Influences of Outcome in CHD

The Blue Print: Our Genes

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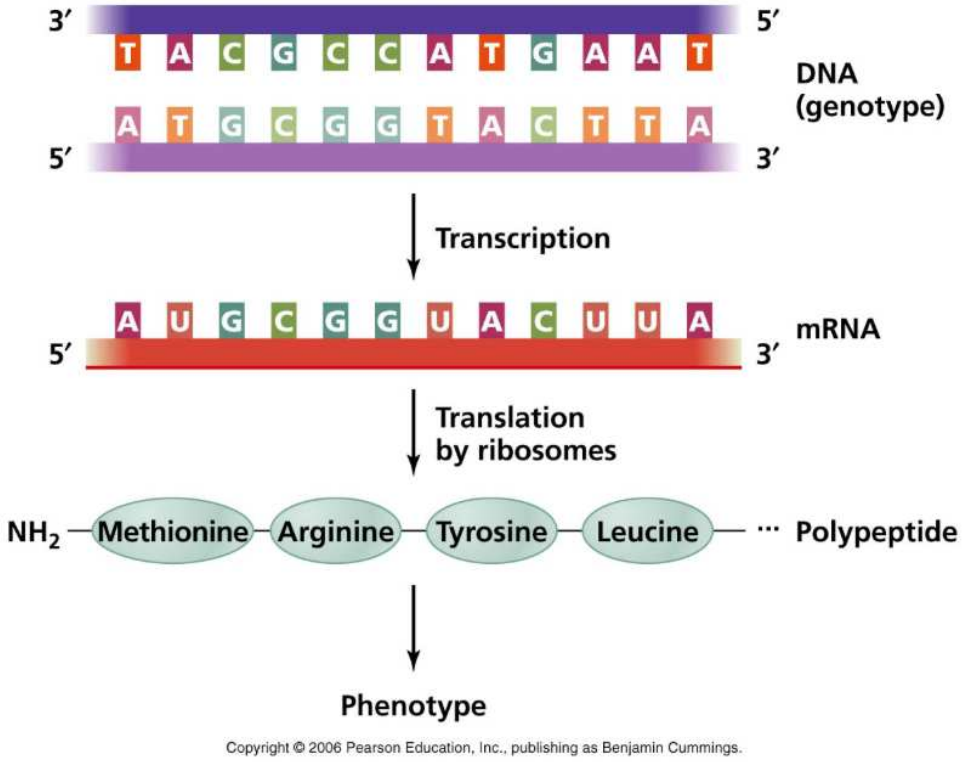
Cardiology 2018

Central Dogma Circa 1980's

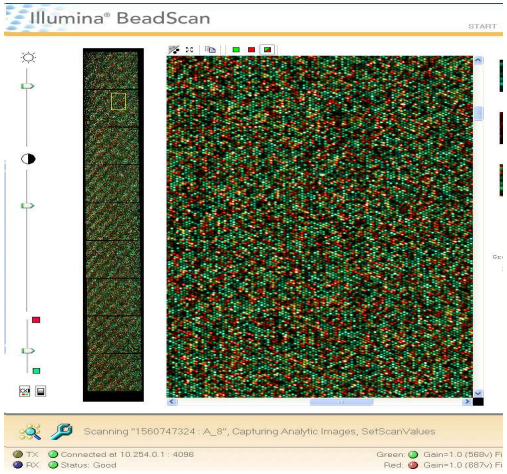


Single Nucleotide Polymorphisms (SNPs): "Common Variants" > 1% population

Genomic DNA

TAGCCATCGGTA A/G GTACTCAATGAT

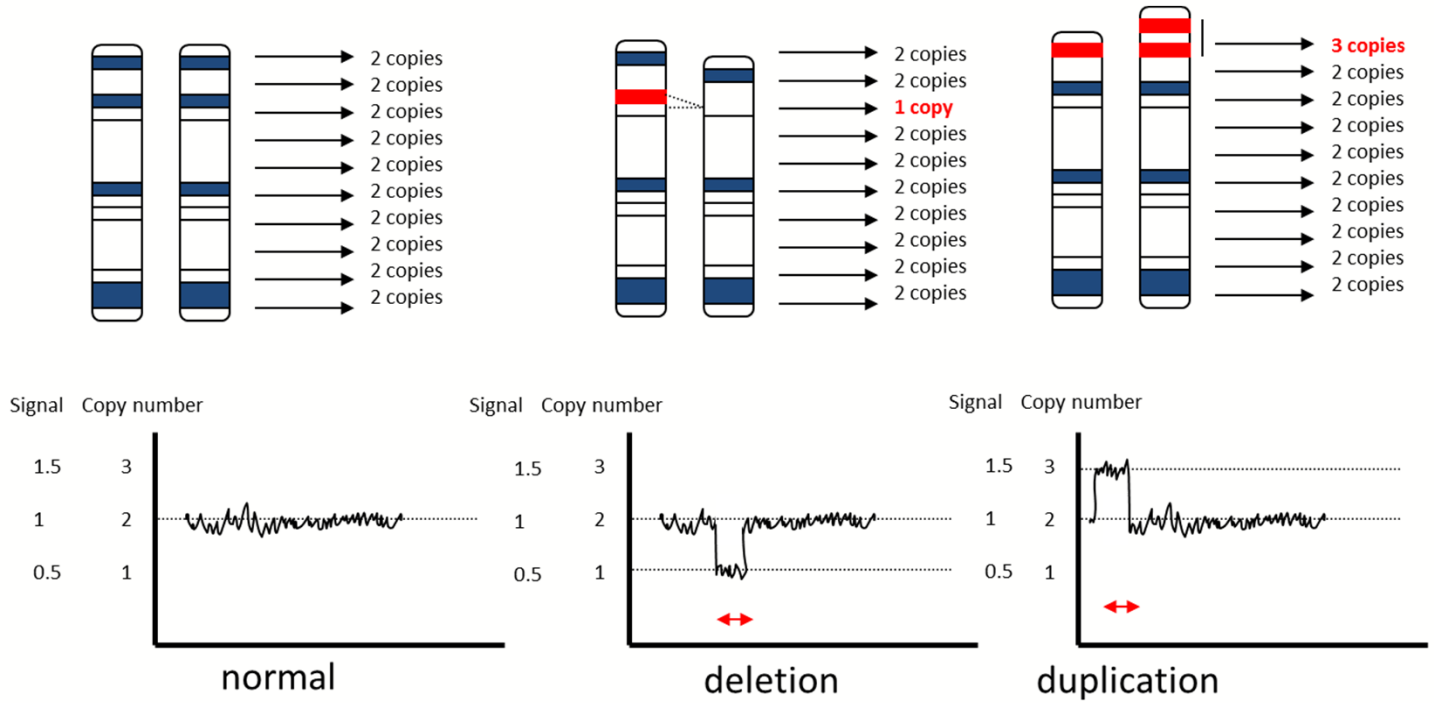
Micro Array



Allele A ATCGGTAGCCATTCATGAGTTACTA

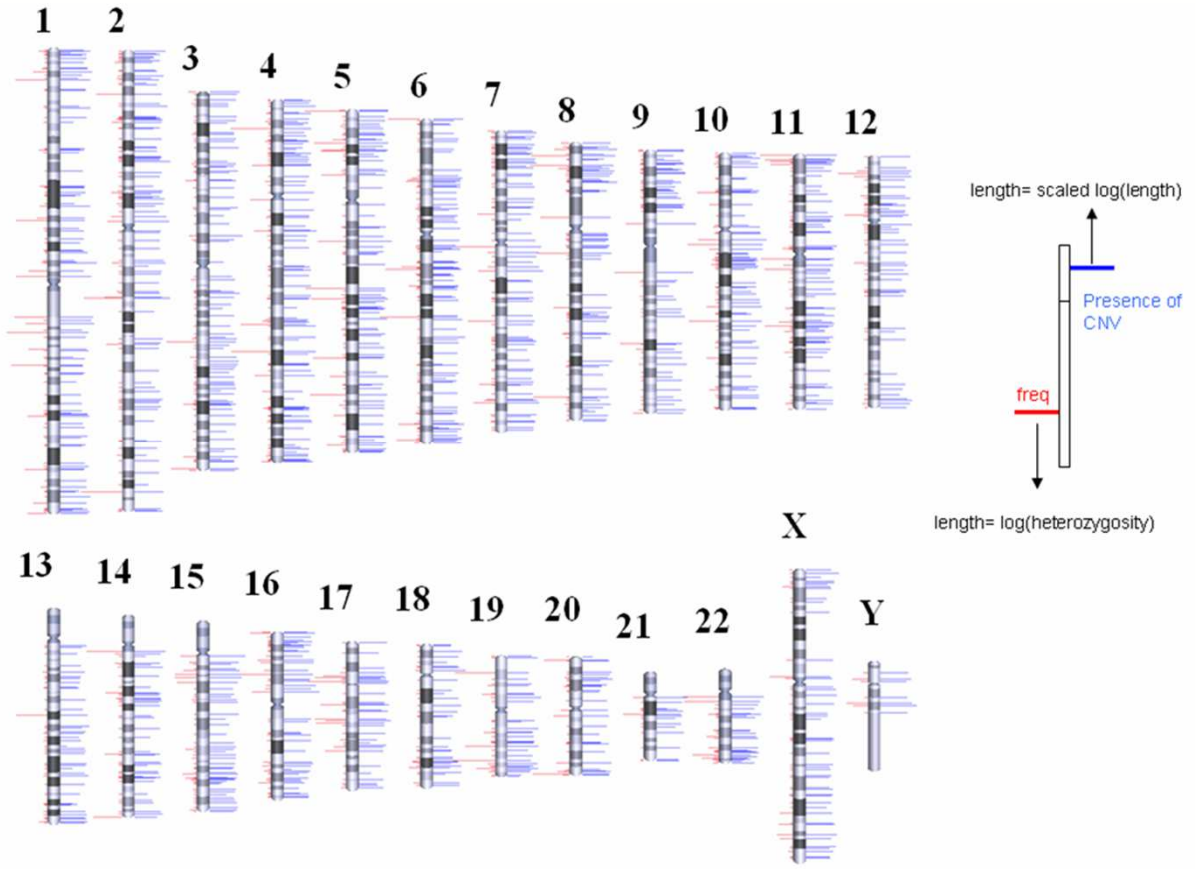
Allele B ATCGGTAGCCATCCATGAGTTACTA

Copy Number Variants



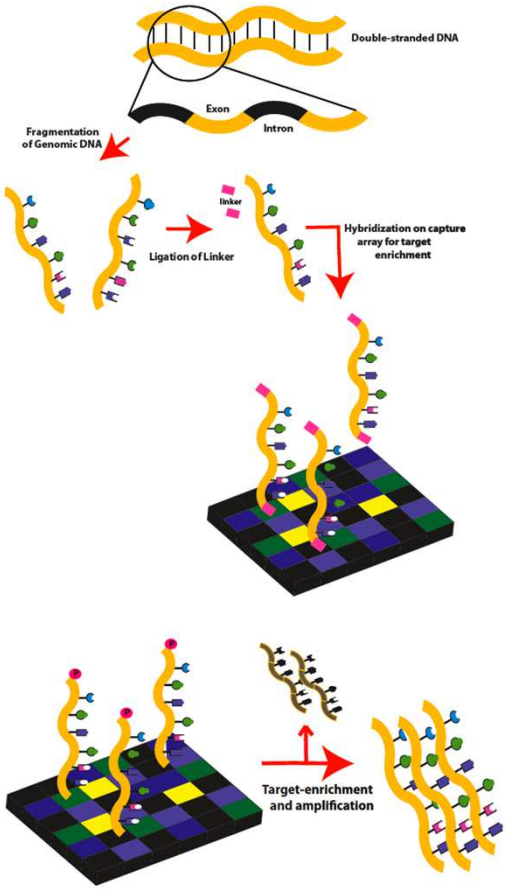
Courtesy of Wendy Chung

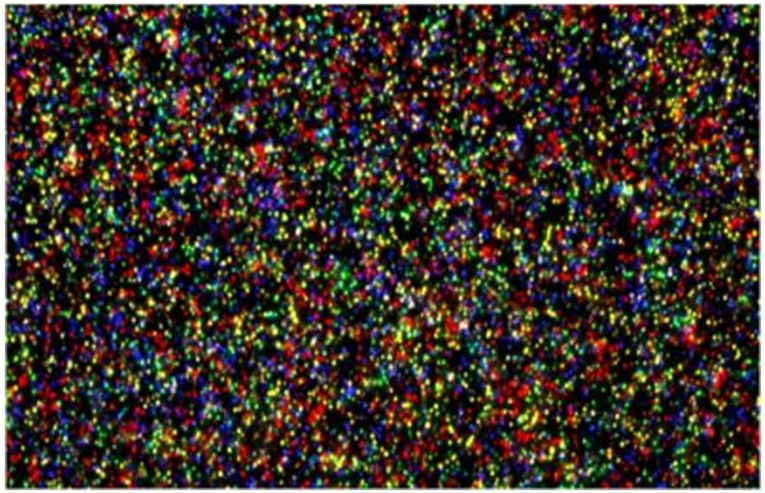
Copy Number Variants



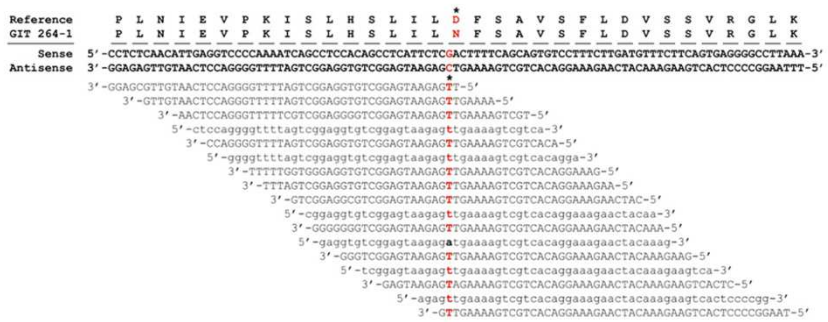
We have produced a dataset of array-based comparative genome hybridization from EBV-transformed lymphoblastoid cell lnes from all 270 HapMap individuals used in the phase I and phase II of the project (populations: CEU, CHB, JPT and YRI). www.sanger.ac.uk/humgen/cnv/data/

Next Generation “Massively Parallel” Sequencing

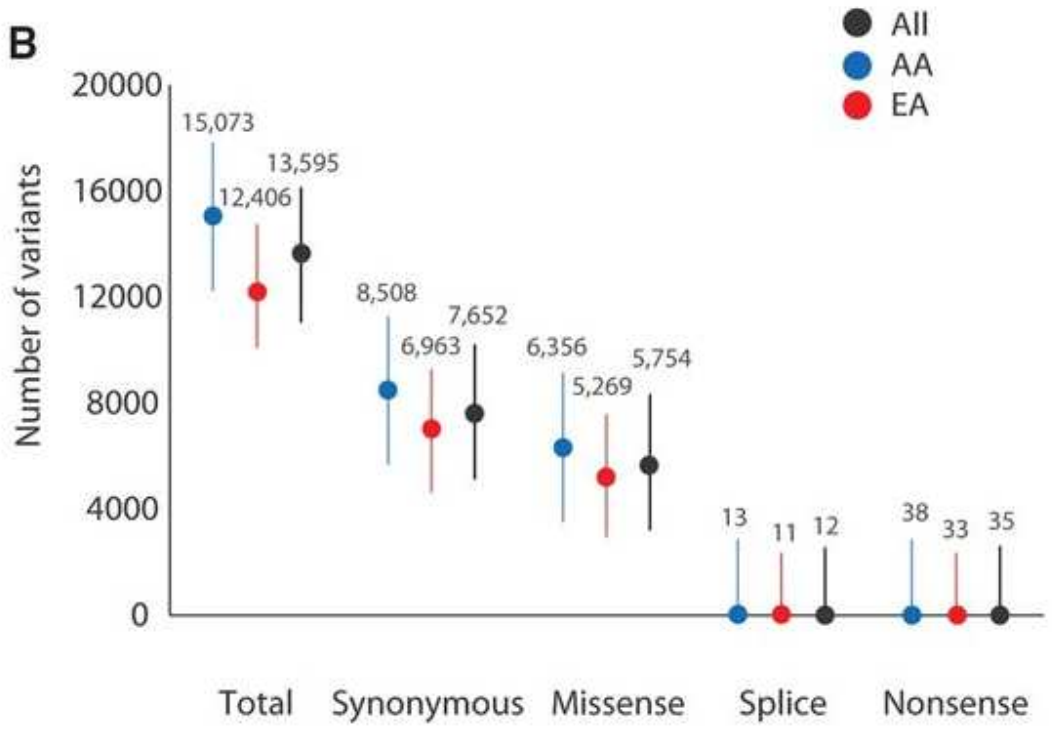




A



Single Nucleotide Variants in One Exome



Tennessen et al., Science 337(6090):64-69, 2012

Harness Genomics to Inform Practice

Pictures Removed

How Does Genomics Inform Outcome?

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Outline

- Genetic etiology and outcome
- Genetic modifiers of outcome
- Promises and challenges

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- Genetic modifiers of outcome
- Promises and challenges

Genetic Syndrome and Mortality

Table 1 Distribution of outcome for 240 fetuses with hypoplastic left heart syndrome (HLHS)

	Total (n)	*TOP* (n)	*IUFD* (n)	*NI* (n)	*WS* (n)	*Tx* (n)	*Norwood operation* *Performed* (n)	*Survived* (n (%))	*Died* (n)	*Total survival after fetal diagnosis of HLHS (%)*
Risk										
Standard	162	18	—	5	—	—	139	129 (92.8)	10	79.6
High	78	10	3	5	11	3	46	26 (56.5)	20	37.2
Total	240	28	3	10	11	3	185	155 (83.8)	30	65.8
Specific risk factors in high-risk group										
Extracardiac, genetic or chromosomal anomaly	42	9	3	5	8	1*	16	9 (56.2)	7	23.8
Prematurity	7	—	—	—	—	—	7	3 (42.9)	4	42.9
Severe tricuspid regurgitation	9	1	—	—	—	1†	7	7 (100)	—	88.9
Intact atrial septum	8	—	—	—	1	—	7	4 (57.1)	3	50.0
Ventricular dysfunction	4	—	—	—	—	—	4	1 (25.0)	3	25.0
Intact atrial septum and extracardiac, genetic or chromosomal anomaly	5	—	—	—	1	—	4	1 (25.0)	3	20.0
Intact atrial septum and ventricular dysfunction	3	—	—	—	1	1‡	1	1 (100)	—	66.7

Rychik et al., Ultrasound Obstet Gynecol 2010

Genetic Syndromes and Mortality

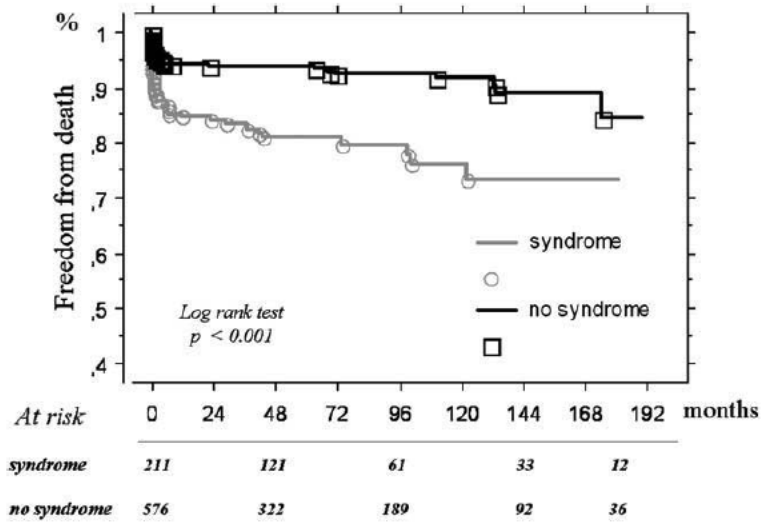
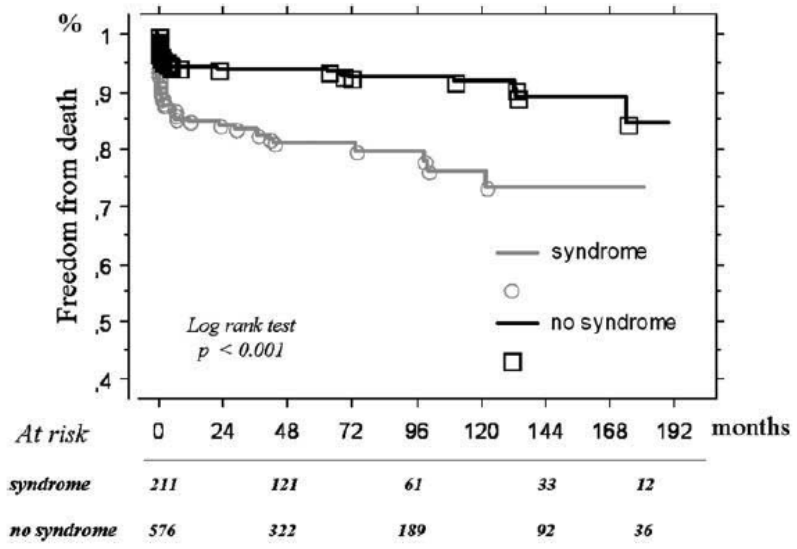


FIGURE 1. Kaplan–Meier survival plot in syndromic and nonsyndromic patients after repair of CTHD.

Michielon et al., JTCVS 2009

Genetic Syndromes and Mortality



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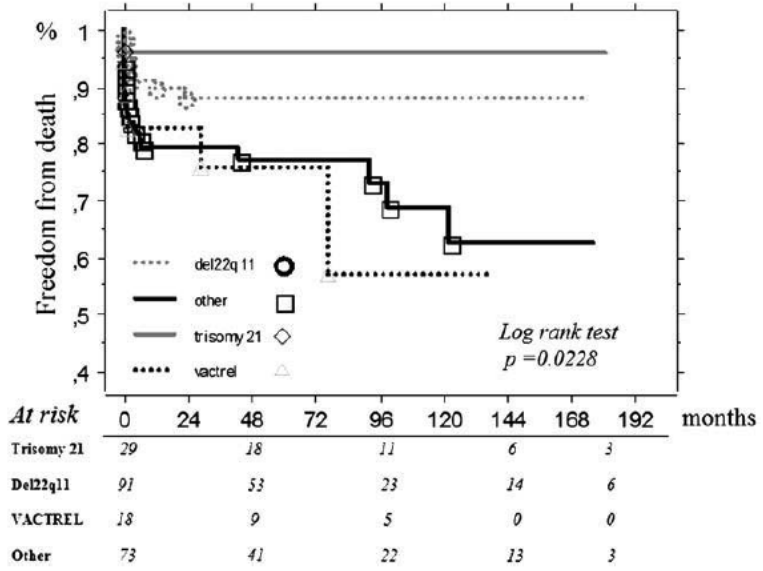
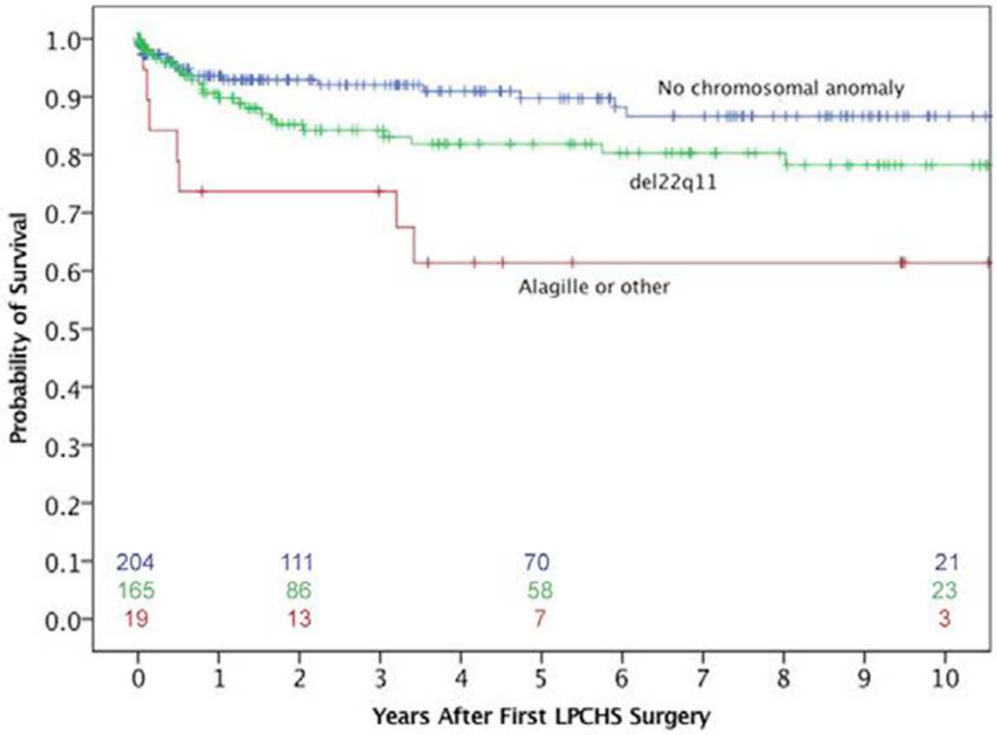


FIGURE 3. Kaplan–Meier survival plot in syndromic patients stratified by type of genetic syndrome.

Michielon et al., JTCVS 2009

Genetic Syndromes and TOF/PA + MAPCAs



Bauser-Heaton et al., Circ Cardiovasc Interv 2017

Trisomy 21 and CAVC

TABLE 4. Distributions of outcomes by patient factors

	Frequency, n (%)	In-hospital mortality (%)	Postoperative length of stay (median, IQR)	Complications (%, with 1 or more major complications*)	Reoperations with cardiopulmonary bypass before discharge (%)
Weight at surgery					
<3.5 kg	151 (6.3)	15.2	14 (8-32)	16.6	4.0
≥3.5 kg	2247 (93.7)	2.2	8 (5-14)	9.4	3.0
P value	—	<.0001	<.0001	<.01	.49
Age at surgery					
≤2.5 mo	284 (11.8)	9.5	12 (8-25)	13.4	7.0
>2.5 mo	2115 (88.2)	2.1	7 (5-13)	9.4	2.5
P value	—	<.0001	<.0001	.03	<.0001
Down syndrome					
No	517 (21.6)	4.4	8 (5-16)	15.1	5.4
Yes	1882 (78.4)	2.6	8 (6-14)	8.4	2.4
P value	—	.03	.34	<.0001	<.001
Overall	2399 (100)	3.0	8 (5-14)	9.8	3.0

IQR, Interquartile range. *Major complications include renal failure requiring dialysis, neurologic deficit persisting at discharge, atrioventricular block/arrhythmia requiring permanent pacemaker, postoperative mechanical circulatory support, phrenic nerve injury, and unplanned reoperations.

St. Louis et al, JTCVS 2014

Morbidity: Truncus/IAA Peri-Op Survivors Analysis

Outcome	No Deletion (N = 55)	22q11.2 del (N = 40)	p
Hosp stay (days)	14 (7-96)	18 (2-103)	0.02
ICU Care (days)	4 (2-56)	7 (3-78)	0.02
Ventilation (hours)	74 (26-2236)	123 (10-968)	0.08
# Cardiac events	1 (0-9)	2 (0-15)	0.04
# Non-cardiac events	1 (0-9)	2 (0-19)	0.26
# Consults	1 (0-5)	4.5 (2-12)	<0.01
# Discharge meds	2 (0-10)	4 (0-8)	<0.01

TOF Peri-Op Survivors Analysis

Variable	Non-Deleted (n=164) Median (Q1, Q3)	22q11.2 Deleted (n=44) Median (Q1, Q3)	P-value
Death (%)	3 (2)	1 (2)	0.9
Length of Hosp Stay* (days)	6 (4,11)	9 (5, 15)	0.02
Length of ICU care (days)¶	4 (3, 7)	6 (4, 12)	0.007
Cardiopulmonary bypass (minutes)§	74 (±30)	84 (± 31)	0.02
Length of ventilation (hours)	26.2 (11, 77)	31.6 (23;97)	0.1870

*subset discharged home
¶ intensive care = mechanical ventilation, intravenous inotropes, chest tube
§ remained significant after controlling for pulmonary valve anatomy, BTS

TOF Peri Op Survivors Analysis

- No difference in the number of:
  - major anomalies
  - infections
  - major cardiac events
  - hospital days before surgery
  - use of nasogastric feeding at discharge

TOF: Genotype and Intermediate Outcome (8-18 yo)

- Assess genotype and intermediate outcome:
  - interim events
  - current cardiovascular status
  - quality of life
- Cross sectional design:
  - Echocardiogram
  - Exercise stress test
  - Cardiac MRI
  - Quality of Life Questionnaires (CHQ and PCQLI)
  - Review of medical records/history
- Funded by NHLBI (SCCOR)

TOF: Exercise Performance (n=149)

	Non syndromic (n=124)	22q11.2 DS (n=25)	p value
RQ≥1.1	84 (68)	10 (40)	0.01
Max HR (BPM)	182 (14)	175 (17)	0.08
% Predicted mVO_2	80±17	61±17	<0.0001
% Predicted mVO_2 at AT	79±16	61±17	<0.0001
% Predicted Max work	86±22	64±18	0.0002
O_2 pulse/m^2	5.8±1.3	4.4±1.2	<0.0001

Mercer-Rosa et al., Circ Cardiovasc Genet 2015

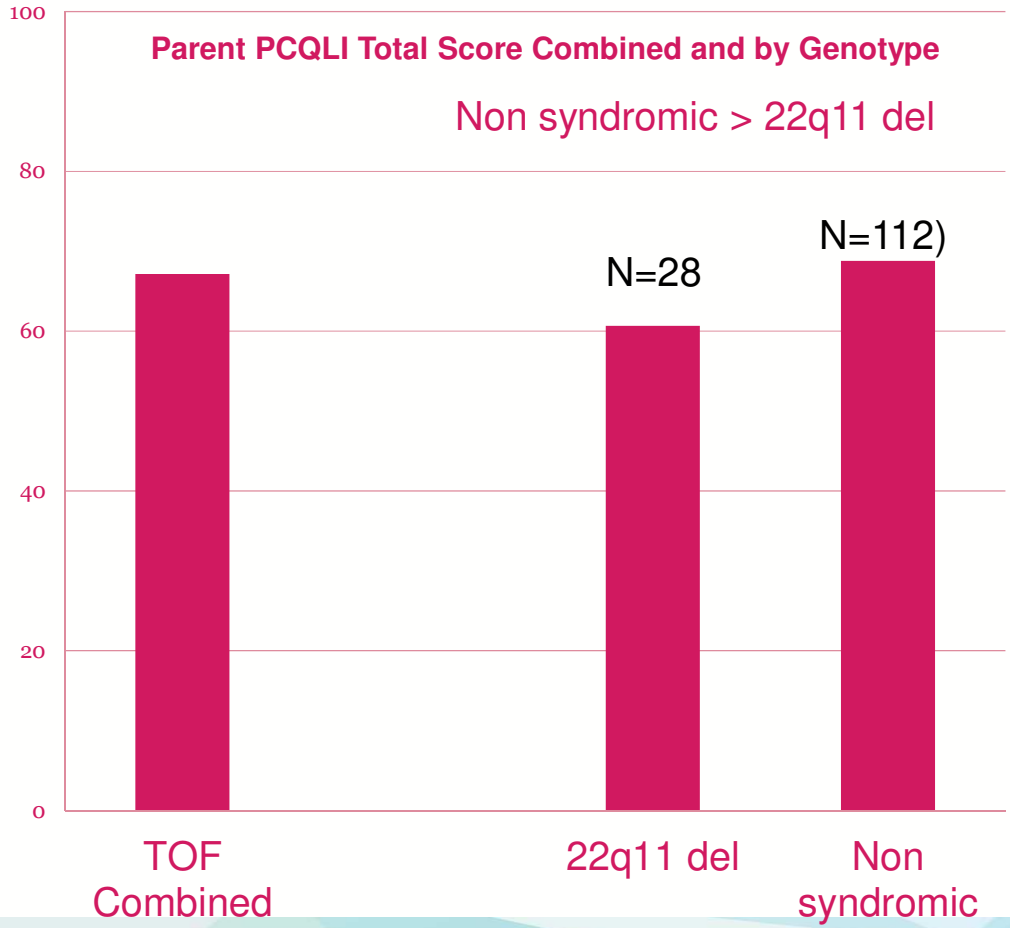
TOF: Genotype and Medical Burden

	Non syndromic			22q11.2 deletion syndrome			p value*
	Median	IQR	Range	Median	IQR	Range	
Hosp	3	(2.0,5.0)	(1,20)	6.5	(5.0,10.0)	(2,28)	<0.0001
cardiac dx	2	(1.0,3.0)	(1,11)	3	(1.0,4.0)	(1,15)	0.03
noncardiac	1	(0.0,3.0)	(0,16)	4	(2.0, 7.0)	(1,19)	<0.0001
Specialists	0	(0.0,1.0)	(0,12)	3.5	(2.0,9.0)	(0,19)	<0.0001
Meds	0	(0.0,1.0)	(0,6)	1	(0.0,3.0)	(0,9)	<0.0001
cardiac	0	(0.0,0.0)	(0,3)	0	(0.0,0.0)	(0,5)	0.04
noncardiac	0	(0.0,1.0)	(0,3)	1	(0.0,2.0)	(0,6)	<0.0001

* Poisson regressions were used to compare each variable between the NS and 22q11.2DS adjusting for patient age

Mercer-Rosa et al., Circ Cardiovasc Genet 2015

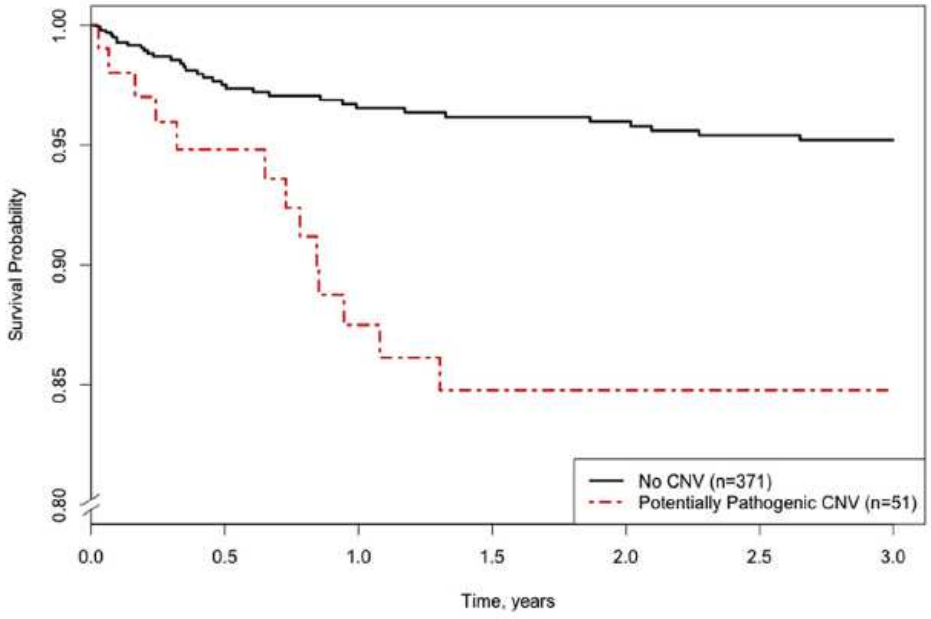
TOF: 22q11.2 Deletion Status and HRQOL



Goldmuntz et al., J Peds 2017

CNVs and Outcome in CHD

- Increased burden of putative pathogenic CNVs in CHD cases as compared to controls (12% vs 5%)
- CNV carriers have 2.5 fold increased risk of transplant or death



Number At Risk						
No CNV	371	350	344	342	341	338
CNV Carriers	51	46	40	38	38	38

Kim et al., J Thorac Cardiovasc Surg 2015

Copy Number Variants and Outcome in SV (N=223)

- ~10% of CHD cases have putatively pathogenic copy number variants (CNVs)

Table 2. Fourteen-Month Outcomes for Subgroups by Genotype

	n‡	MDI	PDI	Weight Z	Length Z	HC Z
Genotype–	192	91.4 (17.0)	77.6 (19.1)	–0.61 (1.15)	–1.00 (1.37)	–0.18 (1.32)
Genotype+	31	90.6 (17.8)	71.4 (20.4)	–0.61 (1.37)	–1.65* (1.82)	–0.16 (1.66)
Gain CNV	25	92.5 (16.3)	74.7 (20.9)	–0.80 (1.24)	–1.81* (1.87)	–0.30 (1.66)
Loss CNV	6	83.2 (23.1)	59.1* (13.5)	0.16 (1.77)	–0.94 (1.55)	0.46 (1.70)
Known CNV	11	79.8* (18.3)	56.8† (7.7)	–0.87 (1.57)	–1.89* (2.16)	–0.43 (1.69)

All data are shown as mean (SD). CNV indicates copy number variant; HC Z, head circumference *Z* score at 14 mo; length Z, length-for-age *Z* score at 14 mo; MDI, mental developmental index; PDI, psychomotor developmental index; and weight Z, weight-for-age *Z* score at 14 mo.

*$P<0.05$ compared with genotype–.

†$P<0.005$ compared with genotype–.

‡Size of each cohort. Incomplete data for outcomes resulted in lower n.

Carey et al., Circ Cardiovasc Genet 2013

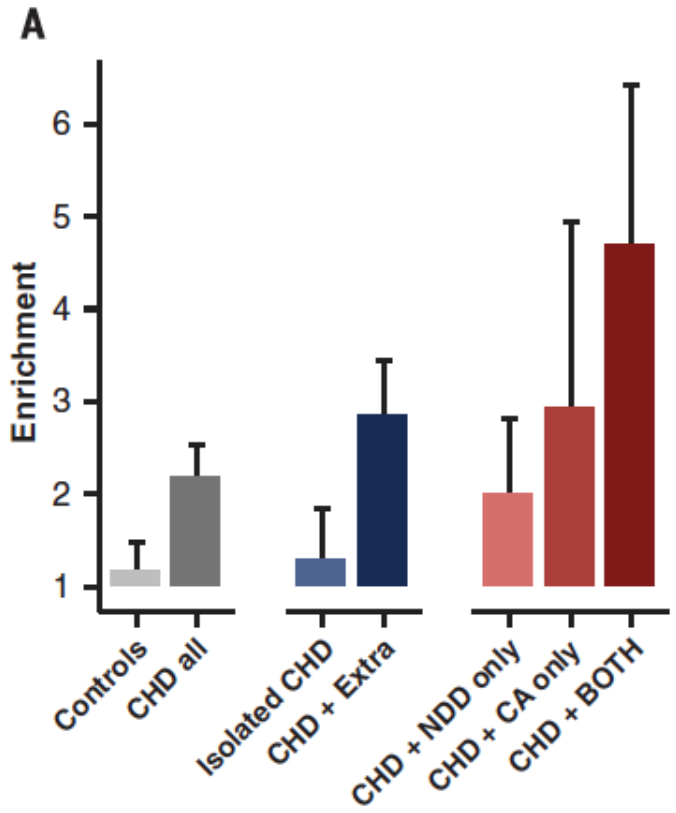
De Novo Damaging Single Nucleotide Variants in CHD

- Pediatric Cardiac Genomics Consortium (PCGC)
  - Funded by NHLBI and now also NICHD
- Whole exome sequencing case-parent trios (n=362)
- De novo mutations thought to be pathogenic found in ~10% CHD cases with critical, sporadic CHD

Zaidi et al., Nature 2013

De Novo Damaging Mutations by CHD Subgroups

- 1213 case-parent trios
- NDD, neurodevelopmental disabilities
- CA, extracardiac congenital anomalies



Homsy et al., Science 2015

Prevalence of De Novo Damaging Mutations in CHD

- 20% with CHD and both NDD/CA
- 10% with CHD and CA
- 6% with CHD and NDD
- 2% with isolated CHD

Homsy et al., Science 2015

De Novo Damaging Mutations in CHD

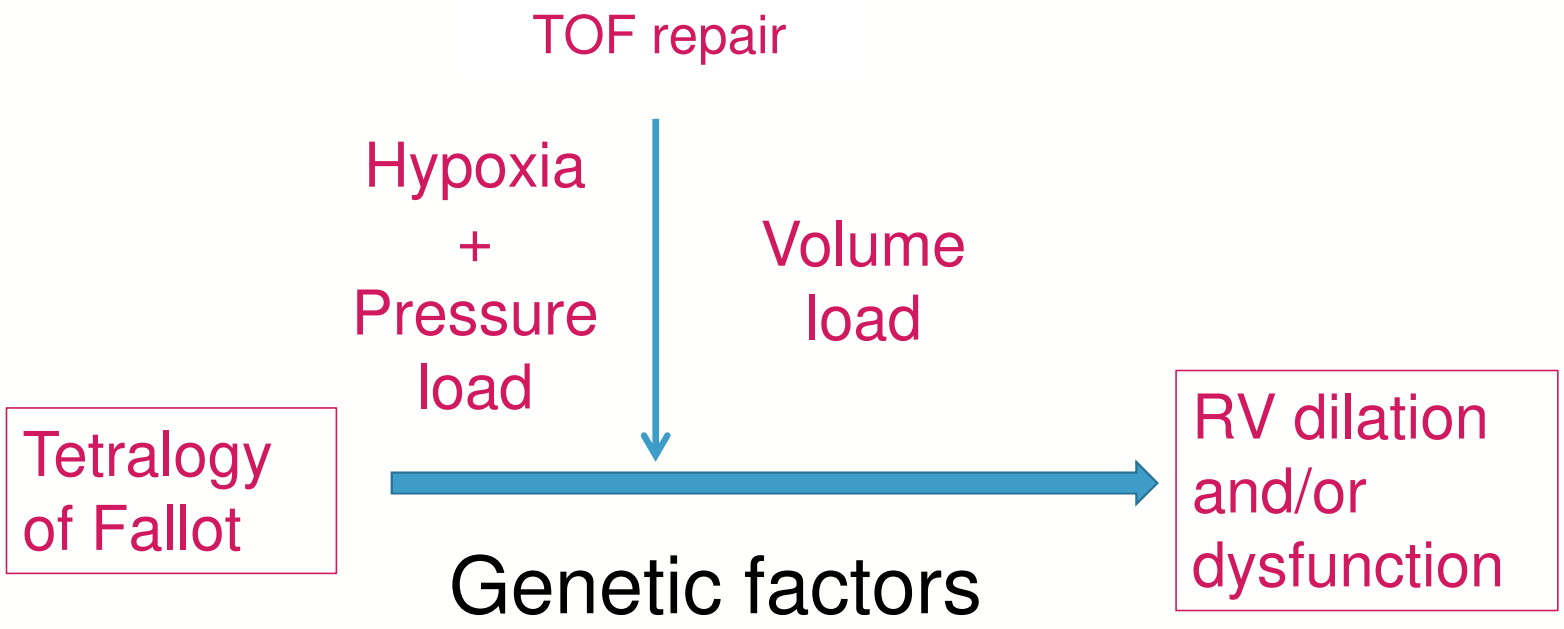
- Disease genes for CHD overlapped with disease genes in cohorts with neurodevelopmental disorders (pleotropic effects)
- Suggests: CHD cases with damaging de novo mutations in overlapping genes at *highest risk* for neurodevelopmental disorders
- Clinical study to assess ND status in patients with and without damaging de novo mutations (NHLBI and NICHD)

Homsy et al., Science 2015

Outline

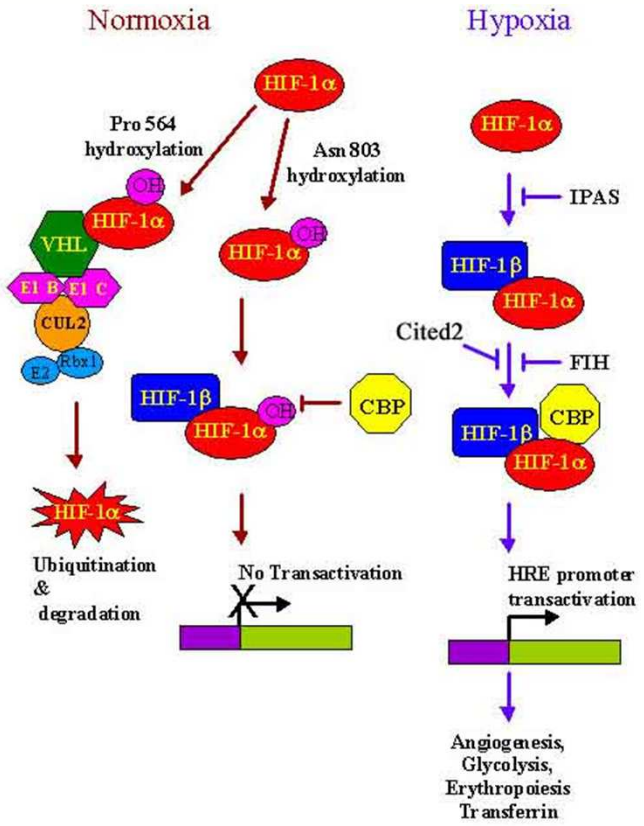
- Genetic etiology and outcome
- Genetic modifiers of outcome
- Promises and Challenges

Modifiers of RV Remodeling in TOF



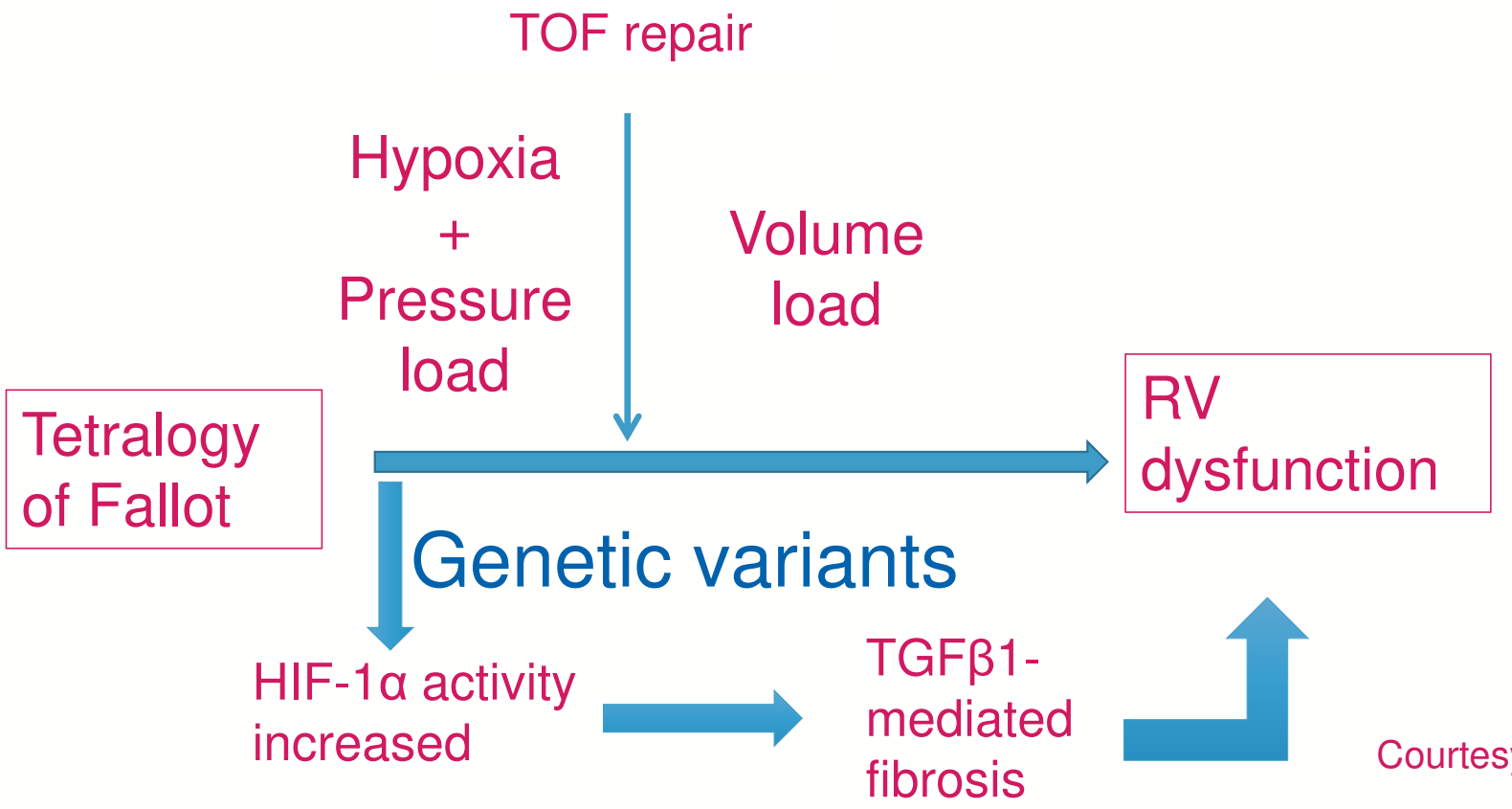
Courtesy of S. Mital

Hypoxia Inducible Factor 1 Alpha (HIF-1α)



- Regulates response to hypoxia; binds to tissue specific *hypoxia response elements* (HREs) to regulate gene expression
- Acute hypoxia: induces cell proliferation, angiogenesis, metabolic adaptation
- Chronic hypoxia: apoptosis, TGFβ1-mediated fibrosis

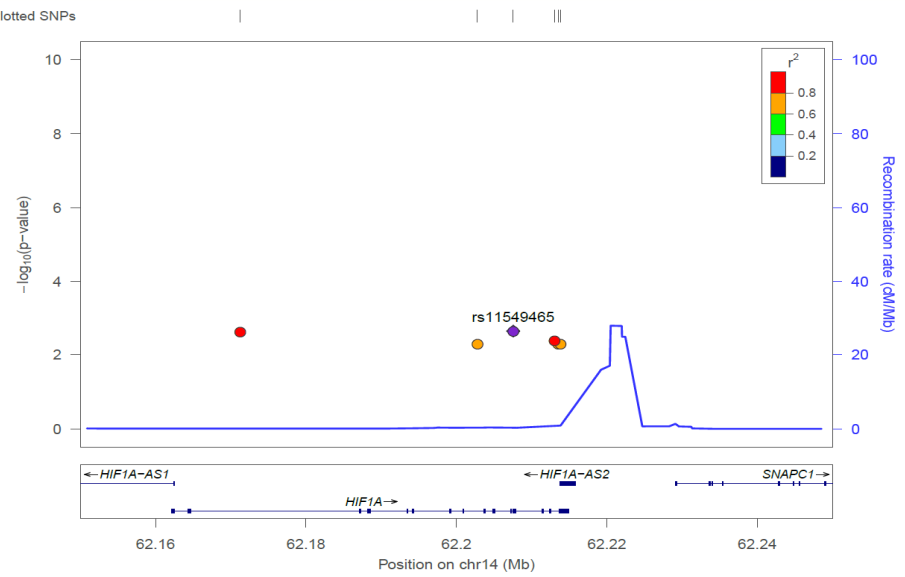
Modifiers of RV Remodeling in TOF



Courtesy of S. Mital

HIF1α Variants Associated with Fibrotic Score in TOF

- Measured RV fibrosis on CMR in 237 cases with repaired TOF
- Fibrotic load inversely assoc with RVEF
- 6 of 48 SNPs in *HIF1α* assoc with fibrotic score
- Could we modify response in at-risk populations with anti-fibrotic medications?



Hoang et al., submitted

Genotype and Risk for JET in CHD

- Angiotensin-converting enzyme (ACE) I/D polymorphism associated with arrhythmias
- Hypothesis: ACE I/D polymorphism associated with risk of JET post op CHD repair
- 174 cases Norwood, ASO, TOF, VSD or VSD genotyped
- 21% developed JET
- D/D genotype independently associated with JET
- Identify at-risk patient and modify outcome?

Table 2 Multivariable analysis of covariates associated with the development of junctional ectopic tachycardia

Adjusted variable	Adjusted odds ratio (95% confidence interval)	*P*
D/D genotype	2.4 (1.04–5.34)	.04
Aortic cross-clamp time	1.02 (1.00–1.04)	.04
Cardiopulmonary bypass time	1.00 (0.99–1.01)	.99
Age	0.99 (0.98–0.99)	.01
Inotrope score	0.92 (0.85–0.99)	.03

Borgman et al., Heart Rhythm, 2011

ApoE and Behavior in CHD

- ApoE important for neuronal repair
- Tested 3 genotypes and neurobehavioral outcomes in 380 preschool children
- ε2 genotype associated with:
  - Increased behavior problems
  - Impaired social interactions
  - Restricted behavior patterns

Gaynor et al., Pediatrics, 2013

Pharmacogenetics

- Warfarin (CYP2C9 and VKORC1 genotypes, dosing and increased risk of hemorrhage)
- Clopidogrel (CYP2C19 genotype and reduced efficacy)
- Not currently used in pediatrics

Genetic Risk Score (GRS)

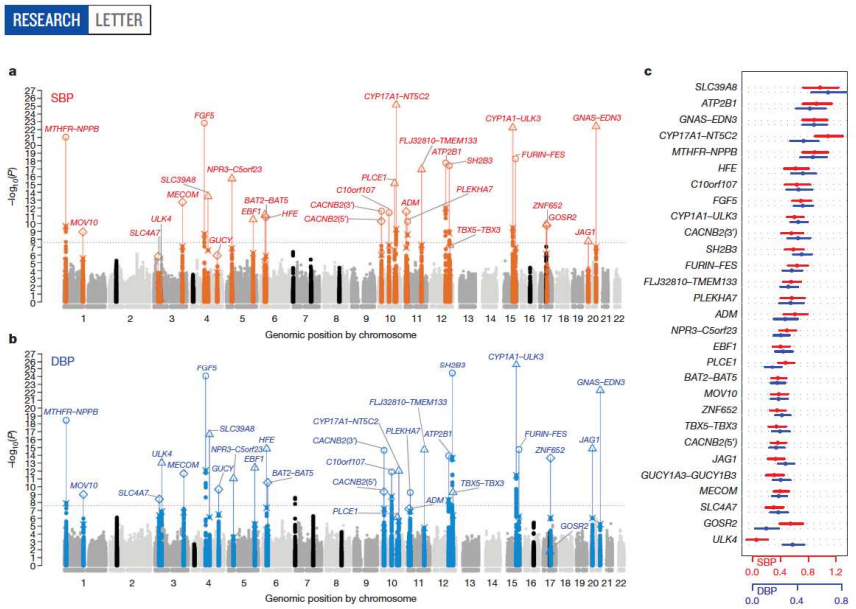


Figure 1 | Genome-wide $-\log_{10}$ *P*-value plots and effects for significant loci. a, b, Genome-wide $-\log_{10}$ *P*-value plots are shown for SBP (**a**) and DBP (**b**). SNPs within loci reaching genome-wide significance are labelled in red for SBP and blue for DBP (±2.5 Mb of lowest *P* value) and lowest *P* values in the initial genome-wide analysis as well as the results of analysis including validation data are labelled separately. The lowest *P* values in the initial GWAS are denoted with a X. The range of different sample sizes in the final meta-analysis including the validation data are indicated as: circle (96,000–140,000), triangle (>140,000–180,000) and diamond (>180,000–220,000). SNPs near unconfirmed loci are in black. The horizontal dotted line is $P = 2.5 \times 10^{-8}$. *GUCY* denotes *GUCY1A3–GUCY1B3*. **c,** Effect size estimates and 95% confidence bars per blood-pressure-increasing allele of the 29 significant variants for SBP (red) and DBP (blue). Effect sizes are expressed in mm Hg per allele.

- Individual SNPs confer small risk
- Composite index:
  - Cumulative predictive ability of genetic variation
  - E.g. 29 SNPs that assess risk for adult HTN
- Combination of common and rare variants
- Risk Stratification

Implications: Precision Medicine

- Genotype explains some of the variability in observed outcomes
- Important to counsel the families about short and long term expectations
- Helps plan for resource utilization
- Will help us identify genotype-guided approaches to improve upon morbidity and quality of life
- Many unanswered questions and significant challenges

Challenges: Genetic

- Enormous genetic heterogeneity of CHD
  - Over 400 disease-related genes predicted
  - Very few recurrent findings to date
  - May need to be addressed by pathway level analyses
  - More data than we know how to interpret
  - At the same time, insufficient power to detect associations
- Complex traits
  - Multiple levels of variability: SNPs, CNVs, SNVs, common and rare.
  - Likely multigenic etiology

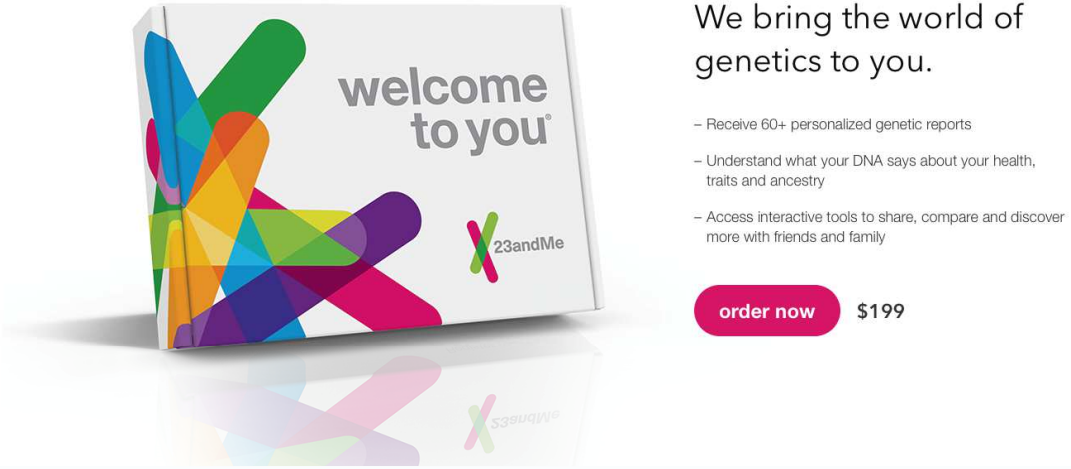
Challenges: Cardiac

- Defining the outcome
  - Some are very rare (e.g. sudden death in TOF, PLE in HLHS)
  - Some are difficult to define or measure (e.g. RV function)
  - Some require long-term follow up
- Careful clinical phenotyping
- Multicenter collaboration
- Funding for large scale, multicenter studies
- Equipoise

Starting Points

- Merging existing genomic and clinical datasets
- STS and PCGC

What Does the Future Hold?



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