Rapid Identification and Validation of Human Craniofacial Development Genes

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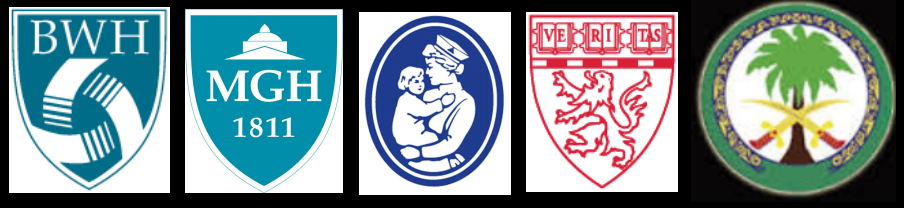
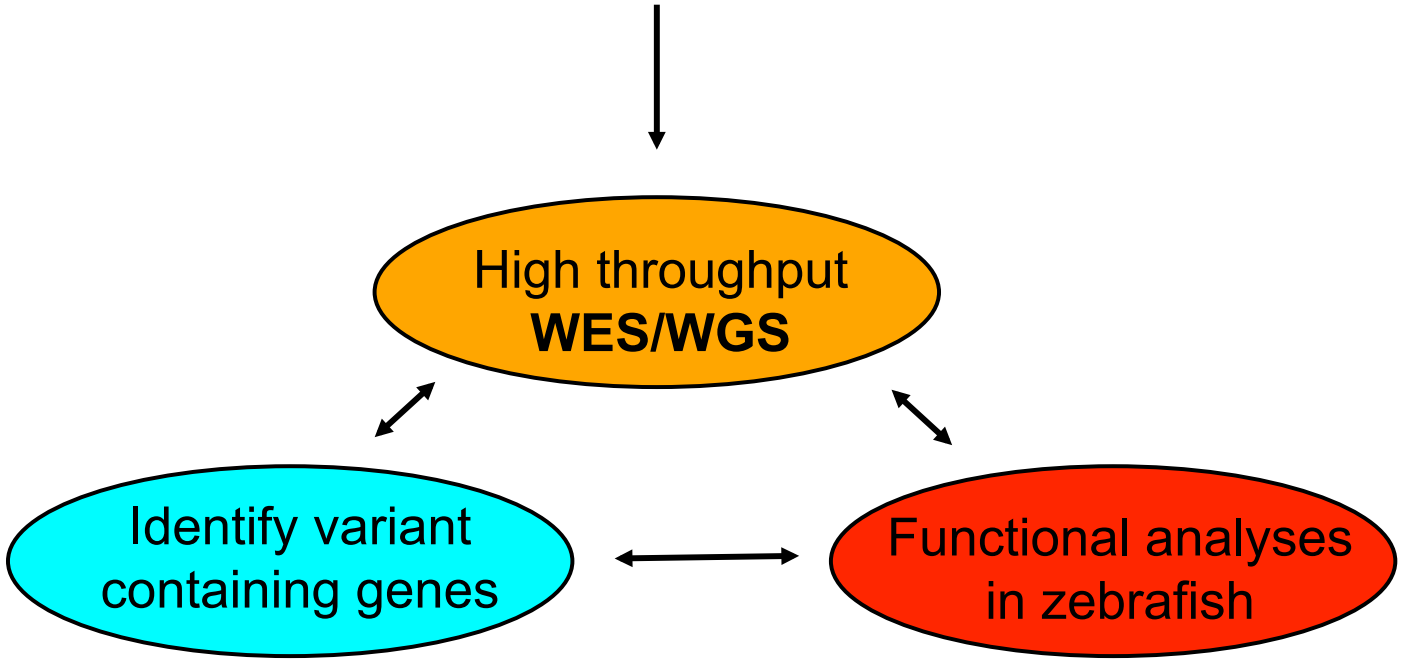


Discovering Human Birth Defect Genes

Identify genes in patients with craniofacial developmental defects *and* bioinformatically tractable monogenic inheritance



Rapid Craniofacial Gene Discovery: Specific Aims

1. Ascertain and recruit patients with a wide range of craniofacial dysmorphoses of likely monogenic etiology.

2. Rapid identification of genes regulating human craniofacial development (WES, WGS and seq. analysis).

3. Rapid expression and functional analysis of human candidate genes (zebrafish > mouse).

Case ascertainment

1. Goal of discovering new gene functions
   Reported rare phenotypes with unknown genetic etiology
   Rare unreported genetic phenotypes

2. High confidence of solving the case
   Assume complete penetrance of a monogenic disease
   Sufficient individuals to solve this case

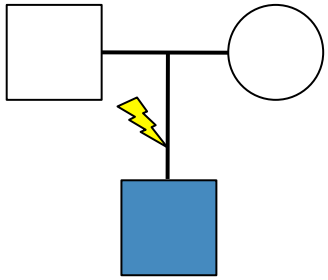
3. Variant interpretation
   Limit analysis to protein coding mutations, splice site mutations, and structural variants (~70-100 per individual)

Assign: 1 Clinician, 1 Bioinformatician, 1 Biologist per case

Three (3) bioinformatically solvable genetic paradigms

1. Dominant phenotypes

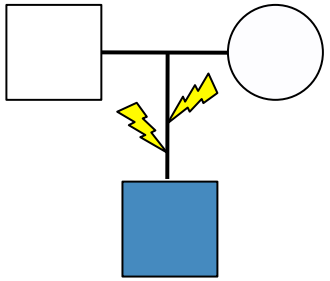
De novo mutations



0-3 coding non-synonymous mutations per individual

2. Recessive phenotypes

Rare compound heterozygote and homozygote mutations



3-5 compound heterozygote and 1-2 homozygote mutations per individual

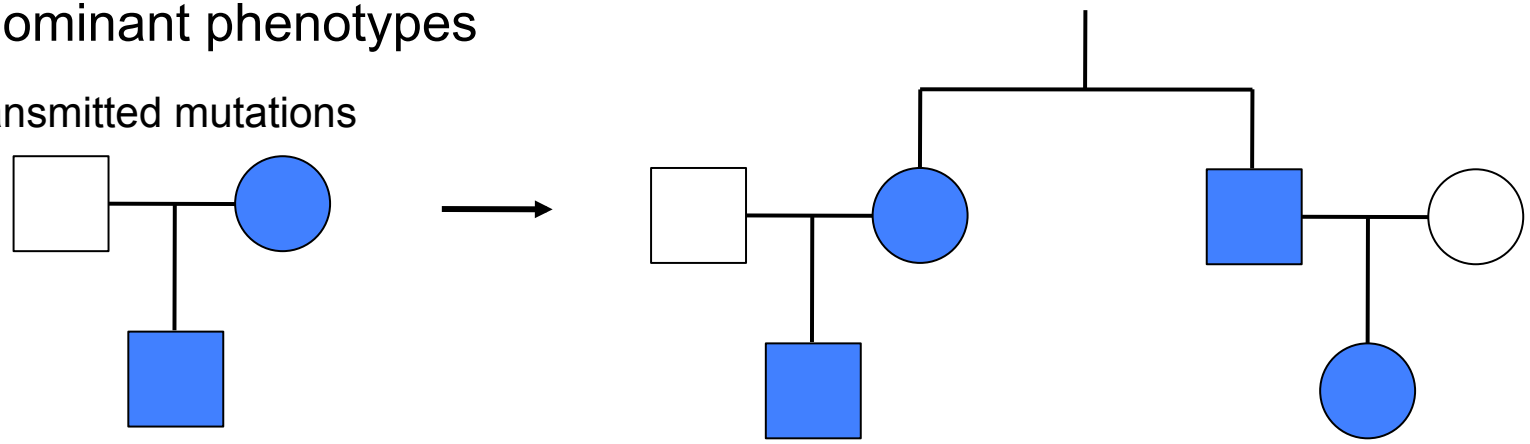
Assumptions

- Monogenic inheritance
- Complete penetrance
- Limit the first order analysis to protein coding mutations, splice site mutations and structural variants

Three (3) genetic paradigms, continued …

3. Dominant phenotypes

Transmitted mutations



~50 coding non-synonymous variants transmitted

A chance to solve the case on statistical grounds!

Strategy

Filter number of candidate SNVs down to a manageable number (*e.g.,* <10), then use an integrated, interdisciplinary approach.

Exceptions and Complications

Missing family members, X-linked and mitochondrial disorders, digenic and complex inheritance, incomplete penetrance, non-exonic mutations*, etc.*

Aim 1: Ascertain and recruit patients w/ a wide range of craniofacial dysmorphoses of likely monogenic etiology.

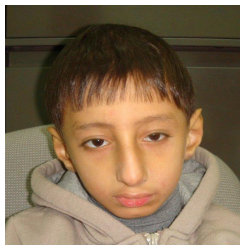
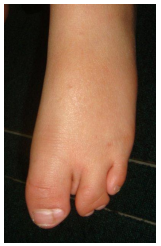
- Craniofacial and genetics clinics at Boston Children's Hospital (BCH), King Faisal Specialist Hospital & Research Center (KFSHRC), Riyadh
- Other collaborators, both national and international, including you! = FaceBase 2 colleagues
- FaceBase Biorepository samples (J. Murray *et al.,* U. Iowa)
- NIH Undiagnosed Disease Network (UDN, 7 clinical centers)

Craniofacial defect cases solved:

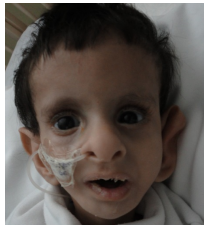
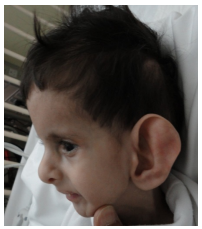
BWH, MGH and KFSHRC experience as of FB2 Kick-Off Meeting

Gene	Disorder	Phenotype	Proof	Institution
SUMO1	CL/P	CL/P (low penetrance)	Mouse	BWH
SPECC1L	ObFC	Oblique Facial Clefting, Tessier IV	Human, Fish, Fly	BWH, MGH
CAPZB	Pierre Robin syndrome	CP, Macrglossia, Micrognathia, Hypotonia	Human, Mouse, Fish	MGH, BWH
ZEB2	Mowat Wilson syndrome	Mandibular prognathism, type III malocclusion	Human, Mouse	Others, BWH
ATG4C	CP	CP	Fish	MGH
PIEZO2	DA5, GS, MWS	Arthrogryposis, characteristic facies, CL/P	Human, Fish, Fly	BWH, MGH
COG6	Novel syndrome	Anhidrosis, intellectual disability, craniofacial dysmorphism	Human	KFSHRC
TMEM231, C5orf42, EXO, C4, TCTN2	Meckel-Gruber syndrome	Skull defect, PCKD, polydactyly	Human	KFSHRC
EOGT, DOCK6	Adams-Oliver synd.	Cutis aplasia (scalp), limb reduct.	Human	KFSHRC
MEOX1	Klippel-Feil synd.	Segmentation defect cervical vert.	Human	KFSHRC
LARP7	Malpuech syndrome	Craniofacial dysmorphism, CP	Human	KFSHRC
C2orf37	Woodhouse-Sakati syndrome	Craniofacial dysmorphism, alopecia, hypogonadism	Human	KFSHRC
CENPJ	Seckel syndrome	Craniofacial dysmorphism, primordial dwarfism	Human	KFSHRC
FREM1	BNAR	Bifid nose, renal anomalies	Human	KFSHRC

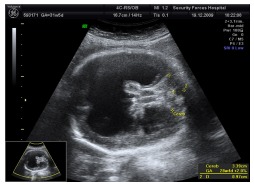
Five new cases since kickoff meeting
(Fowzan Alkuraya and colleagues, KFSHRC)

KF1 is a DNA damage repair gene with homozygous truncation in a patient **with primary microcephaly, Seckel facies** (but no dwarfism) and oligodactyly. He has cancer predisposition and was treated with chemotherapy for HCC.

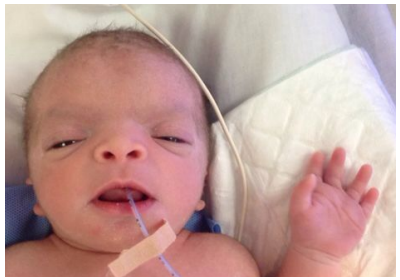



KF2 is a gene with homozygous truncation in a patient with bizarre **craniofacial dysmorphism, large ears** and profound postnatal growth retardation.



KF3 is a gene with homozygous truncation in a family with a novel syndrome including **massive congenital hydrocephalus** and Hirschsprung disease.

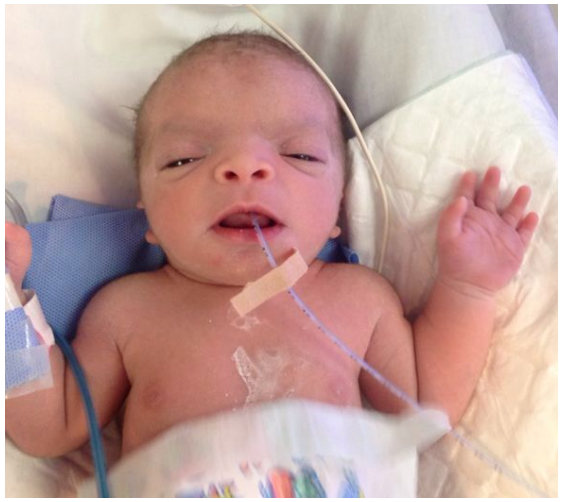
KF4 in a case with **facial dysmorphism** and skeletal dysplasia.



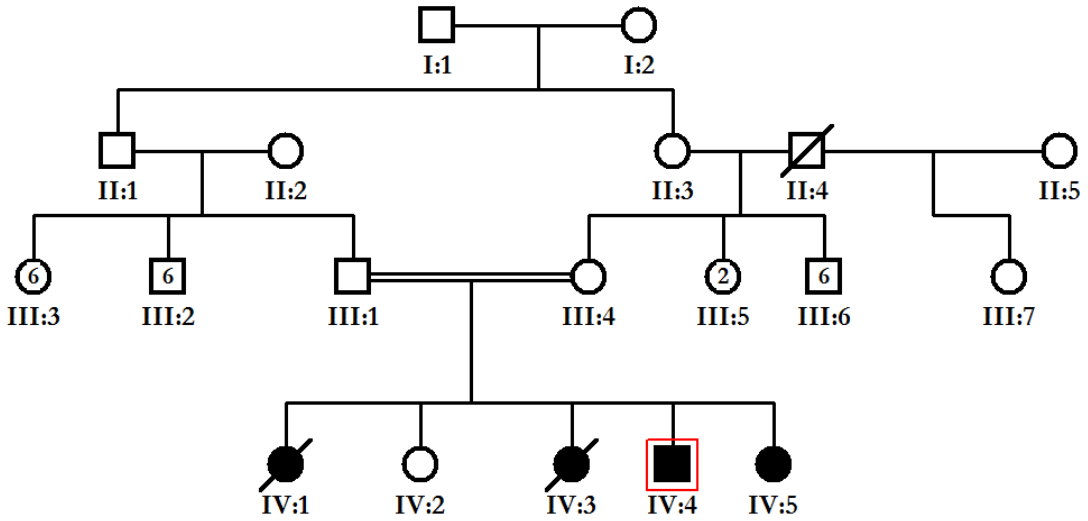
MYBPC2 novel gene with a missense mutation for **Frank ter Haar syndrome.** Only other known gene for this condition is *SH3PXD2B* but there is acknowledged genetic heterogeneity.

Frank ter Haar syndrome

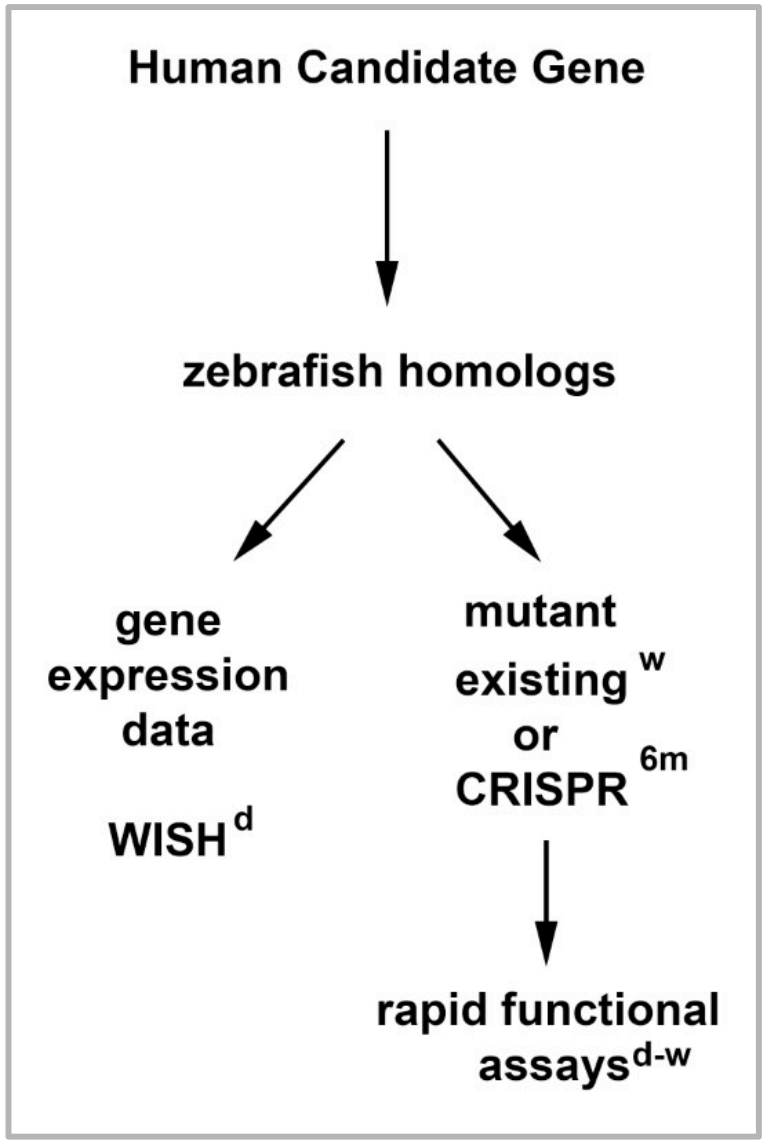
(Dermato-cardio-skeletal syndrome)



- Brachycephaly
- Wide fontanelles
- Prominent forehead
- Hypertelorism
- Flexion contractures
- Congenital glaucoma



FaceBase 2 Functional Genomics Pipeline

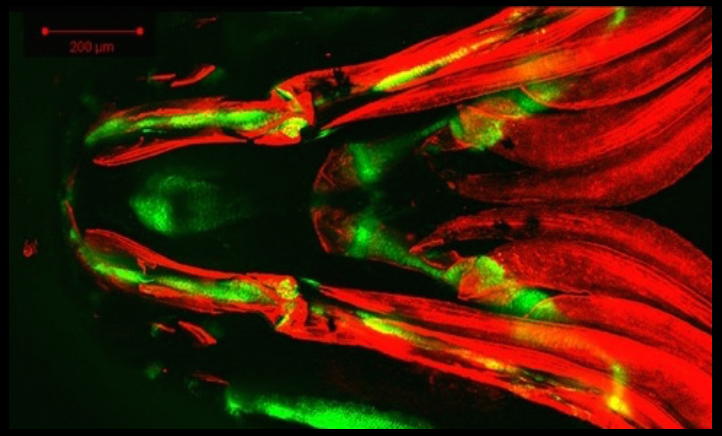


- Functional evidence for causality
  - Phenocopy clinical presentation
- Rapid gene editing
  - mutagenesis
  - knock-in

- Mechanism

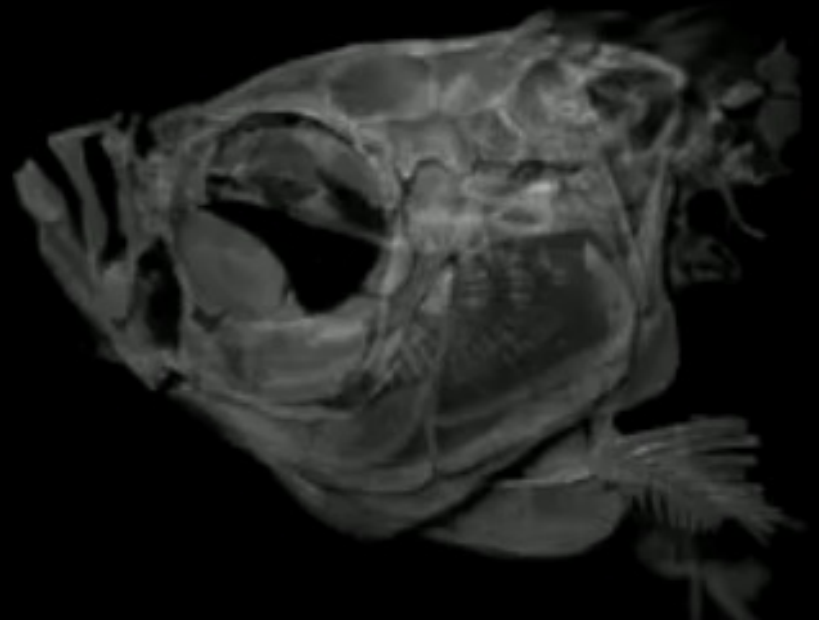
- Identify new pathways

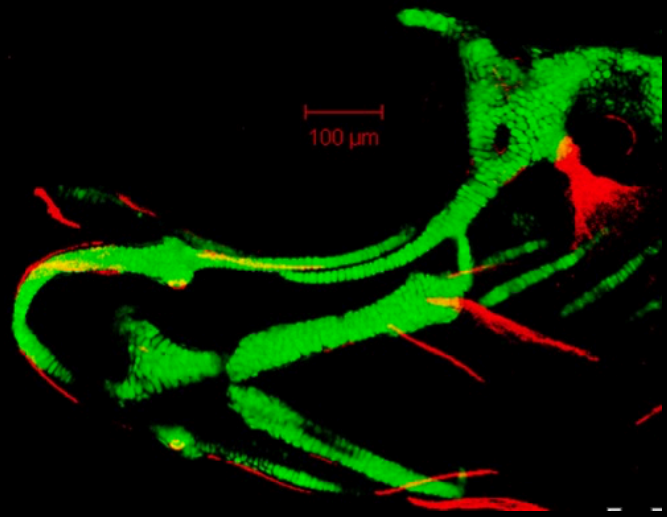
FaceBase



Welcome to FishFace: An Atlas of zebrafish craniofacial development

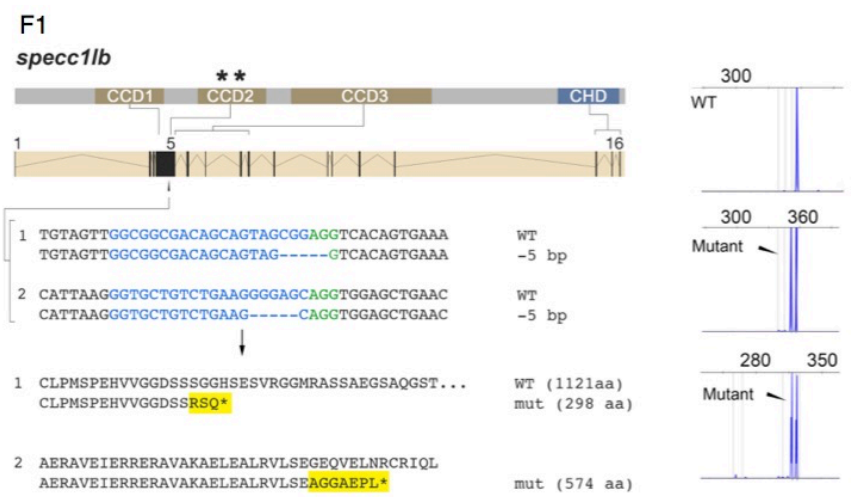
DeLaurier, A.F.	Eames, B.F.	Kimmel, C.B.	Nichols, J.T.	Ullmann, B.
Dowd, J.	Huycke, T.	McFadden, M.	Sasaki, M.M.	Walker, C.



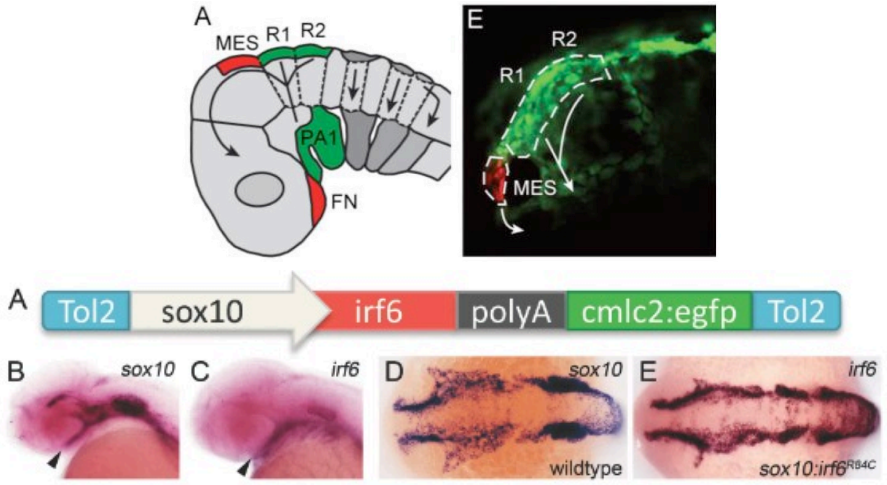


Efficient gene editing

Targeted mutagenesis



Transgenesis



Homologous recombination / Knock-in

Precise and efficient genome editing in zebrafish using the CRISPR/Cas9 system

Uwe Irion*, Jana Krauss and Christiane Nüsslein-Volhard

Mutagenesis projects

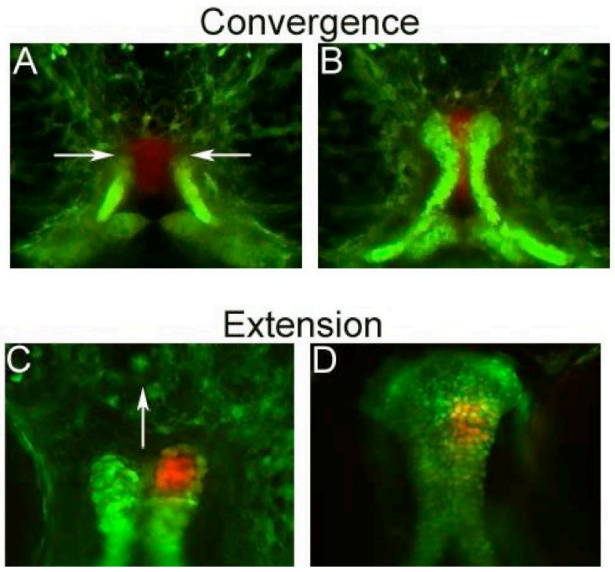
Retroviral Tg
ENU

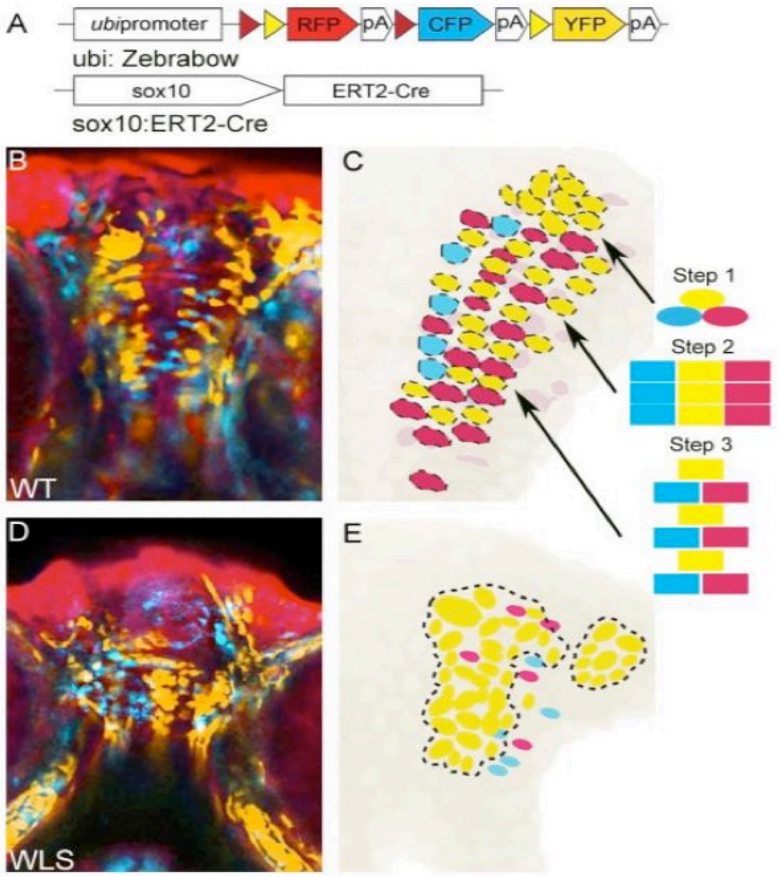
Rapid and detailed phenotype assays

Rapid gene expression analysis
Expression database

ZFIN
The Zebrafish Model Organism

In vivo visualization of cranial neural crest





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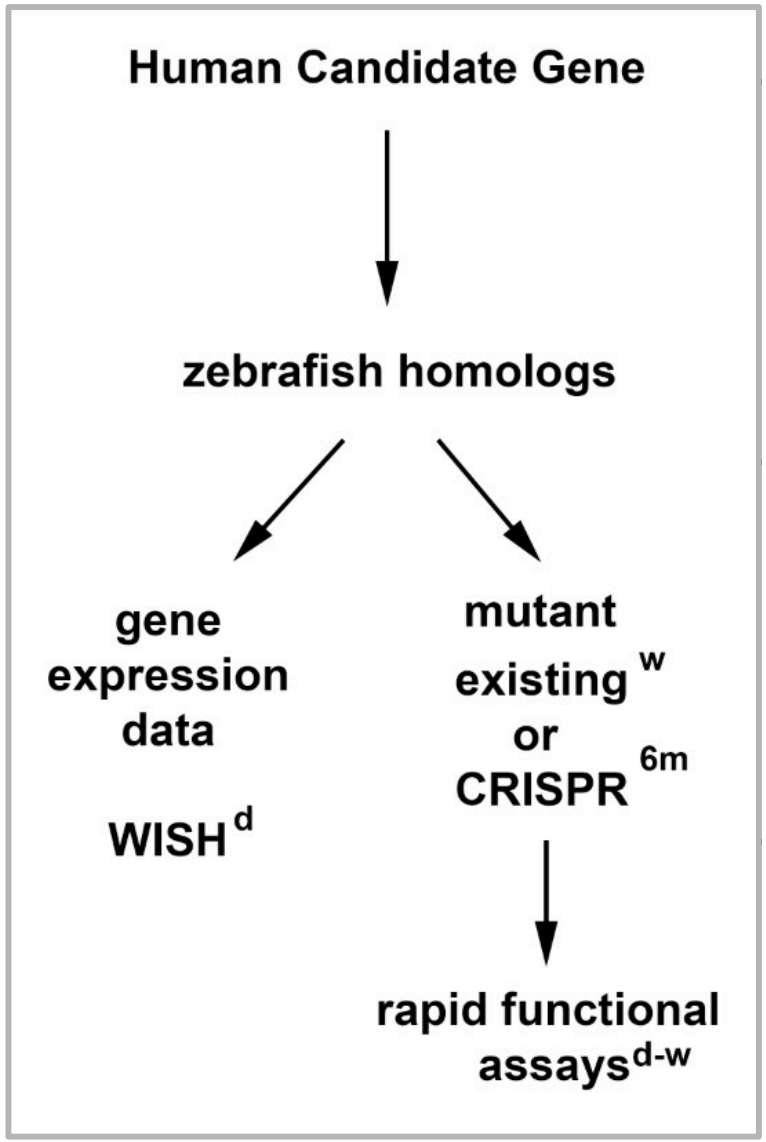

Functional evidence for causality
- new models of ObFC
- CLP / CP
- micrognathia

Rapid gene disruption
- *SPECC1L*
- *CAPZB*
- *ATG4C*

Identify new pathways
- cytoskeleton / wave complex
- autophagy

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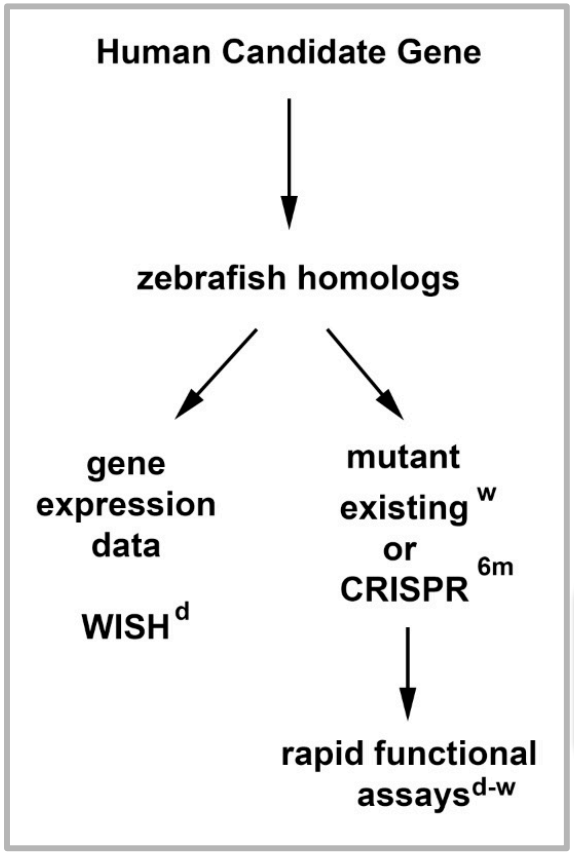

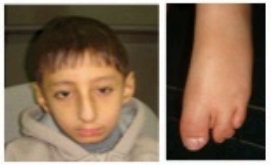

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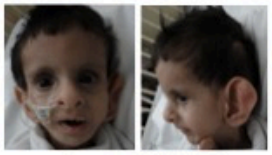

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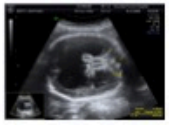

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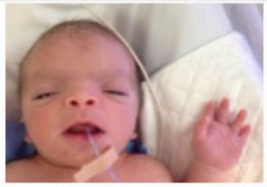

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FaceBase 2 Functional Genomics Pipeline

Candidate gene: *MYBPC2*
Zebrafish homologs: *mybpc2a, mybpc2b*

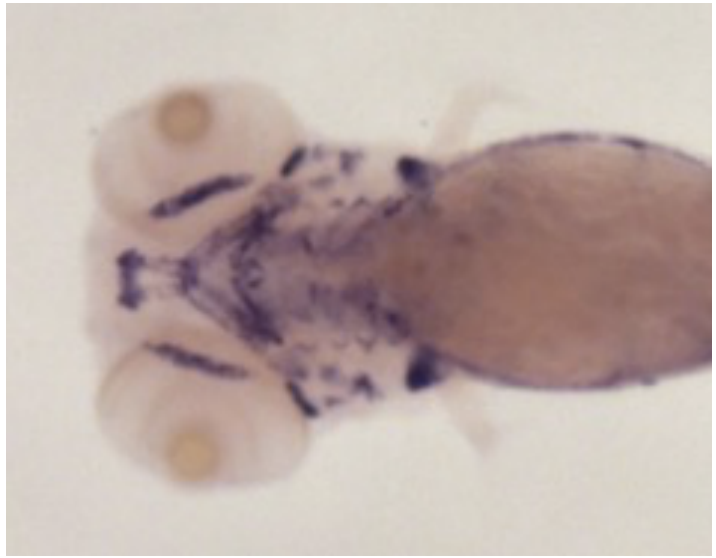
Dermato-cardio-skeletal
(Frank ter Haar)
Cleft palate
Skeletal dysmorphism
myosin binding protein C

Rapid gene expression analysis

Wealth of existing mutants

Gene editing

Rapid phenotype assays



mybpc2b

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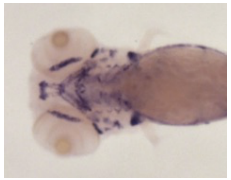

mybpc2b

Wealth of existing mutants

Gene edi

Rapid ph

Genomic Feature:	**la024094Tg**
Synonyms:	la024094 (2)
Affected Genes:	*mybpc2a* (1)
Construct:	Tg(nLacz-GTvirus) (1)
Type:	Transgenic Insertion (2)
Protocol:	embryos treated with DNA
Lab Of Origin:	Burgess & Lin Lab
Location:	Unmapped
Sequence:	GenBank:JS885776 (2)
Current Sources:	Zebrafish International Resource Center (ZIRC)

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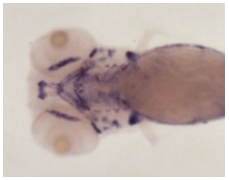


mybpc2b

Wealth of existing mutants

mybpc2a: la024094Tg

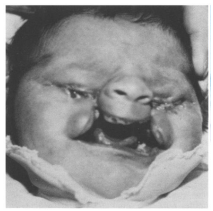
Gene editing

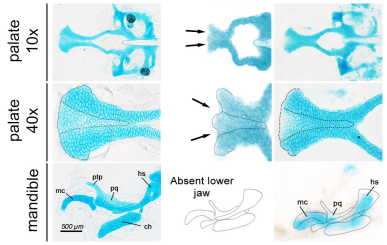
CRISPR *mybpc2b*

Rapid phenotype assays

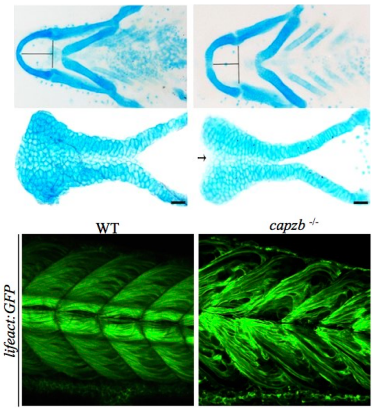
Functional Genomics -------- Pathways

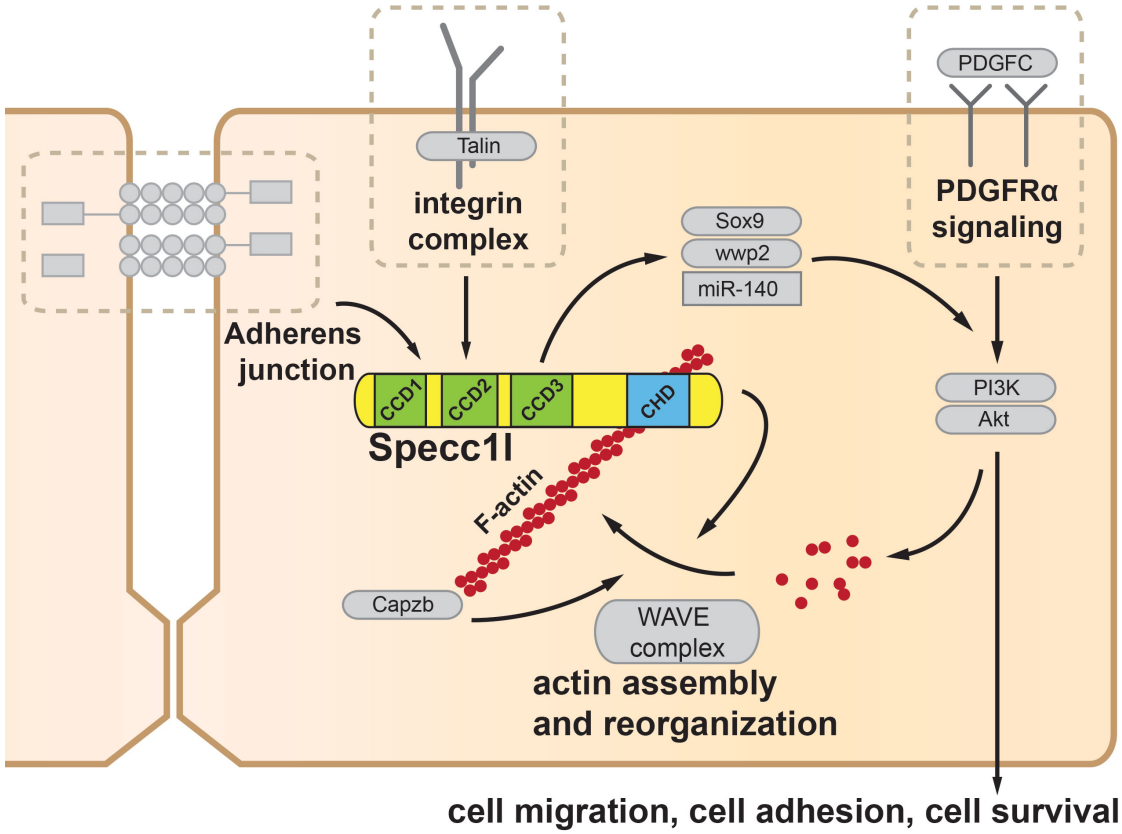
SPECC1L





CAPZB





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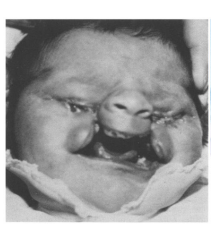
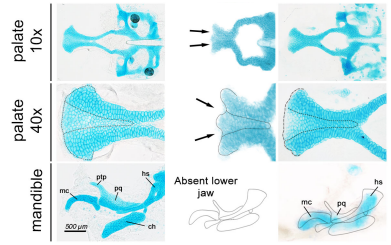
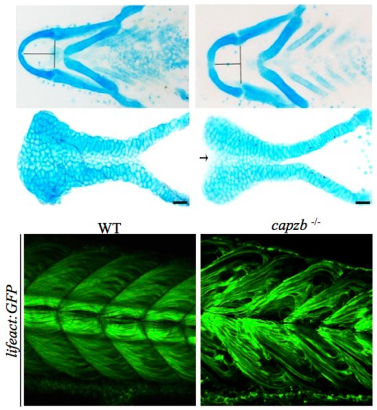
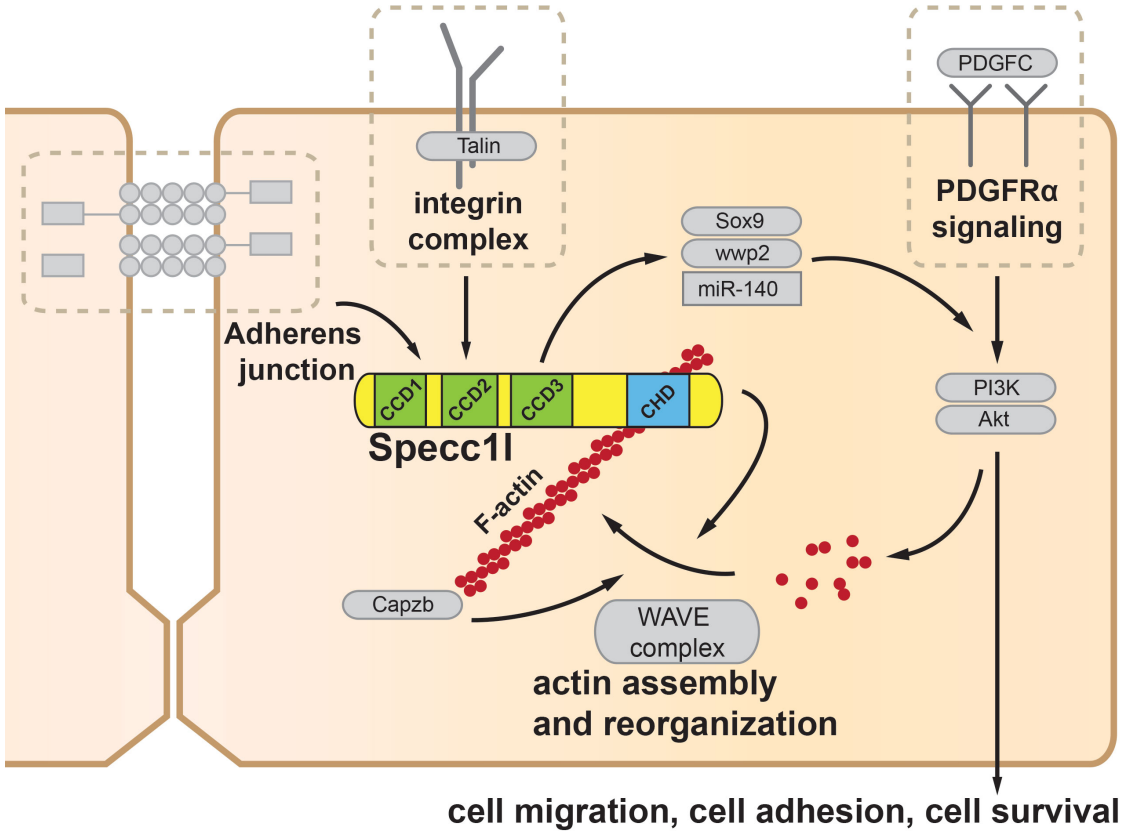




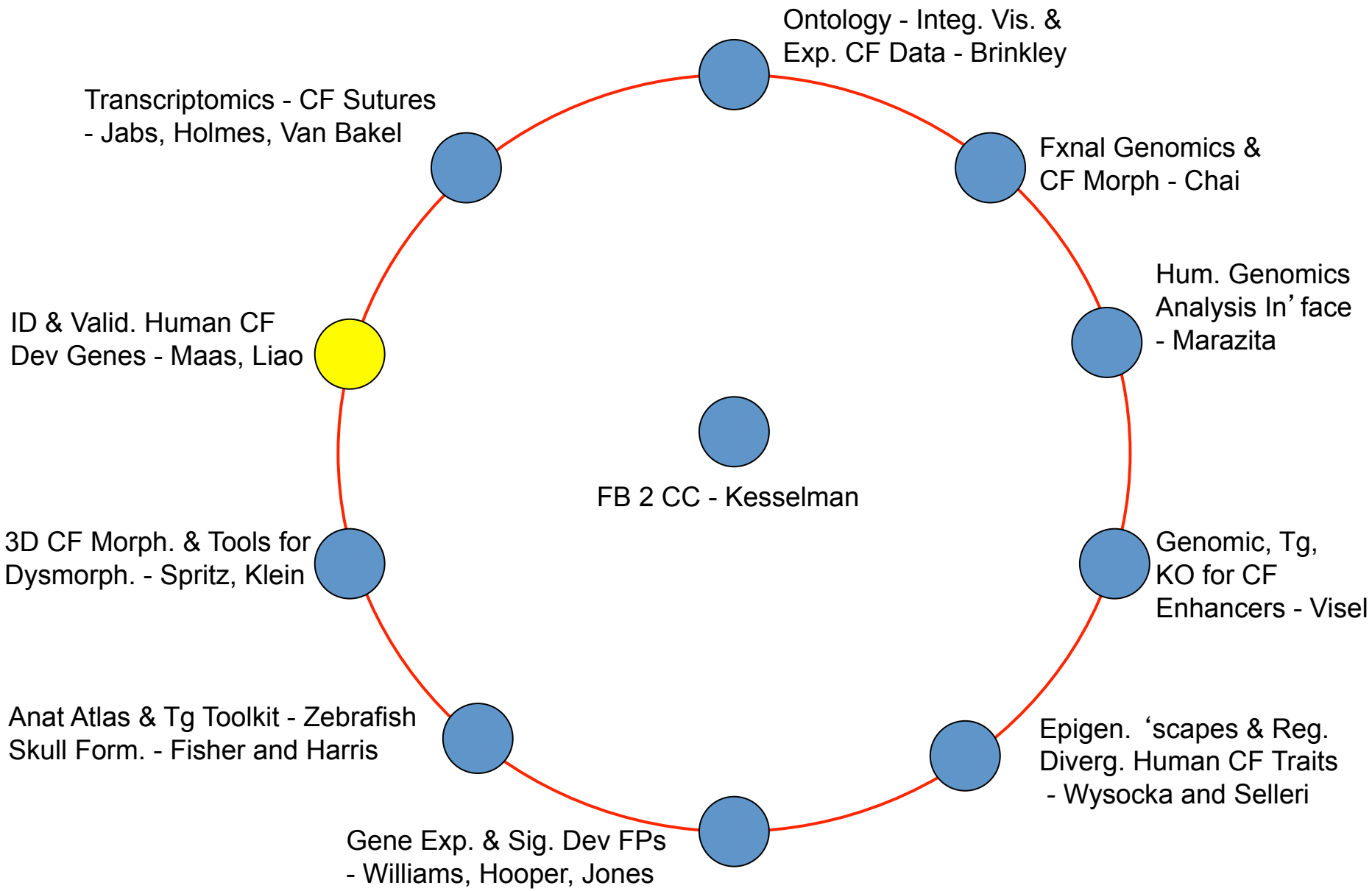
MYBPC2

CAPZB





Potential Interactions with Other Spoke Projects



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Brigham and Women's Hosp.
(Maas and Sunyaev Laboratories

Shamil Sunyaev, PhD
Dana Vuzman, PhD
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Peter Park, PhD
Hichem Miraoui, PhD

Boston Children's Hosp.

Joan Stoler MD
Catherine Nowak, MD

Massachusetts General Hosp.
(Liao Laboratory)

Kana Ishii, PhD
Irving Ling, MD
Edward Li
Kushi Mukherjee, PhD
Lucie Rochard, PhD

King Faisal Hosp. & Research Ctr.

Fowzan Alkurarya, MD
Ranad Shaheen, MD
Fatma Alzahrani, MD
Nisha Patel, MD

NIDCR Facebase - 2 U01DE024443
And NIDCR Program Staff

Plus many other colleagues!