Où en est la recherche en génétique médicale?

Comment l’appliquer aux maladies cardiaques?

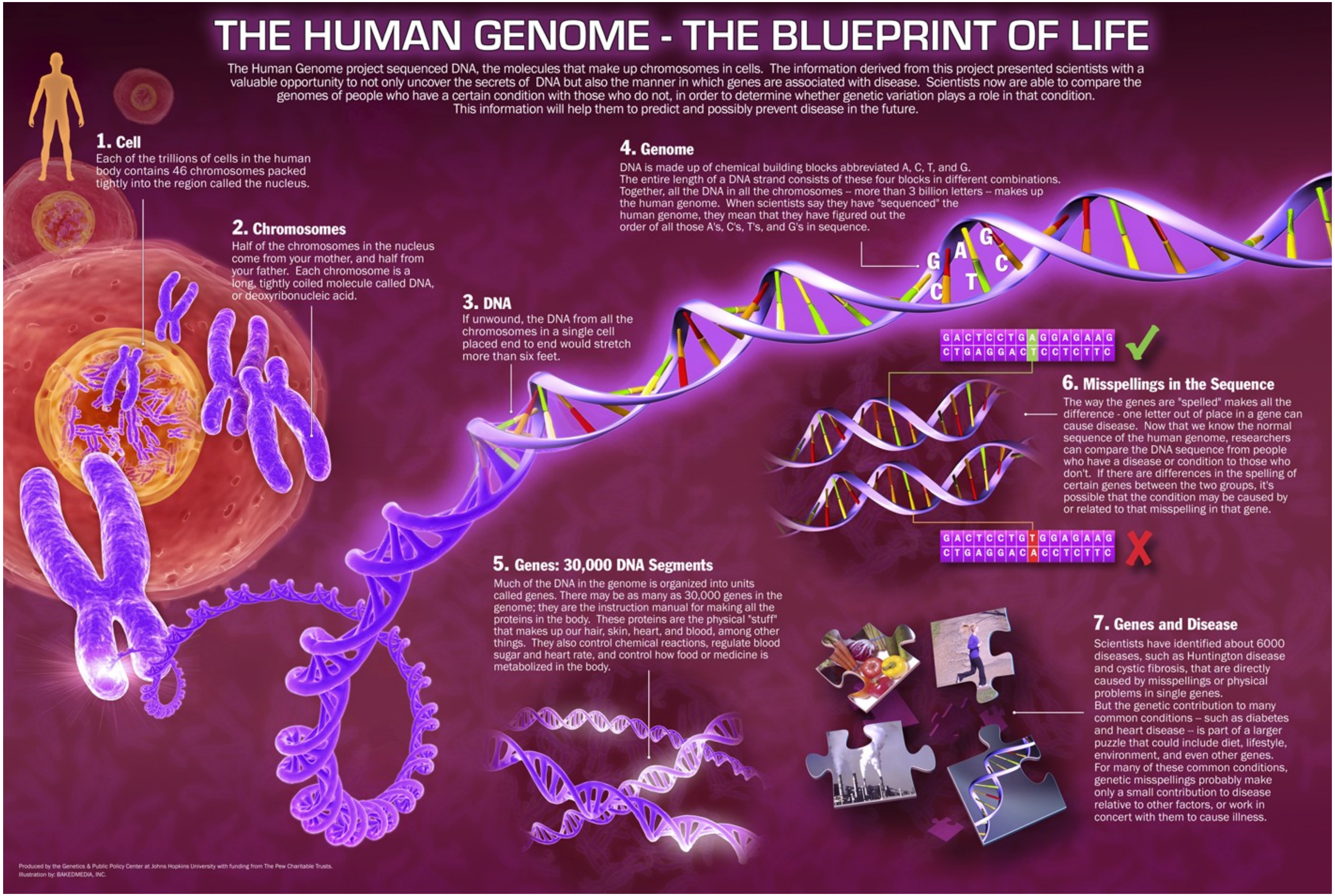
l'institut du thorax

Inserm

CENTRE HOSPITALIER
UNIVERSITAIRE DE NANTES

Richard REDON

2001: le génome humain est séquencé


G
A
G
C
C
T
G A C T C C T G A G G A G A A G
C T G A G G A C T C C T C T T C
✓
G A C T C C T G T G G A G A A G
C T G A G G A C A C C T C T T C
✗

THE HUMAN GENOME - THE BLUEPRINT OF LIFE

The Human Genome project sequenced DNA, the molecules that make up chromosomes in cells. The information derived from this project presented scientists with a valuable opportunity to not only uncover the secrets of DNA but also the manner in which genes are associated with disease. Scientists now are able to compare the genomes of people who have a certain condition with those who do not, in order to determine whether genetic variation plays a role in that condition. This information will help them to predict and possibly prevent disease in the future.

1. Cell

Each of the trillions of cells in the human body contains 46 chromosomes packed tightly into the region called the nucleus.

2. Chromosomes

Half of the chromosomes in the nucleus come from your mother, and half from your father. Each chromosome is a long, tightly coiled molecule called DNA, or deoxyribonucleic acid.

3. DNA

If unwound, the DNA from all the chromosomes in a single cell placed end to end would stretch more than six feet.

4. Genome

DNA is made up of chemical building blocks abbreviated A, C, T, and G. The entire length of a DNA strand consists of these four blocks in different combinations. Together, all the DNA in all the chromosomes -- more than 3 billion letters -- makes up the human genome. When scientists say they have "sequenced" the human genome, they mean that they have figured out the order of all those A's, C's, T's, and G's in sequence.

5. Genes: 30,000 DNA Segments

Much of the DNA in the genome is organized into units called genes. There may be as many as 30,000 genes in the genome; they are the instruction manual for making all the proteins in the body. These proteins are the physical "stuff" that makes up our hair, skin, heart, and blood, among other things. They also control chemical reactions, regulate blood sugar and heart rate, and control how food or medicine is metabolized in the body.

6. Misspellings in the Sequence

The way the genes are "spelled" makes all the difference - one letter out of place in a gene can cause disease. Now that we know the normal sequence of the human genome, researchers can compare the DNA sequence from people who have a disease or condition to those who don't. If there are differences in the spelling of certain genes between the two groups, it's possible that the condition may be caused by or related to that misspelling in that gene.

7. Genes and Disease

Scientists have identified about 6000 diseases, such as Huntington disease and cystic fibrosis, that are directly caused by misspellings or physical problems in single genes.
But the genetic contribution to many common conditions -- such as diabetes and heart disease -- is part of a larger puzzle that could include diet, lifestyle, environment, and even other genes. For many of these common conditions, genetic misspellings probably make only a small contribution to disease relative to other factors, or work in concert with them to cause illness.

Produced by the Genetics & Public Policy Center at Johns Hopkins University with funding from The Pew Charitable Trusts.
Illustration by: BAKEDMEDIA, INC.

Recherche en génétique humaine

* Maladies monogéniques

maladies rares causées chacune par l'altération d'un seul gène

Gène muté
[erreur(s) de séquence]

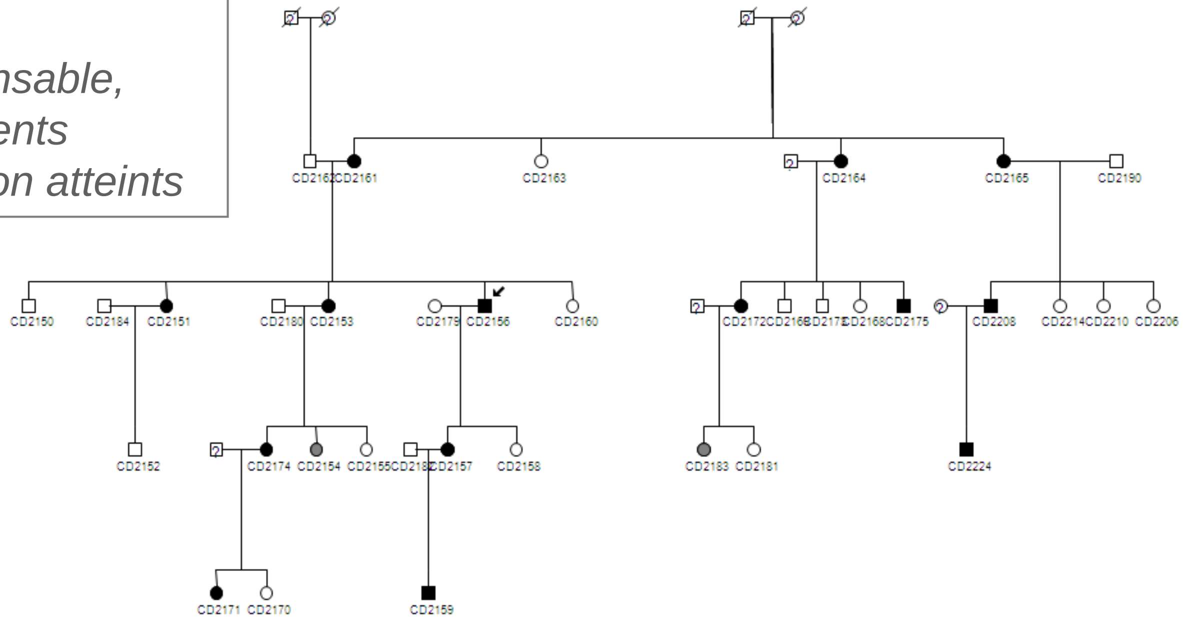
→

Maladie

Exemple: Syndrome du QT-Long

Etudes familiales

pour découvrir le gène responsable,
endommagé chez les patients
mais pas chez les apparentés non atteints



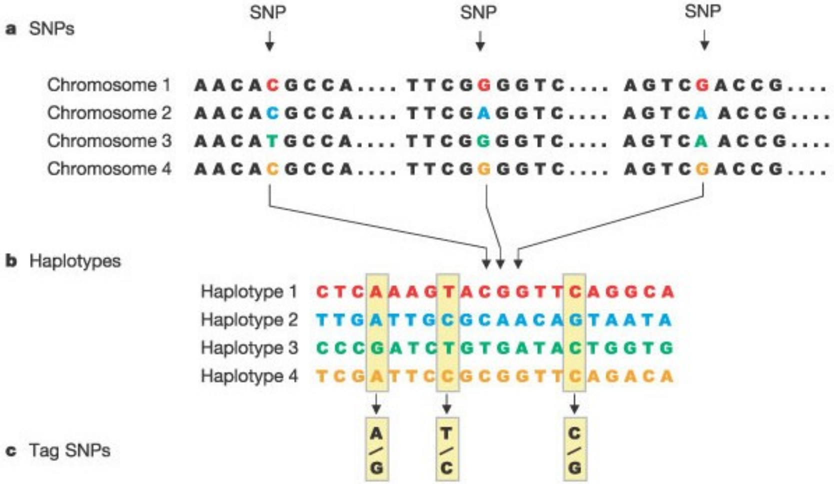
CD2161
CD2163
CD2164
CD2165
CD2190
CD2150
CD2184
CD2151
CD2180
CD2153
CD2179
CD2156
CD2160
CD2172
CD2168
CD2175
CD2208
CD2214
CD2210
CD2206
CD2152
CD2174
CD2154
CD2155
CD2157
CD2158
CD2183
CD2181
CD2224
CD2171
CD2170
CD2159

☐ ○ Non atteint
■ ● Atteint
▨ ● Indéterminé

Variations génétiques entre individus

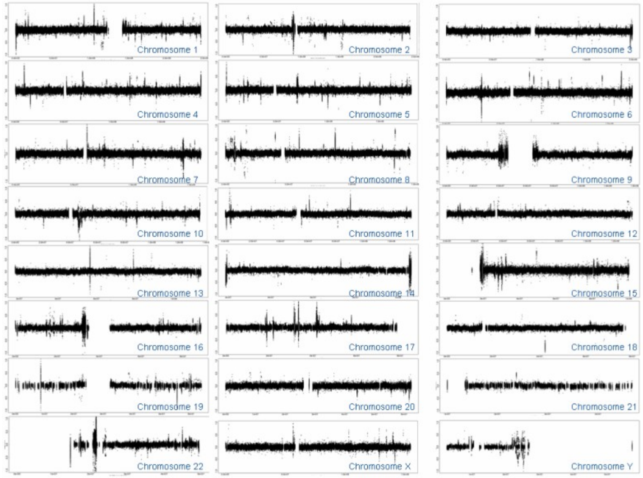
2005

International
HapMap
Project


a SNPs
SNP
SNP
SNP
Chromosome 1 AACACGCCA.... TTCGGGGTC.... AGTCGACCG....
Chromosome 2 AACACGCCA.... TTCGAGGTC.... AGTCA ACCG....
Chromosome 3 AACATGCCA.... TTCGGGGTC.... AGTCA ACCG....
Chromosome 4 AACACGCCA.... TTCGGGGTC.... AGTCGACCG....
b Haplotypes
Haplotype 1 CTCAAAGTACGGTTCAGGCA
Haplotype 2 TTGATTGCGCAACAGTAATA
Haplotype 3 CCCGATCTGTGATACTGGTG
Haplotype 4 TCGATTCCGCGGTTCAGACA
c Tag SNPs
A/G
T/C
C/G

Catalogue de variations génétiques ponctuelles dans les populations humaines

2006



Redon *et al*, *Nature* **444**:444-54

Premier catalogue
des variations de nombre de copies d'ADN

(pertes et gains de fragments d'ADN
observables dans la population générale)

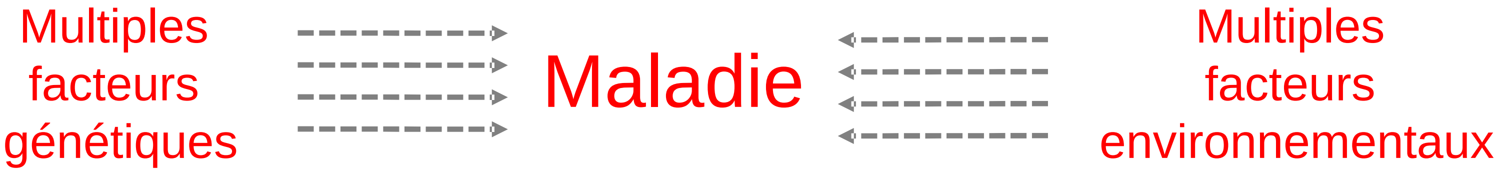
Recherche en génétique humaine

* **Maladies monogéniques**

maladies rares causées chacune par l’altération d’un seul gène

* **Maladies multifactorielles, ou complexes**

maladies plus fréquentes, dont l’apparition peut être associée
à de multiples facteurs génétiques et environnementaux



Multiples facteurs génétiques

Maladie

Multiples facteurs environnementaux

Exemples: diabète, hypertension artérielle

⇒ *De larges études comparant les gènes de patients*
avec ceux d’individus non atteints
permettent d’identifier des facteurs de risque

nature genetics

Genome-wide association study identifies a susceptibility locus at 21q21 for ventricular fibrillation in acute myocardial infarction

Connie R Bezzina[1,17], Raha Pazoki[1,2,17], Abdennasser Bardai[1,17], Roos F Marsman[1,17], Jonas S S G de Jong[3,17], Marieke T Blom[1], Brendon P Scicluna[1], J Wouter Jukema[4,5], Navin R Bindraban[3,6], Peter Lichtner[7], Arne Pfeufer[7,8], Nanette H Bishopric[9–11], Dan M Roden[12], Thomas Meitinger[7,8], Sumeet S Chugh[13], Robert J Myerburg[9], Xavier Jouven[14], Stefan Kääb[15], Lukas R C Dekker[3,16], Hanno L Tan[1,3], Michael W T Tanck[2] & Arthur A M Wilde[1,3]

Table 1 Baseline characteristics of the AGNES case-control set

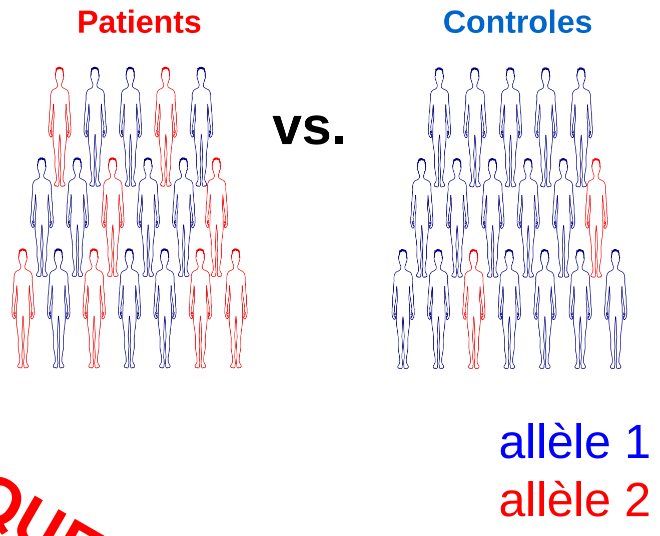
Characteristic	N^a	Total[b]	Cases[c]	Controls[d]	P^e
Sex (male)[f]		783 (80.6)	412 (80.0)	371 (81.2)	0.6850
Mean age at myocardial infarction[g]		56.4 ± 11	55.9 ± 11.2	56.9 ± 10.8	0.1740
Median ST segment deviation-mm (IQR)	735, 379, 356	17 (19)	22 (22)	14 (14)	<0.0001
Myocardial infarction location[f]	962, 505, 457				
Inferior myocardial infarction		408 (42.4)	203 (40.2)	205 (44.9)	0.1442[h]
Anterior myocardial infarction		554 (57.6)	302 (59.8)	252 (55.1)	
Median CK-MB-μg/l (IQR)	786, 359, 427	215 (299.2)	225.6 (365.2)	211.4 (257.7)	0.0210
Family history of sudden death[f]	909, 459, 450	291 (32.0)	174 (37.9)	117 (26.0)	<0.0001
Beta blocker usage[f]	936, 482, 454	85 (9.1)	50 (10.4)	35 (7.7)	0.1726
Cardiovascular risk factors[f]					
Current smoking	914, 473, 441	565 (61.8)	303 (64.1)	262 (59.4)	0.1531
Diabetes mellitus	893, 462, 431	62 (6.94)	21 (4.6)	41 (9.5)	0.0040
Hypertension		294 (30.2)	149 (28.9)	145 (31.7)	0.3633
Hypercholesterolemia		297 (30.6)	127 (24.7)	170 (37.2)	<0.0001
BMI[i]	879, 439, 440	26.5 ± 3.9	26.1 ± 3.8	26.9 ± 4.0	0.0042

IQR, interquartile range; CK-MB, creatine kinase-MB.

[a] In case of missing values, the sample sizes of the total, case and control sets (total, case, control) for whom information was available are given. [b] $n = 972$. [c] $n = 515$. [d] $n = 457$. [e] P value for comparison of cases and controls for each item. [f] Number (%). [g] Age (years) ± s.d. [h] P value for comparison of inferior and anterior myocardial infarction between cases and controls. [i] Kg/m^2 ± s.d.

ATTENTION : CES RESULTATS N'EXPLIQUENT PAS TOUT !

Analyse génétique
de centaines de patients
et de contrôles



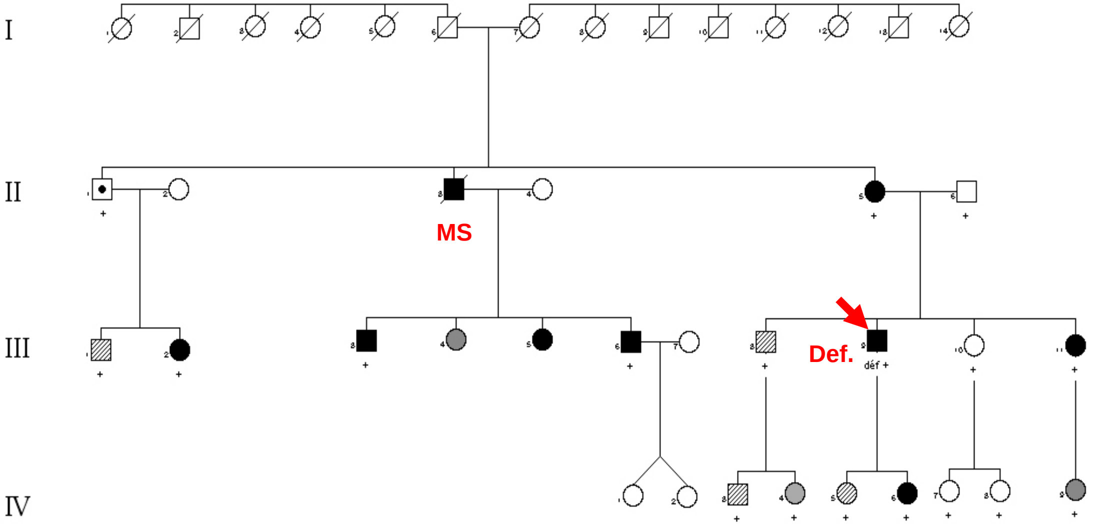
Marqueurs génétiques
du risque de mort subite
(effet très modéré)

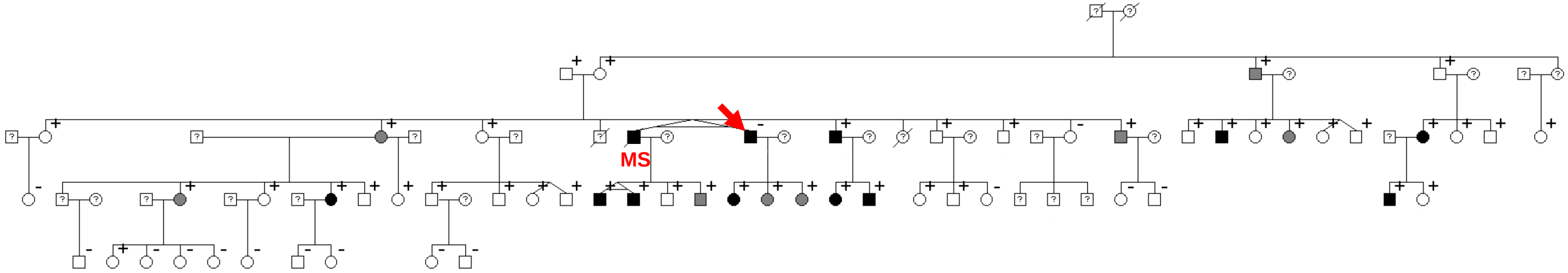
Table 2 Leading SNPs at the three loci most strongly associated with ventricular fibrillation in the GWAS in the AGNES case-control set

SNP[a]	Chromosome position	Position[b]	Minor allele	Major allele	Frequency of minor allele: Total	Cases	Controls	Odds ratio (95% CI)[c]	P^d	Closest genes[e]
rs2824293	21q21	17,709,385	G	A	0.47	0.53	0.39	1.78 (1.47–2.13)	3.34×10^{-10}	*CXADR, BTG3*
rs2824292	21q21	17,709,047	G	A	0.47	0.53	0.39	1.78 (1.47–2.13)	3.36×10^{-10}	*CXADR, BTG3*
rs1353342	9q21	78,064,589	A	C	0.23	0.07	0.14	0.46 (0.34–0.63)	3.34×10^{-7}	*PCSK5, RFK, GCNT1*
rs12090554	1q25	183,818,971	A	G	0.23	0.19	0.28	0.58 (0.47–0.72)	7.89×10^{-7}	*HMCN1, IVNS1ABP*

[a] Only rs2824293 was imputed; the rest of the SNPs were genotyped directly. [b] Based on Build 36.2. [c] Odds ratios are per copy of minor allele and are adjusted for age and sex. [d] The P values are corrected for genomic control factor. [e] Genes within an area of 1 Mb centered at the SNP are listed.

L’approche familiale: une bonne alternative

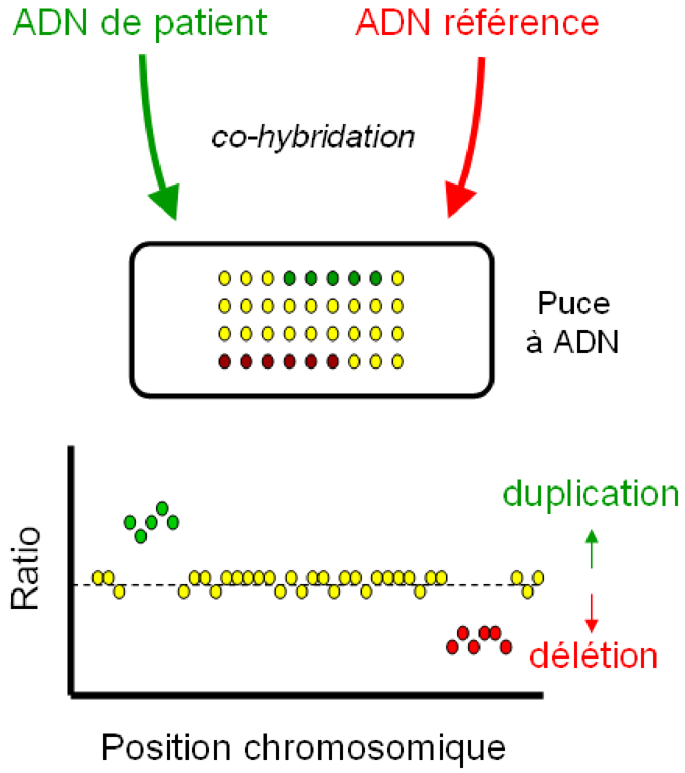

I
II
III
IV
MS
Def.
déf +



Recherche de nouveaux gènes endommagés chez les malades

Recherche de pertes / gains de gènes
chez les patients **par array-CGH**

Array-CGH:
Technique qui permet de détecter des pertes ou des gains de gènes sur l'ensemble du génome du patient analysé


ADN de patient
ADN référence
co-hybridation
Puce
à ADN
duplication
Ratio
délétion
Position chromosomique

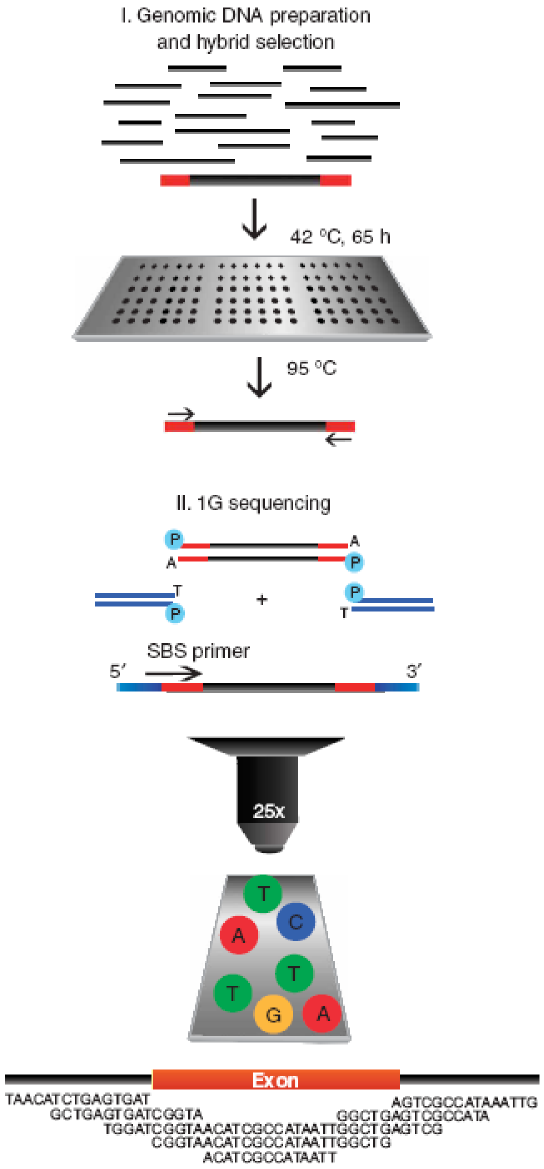
Recherche de nouveaux gènes endommagés chez les malades

Recherche de pertes / gains de gènes
chez les patients **par array-CGH**

Séquençage de (presque) tous les gènes

Analyse de quelques patients
sélectionnés dans de grandes familles
avec plusieurs patients atteints

➢ **identification de nouveaux gènes candidats**
à tester sur de larges cohortes de patients


I. Genomic DNA preparation and hybrid selection
42 °C, 65 h
95 °C
II. 1G sequencing
P
A
A
P
T
P
+
P
T
SBS primer
5′
3′
25x
T
C
A
T
T
G
A
Exon
TAACATCTGAGTGAT
GCTGAGTGATCGGTA
TGGATCGGTAACATCGCCATAATTGGCTGAGTCG
CGGTAACATCGCCATAATTGGCTG
ACATCGCCATAATT
AGTCGCCATAAATTG
GGCTGAGTCGCCATA

Génétique des maladies cardiovasculaires

Unité de Recherche:
Inserm unité 1087
JJ Schott
R Redon

Hôpital:
CHU de Nantes
H Le Marec
V Probst

l'institut du thorax

Axes de recherche

Nouvelles stratégies d'analyse sur formes familiales non élucidées

Nouvelles approches sur les formes sporadiques, plus fréquentes