Using WES in distant relationships to identify cardiomyopathy genes

Robert M. (Bob) Hamilton
Professor and Senior Associate Scientist
The Hospital for Sick Children & Research Institute

Genetics Home Reference

Your Guide to Understanding Genetic Conditions

familial dilated cardiomyopathy

Learn more about the genes associated with familial dilated cardiomyopathy

ABCC9 ACTC1 ACTN2 ANKRD1 BAG3 CRYAB CSRP3 DES DMD

DSG2 EYA4 LAMA4 LDB3 LMNA MYBPC3 MYH6 MYH7 MYPN

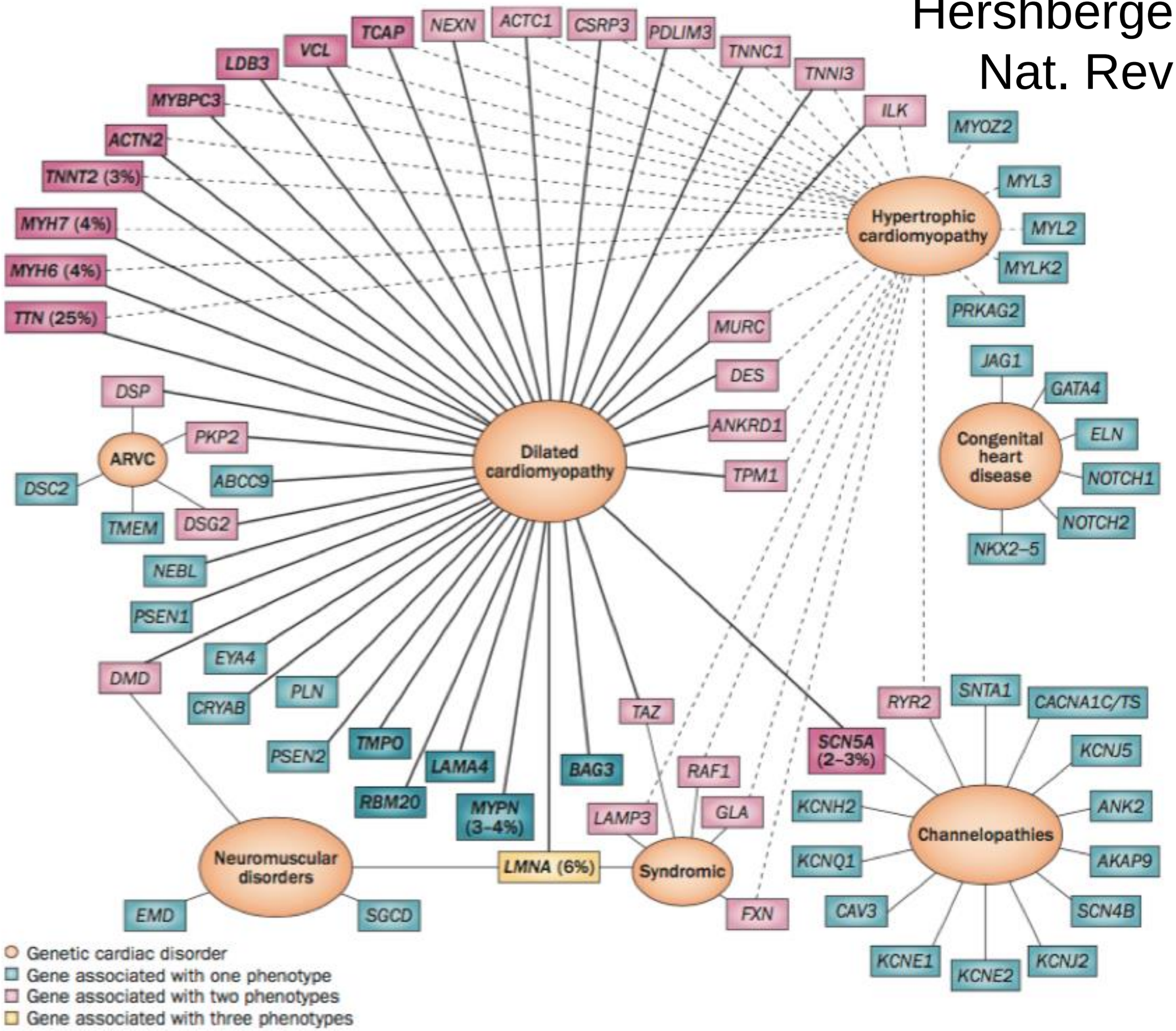
PLN PSEN1 PSEN2 RBM20 SCN5A SGCD TAZ TCAP TMPO

TNNC1 TNNI3 TNNT2 TPM1 TTN VCL

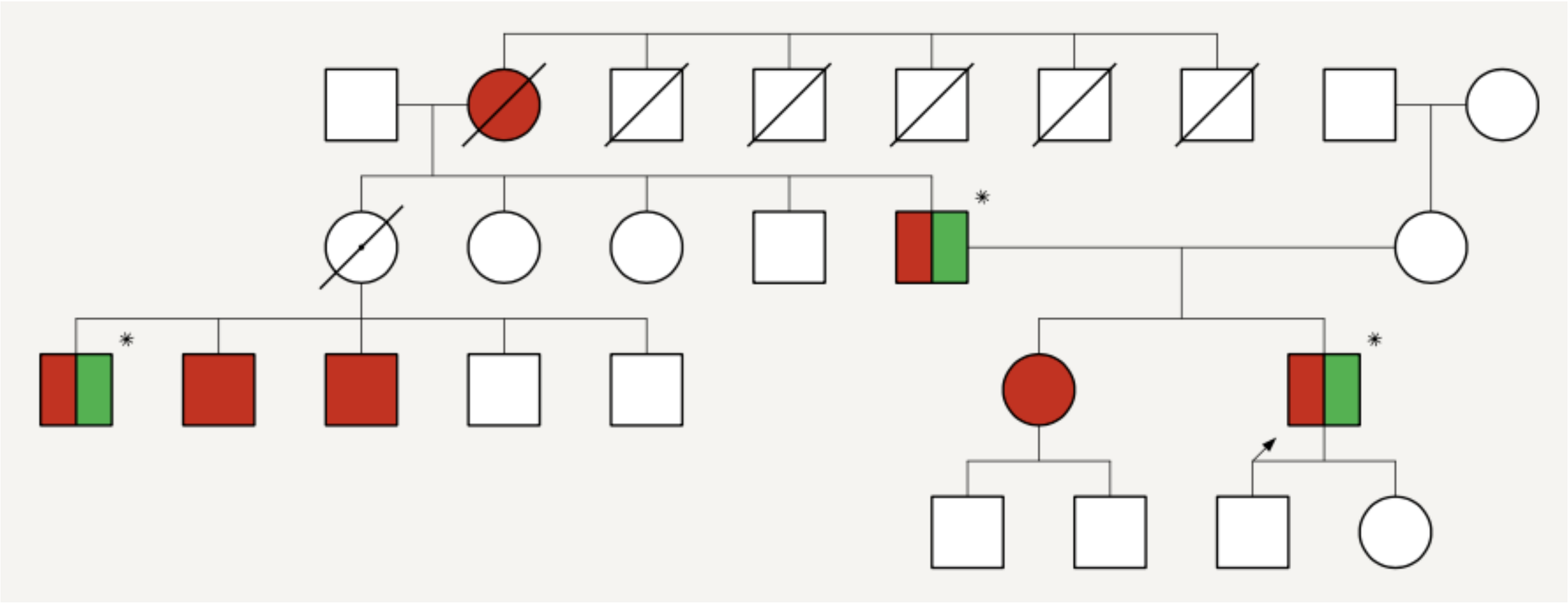
Hereditary Cardiomyopathies

- Dilated Cardiomyopathy (dilated LV and ↓ function)
  - Ischemic, Inflammatory/Immune, Hereditary (25-50% of DCM)
  - Identified Genes in 40% of Hereditary DCM
- Arrhythmogenic Cardiomyopathy
  - Ventricular Arrhythmias (predominantly from RV)
  - Fibrofatty Myocardial Replacement (predominantly of RV)
- Other:Hypertrophic (~75% gene-ID’ed), Restrictive (rare)

Hershberger et al.
Nat. Rev. 2013



DCM Family 1



- A multiplex family with dilated cardiomyopathy (red) resulting in cardiac transplantation in 3 (green)
- Oldest generation died without sampling; youngest generation does has not yet manifest a phenotype; * indicates DNA available
- Thus, traditional segregation analysis will not inform gene discovery

Select the reference allele field from the marker map

C Reference | Select Map Field

Check genotype patterns for at least one sample

249468

☐ Ref_Ref ☑ Alt_Ref ☐ Alt_Alt ☐ ?_?

249552

All: 3 x 110,793
Active: 3 x 8,031

Unsort		G 57994	G 57995	G 57996	G 57997
Map	Samples	10:121429256-SNV	10:121435955-SNV	10:121435979-Del	10:121436362-SNV
1	249468	?_?	C_C	-_C	G_G
2	249552	A_G	C_C	-_C	G_G
3	250032	?_?	C_C	-_C	G_G

All variants will be annotated against:
ExACVariantFrequencies0.3-BROAD_2015-01-13_GRCh_37_(

☑ Filter

Remove variants
- ○ Found in source
- ○ Absent in source
- ◉ According to frequency/MAF thres

Alt Allele Freq (AF) | is | >= | 0.00005

All: 3 x 2,998
Active: 3 x 2,998

Unsort		G 1592	G 1593	G 1594	G
Map	Samples	10:121429256-SNV	10:121435979-Del	10:123277667-Ins	
1	249468	?_?	-_C	-_TA	
2	249552	A_G	-_C	-_TA	
3	250032	?_?	-_C	-_TA	

Coding Variant Classification filtering options

☑ Remove Non-coding variants

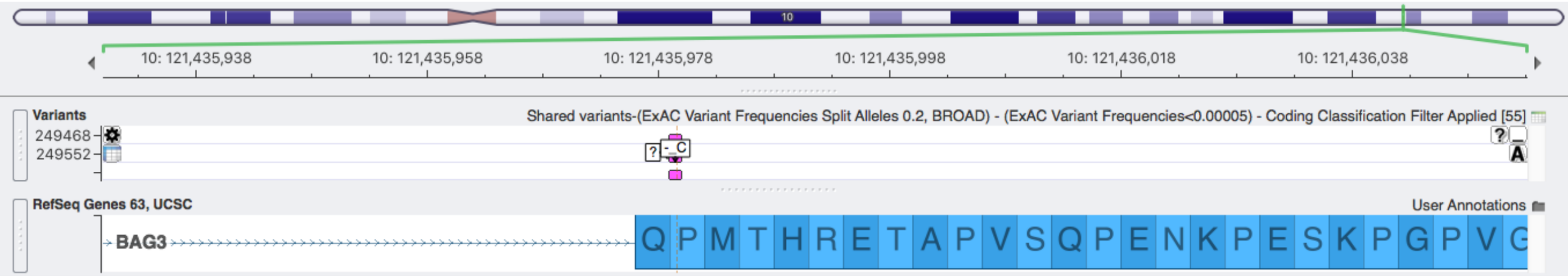
Keep the following c

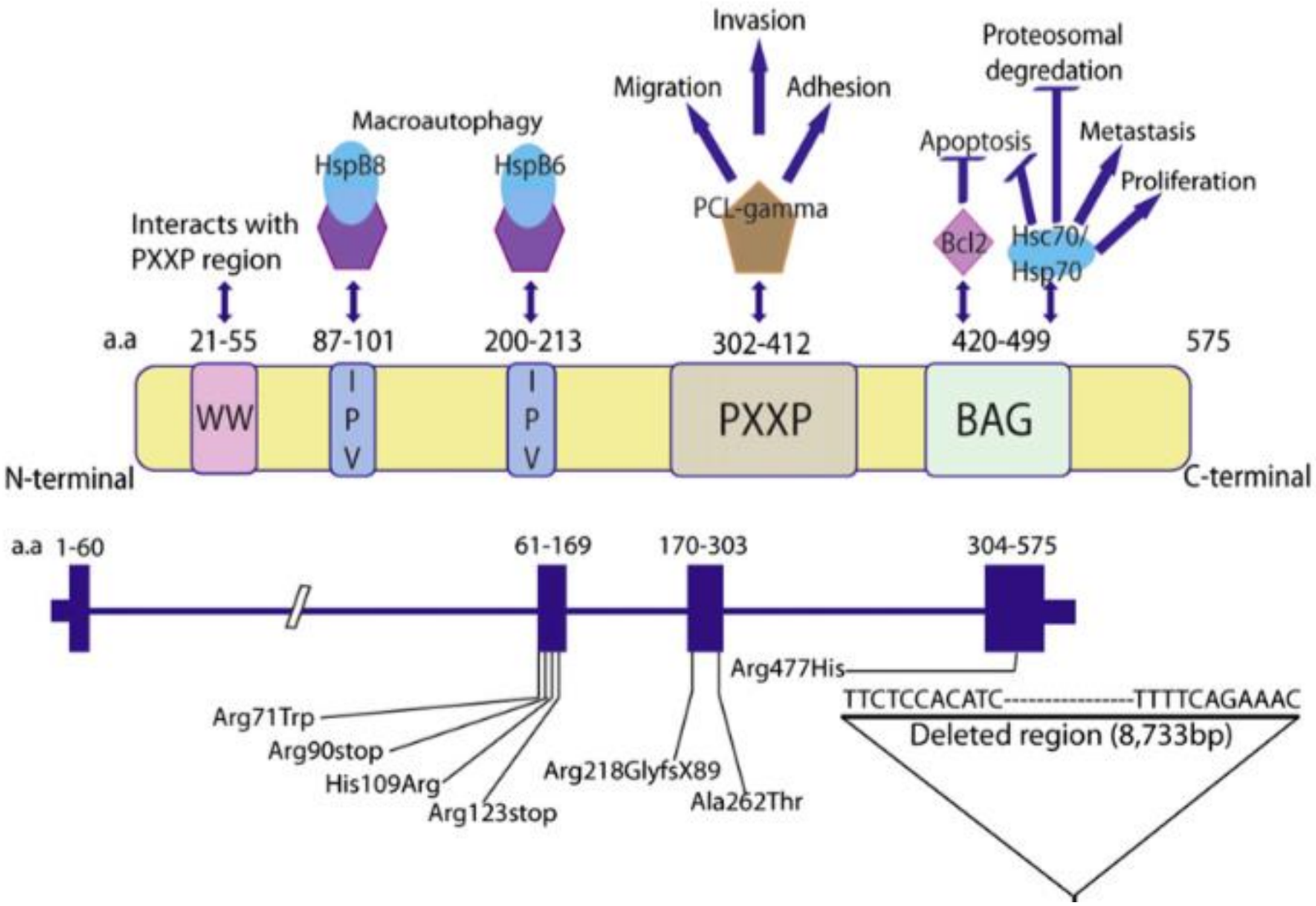
- ☐ Unknown
- ☐ Synonymous
- ☑ Nonsyn SNV
- ☑ Sub
- ☑ Del
- ☑ Ins
- ☑ Stoploss
- ☑ Stopgain
- ☑ Frameshift Su
- ☑ Frameshift De
- ☑ Frameshift Ins
- ☑ Init Codon
- ☑ Splicing

All: 51 x 22
Active: 51 x 22

Unsort		C 1	I 2 ▼	C 3	C 4	I 5
Map	Variant	Classification	Priority	Gene 1	Transcript 1	Exon 1
1	10:121435979-Del	Frameshift Del	7	BAG3	NM_004281	4
2	16:70896016-Del	Frameshift Del	7	HYDIN	NM_001270974	69
3	12:11461554-Ins	Frameshift Ins	7	PRB4	NM_002723	3
4	16:71061495-SNV	Stoploss	5	HYDIN	NM_017558	20
5	4:13582822-SNV	Nonsyn SNV	3	BOD1L1	NM_148894	20
6	11:7694019-SNV	Nonsyn SNV	3	CYB5R2	NM_016229	2
7	X:34148844-SNV	Nonsyn SNV	3	FAM47A	NM_203408	1
8	16:70884524-SNV	Nonsyn SNV	3	HYDIN	NM_001270974	74
9	16:70894087-SNV	Nonsyn SNV	3	HYDIN	NM_001270974	71
10	16:70896122-SNV	Nonsyn SNV	3	HYDIN	NM_001270974	69
11	16:70897039-SNV	Nonsyn SNV	3	HYDIN	NM_001270974	68
12	16:70902559-SNV	Nonsyn SNV	3	HYDIN	NM_001270974	66

BCL2-associated athanogene 3 (BAG3)



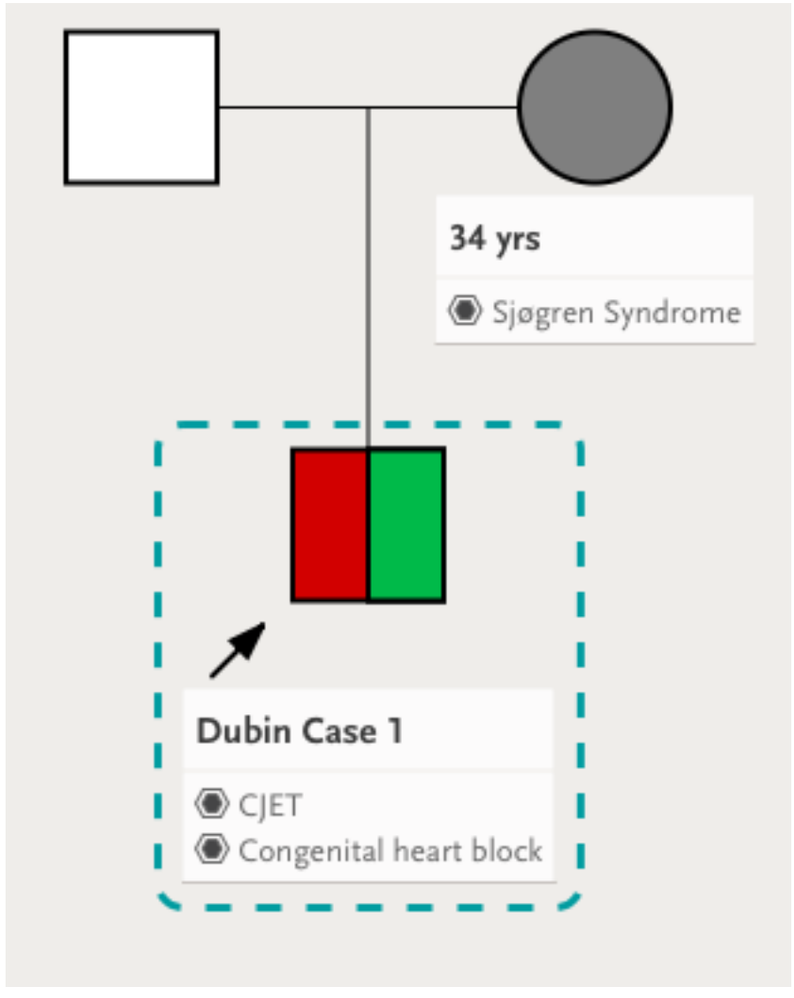


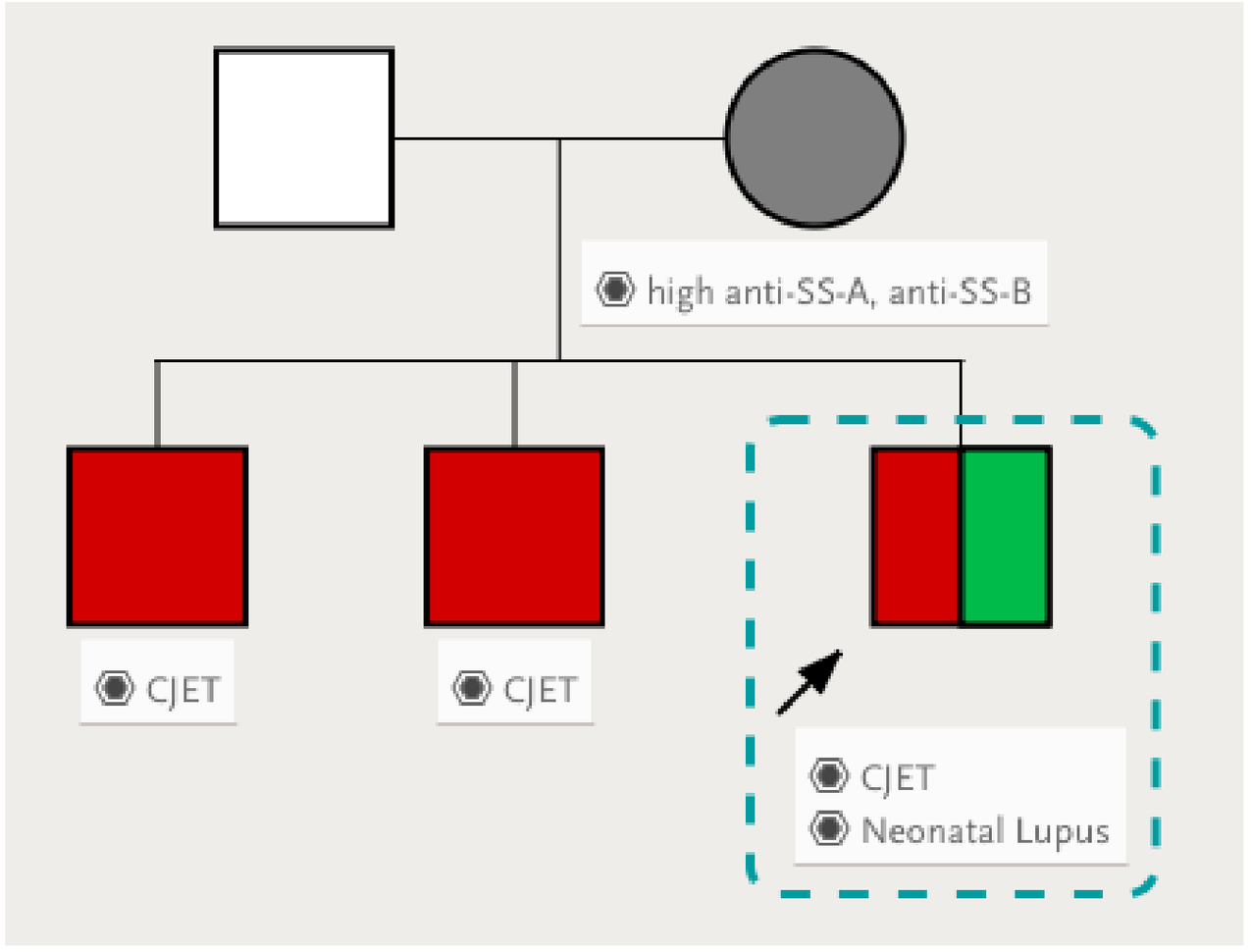
Case series of CJET: Familial Occurences

- Villain and colleagues (1990): 26 subjects
  - 5 sib-pairs (4 non-identical twin pairs)
  - 1 cousin relationship
    - 1 subject’s father had accelerated
- Collins and colleagues (2009): 99 subjects
  - familial association in 20%

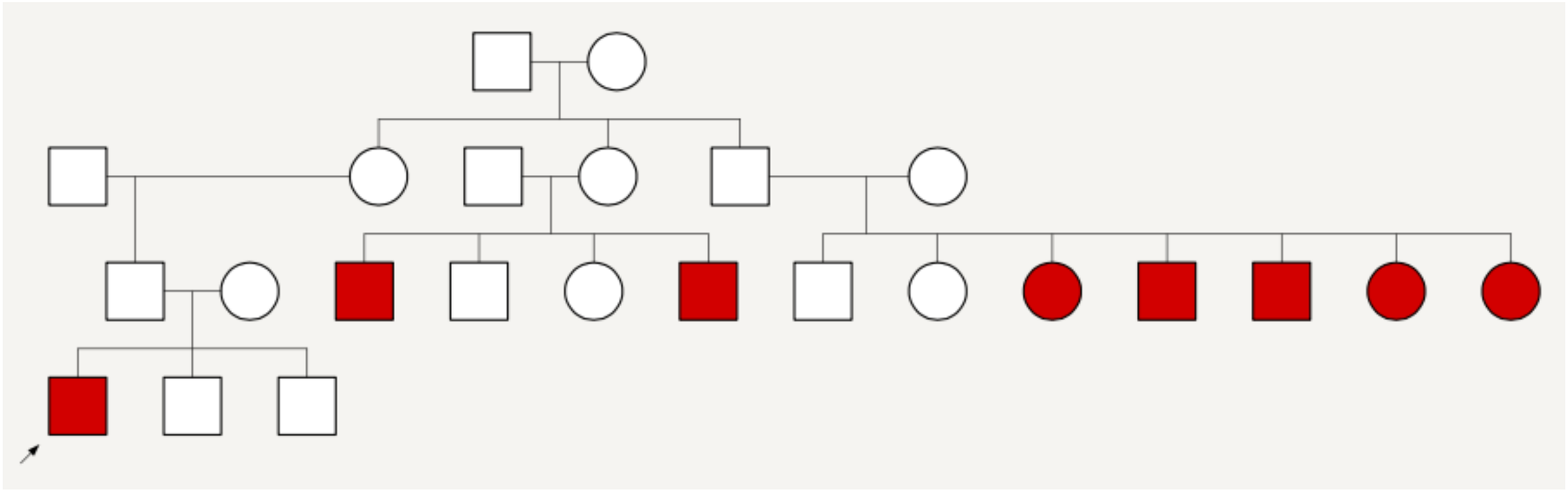
Congenital junctional ectopic tachycardia and congenital complete atrioventricular block: A shared etiology?

Anne M. Dubin, MD,[a] Bettina F. Cuneo, MD,[b] Janette F. Strasburger, MD,[c] Ronald T. Wakai, PhD,[d] George F. Van Hare, MD,[a] David N. Rosenthal, MD[a]





Congenital Junctional Ectopic Tachycardia (Lancaster Amish Family)

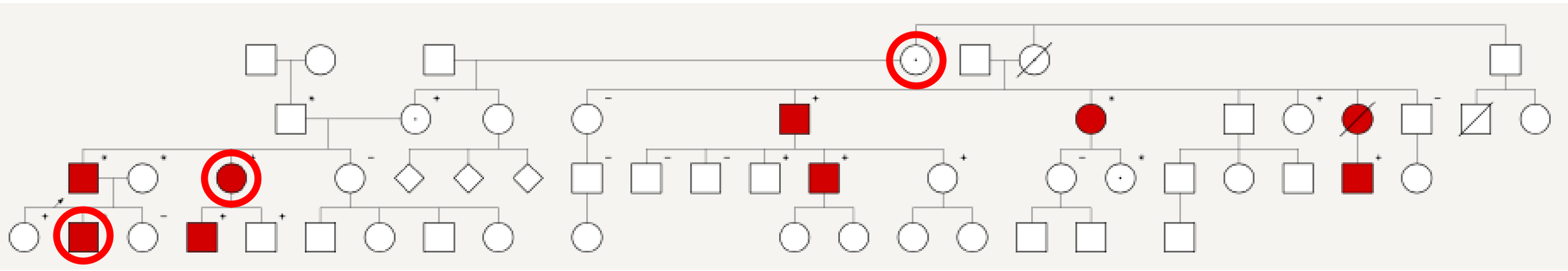


- Kevin Strauss and Eric Puffenberger
  (Clinic for Special Children, Lancaster PA)

- SNP mapping identified a putative locus

- WES (Broad Institute) has not identified a candidate to date

Congenital Junctional Ectopic Tachycardia (Upstate NY Family)



- Dr. Jeff Vinocur, Golisano Children’s Hospital, Rochester
- Extended family drove to Toronto as research volunteers
- WES performed on 3/17 samples: 3 candidate genes
- Sanger sequencing to confirm and expand segregation

Congenital Junctional Ectopic Tachycardia Gene Candidates

Stoploss	5	HYDIN	NM_017558	20	c.3052T>C	p.*1018Glne
Nonsyn SNV	3	CLASP2	NM_001207(	25	c.2651A>G	p.Asn884Ser
CLASP2	NM_001207(	25	c.2651A>G	p.Asn884Ser		
CP	NM_000096	8	c.1426G>A	p.Glu476Lys		
FAM83B	NM_001010{	2	c.28T>A	p.Leu10Met		
TNNI3K	NM_015978	17	c.1729C>T	p.Leu577Phe		
GYG1	NM_001184{	6	c.683C>T	p.Thr228Ile		
HACE1	NM_020771	21	c.2391A>G	p.Ile797Met		
KIR3DL2	NM_001242{	3	c.322G>A	p.Ala108Thr		
RAD54L2	NM_015106	16	c.2571G>T	p.Lys857Asn		
Nonsyn SNV	3	HYDIN	NM_001270{	16	c.2170A>G	p.Asn724Asp
Nonsyn SNV	3	KIR3DL1	NM_013289	3	c.235A>G	p.Ser79Gly
Nonsyn SNV	3	KIR3DL1	NM_013289	4	c.475G>T	p.Gly159Trp
Nonsyn SNV	3	KIR3DL1	NM_013289	4	c.550C>T	p.Pro184Ser
Nonsyn SNV	3	KIR3DL2	NM_001242{	3	c.322G>A	p.Ala108Thr
Nonsyn SNV	3	RAD54L2	NM_015106	16	c.2571G>T	p.Lys857Asn
Synonymous	2	HYDIN	NM_001270{	15	c.1998G>A	p.=
Synonymous	2	HYDIN	NM_001270{	14	c.1875A>G	p.=

Congenital Junctional Ectopic Tachycardia

ELSEVIER

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Letter to the Editor

Online 11 March 2015

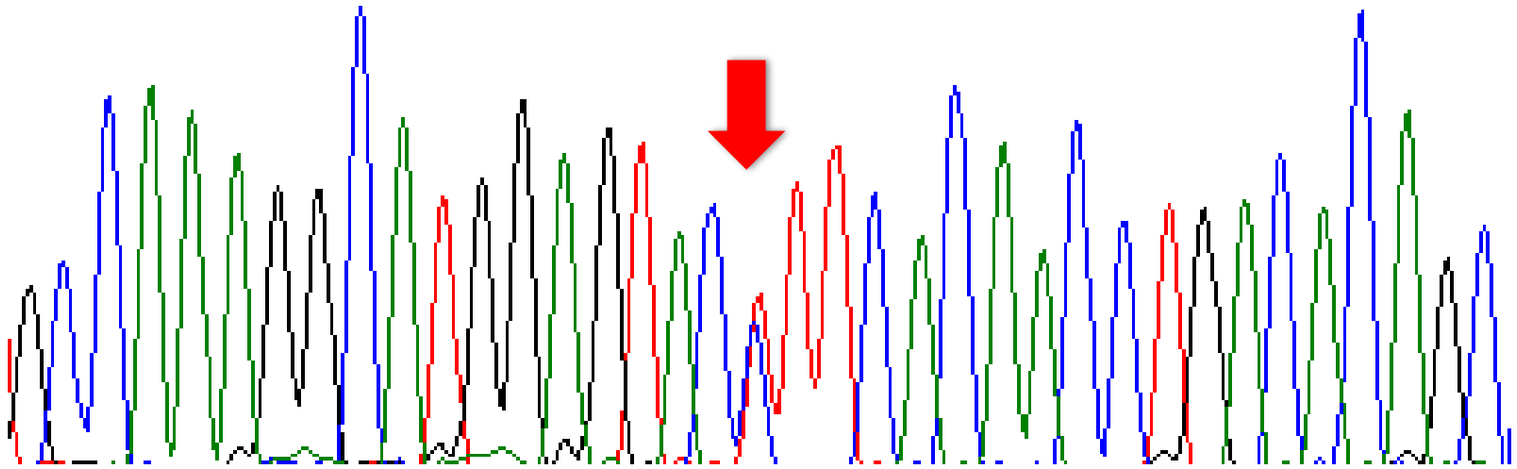
Whole exome sequencing identifies the *TNNI3K* gene as a cause of familial conduction system disease and congenital junctional ectopic tachycardia ☆

Yanwei Xi [a,1], Christina Honeywell [b,1], Dapeng Zhang [c], Jeremy Schwartzentruber [d], Chandree L. Beaulieu [b], Martine Tetreault [d,e], Taila Hartley [b], Jennifer Marton [e], Silvia M. Vidal [e], Jacek Majewski [d,e], L. Aravind [c], Care4Rare Canada Consortium [a], Michael Gollob [f], Kym M. Boycott [a,b,*,1], Robert M. Gow [b,**,1]

[a] *Children's Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, Ontario, Canada*

Causal gene mutation

- TNNI3K: c.1729C>T; p.Leu577Phe



- Gene had just previously been associated with CJET and familial conduction system disease

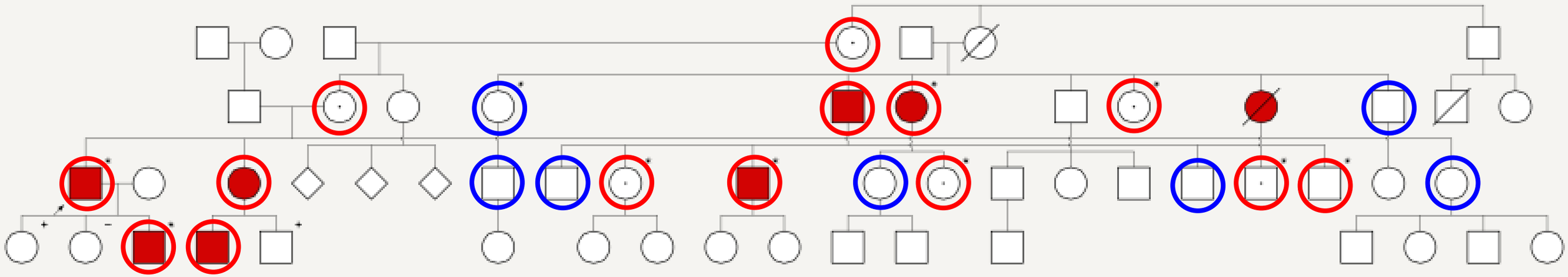
(Xi et al., Int J Cardiol 2015)

SickKids®

TNNI3K

- Troponin I-interacting kinase
- Kinase with cardiac-restricted expression
- Has been implicated in various cardiac phenotypes and diseases including heart failure, cardiomyopathy, ischemia/reperfusion injury and conduction of the cardiac electrical impulse

Family screening



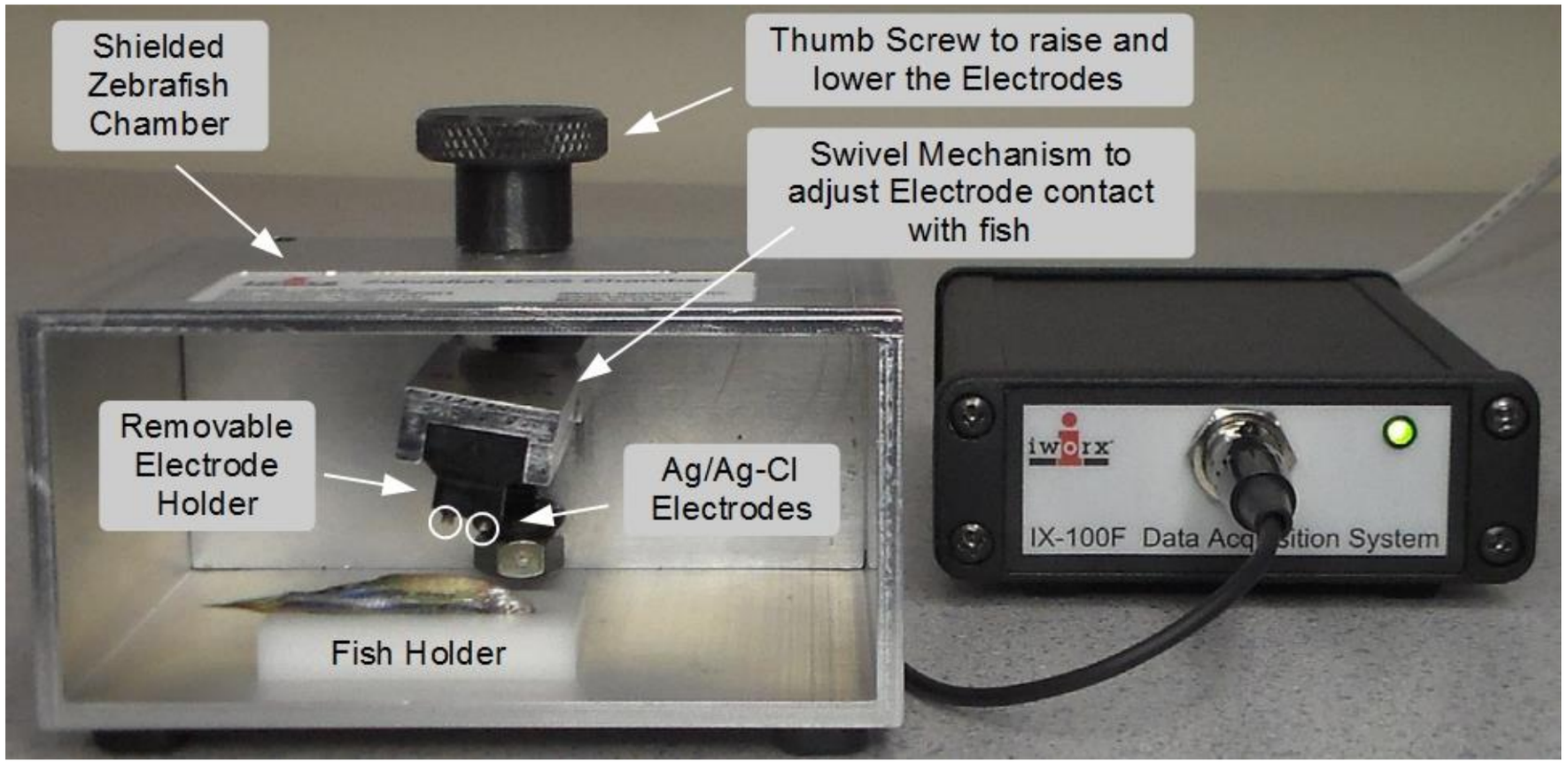
Mutation carrier

Non mutation carrier

SickKids®

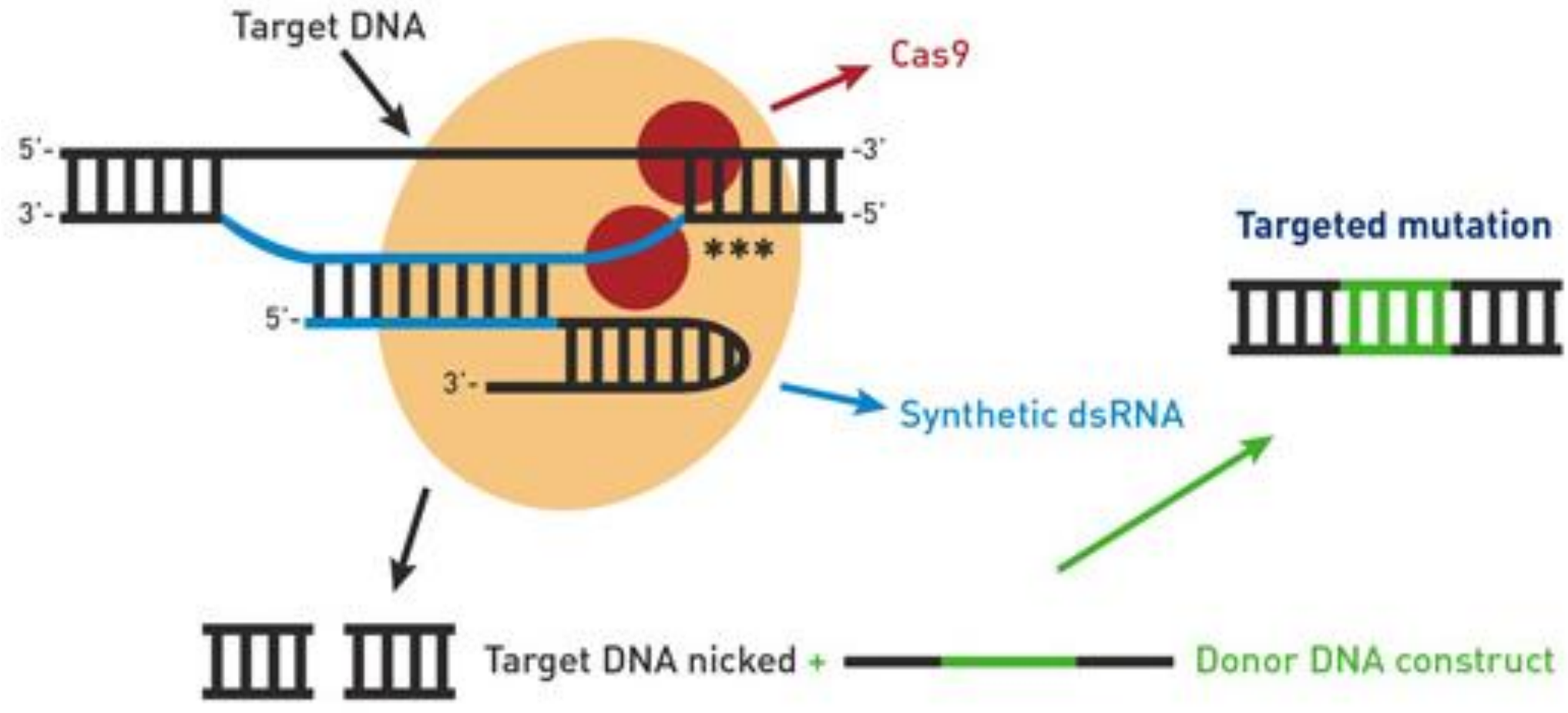
Functional study

- The identified gene mutation is inserted into a zebrafish model (using CRISPR/Cas gene editing)
- Zebrafish will be assessed for tachyarrhythmia



SickKids®

Basic DNA editing using CRISPR/Cas9



From: The Jackson laboratory

SickKids®

Zebrafish model

- Gain or loss of function?
- Screen small molecule libraries focused on TNNI3K inhibitors (or alternately kinase activators) to influence this pathway involved in ischemia/reperfusion injury, oxidative stress, and myocyte death
- Assessment of FDA-approved generic drugs

SickKids®

Contributors

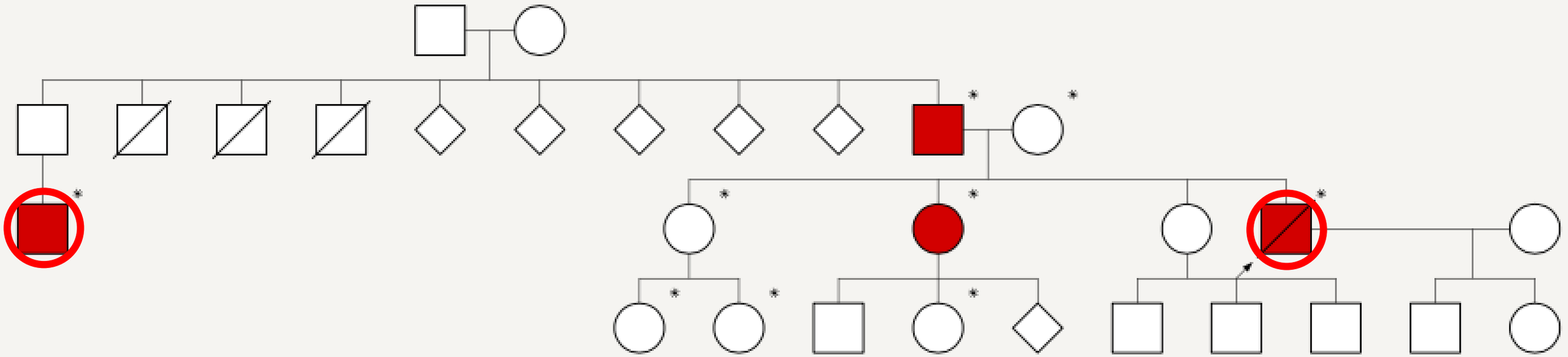
- University of Rochester:
  - Jeffrey Vinocur
- The Hospital for Sick Children:
  - Tamara Koopmann
  - Meena Fatah
  - Sarah Hutchinson
  - James Dowling
  - Robert Hamilton

robert.hamilton@sickkids.ca

Supported by the
Paul C. Gillette fund
Ped. & Cong. EP Soc.

SickKids®

Gene Discovery in Arrhythmogenic Cardiomyopathy 1

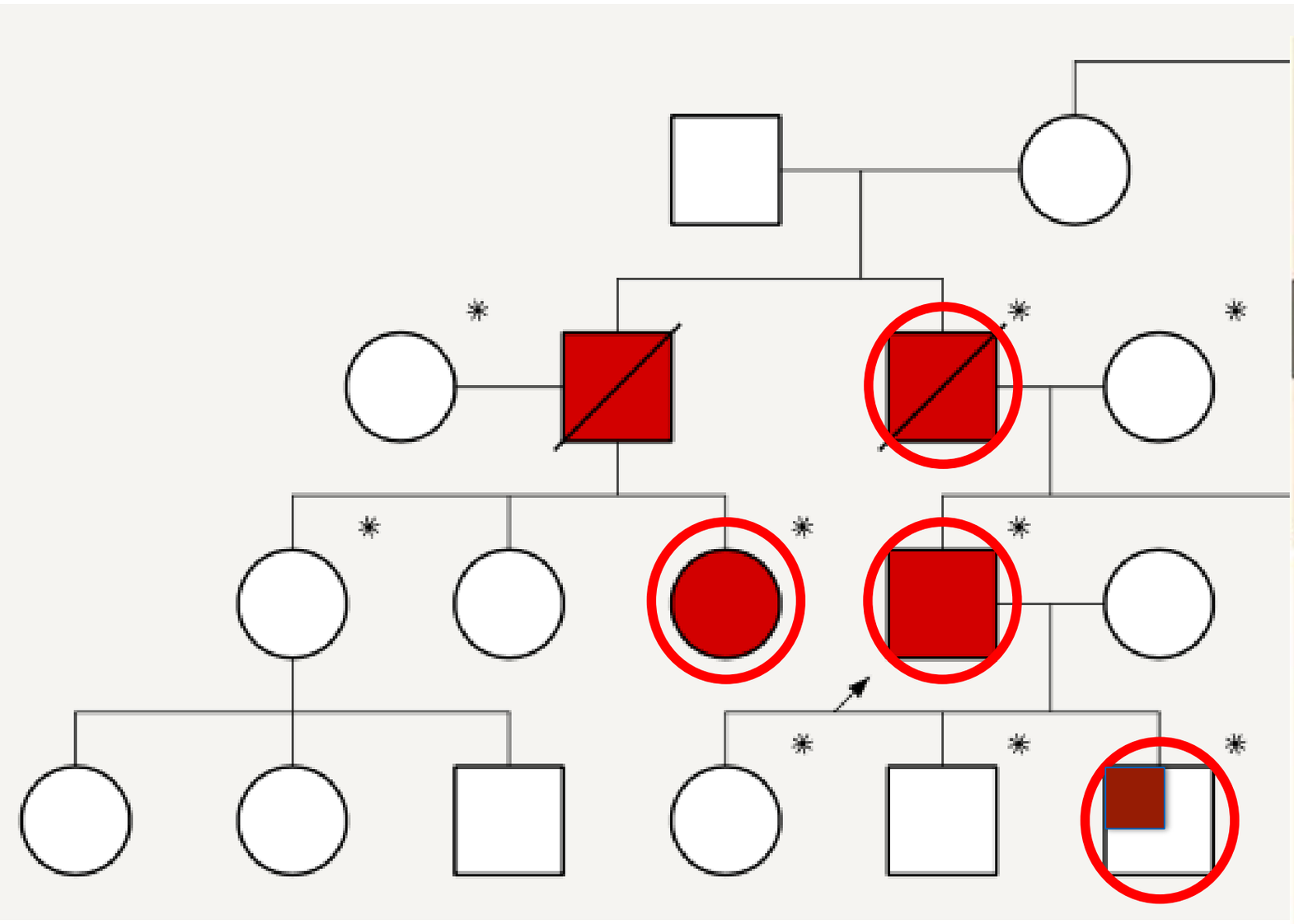


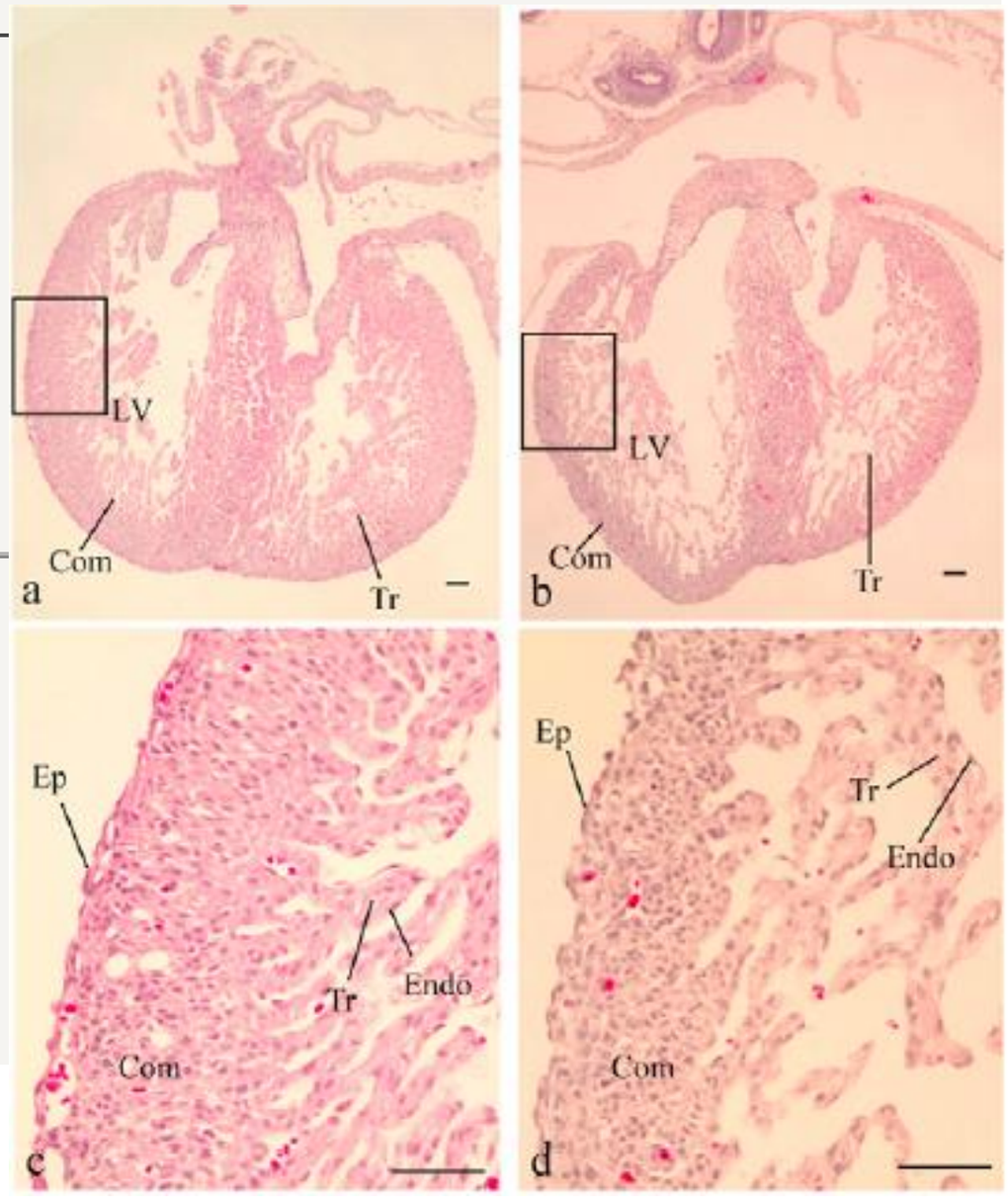
- “Although the QT prolonged from 25 ± 1.0 to 30 ± 1.1 ms in mice, the difference could be due to delayed conduction”

- “PR intervals increased from 35.9 ± 1.0 to 39.6 ± 0.7 ms, QRS intervals increased from 8.3 ± 0.1 to 11.2 ± 0.2 ms, and P-wave duration increased from 8.2 ± 0.7 to 13.4 ± 0.5 ms for wild-type v. mutant mice; all differences statistically significant, $P < 0.05$)”

- “Given that action potentials of adult mutant cardiomyocytes are not substantially prolonged, the increase in QT interval observed in mice is probably due to delayed conduction”

Gene Discovery in Arrhythmogenic Cardiomyopathy 2



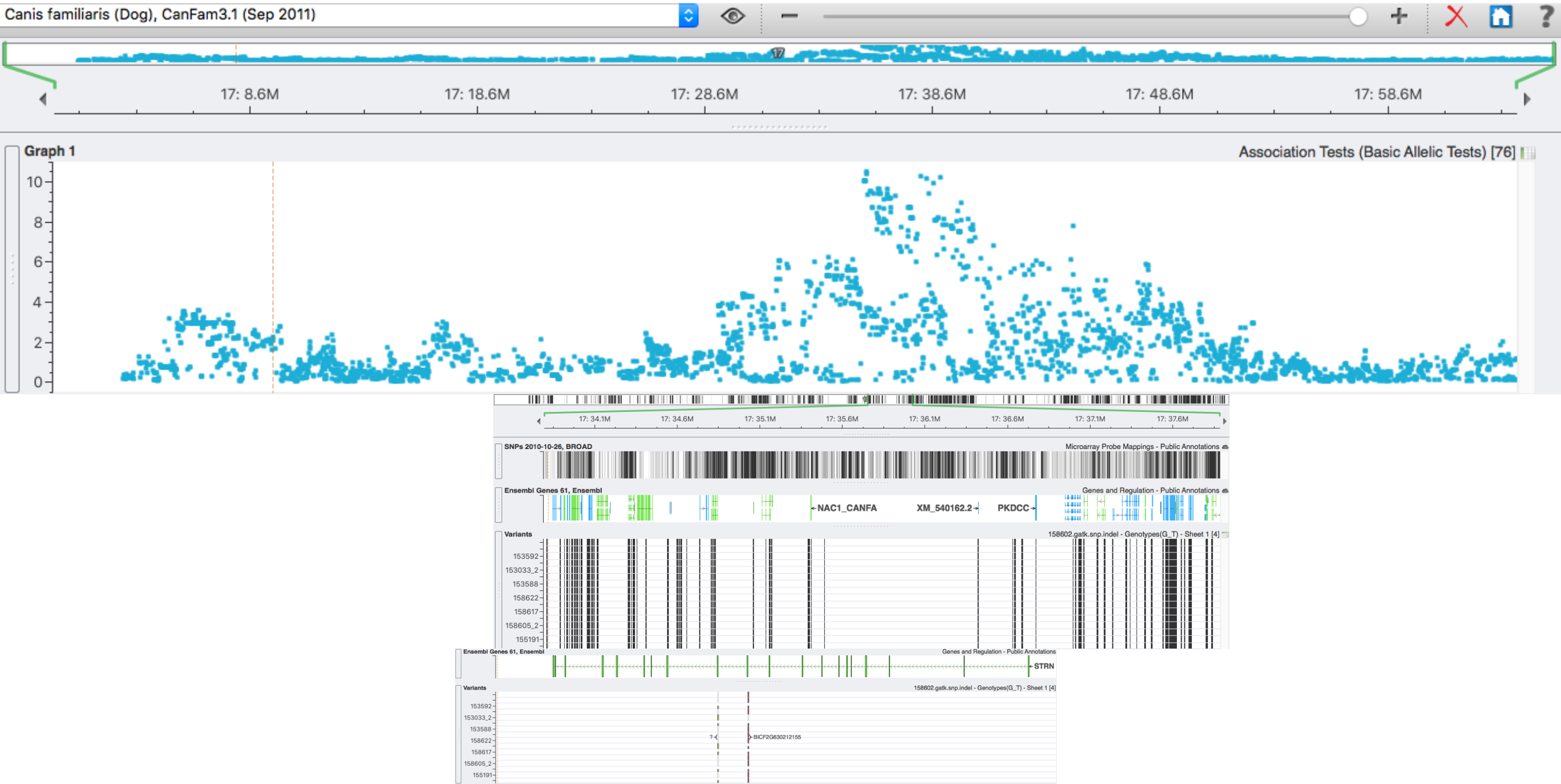


WT Mutant

Embryonic Mouse Heart

Gene Discovery in Canine Arrhythmogenic Cardiomyopathy

- Associated with Striatin 3’UTR mutation in American dogs
- Does not segregate among Canadian, UK or European dogs



Golden Helix SVS

- Provides for facile analysis of SNP, Exome & Genome data to users without major bioinformatic background
- Relatively easy to learn
- Things I like:
  - Many analyses in one program (Swiss Army Knife)
  - Rapid implementation of new reference data
  - Integration with & free BAM viewer
  - Amazing company support

Golden Helix SVS

- Examples of company support (things I asked for)
  - Multiplex pedigree function (Greta suggested importing pedigree file and then assigning genotype data)
  - Demonstration of protein-level effects (Greta showed me how to apply feature labels from protein coding field)
  - User-friendly tools for file conversions: (FASTA/Q > SAM > BAM > VCF) (Offered to perform file conversions)
  - Implement Estimation of Recent Shared Ancestry (Offered to look at algorithms