Association studies on dilated cardiomyopathy

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Dilated Cardiomyopathy (DCM)

Familial form : ~35%

Linkage studies **Candidate genes**

> 50 genes involved in several pathways…

- Z Band
- Sarcomere
- Calcium Cycle
- Mb anchoring
- Stretch sensing …

…but no clear physiopathology

Sporadic form : ~65%

Unsuccessful/unreplicated candidate gene approaches

Need for **hypothesis free** methods

= Genome Wide Association Studies (GWAS)

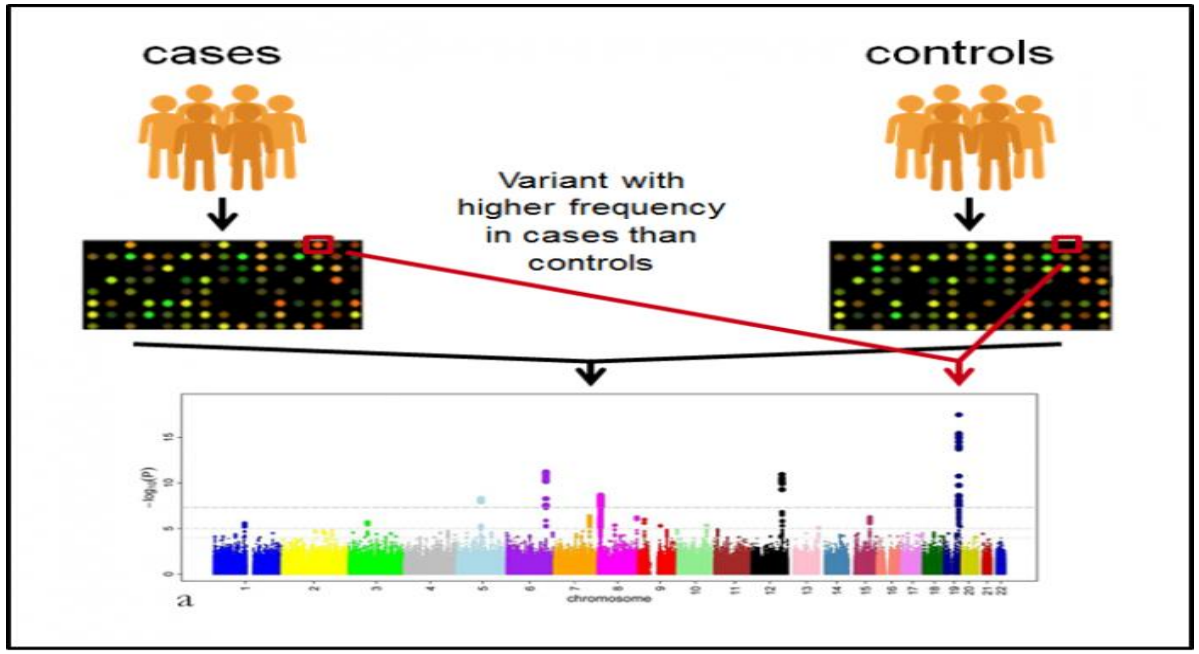
GWAS principle

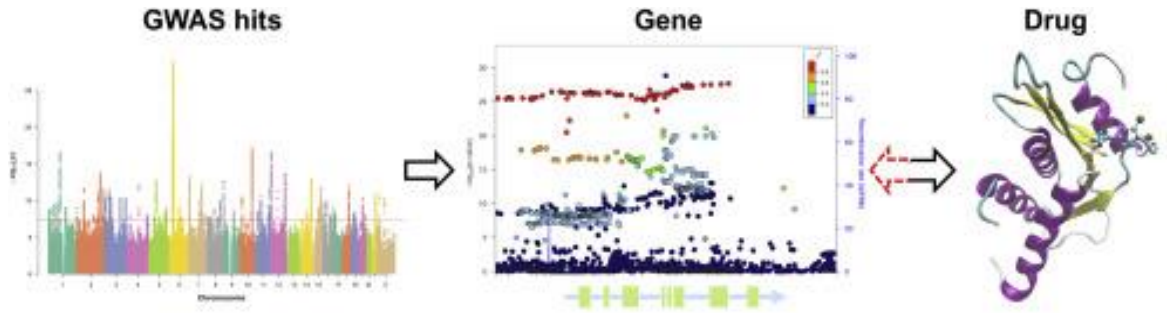
Compare allele frequency of millions of human polymorphisms between cases and controls for a given disease.

If a polymorphism or an haplotype is statistically at different frequency it suggested that the loci is implicated in the disease

Results are plotted as p-value for association vs genomic position, associated regions appears at peaks on the so-called Manhattan plot

Candidate gene in the regions are subjected to in silico/experimental analysis to identify pathways, mechanisms and drug targets



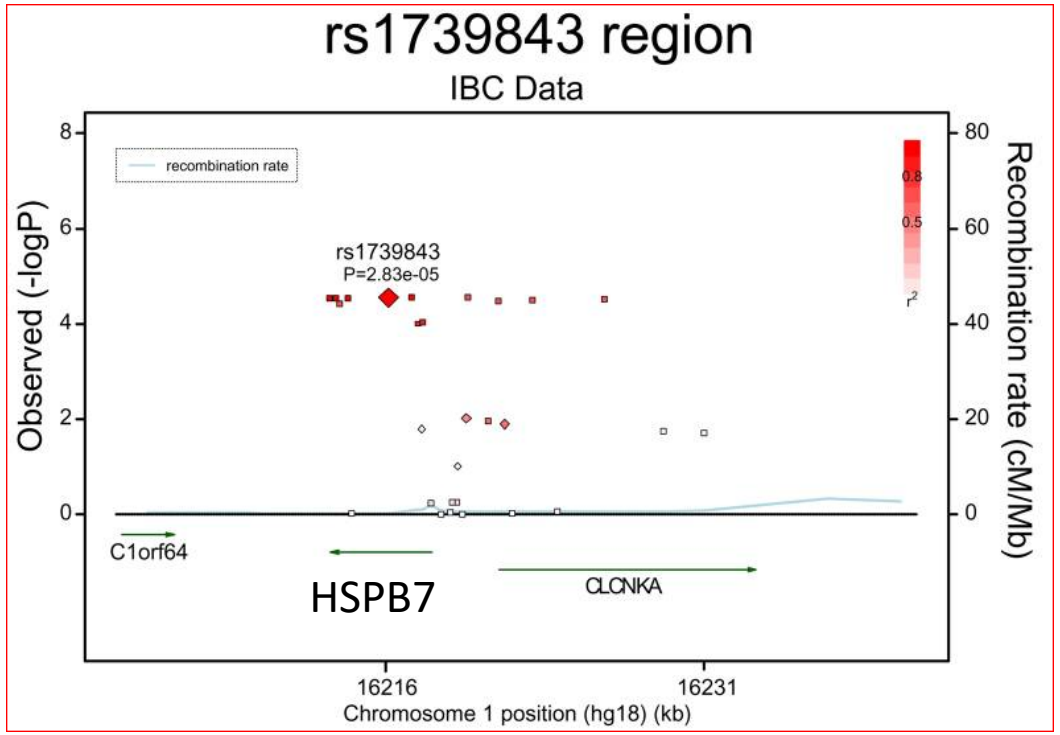


Trait	Gene with GWAS hits	Known or candidate drug
Type 2 Diabetes	*SLC30A8/KCNJ11*	ZnT-8 antagonists/Glyburide
Rheumatoid Arthritis	*PADI4/IL6R*	BB-Cl-amidine/Tocilizumab
Ankylosing Spondylitis(AS)	*TNFR1/PTGER4/TYK2*	TNF-inhibitors/NSAIDs/fostamatinib
Psoriasis(Ps)	*IL23A*	Risankizumab
Osteoporosis	*RANKL/ESR1*	Denosumab/Raloxifene and HRT

First large scale association studies on heart failure or DCM

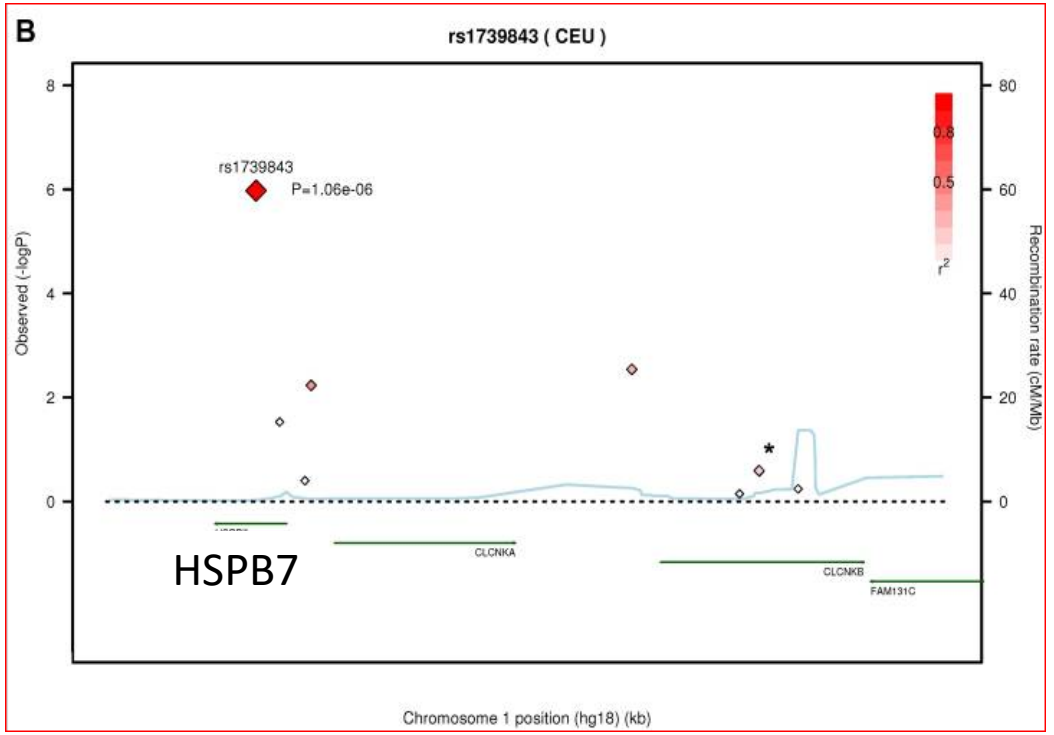
On 50K CardioChip (2000 « cardiovascular » genes)

1,590 HF (all causes) cases and 577 controls



Cappola et coll, Circ Cardiovasc, 2010

1,910 DCM cases and 3,630 controls



Stark et coll. PloS Genet, 2010

HSPB7 loci associated with all causes heart failure and DCM

First GWAS in DCM : *DNA Pooling & discovery/replication design*

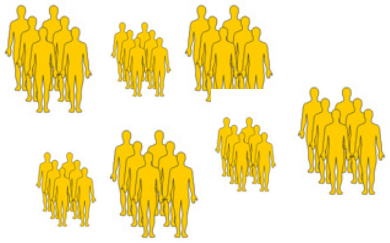
Discovery Phase
Pooled DNA

Discovery Phase
Individual DNA

Replication Phase
Individual DNA

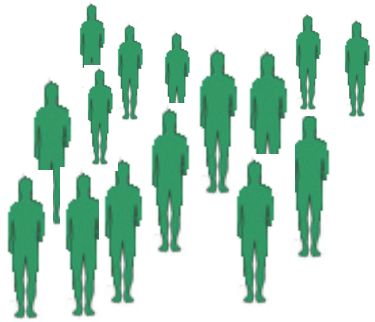
RESULT

1179 DCM
(Fr,Ger & It)

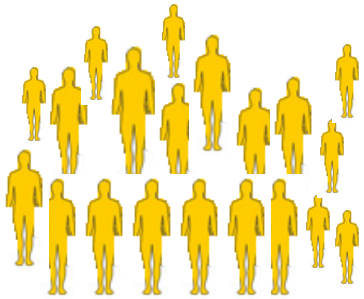


1108 controls

Illumina genotyping quad 610
(Allelic signal ratio frequency estimate)
SNP with p<5x10-7

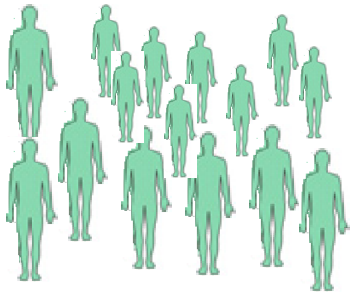


1179 DCM

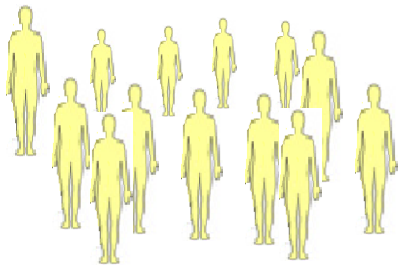


1108 controls

TaqMan individual genotyping for
SNP with p<5x10-7



1165 DCM
(Ger & UK)

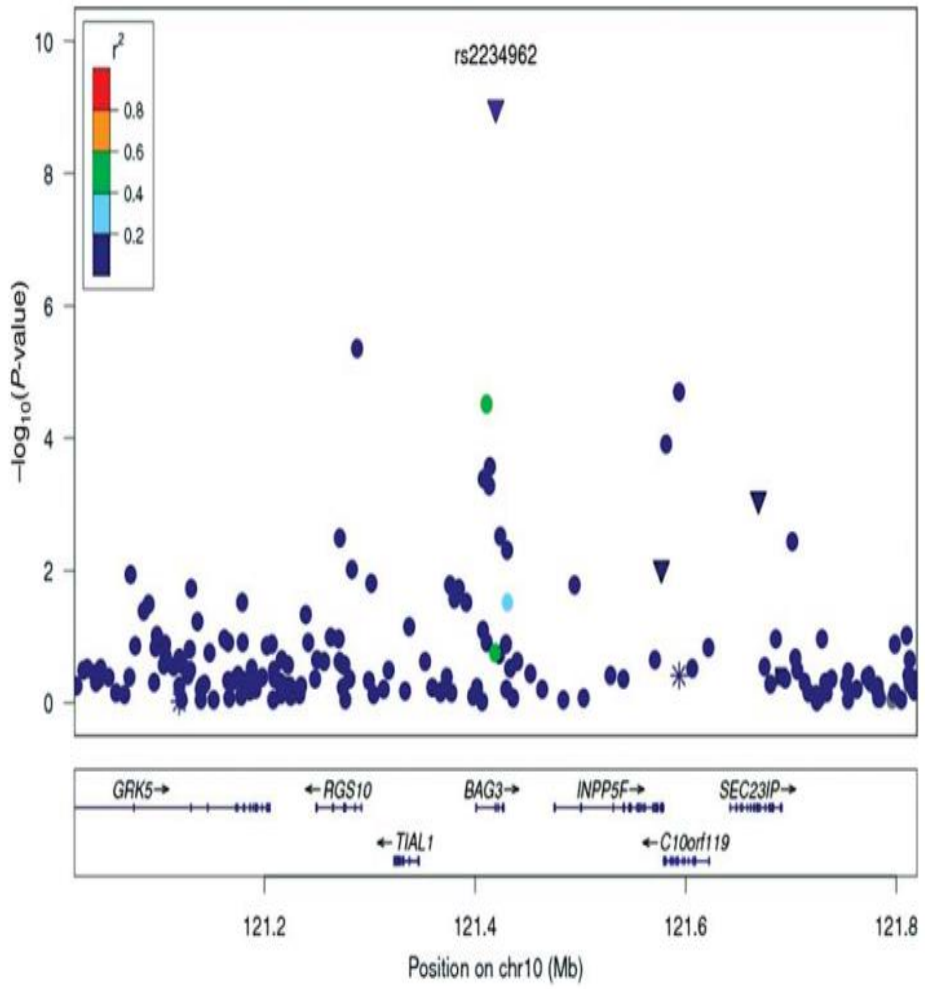


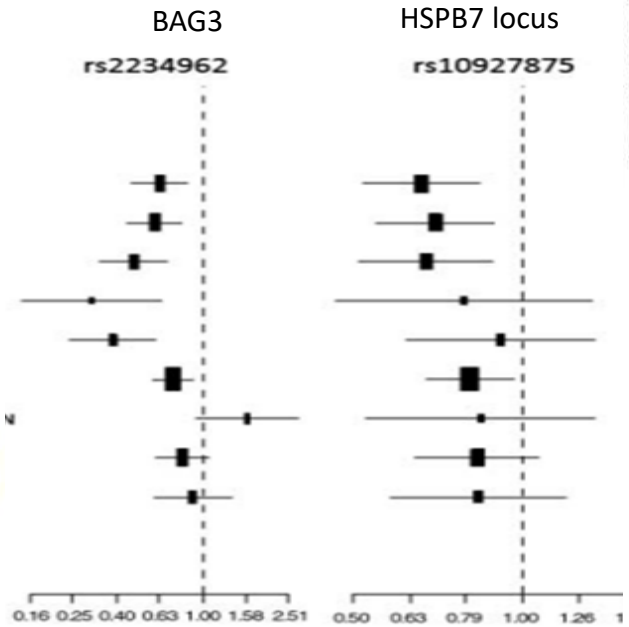
1302 controls

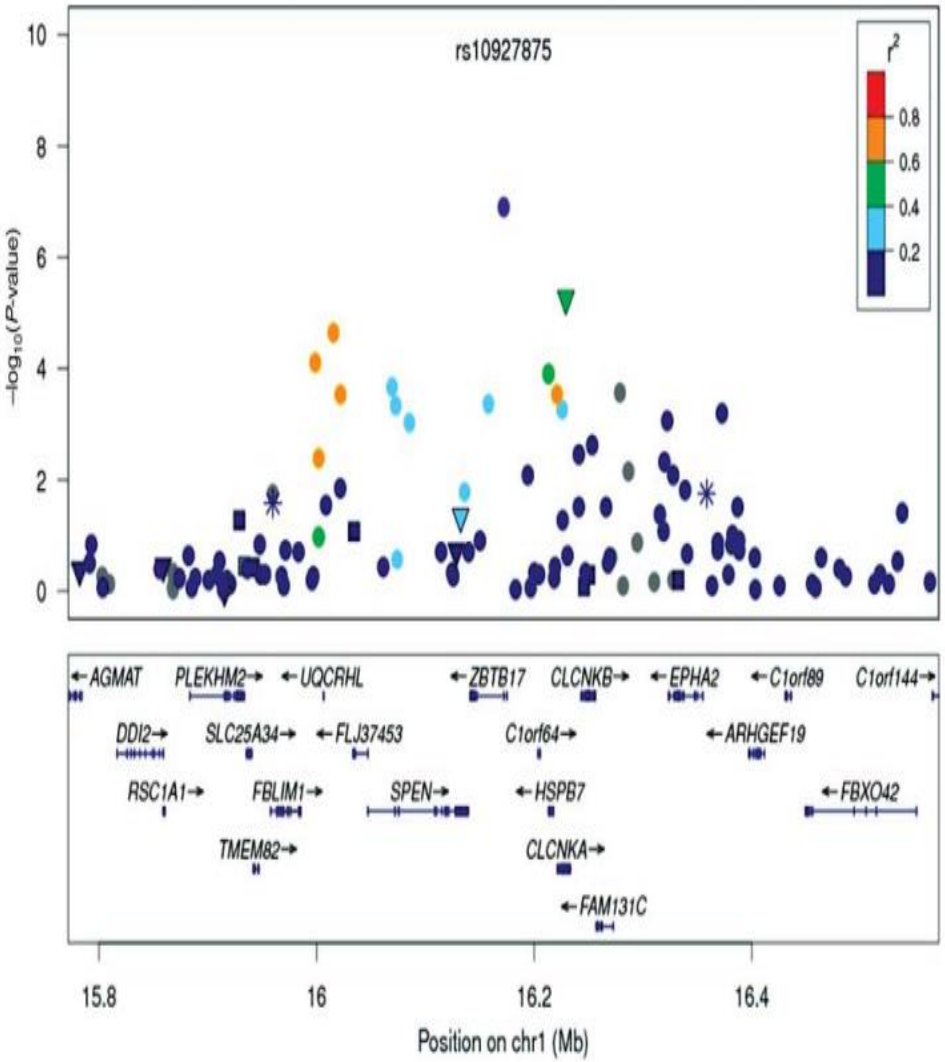
TaqMan genotyping for confirmed
SNP

Villard et al EHJ 2011

Pooled DNA GWAS on DCM: Results







OR:0.66
(95%CI:0.53-0.82)
P=4.0x10^{-12}

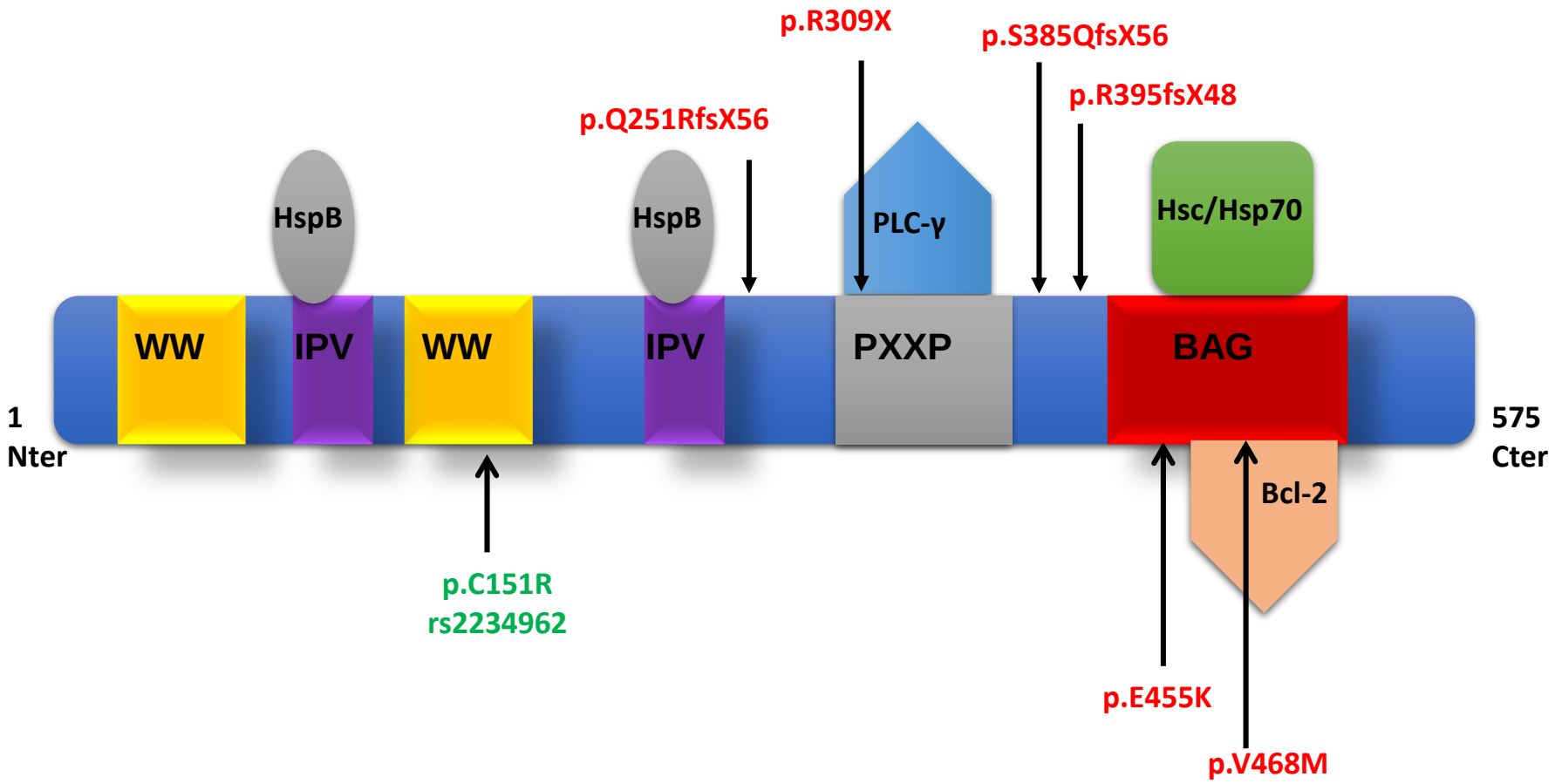
OR:0.76
(95%CI:0.70-0.84)
P=9.5x10^{-10}

Limitation: limited power due to pool-genotyping strategy

Villard et al EHJ 2011

BAG3 sequencing in FDCM

- 6 mutations found in 168 DCM sequenced individuals



BAG3 also involved in familial DCM.

Loss of autophagy function of BAG3 involved?

Exome-WAS in DCM

- Illumina Exome Chip (12v1.1) recapitulating human non-synonymous SNP content. This exome array targets putatively functional variants but it provides a limited coverage of genome variability (< 10% of the variants with a MAF > 1%)
- 89659 polymorphic variants analysed
- Mixed linear Model statistics accounting for genetic relatedness (principal component) and gender applied on each populations (n=6) separately and combined by **meta-analysis** (FDR Q-value < 0.01)

Table 1. Number of DCM patients and controls

	DCM n (women)	CONTROLS n (women)
France	706 (149)	3677 (1394)
Germany	1161 (205)	1830 (959)
UK	96 (20)	89 (19)
USA1	119 (43)	189 (144)
Italy	83 (15)	92 (23)
USA2	631 (211)	1000 (506)
All	2796 (643)	6877 (3045)

Limitations:

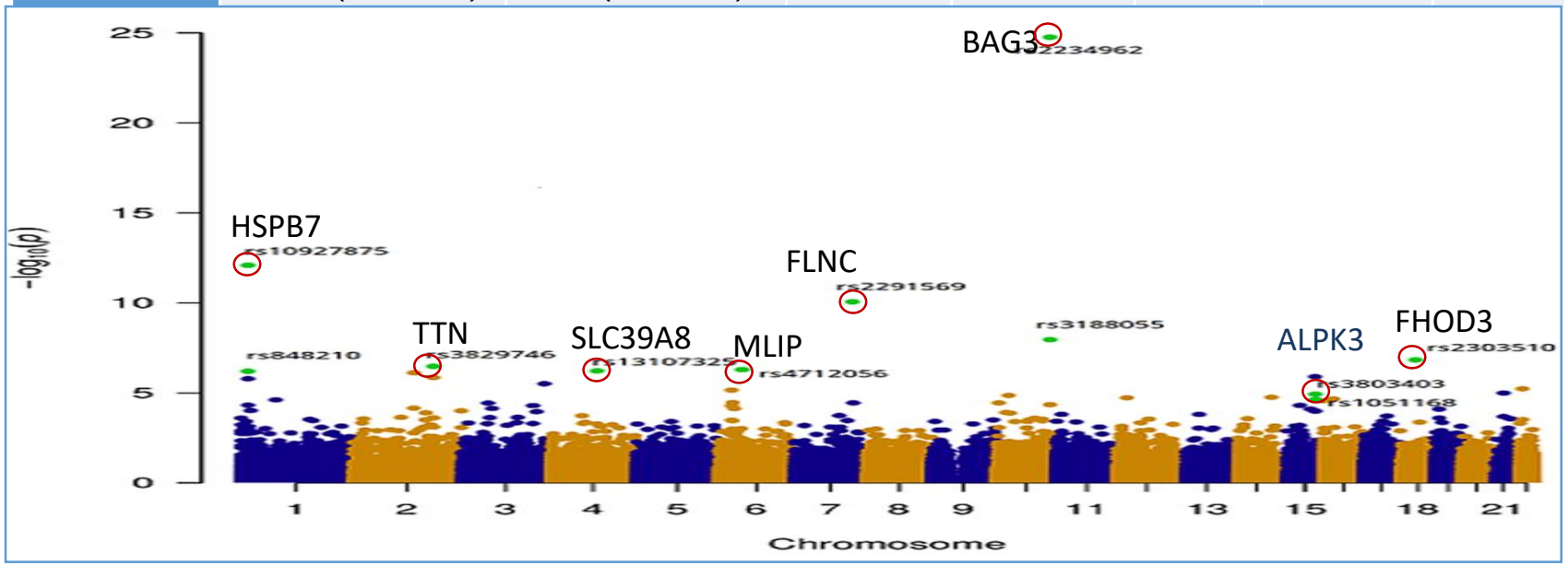
- meta-analysis rather than discovery-replication design
- Partial coverage

Esslinger U Plos One 2017

Results for the 8 lead-SNPs in associated loci

	MAF	OR (95% CI)	P-value	Q-value	P-Het	Gene	AA chg
rs10927875	A: 0.31 (0.26-0.37)	0.768 (0.71-0.83)	$8.1x10^{-13}$	$3.9x10^{-08}$	0.83	HSPB7	-
rs3829746	G: 0.23 (0.21-0.24)	0.810 (0.75-0.88)	$3.4x10^{-07}$	$4.6x10^{-03}$	0.039	TTN	I/V
rs13107325	A: 0.08 (0.07-0.12)	1.348 (1.20-1.52)	$6.0x10^{-07}$	$6.0x10^{-03}$	0.61	SLC39A8	A/T
rs4712056	G: 0.35 (0.34-0.36)	1.191 (1.11-1.28)	$5.1x10^{-07}$	$6.0x10^{-03}$	0.53	MLIP	V/I
rs2291569	A: 0.08 (0.05-0.09)	0.651 (0.57-0.74)	$8.7x10^{-11}$	$2.8x10^{-06}$	0.27	FLNC	R/Q
rs2234962	G: 0.19 (0.15-0.22)	0.620 (0.57-0.68)	$1.7x10^{-25}$	$1.6x10^{-20}$	0.14	BAG3	C/R
rs3803403	G: 0.30 (0.28-0.35)	1.276 (1.16-1.40)	$2.9x10^{-07}$	$4.0x10^{-03}$	0.29	ALPK3*	T/S
rs2303510	A: 0.31 (0.29-0.34)	0.824 (0.77-0.89)	$1.5x10^{-07}$	$2.3x10^{-03}$	0.023	FHOD3	V/I

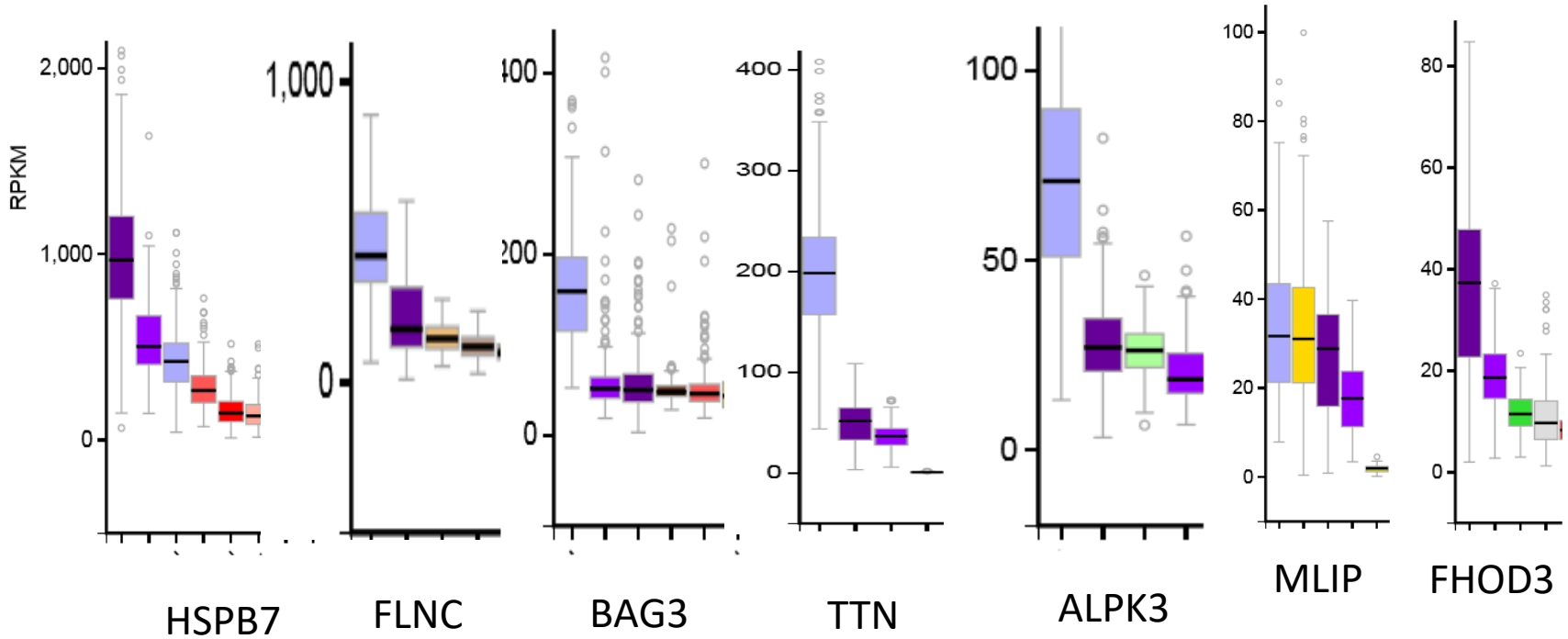
*recessive model



Esslinger U Plos One 2017

Esslinger et coll, PlosOne 2017

Positional candidate expression profils from GTEx RNA-Seq



Ventricle

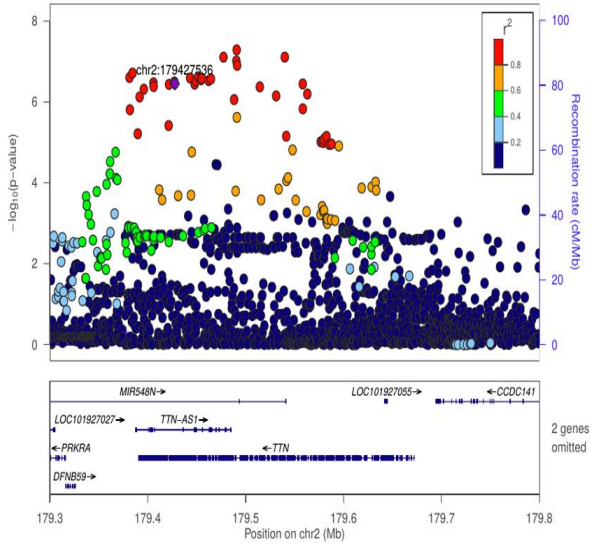
Atrial App.

Sk. muscle

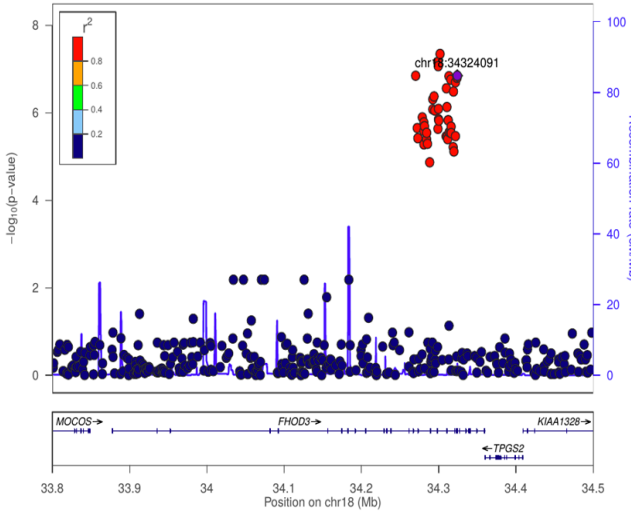
All but one are strongly expressed in striated muscle

Regional Plots after imputation (ExomeWAS)

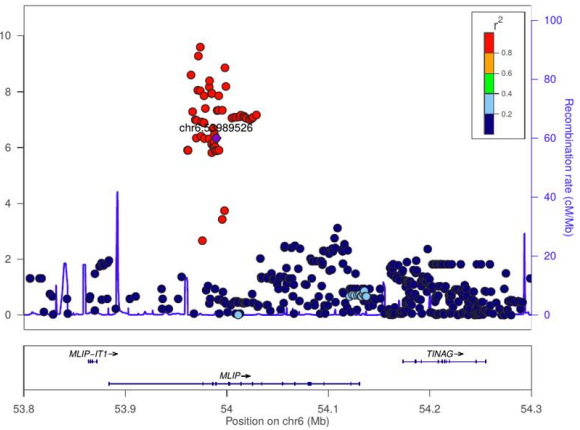
Locus chr2 (TTN)



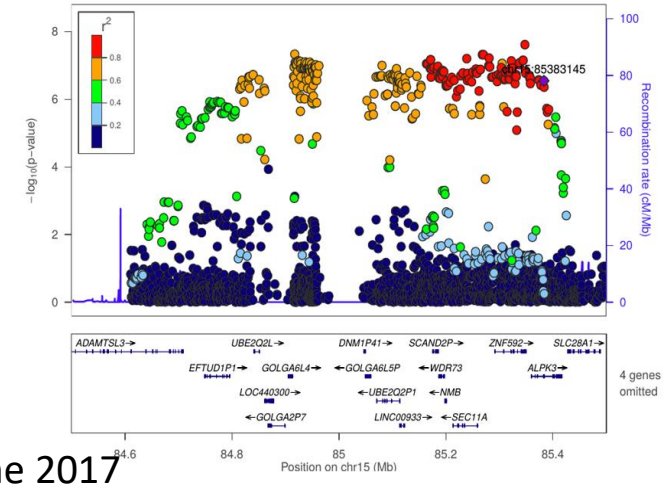
Locus chr18 (FHOD3)



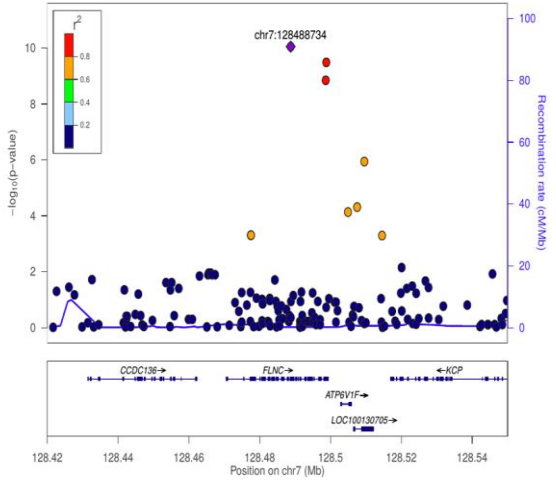
Locus chr6 (MLIP)



Locus chr15 (ALPK3)



Locus chr7 (FLNC)

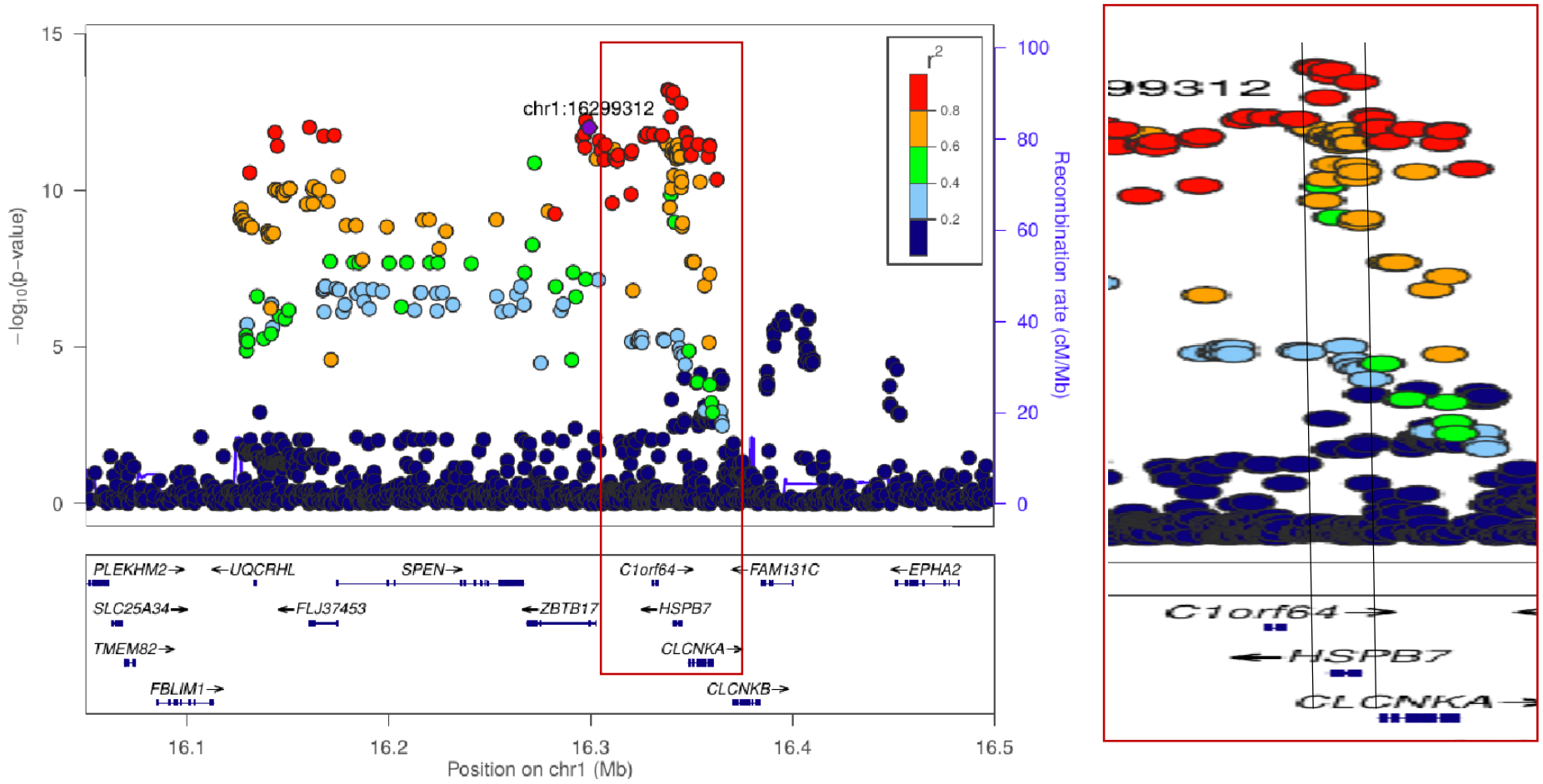


All leaders are Coding SNPs

4 are in a unique-gene restricted LD block (except ALPK3)

Esslinger U Plos One 2017

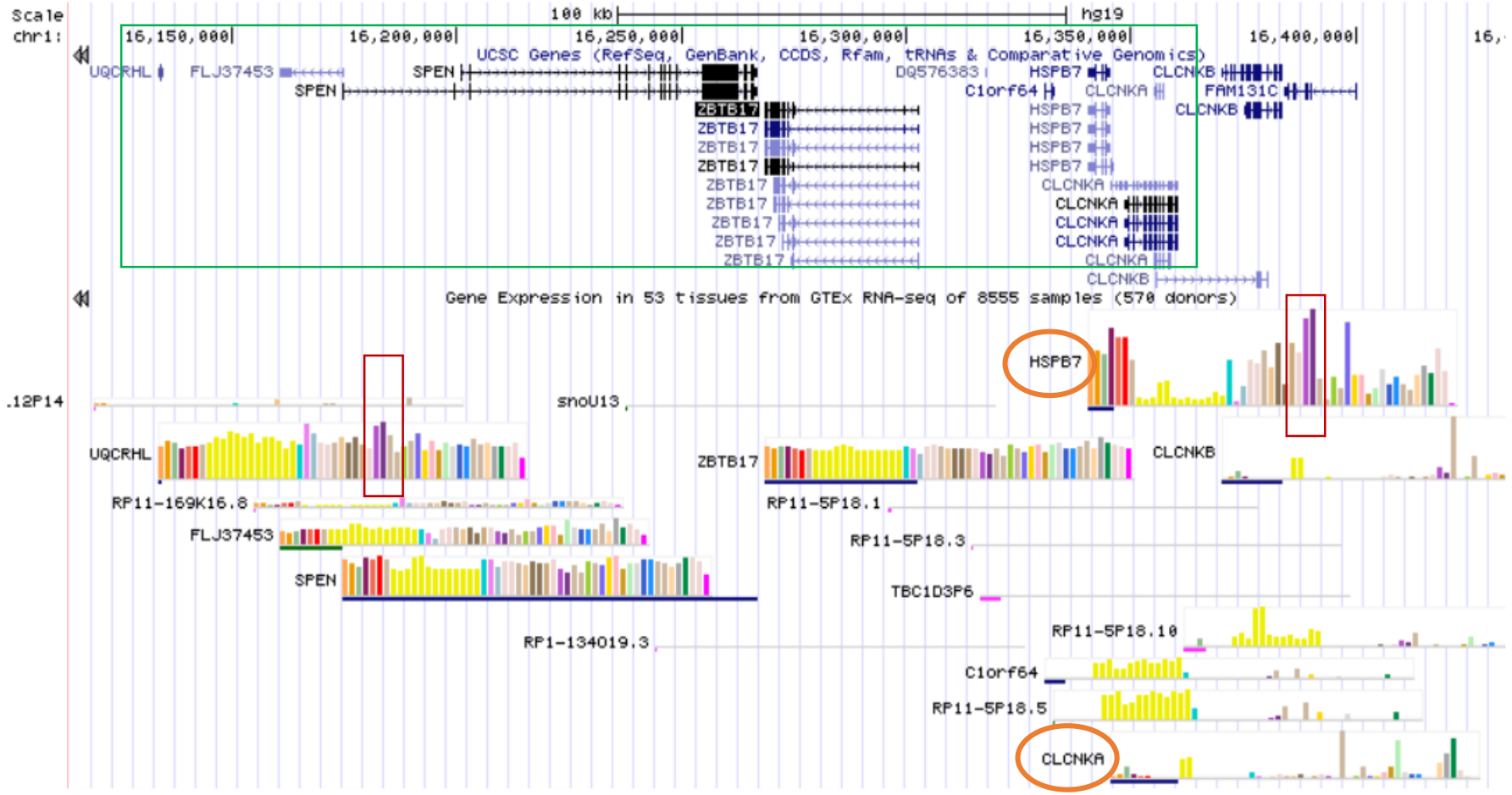
Regional plot after imputation at HSPB7/ZBTB17 locus



HSPB7 SNPs : the strongest associations

Esslinger et coll, PlosOne 2017

Comparative expression at the HSPB7 locus



HSPB7, the best regional candidate?

- Cardiac expression, but no eQTL
- Bibliographic evidencies

In Zebrafish: Hspb7 is a cardioprotective chaperone facilitating sarcomeric proteostasis (Mercer et al ; Dev Biol; 2018)

In Mice: Cardiac-specific loss of HSPB7 globally or specifically in cardiomyocytes resulted in embryonic lethality (Wu PNAS 2017)

In Mice: loss of HSPB7 in cardiomyocytes results in rapid heart failure and sudden death […] filamin C, an interacting protein of HSPB7, is upregulated and aggregated in HSPB7 mutant cardiomyocytes (Liao; Plos Genet 2017)

Exome-WAS: summary

- Identification of 6 novel loci associated with sporadic DCM
- Confirmation for two previously reported associations with the HSPB7 locus and BAG3 gene.
- 7 of the DCM-associated genes are very plausible candidates from a pathogenic perspective.
- In addition, HSPB7, BAG3, TTN, FHOD3 and FLNC share functional roles suggesting that proteostasis plays a central role in DCM pathophysiology.

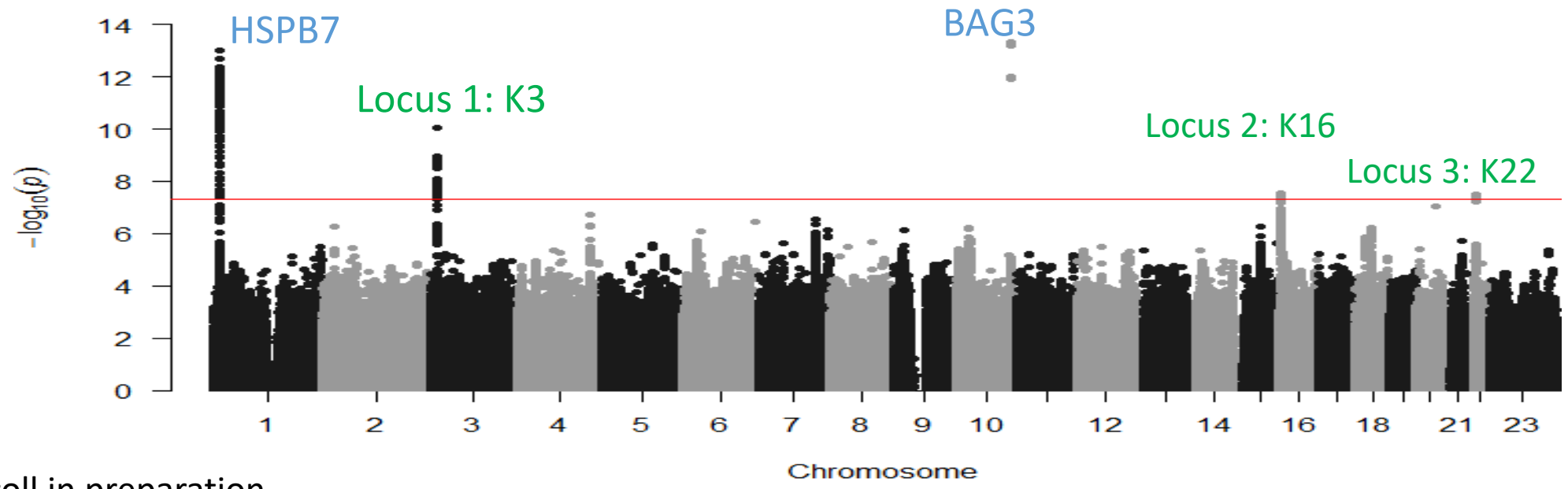
GWAS followed by Imputation : ongoing project

Rational for a 3rd GWAS

- Increased cases and controls numbers (vs GWAS1)
- Individual genotyping (vs GWAS1)
- Genome-Wide chip OmniExpress (vs Exome-WAS)
- Discovery/Replication design (vs Exome-WAS)

Standard GWAS on 2651 Cases / 4329 Controls on Whole Genome Chip and imputation with 1000Genomes (~10M SNPs); mixed linear model & adjustment on sex and 12 PCs

Manhattan plot: 9,152,884 SNPs



Garnier et coll in preparation

Cohorts description

Country	Case	Array/Centre	Controls	Array/Centre
France	676	Illumina HumanOmniexpress24 713, 014 SNPs (CNG, GENMED)	1085	Illumina HumanOmniexpress24 713, 014 SNPs (CNG, GENMED)
England	112	Illumina HumanOmniExpressExome8-v1-2 964 193 (273 246 in exon) (C. Proust)		
Germany	1118	Illumina HumanOmniexpress24 713, 014 SNPs (CNG, GENMED)	3264 (KORA)	Illumina Omni2.5 & omniExpress 587 050 genotyped SNPs 17 842 083 imputed SNPs
	82	Illumina HumanOmniExpressExome8-v1-2 964 193 (273 246 in exon) (CNG, GENMED)		
USA	631	Illumina HumanOmniExpressExome8-v1_A 951 117 SNPs		
Italy	82	Illumina HumanOmniexpress24 713, 014 SNPs (CNG, GENMED)	92 (EHF)	Illumina HumanOmniexpress24 713, 014 SNPs (CNG, GENMED)
Total	2641		4441	

Associated Loci :discovery and replication

Discovery Phase

Loci				
Chromosome	K3	K3	K16	K22
Discovery				
Allelic OR [95% CI]	1.36 [1.24-1.49]	1.28 [1.17-1.40]	1.35 [1.21-1.50]	1.32 [1.20-1.46]
P	**$8.7\ 10^{-11}$**	**$8.4\ 10^{-9}$**	**$3.0\ 10^{-8}$**	**$3.3\ 10^{-8}$**

Replication Phase

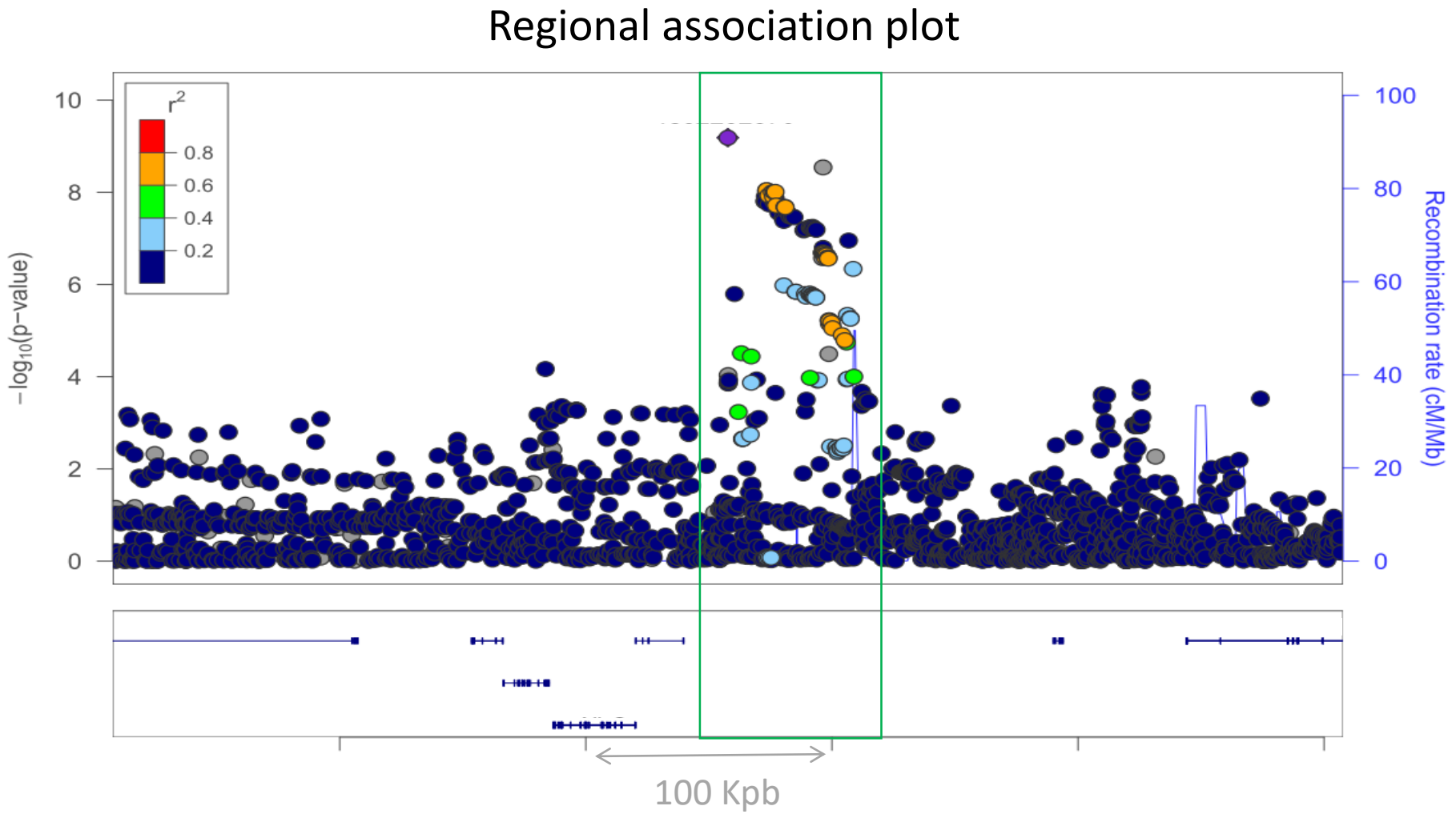
Loci					
	Chromosome	K3	K3	K16	K22
Replication					
Dutch study **145 cases** **527 controls**	AF	0.28	0.61	0.80	0.79
	Imputation r2	0.95	0.99	0.92	0.99
	Allelic OR [95% CI]	1.42 [0.99-2.04]	1.47 [1.07-2.03]	1.14 [0.76-1.72]	1.58 [1.08-2.31]
	P	**0.030**	**$7.7\ 10^{-3}$**	0.258	**$8.2\ 10^{-3}$**
German study **439 cases** **439 controls**	AF	0.22	0.71	0.84	0.82
	Imputation r2				
	Allelic OR [95% CI]	1.36 [1.08-1.71]	1.19 [0.96-1.46]	1.13 [0.88-1.46]	1.26 [0.99-1.61]
	P	**$5.6\ 10^{-3}$**	0.046	0.172	0.031

Combined

Allelic OR [95% CI]	1.36 [1.25-1.48]	1.28 [1.18-1.38]		1.33 [1.21-1.45]
P	**$5.9\ 10^{-13}$**	**$2.9\ 10^{-10}$**		**$6.3\ 10^{-10}$**

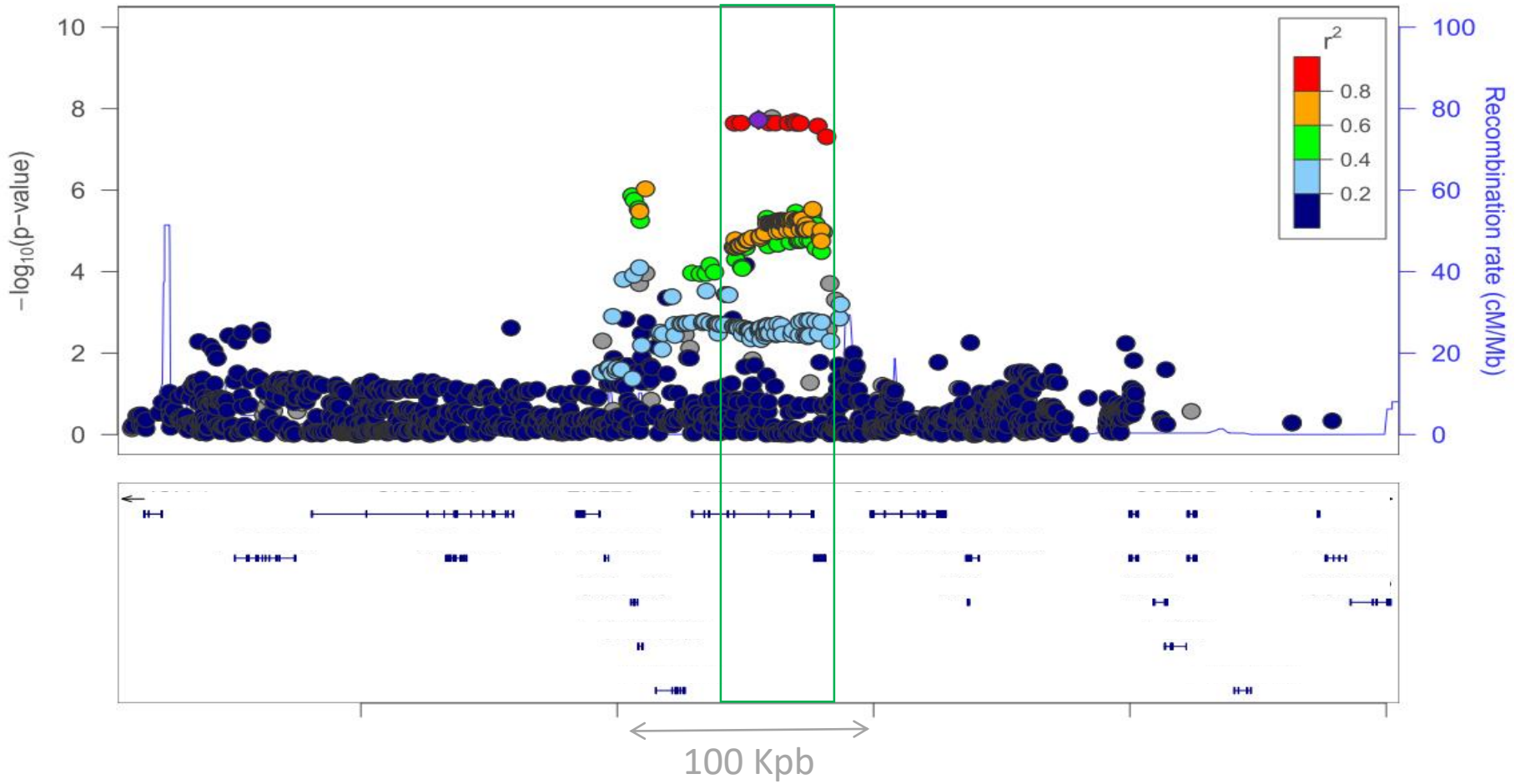
Garnier et coll in preparation

GWAS3-association region K3



GWAS3-association region K22

Regional association plot

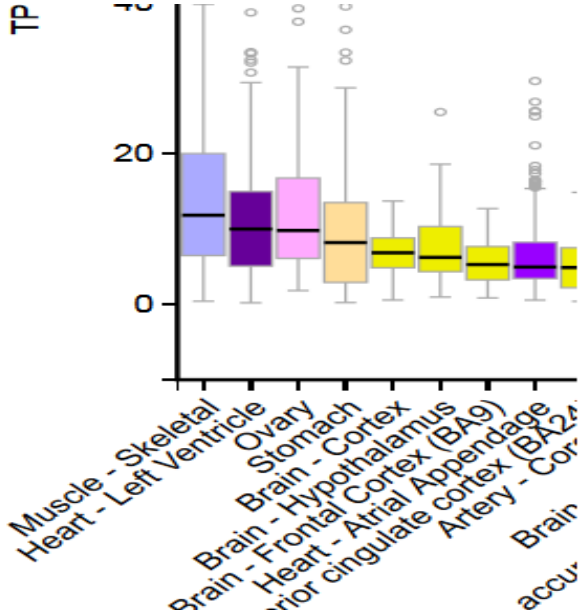


Follow-up strategy on GWAS3

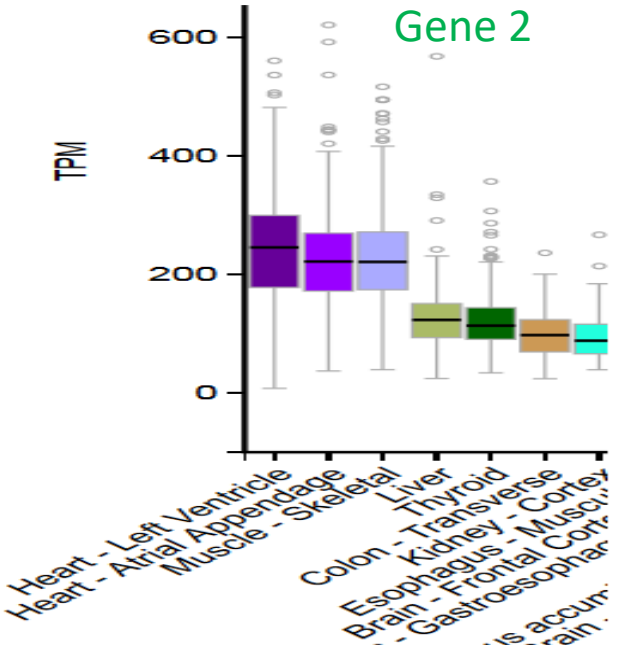
Lead SNPs at K3 and K22: are they

- In “good” regional candidate genes (expression pattern, known function…)

Gene 1



Gene 2



Some are significantly expressed in Heart

Follow-up strategy on GWAS3

Lead SNPs at K3 and K22: are they

- In “good” candidate genes (expression pattern, known function…)

  Some genes are significantly expressed in Heart

- Coding or in high LD (r2 > 0.8) with coding SNPs?

  None of the LD SNPs are coding at K3 or K22

Follow-up strategy on GWAS3

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- **eSNPs** or in high LD ($r^2 > 0.8$) with eSNPs?

  Information to be extracted from Gtex database or other RNA seq effort on heart samples.

Follow-up strategy on GWAS3

Lead SNPs at K3 and K22: are they

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  Some genes are significantly expressed in Heart

- Coding or in high LD (r2 > 0.8) with coding SNPs?

  None of the LD SNPs are coding at K3 or K22

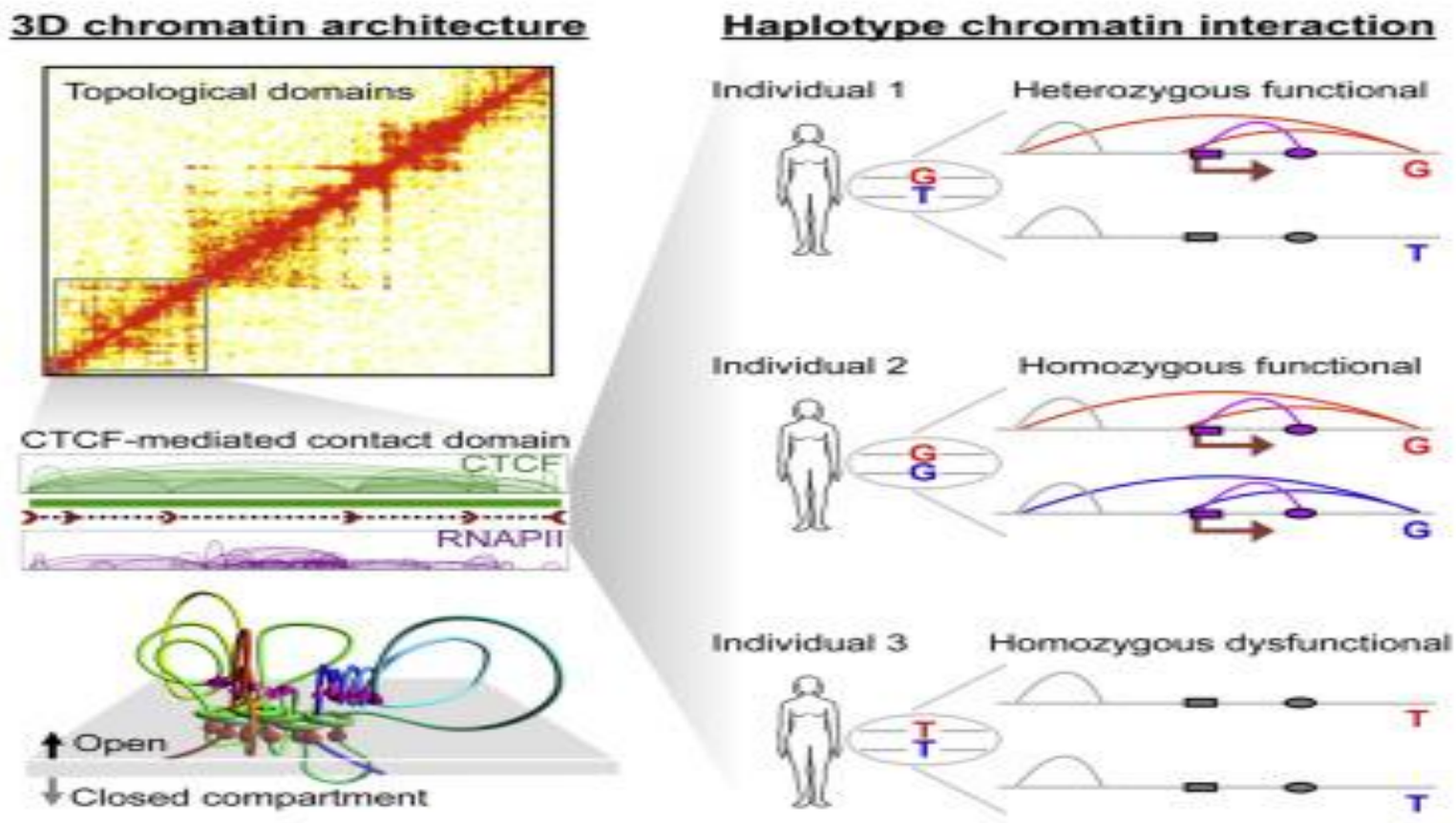
- **eSNPs** or in high LD ($r^2 > 0.8$) with eSNPs?

  Information to be extracted from Gtex database or other RNA seq effort on heart samples

- Involved in **chromatin interactions** between SNPs and positional candidate define by TAD (topology associated domains)?

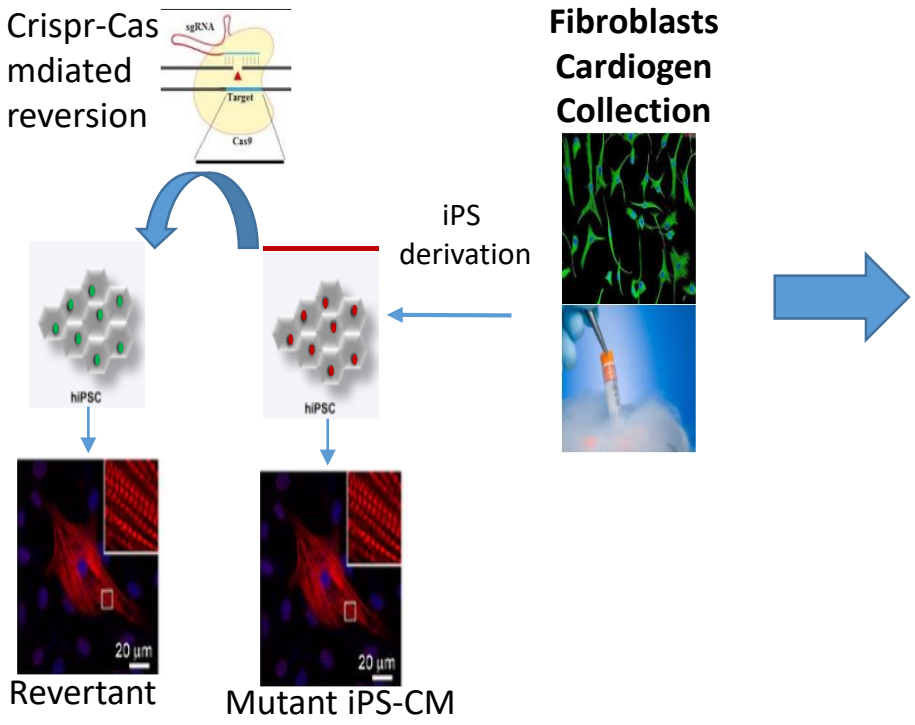
Chromatine interactions in TAD delimited candidates

Objective: identify chromatin interaction and differencies at the allele specific level

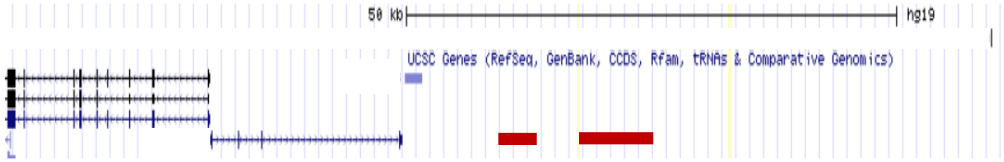


iPS clones selection for Allele specific chromatin interactions

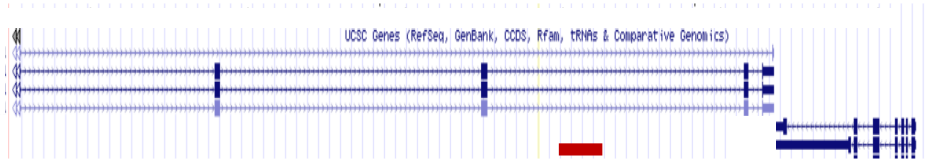
iPS project on HCM



12 salmples DNA **genotyped** (PCR +Sanger)
for K3 and K22 locus



	RsX	RsY
myH05	G/A	G/C
myH08	G/A	G/G



	rsW	rsZ
myH05	C/G	T/C
myH06	C/G	T/C
myH07	C/G	T/C
myH08	C/G	T/C

Clones 5 & 8 Cardiomyocytes (3 wk differentiation)
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F Cambien
E Villard

Patients, controls

Cardiogenics
Eurogene Heart Failure
PHRC-CMD
CARDIGENE
Inheritance EU-FP7
Insight-DCM

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