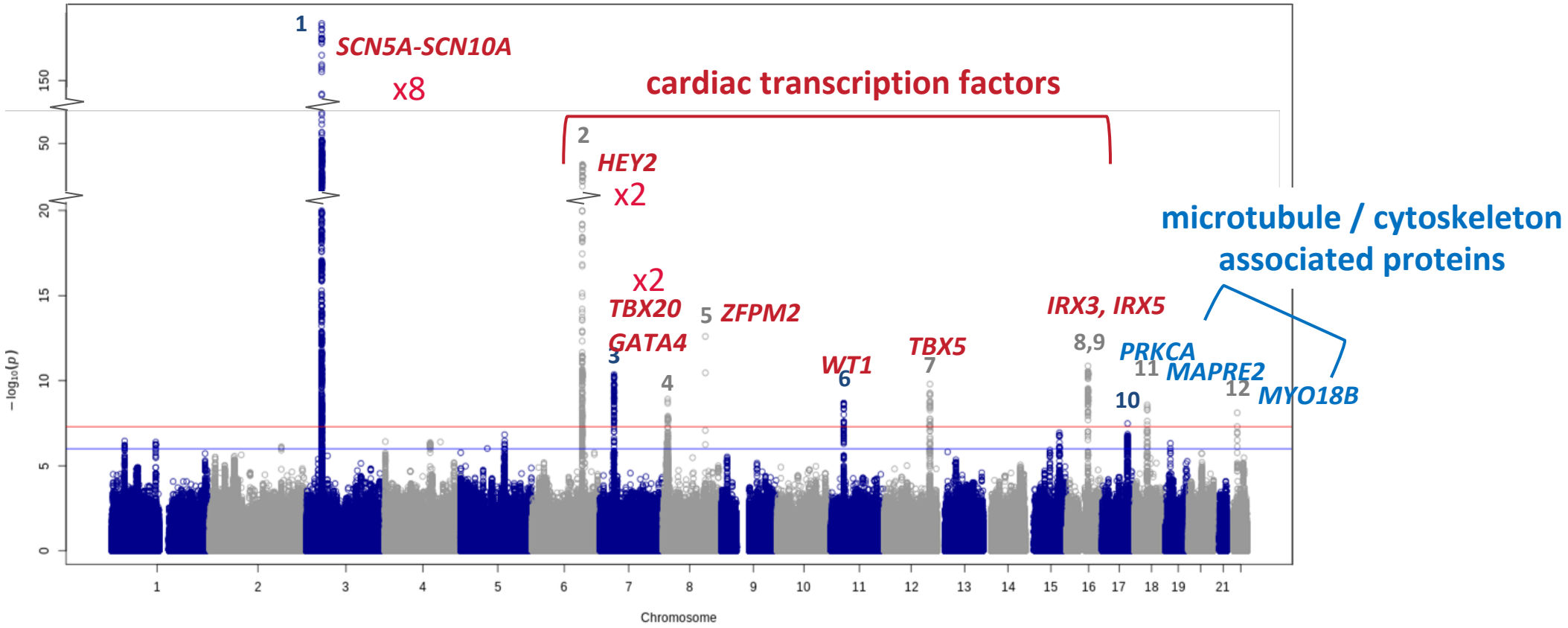
Brugada syndrome Genome-wide association meta-analysis

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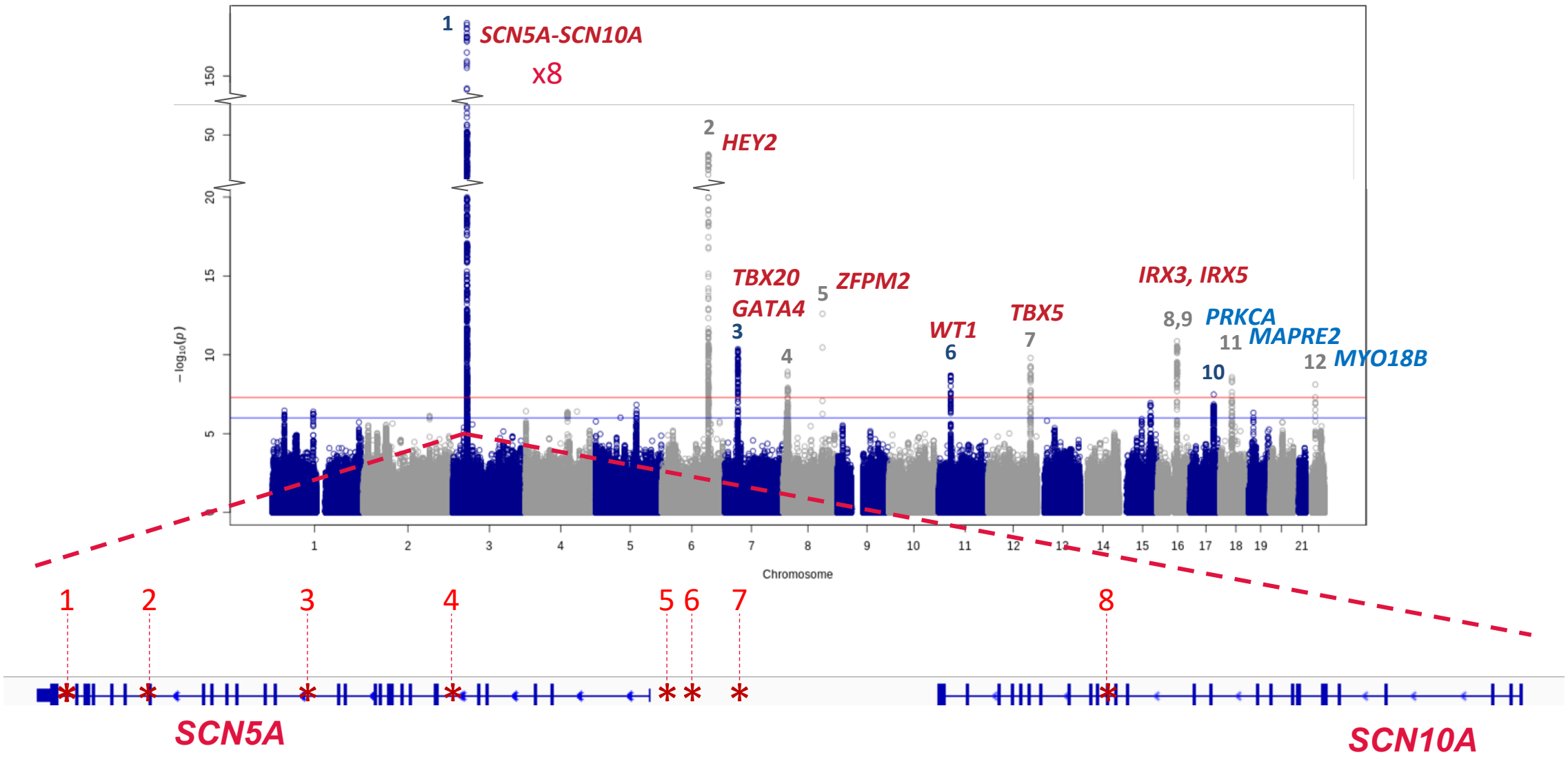
A predominant role of *SCN5A/SCN10A locus risk alleles*



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

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SCN5A/SCN10A locus: 8 independant loci

Locus	Lead SNP	Genomic position (hg19)	Risk allele	Other allele	Risk allele frequency in cases	Risk allele frequency in controls	OR [95% CI]	P value	Nearest gene
1	rs7638909*	3:38594973	G	T	0.32	0.24	1.28 [1.17 - 1.40]	2.79E-08	*SCN5A*
	rs62241190*	3:38607468	G	A	0.06	0.03	1.96[1.63 - 2.32]	8.56E-14	*SCN5A*
	rs7374540*	3:38634142	C	A	0.51	0.39	1.72 [1.61 - 1.81]	3.56E-57	*SCN5A*
	rs7433206*	3:38657708	A	T	0.45	0.42	1.48 [1.37 - 1.60]	9.52E-24	*SCN5A*
	rs34760424*	3:38683018	G	T	0.98	0.94	2.32 [1.96 - 2.70]	3.03E-23	*SCN5A*
	rs41310232*	3:38689242	A	G	0.16	0.09	1.56 [1.40 - 1.74]	1.19E-15	*SCN5A*
	rs6782237*	3:38696553	C	G	0.78	0.68	1.74 [1.61 - 1.87]	1.05E-47	*SCN5A*
	rs6801957	3:38767315	T	C	0.65	0.42	2.49 [2.34 - 2.65]	1.30E-180	*SCN10A*

7

1

- **All non-coding risk alleles: role in gene regulation**
  - post-GWAS approaches: translate genetic findings into molecular mechanisms
  - characterize and annotate all cardiac regulatory regions

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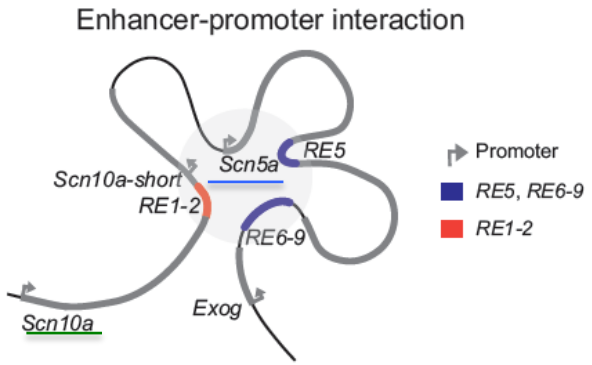
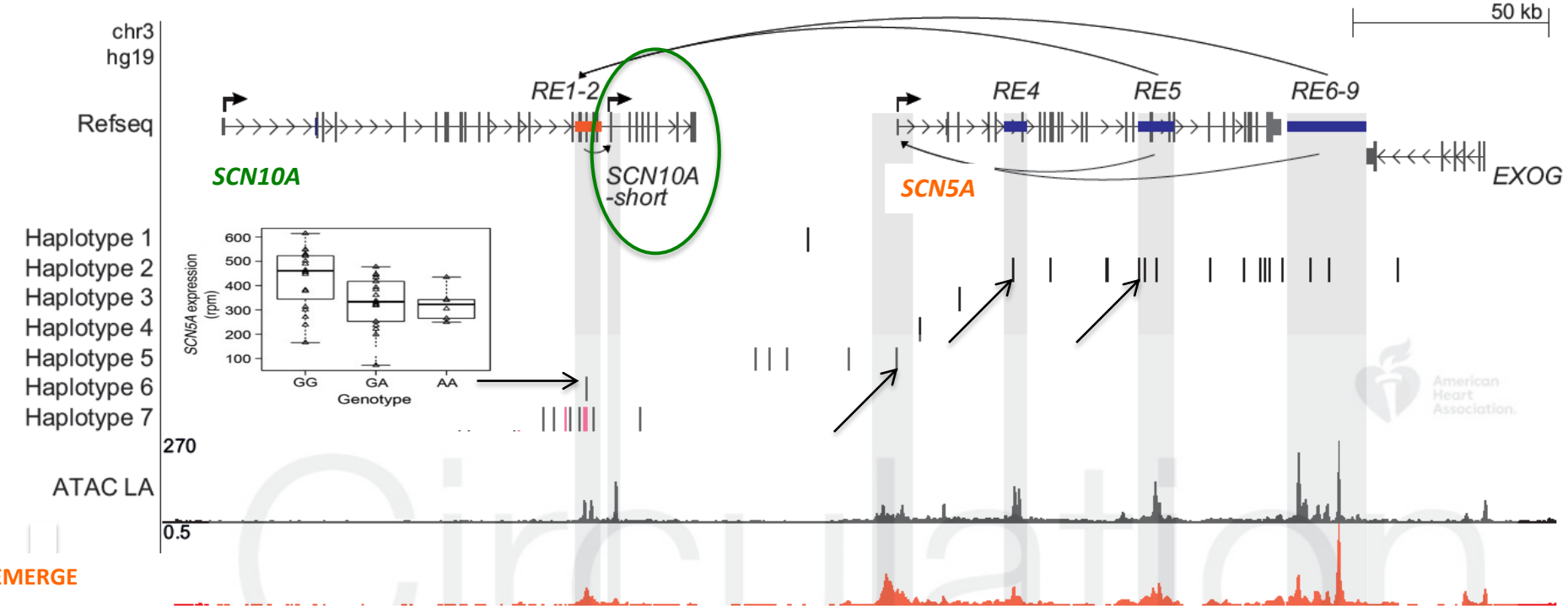
Risk haplotypes co-localize at multiple SCN10A / SCN10A intronic enhancers



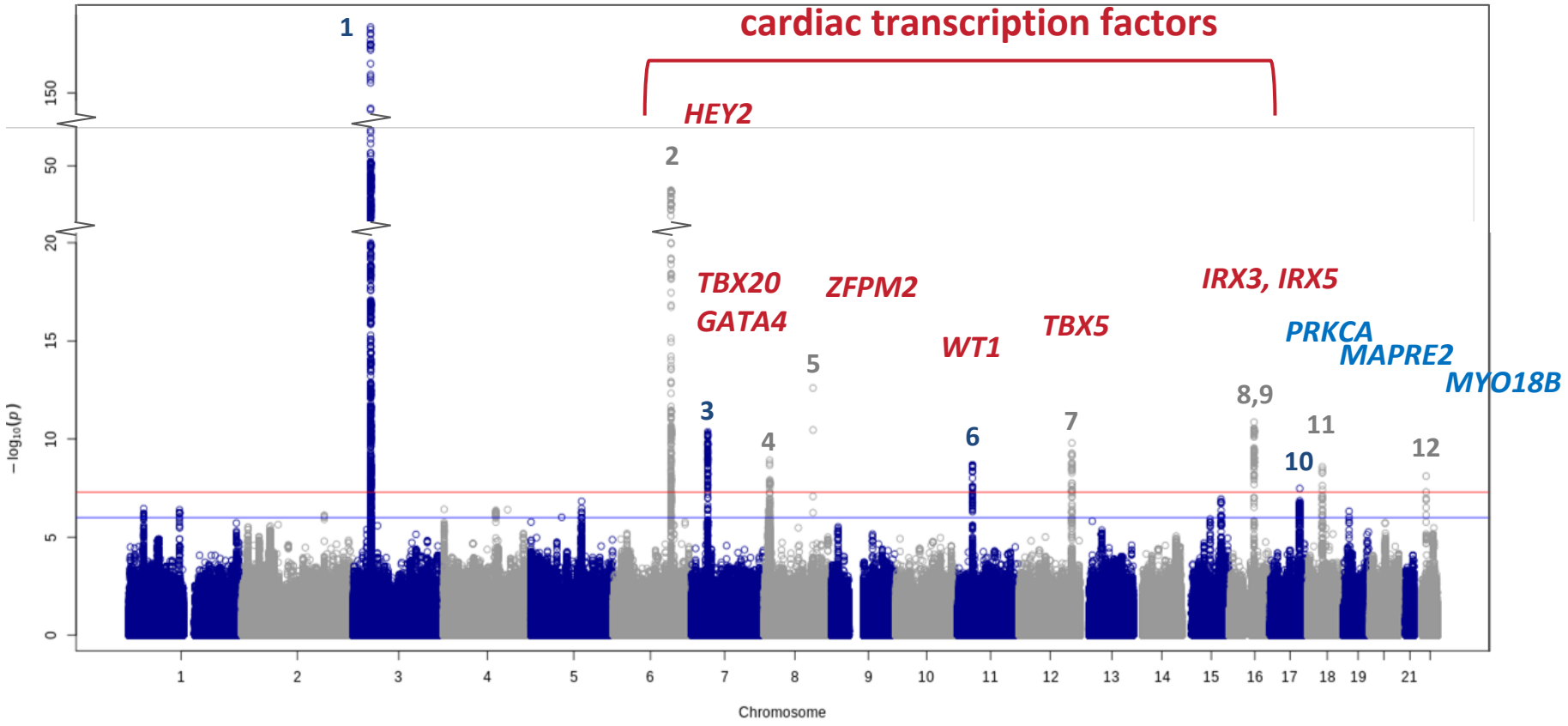


EMERGE

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

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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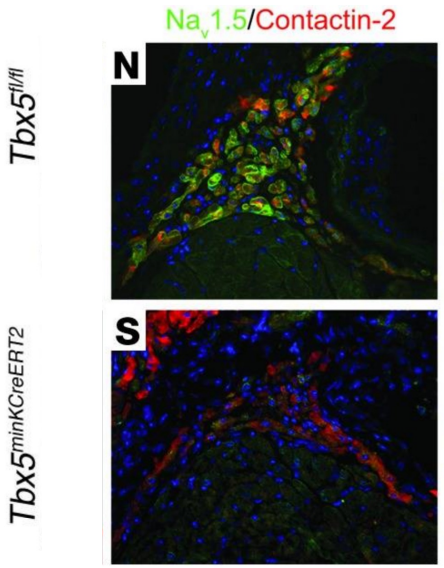
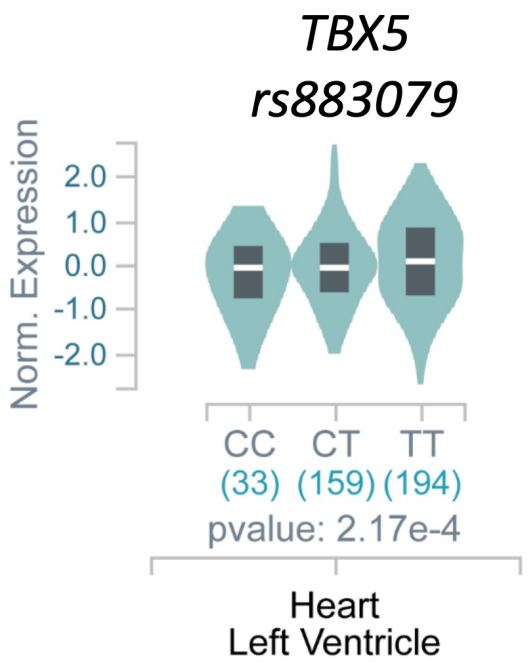
TBX5 drives expression of cardiac conduction system function

TBX5



Arnolds et al. J Clin Invest. 2012

rs 883079 eQTL



➢ Indirect regulation of Nav1.5 expression by TF

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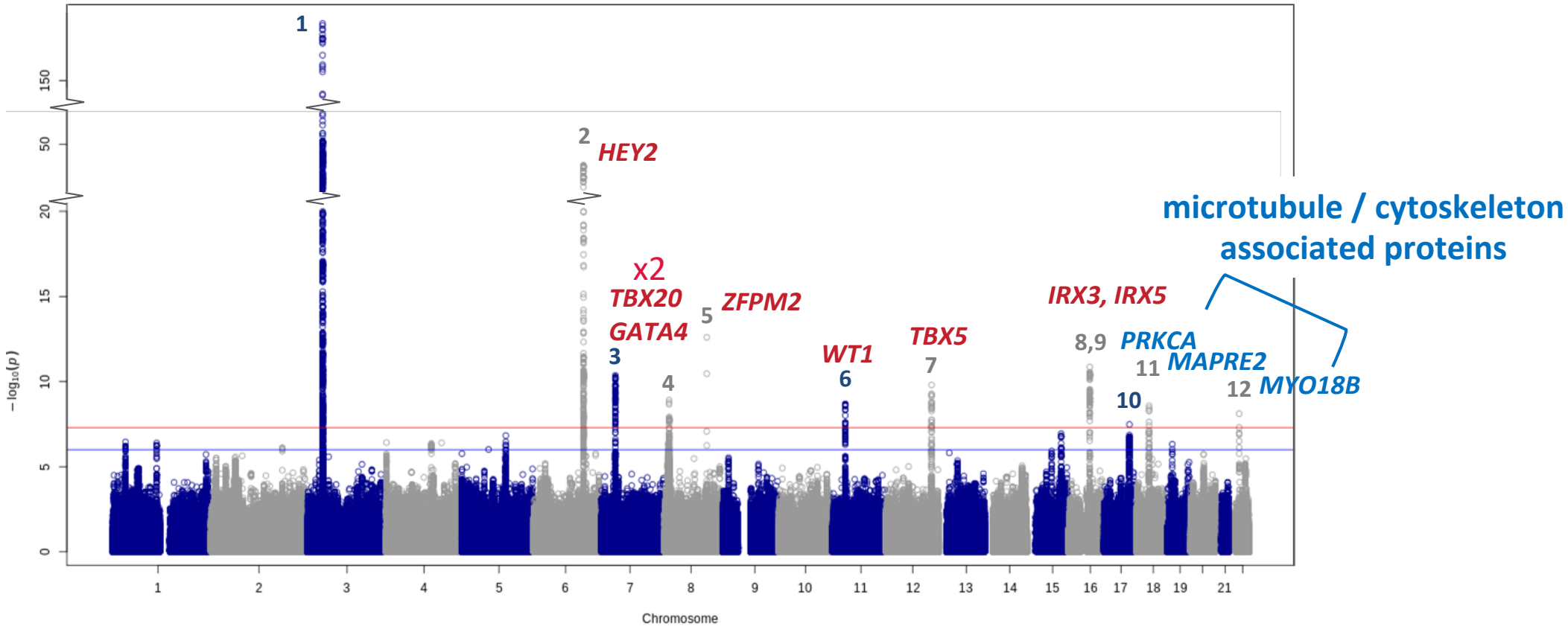
GWAS: identification of 3 loci associated with structural proteins



➢ new molecular mechanisms

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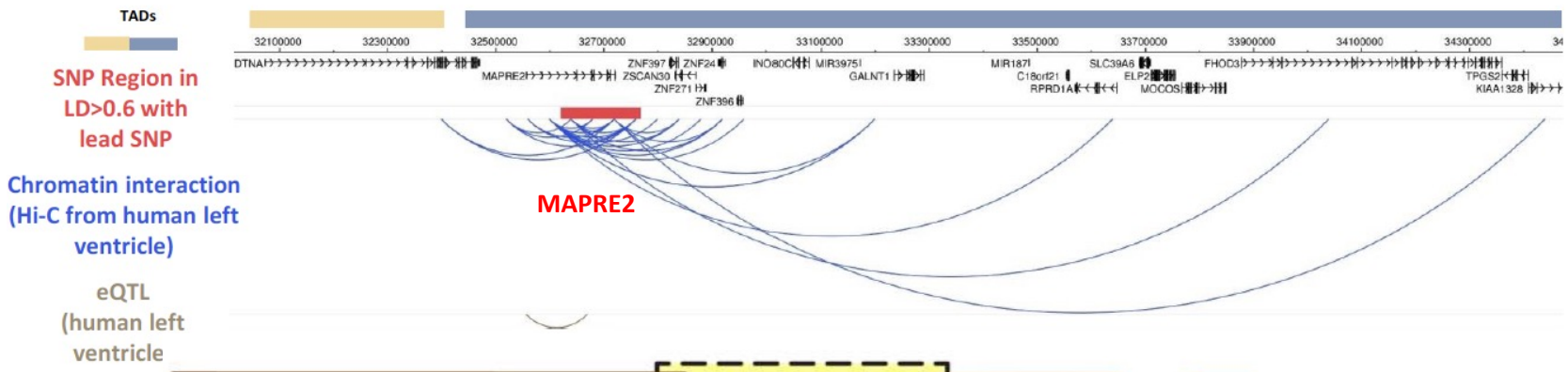
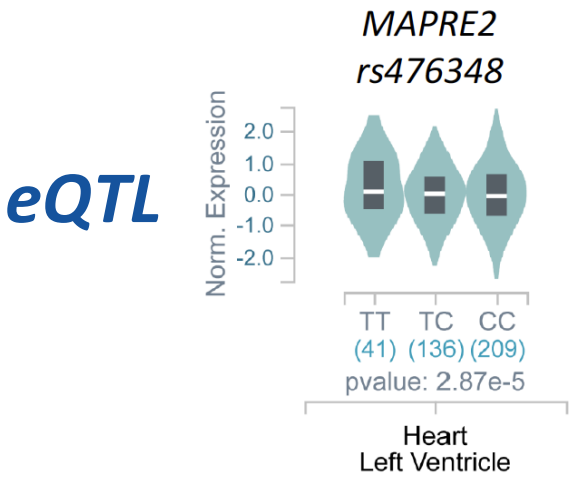
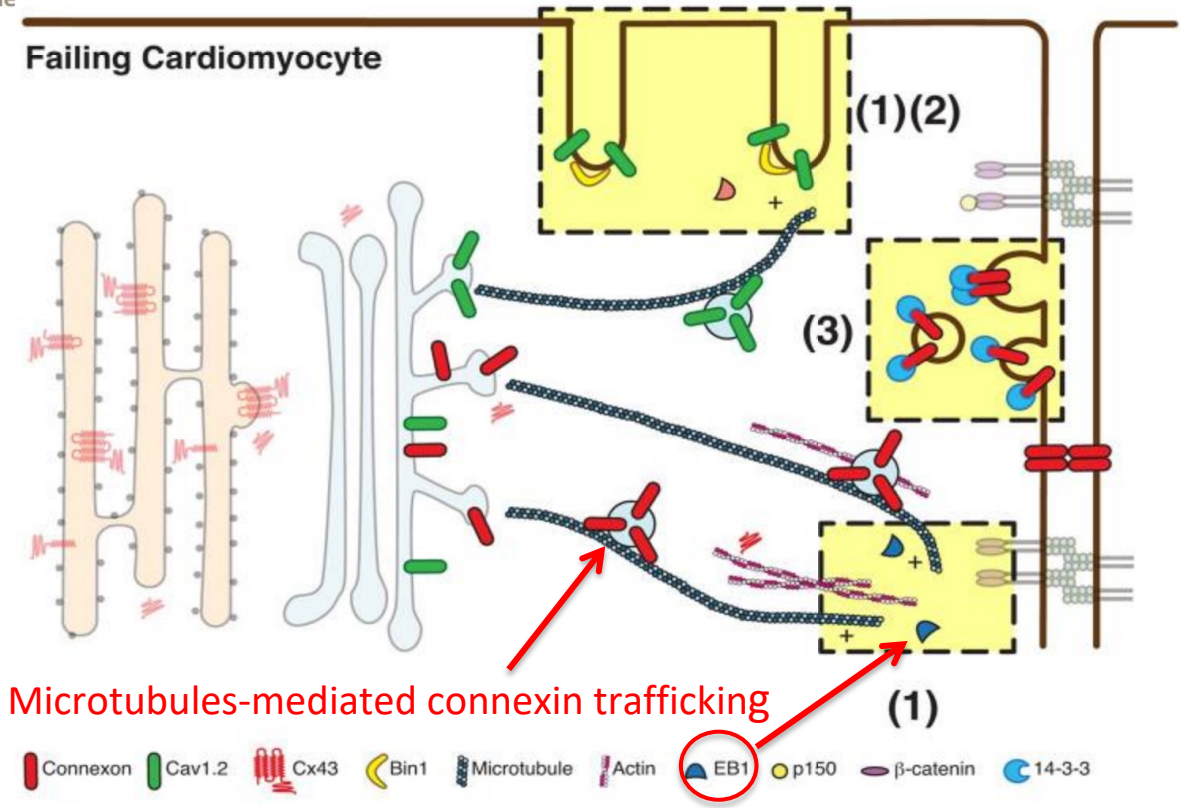
MAPRE2 is a trafficking protein / regulator of microtubules

(microtubule plus-end binding protein (EB2))







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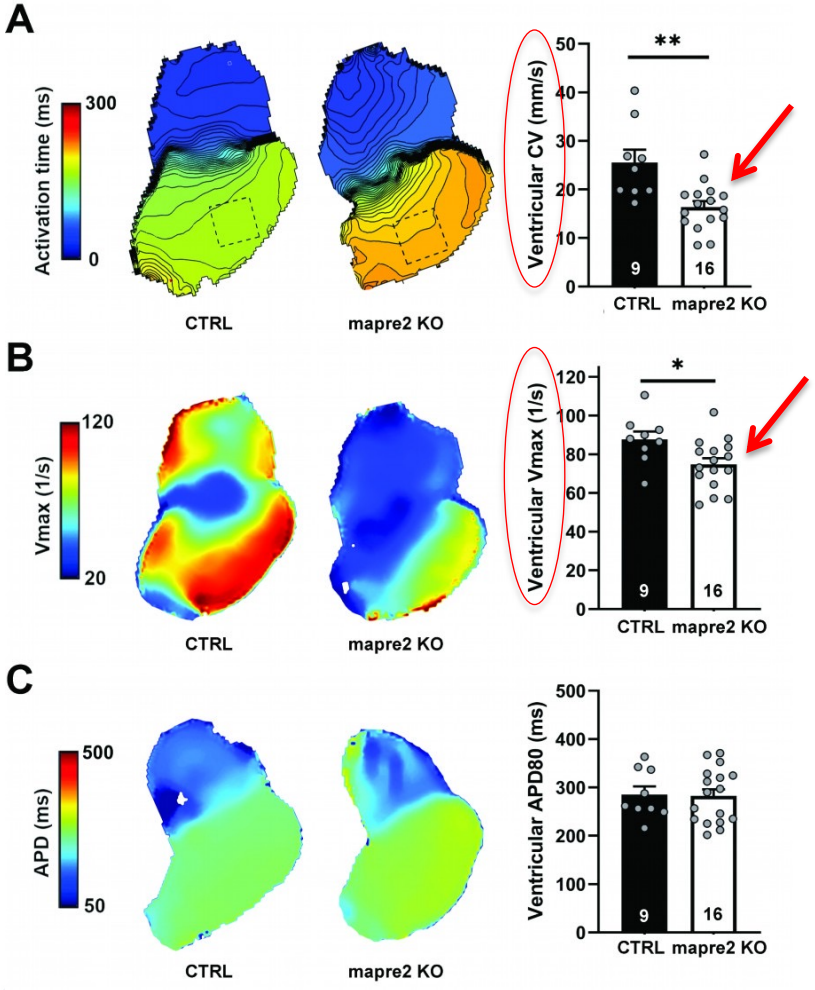
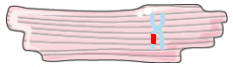
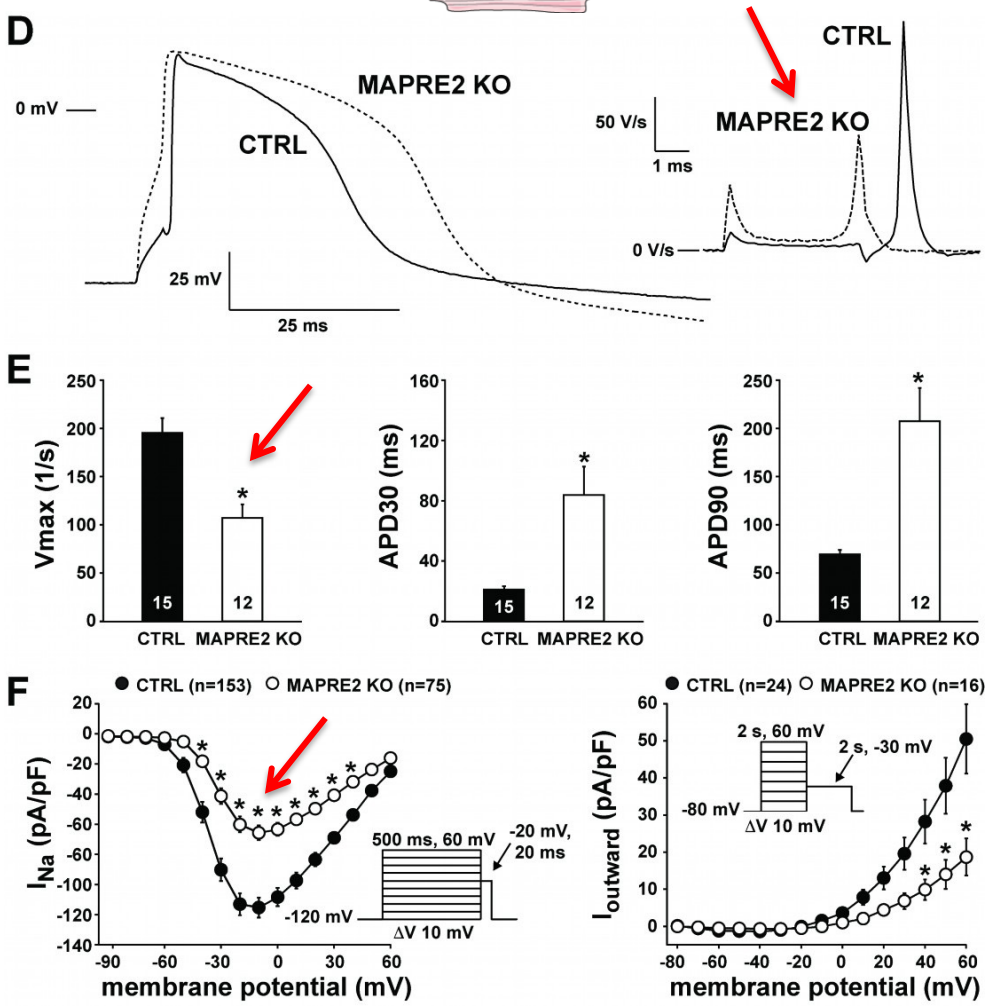
MAPRE2 is a regulator of microtubule organization and impacts on sodium current



iPS-CM





Leducq Foundation

David Chiang
(Boston)

Mariam Jouni
(Chicago)

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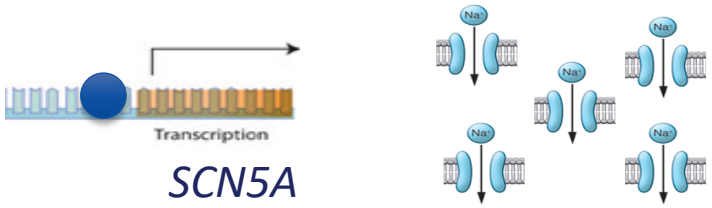
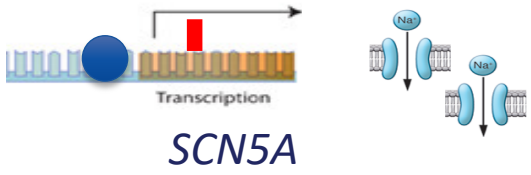
Modulation of microtubule function alters ion-channel trafficking

Multiple levels of sodium current dosage regulation affects in BrS phenotype



Normal sodium current

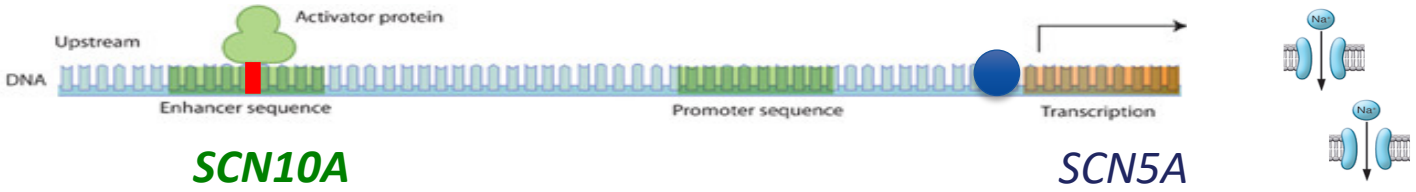
Rare coding mutations
(loss of function)



Reduced sodium current

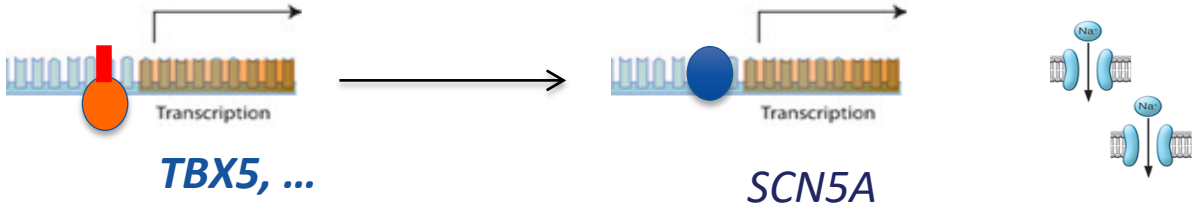
Common non-coding risk alleles

Ion channel control



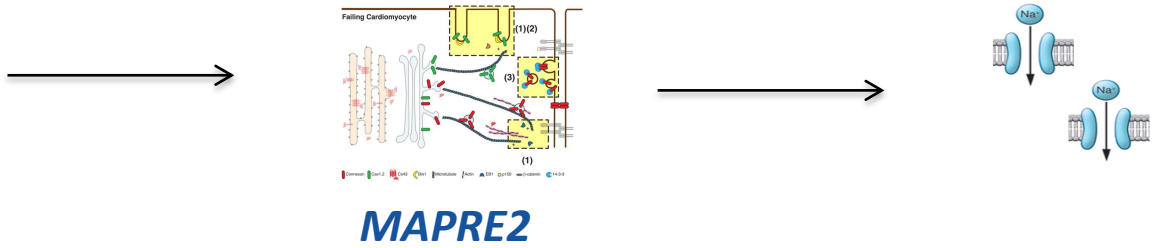
Reduced sodium current

Translational control



Reduced sodium current

Post-translational control



Reduced sodium current

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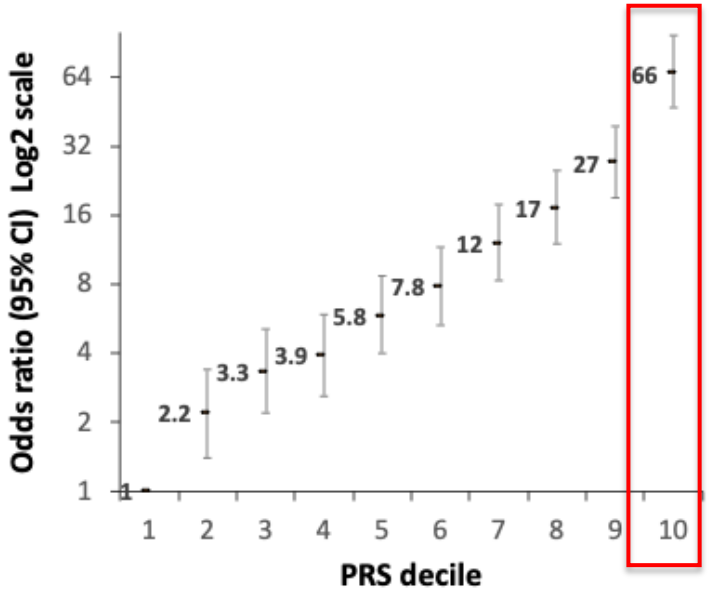
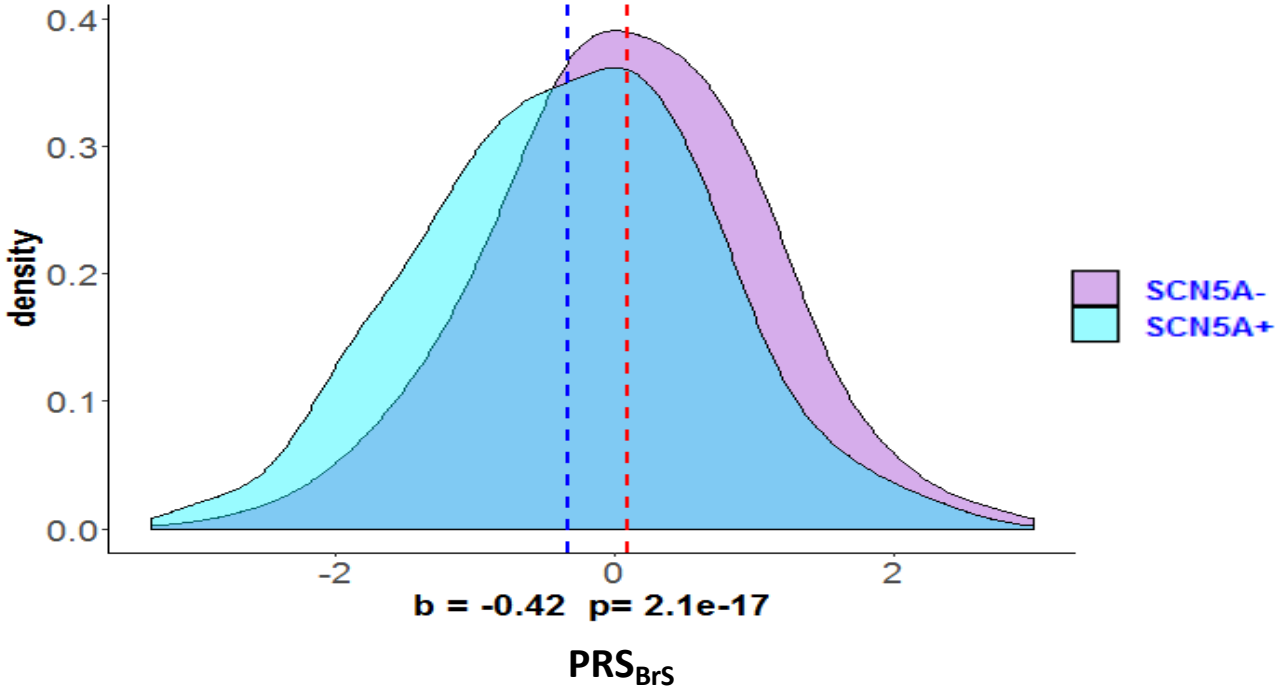
Impact in clinical practice: PRD and risk allele distribution among cases

Cumulative effect of susceptibility variants
(21 loci)

Polygenic risk score



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



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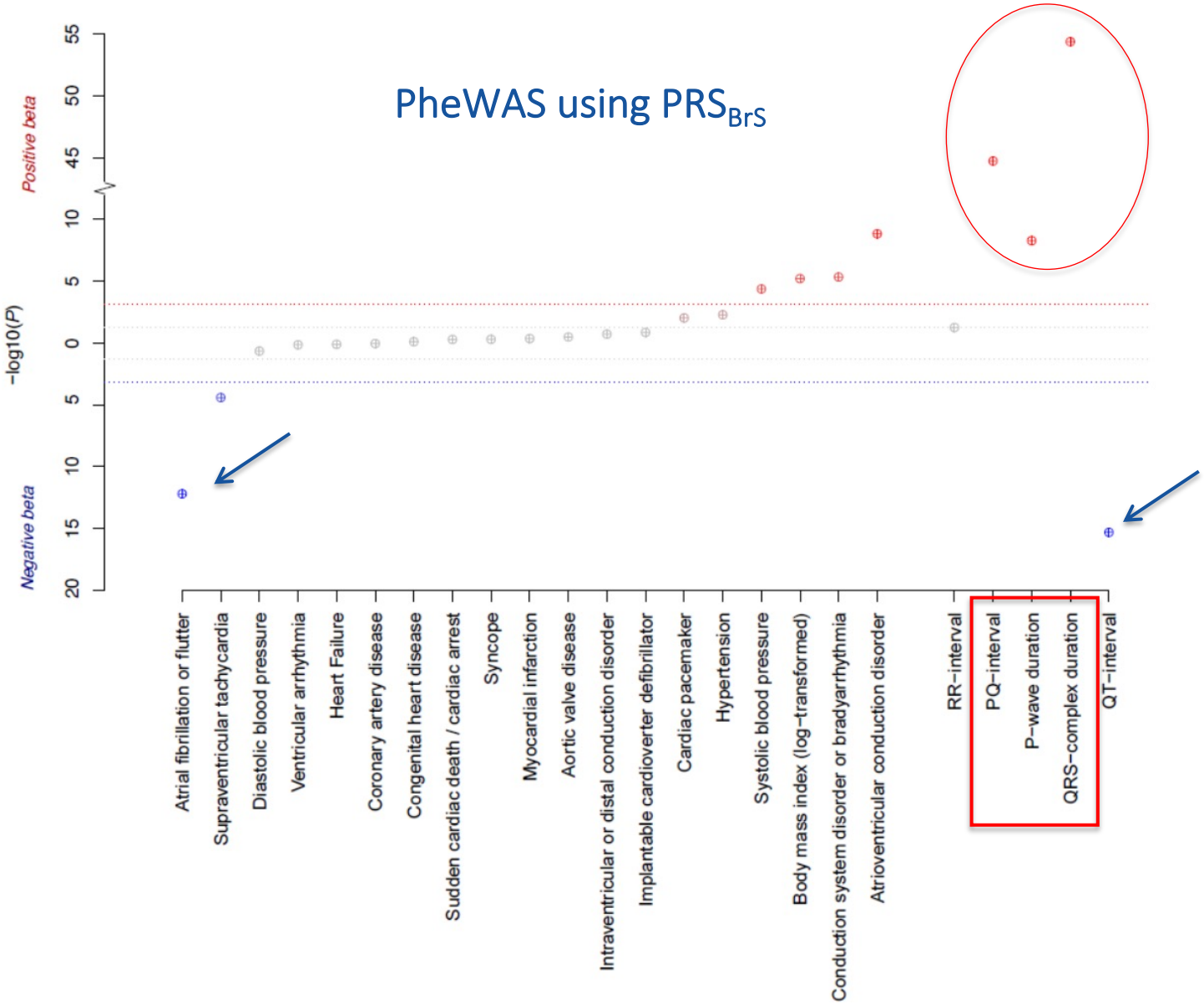
BrS, a relevant model for more complex diseases



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



Julien Barc[1*], Rafik Tadros[2,3*], Charlotte Glinge[2,4*], David Y Chiang[5*], Mariam Jouni[6*], Floriane Simonet[1*], Sean J Jurgens[7], Manon Baudic[1], Michele Nicastro[2], Franck Potet[8], Joost A Offerhaus[2], Roddy Walsh[2], Seung Hoan Choi[7], Arie O Verkerk[2,9], Yuka Mizusawa[2], Soraya Anys[10], Damien Minois[10], Marine Arnaud[10], Josselin Duchateau[11,12,13,14], Yanushi D Wijeyeratne[15], Alison Muir[16], Michael Papadakis[15], Silvia Castelletti[17], Margherita Torchio[18], Cristina Gil Ortuño[19], Javier Lacunza[20], Daniela F Giachino[21,22], Natascia Cerrato[23], Raphaël P Martins[24], Oscar Campuzano[25,26,27,28], Sonia Van Dooren[29,30], Aurélie Thollet[10], Florence Kyndt[10], Andrea Mazzanti[31], Nicolas Clémenty[32], Arnaud Bisson[32], Anniek Corveleyn[33], Birgit Stallmeyer[34], Sven Dittmann[34], Johan Saenen[35], Antoine Noël[36], Sherry Honarbakhsh[37], Boris Rudic[38,39], Halim Marzak[40], Matthew K Rowe[41], Claire Federspiel[42], Sophie Lepage[43], Leslie Placide[44], Antoine Milhem[45], Hector Barajas-Martinez[46], Britt-Maria Beckmann[47], Ingrid PC Krapels[48], Johannes Steinfurt[49], Bo Gregers Winkel[50], Reza Jabbari[51], Moore B Shoemaker[52], Carol Ann Remme[2], Bas J Boukens[9], Doris Škorić-Milosavljević[2], Hennie Bikker[53], Federico C Manevy[2], Peter Lichtner[54], Marta Ribasés[55], Thomas Meitinger[54], Martina Müller-Nurasyid[56,57,58,59], KORA-Study Group[60], Jan H Veldink[61], Leonard H van den Berg[61], Philip Van Damme[62], Daniele Cusi[63], Chiara Lanzani[64], Sidwell Rigade[1], Eric Charpentier[1,65], Estelle Baron[1], Stéphanie Bonnaud[1,65], Simon Lecointe[1], Audrey Donnart[65,1], Hervé Le Marec[1], Stéphanie Chatel[1], Matilde Karakachoff[1], Stéphane Bézieau[10], Barry London[66], Jacob Tfelt-Hansen[67,68,30], Dan Roden[69,70,71], Katja E Odening[49,72], Marina Cerrone[73], Larry A Chinitz[73], Paul GA Volders[74], Maarten P van de Berg[75], Gabriel Laurent[76], Laurence Faivre[77], Charles Antzelevitch[46], Stefan Kääb[78,79], Alain Al Arnaout[45], Jean-Marc Dupuis[43], Jean-Luc Pasquie[80], Olivier Billon[42], Jason D Roberts[41], Laurence Jesel[40,81], Martin Borggrefe[38,39], Pier D Lambiase[82,37], Jacques Mansourati[36], Bart Loeys[83], Antoine Leenhardt[84,30], Pascale Guicheney[85,86], Philippe Maury[87], Eric Schulze-Bahr PhD[34,30], Tomas Robyns[88,89], Jeroen Breckpot[33], Dominique Babuty[32], Silvia G Priori[31,30], Carlo Napolitano[31,30], Nantes Referral Center for inherited cardiac arrhythmia[90], Carlo de Asmundis[91,92,27,30], Pedro Brugada[93], Ramon Brugada[94], Elena Arbelo[95], Josep Brugada[96], Philippe Mabo[24], Nathalie Behar[97], Carla Giustetto[23], Maria Sabater Molina[19], Juan R Gimeno[20,30], Can Hasdemir[98], Peter J Schwartz[17,30], Lia Crotti[99,30], Pascal P Mckeown[100], Sanjay Sharma[15], Elijah R Behr[15,30], Michel Haissaguerre[11,12,13,14], Frédéric Sacher[11,12,13,14], Caroline Rooryck[101,102], Hanno L Tan[103], Pieter G Postema[2], Mario Delmar[104], Patrick T Ellinor[105], Steven A Lubitz[105], Jean-Baptiste Gourraud[10], Michael WT Tanck[106], Alfred L George, Jr.[107], Calum A MacRae[108], Paul W Burridge[109,110], Christian Dina[1], Vincent Probst[10,30*], Arthur A Wilde[2,30*], Jean-Jacques Schott[10*], Richard Redon[10*], Connie R Bezzina[2*]

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**

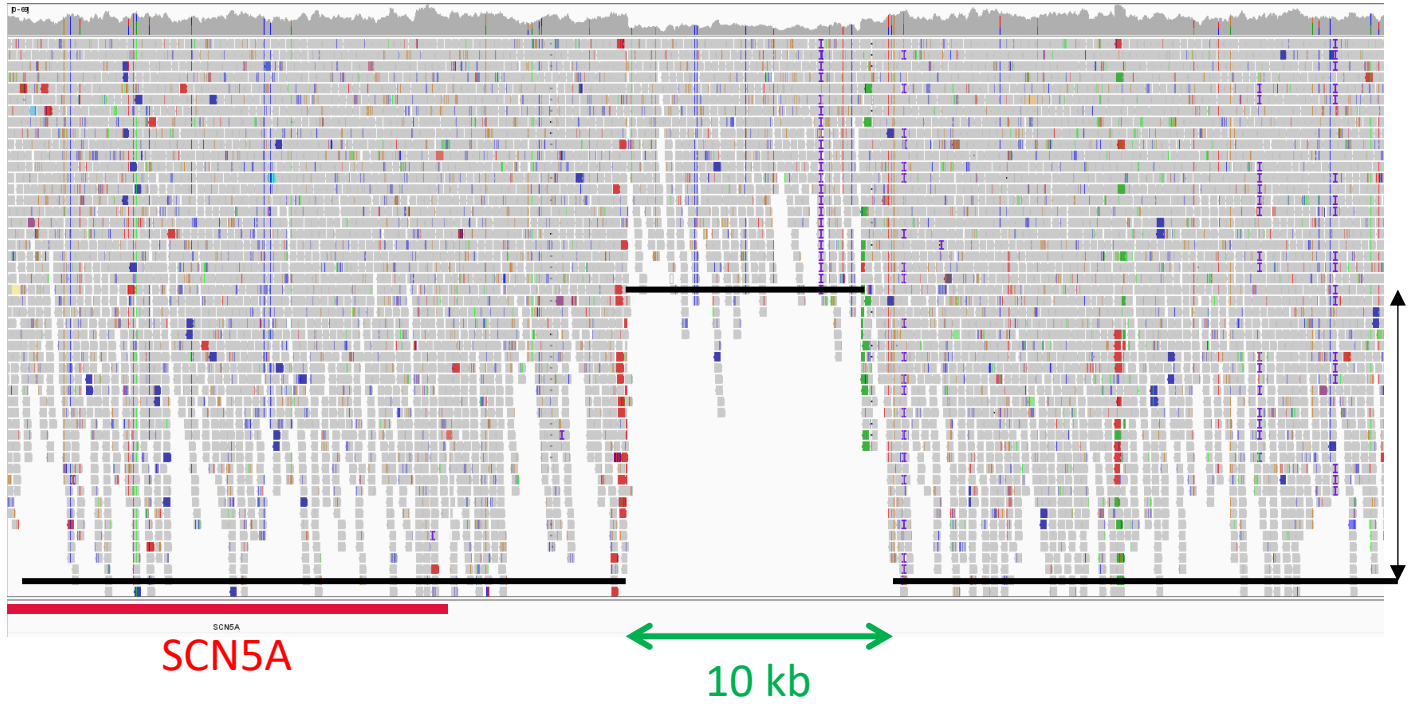
3- Genome-wide analysis of lethal arrhythmic events among BrS patients identifies 1 VF-associated SNP in Japanese and European BrS

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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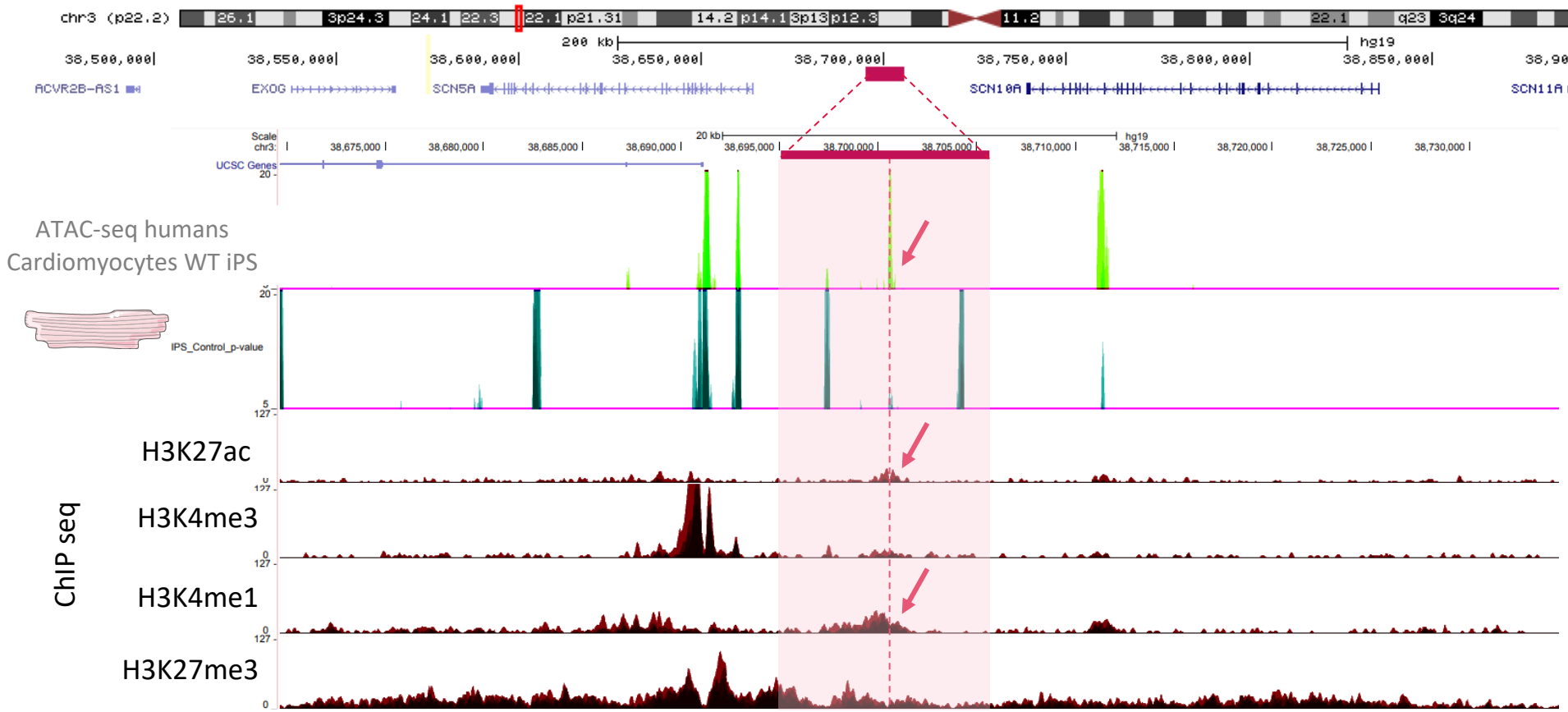
Functioal annotation

ATAC-seq humans
Cardiomyocytes WT iPS

Potential 2 opened chromatin regions

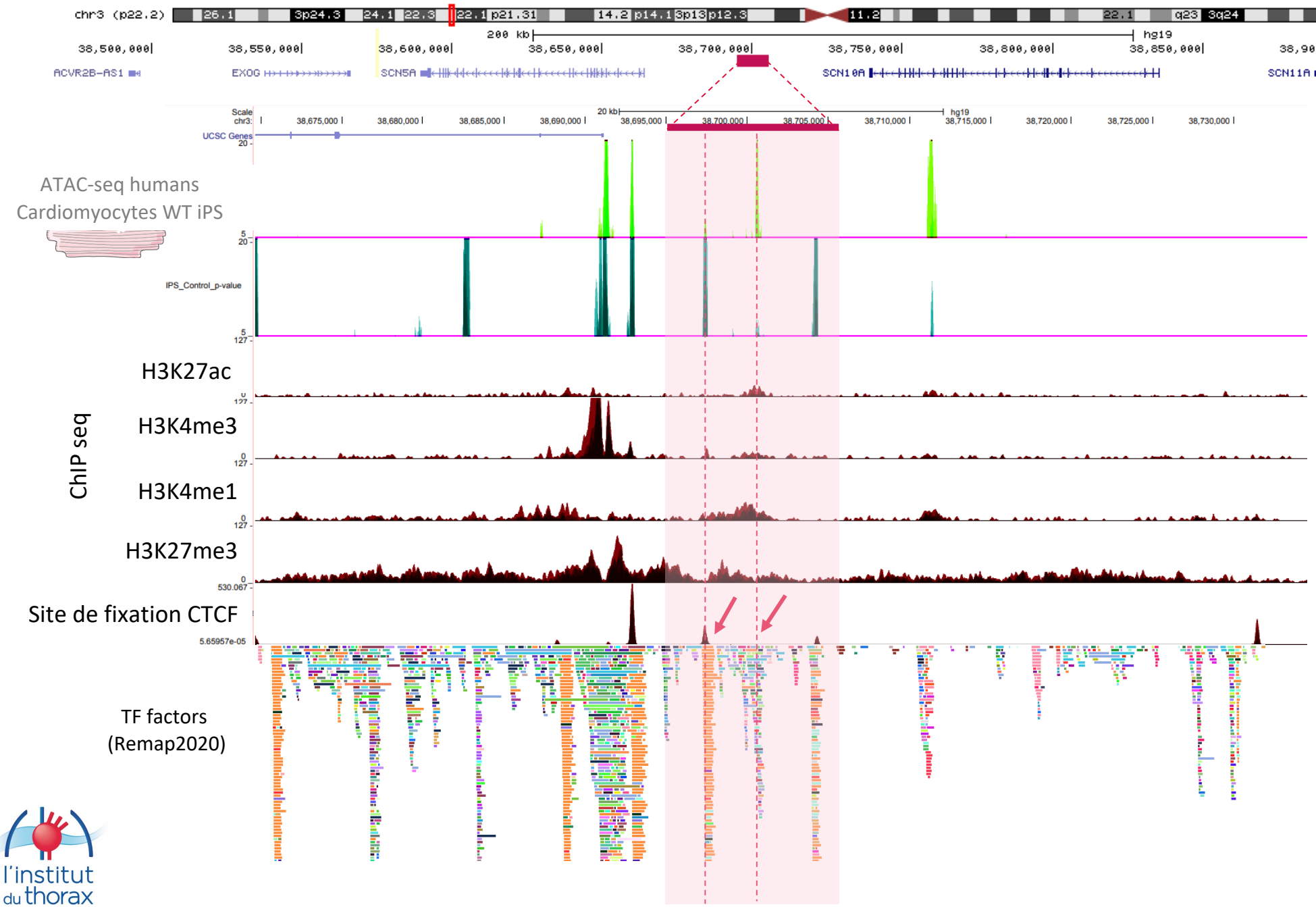
Functional annotation of the deletion region



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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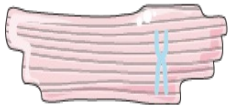
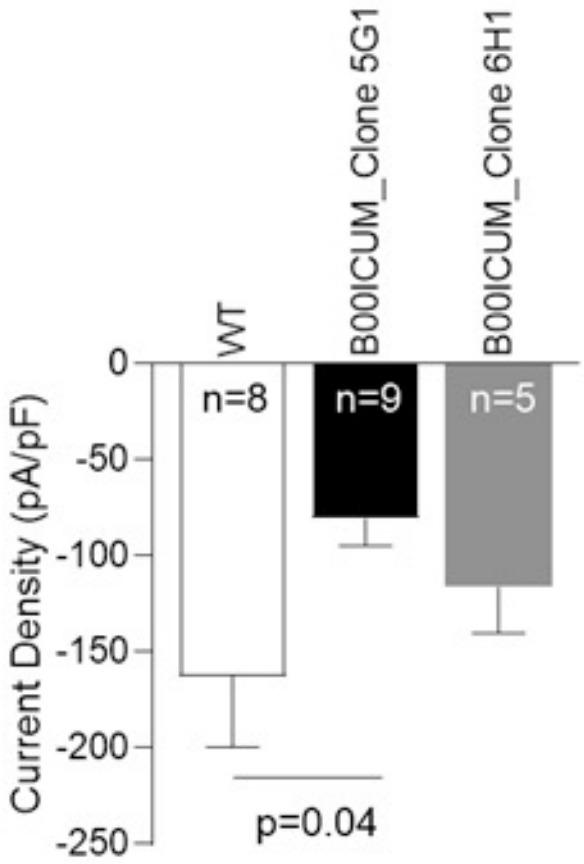
Functional annotation of the 10kb deletion (patch clamping)





➢ Reduction of I_{Na} density

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**

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