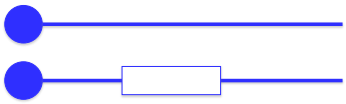
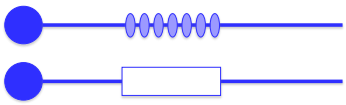
Genetic modifiers of cardiovascular anomalies in 22q11DS

Presented by Bernice Morrow,
Albert Einstein College of Medicine, Bronx, NY

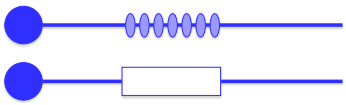
Why does the deletion occur?



What genes explain phenotype on 22q11.2?



What genes explain phenotype elsewhere?



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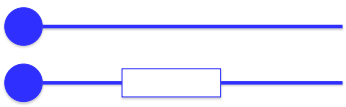
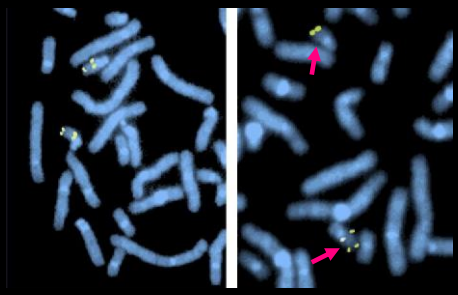
Could some of the same genes alter risk for non-syndromic birth defects?

Why does the deletion occur?

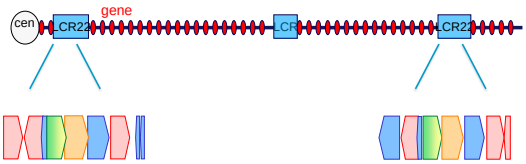


Fluorescence *in situ* hybridization to detect the 22q11.2 deletion

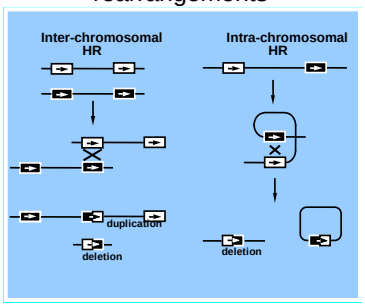


Structural organization of LCR22s

Maria Delio, Tingwei Guo
Collaboration with Deyou Zheng (Xingyi Guo), Wash U. Genome Center

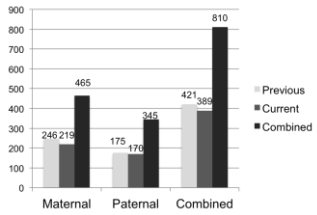


Meiotic NAHR between LCR22s mediate rearrangements



Are there gender differences in risk for the deletion?

35% increase in maternal risk for the 22q11.2 deletion



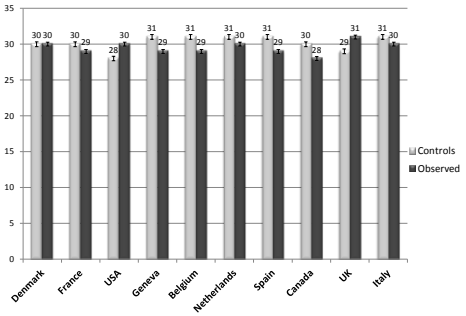
	Previous	Current	Combined
Two-Tailed p-Value	6.5E-4	0.0151	2.8E-5

Site	Address	Collaborators	Number of Samples
University Hospitals Leuven	Belgium	Ann Swillen, Koenraad Devriendt	91
University of Toronto	Canada	Anne Bassett, Eva Chow	109
Centro de Genética Humana	Chile	Gabriela Repetto	102
University of Mediterranee and AP-HM	France	Nicole Philip	30
Tel Aviv University	Israel	Doron Gothelf	152
Bambino Gesu Pediatric Hospital	Italy	Maria Cristina Digilio, Bruno Marino, Bruno Dallapicola	95
University Medical Center Utrecht	Netherlands	Frits Beemer	13
Academic Medical Centre	Netherlands	Therese van Amelsvoort	47
Instytut Centrum Zdrowia Matki Polki	Poland	Wanda Hawula	12
Hospital Universitari Son Dureta	Spain	Damian Heine Suner	29
The Children's Hospital of Philadelphia	USA	Donna McDonald-McGinn, Elaine Zackai, Bev Emanuel, Betsy Goldmuntz	364
Harvard Medical School	USA	Amy Roberts, Raju Kucherlapati	53
Upstate Medical University	USA	Robert Shprintzen, Wendy Kates	397
Children's Healthcare of Atlanta	USA	Karlene Coleman	75
UC Davis MIND Institute	USA	Tony Simon	10
Montefiore Medical Center	USA	Joy Samanich, Bob Marion	1
Medical College of Wisconsin	USA	Aoy Tomita-Mitchell	36
UCLA California	USA	Carrie Bearden	65
		TOTAL =	**1681**

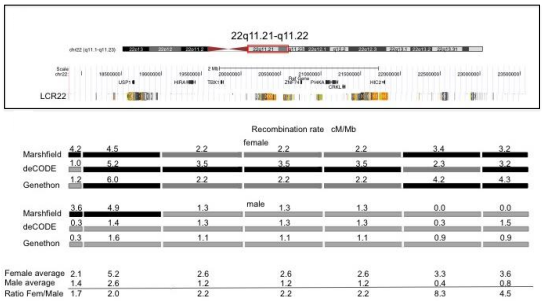
Data from 1,785 families demonstrate that maternal age doesn't influence risk for a 22q11.2 deletion

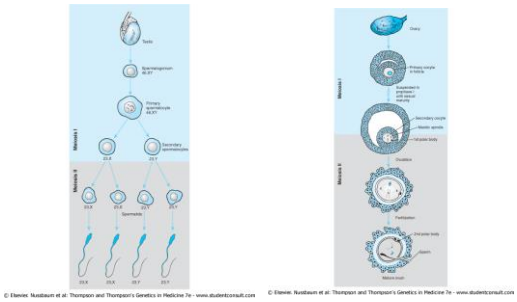
	Belgium	Denmark	Spain	France	Italy	Netherlands	UK	Switzerland	Canada	US	
10-14 years of age	0	0	0	0	0	0	0	1	0	0	1
15-19 years of age	1	2	1	3	3	2	1	2	3	23	41
20-24 years of age	18	6	3	17	13	20	5	14	29	129	254
25-29 years of age	46	20	7	27	29	68	23	44	53	262	579
30-34 years of age	27	18	13	34	30	91	36	39	44	276	608
35-39 years of age	11	9	1	5	14	37	17	10	17	130	251
40-44 years of age	0	1	0	5	6	1	2	2	0	31	48
45-49 years of age	0	0	0	0	0	1	0	0	0	2	3
50 and over	0	0	0	0	0	0	0	0	0	0	0
TOTALS	103	56	25	91	95	220	84	112	146	853	1785

No difference in maternal age of 22q11DS births as compared to normal births (United Nation Data)



Female recombination rate is higher than the male recombination rate

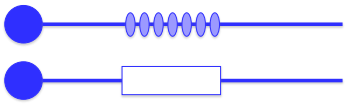




Next steps to further define mechanisms and genetic risk factors for the 22q11.2 deletion:

1. Long read WGS to define deletion breakpoints
2. Candidate gene approach to identify genetic risk factors
3. Gender based GWAS for genetic risk factors in 22q11.2 versus healthy controls

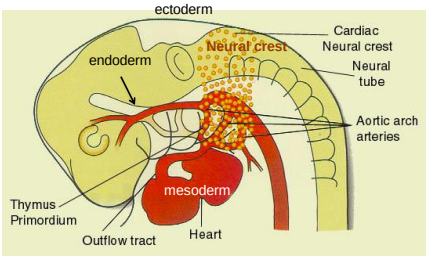
What genes explain phenotype on 22q11.2?

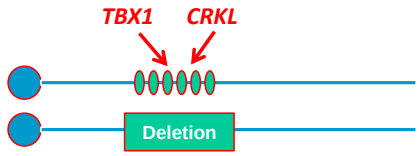


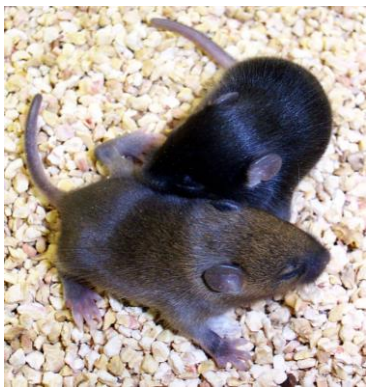
Clinical features:

1. Learning disabilities, behavioral problems
2. Craniofacial
3. Intra-cardiac defects-TOF, VSD, PTA
4. Aortic arch anomalies-IAAB, RAA
5. Immune dysfunction, low T-cells
6. Neonatal hypocalcemia
7. Many other, with reduced penetrance

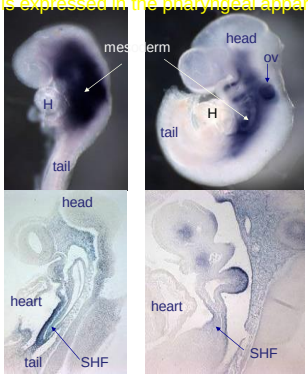
Structures affected derives from the embryo



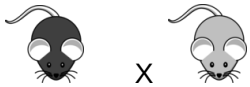




Tbx1 is expressed in the pharyngeal apparatus



Hypothesis: *Tbx1* and *Crkl* have tissue specific roles in forming the embryo

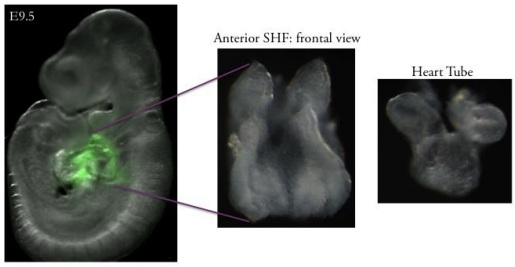


Tbx1 flox/flox; GFP flox/flox x *Mef2c-AHF-Cre; Tbx1 +/-*
Crkl flox/flox; GFP flox/flox x *Mef2c-AHF-Cre; Crkl +/-*

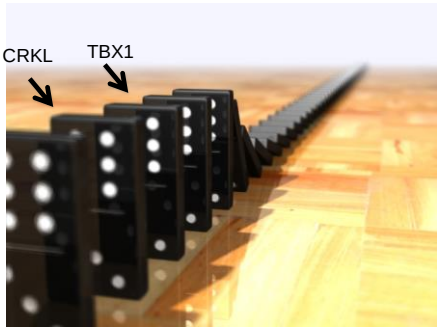
Embryos:
Second heart field inactivation of *Tbx1* or *Crkl* with a reporter

Silvia Racedo
Postdoc +
Collaboration with
Bin Zhou

Dissect tissue: normal versus Tbx1 or Crkl knockout
Find genes acting downstream to explain phenotypes



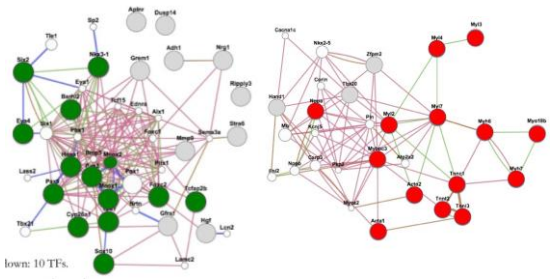
Mef2c-AHF-Cre/+; GFP f/+



From mice:

Genes down

Genes up



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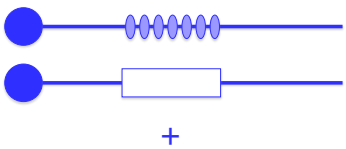

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Overall hypothesis:

2nd Hit Model for 22q11DS

1st hit: deletion

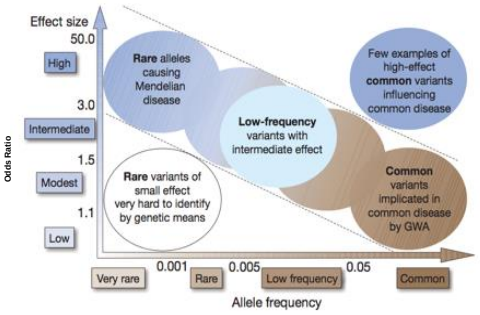
All the 22q11DS patients have a deletion (causative deletion), but why are some mildly affected (30% normal heart) and others severely affected (70%) ?

2nd Hit: Genetic modifiers

Identify the genetic modifiers contribute to congenital heart defects through GWAS/WES in 22q11DS patients

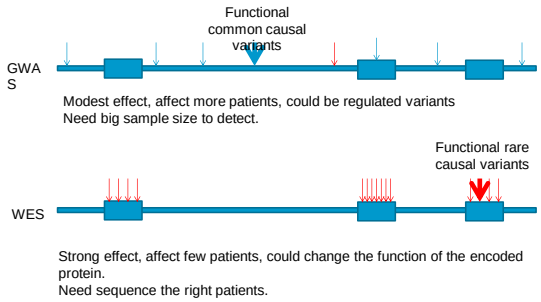
1) DNA variations in deletion region
2) DNA variations (CNVs, SNPs, mutation, rare or common) in the regions outside of deletion.

Of Men: Common Variants vs Rare Variants

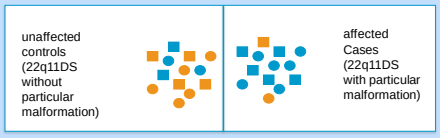


Manolio et al. Nature 461, 747-753 (2009)

Rare/common Variants



Association studies to find modifier genes



Hypothesis: Modifiers in 22q11DS can be identified by GWAS.

Tingwei Guo
Associate

GWAS; 706 DNA samples on Affymetrix 6.0 arrays

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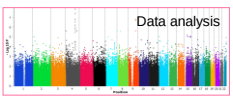
GWAS: 400 Affymetrix 6.0 Arrays

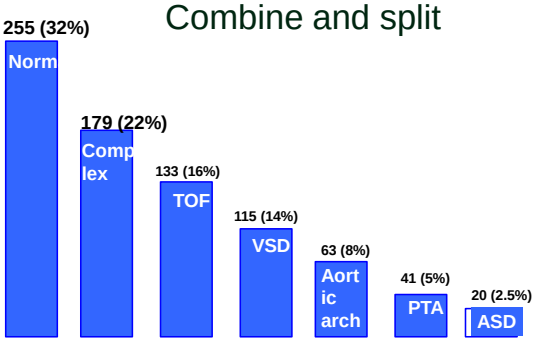


GWAS non-syndromic heart

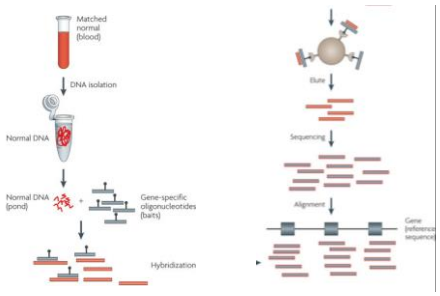
Mouse/zebrafish expression

Targeted capture top loci of genes

Combine and split

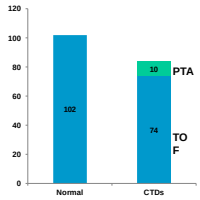


Whole Exome Sequencing (WES)

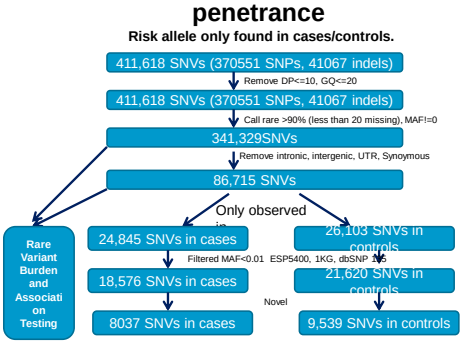


WES projects for 186 samples

1. DNAs from 209 22q11DS were sent to University of Washington. 186 were sequenced using Illumina Hi-seq 2000. NimbleGen SeqCap EZ Exome Library v3.0 was used for solution-based exome capture.
2. Phenotypes for 186 samples:



Risk/protective alleles with high penetrance



GWAS + WES will provide common and rare DNA variants to explain variable phenotypes.

Animal models will be used to determine if genes found are relevant to the phenotype.

In vitro models could explain how the DNA variant affects gene function.

Could some of the same genes alter risk for non-syndromic birth defects?

Acknowledgements:

1) Maria Delio, Tingwei Guo, Silvia Racedo, Jonathan Chung, Stephania Macchiarulo
2) International 22q11.2 consortium
3) Individuals with the syndrome and their parents.