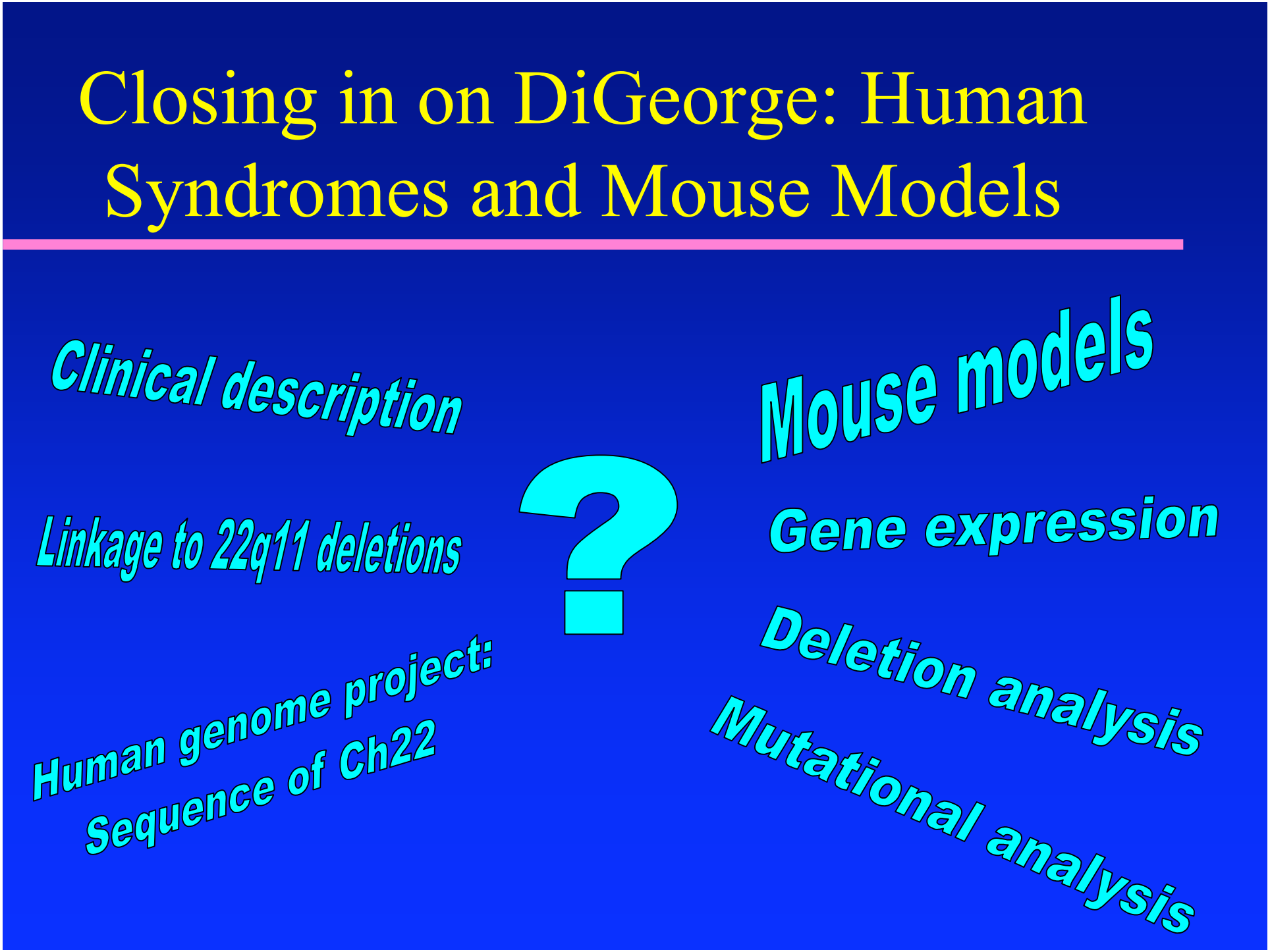
Closing in on DiGeorge: Human Syndromes and Mouse Models



The DiGeorge Syndrome (DGS)

- Cardiac outflow tract & septal defects
- Thymus and parathryoid hypoplasia
- Laryngeotrachial anomalies
- Craniofacial anomalies/facial dysmorphogenesis
- Cleft palate
- Micrognathia
- Low-set, abnormal ears
- Mental retardation

The DiGeorge/Velocardiofacial Syndrome (DGS/VCFS)

- Chr22 deletions
- Overlapping clinical features with Velocardiofacial syndrome, conotruncal face syndrome
- CATCH 22:

  Cardiac, T-cell deficit, Clefting, Hypocalcemia, Chromosome 22

  (it lived up to its namesake!)

Etiology of DGS

Developmental

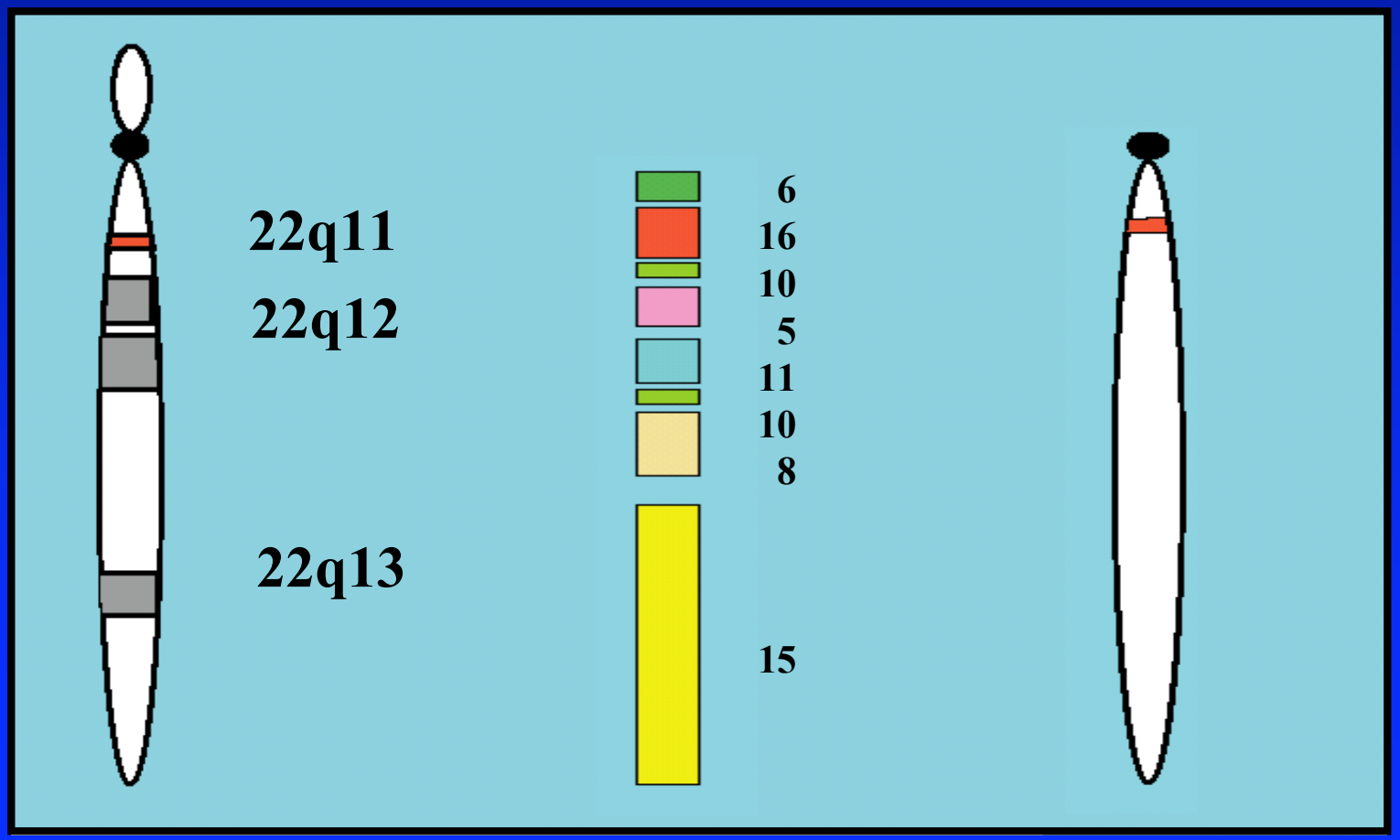
- Pharyngeal arch and pouch developmental defects
- Neural crest defects

Genetic

- Variable size deletions 1.5-3MB
- No common region of overlap
- Contiguous gene syndrome?
- Single gene haploinsufficiency?

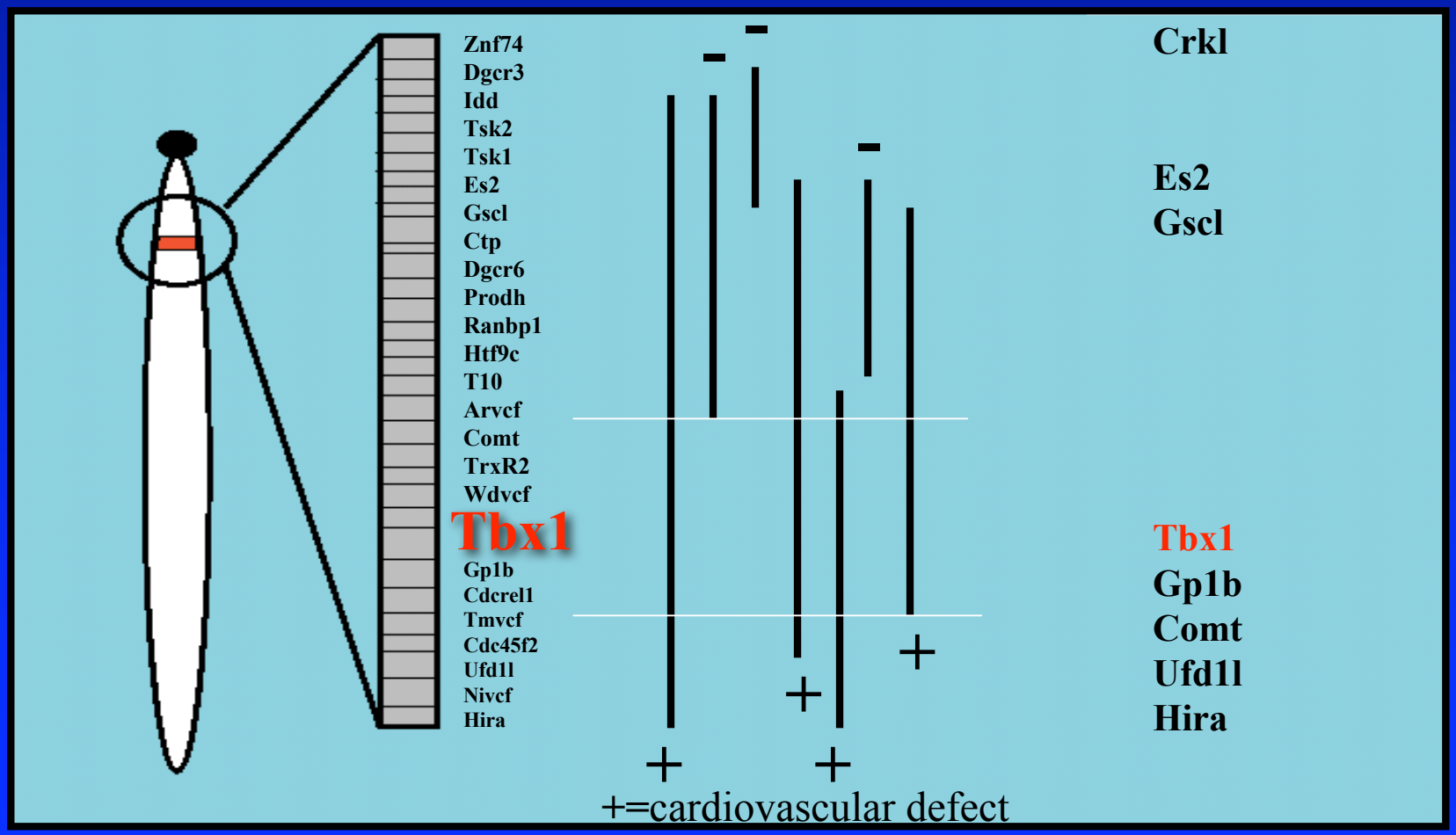
Regions of Conserved Synteny

Human Chromosome 22 | **Mouse Chromosome Synteny** | **Mouse Chromosome 16**



Mouse Chromosome 16

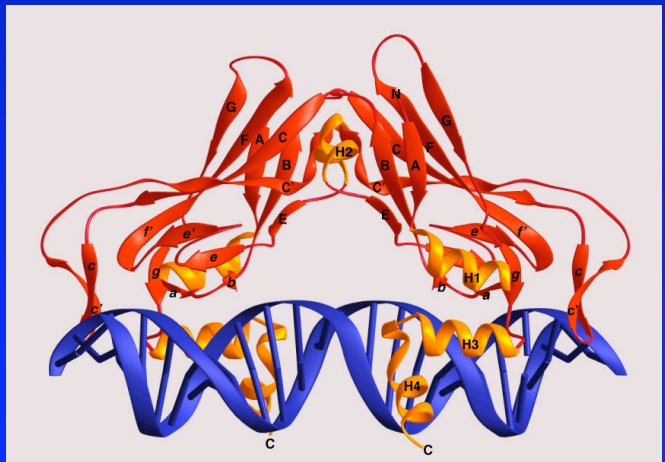
DGS syntenic region genes | **Deletion models** | **Candidate gene approach**



What is *Tbx1*
Is *Tbx1* the key?

The T-box Transcription Factor Gene Family

- 17 genes in mouse and human
- Conserved DNA binding domain - the T-domain
- Bind sequence-specific DNA as dimers
- Affect transcription of target genes
- Conserved developmental functions
- *Tbx1* maps to DGS region



T-domain dimer bound to DNA

Muller & Herrmann (1997) Nature 389:884

T-box gene mutations in human and mouse

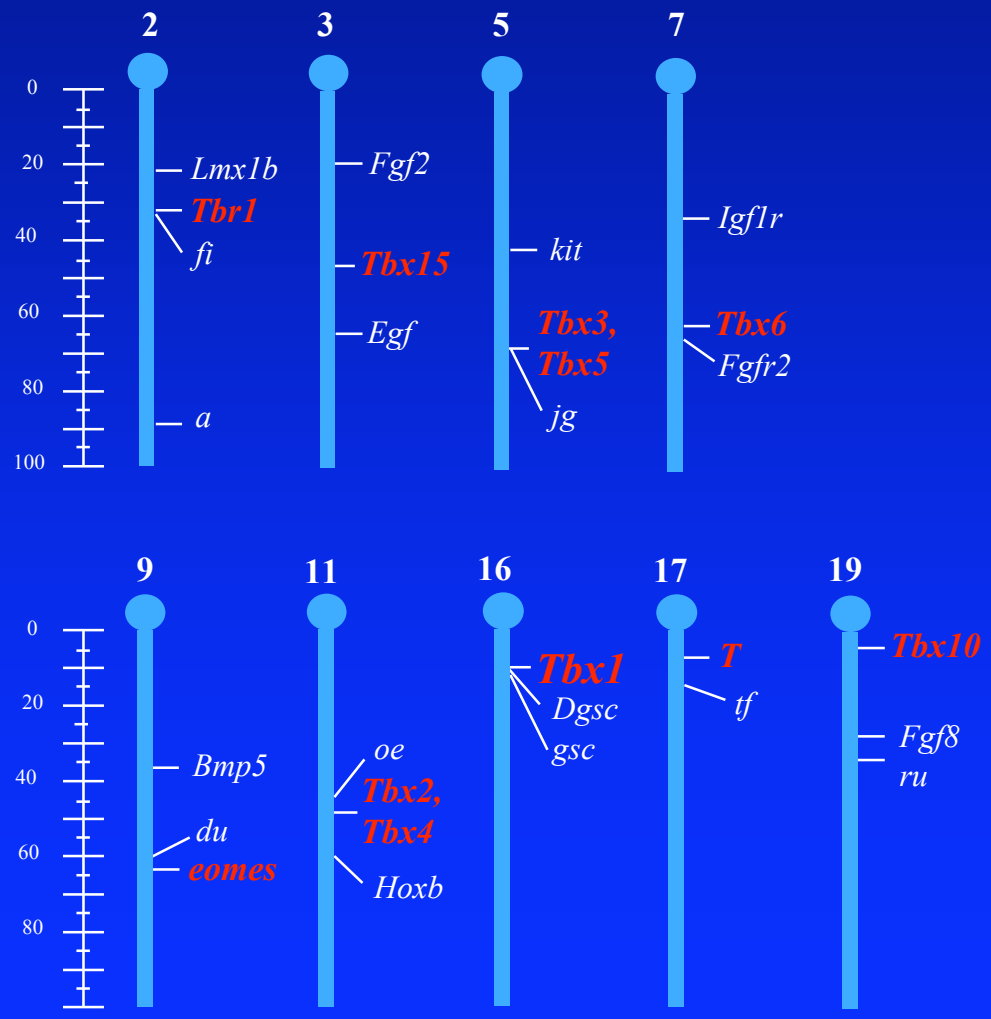
Human

- *TBX3* - ulnar-mammary syndrome
- *TBX4* - small patella syndrome
- *TBX5* - Holt-Oram syndrome
- *TBX19* - pituitary deficiency of ACTH
- *TBX22* - cleft palate with ankyloglossia

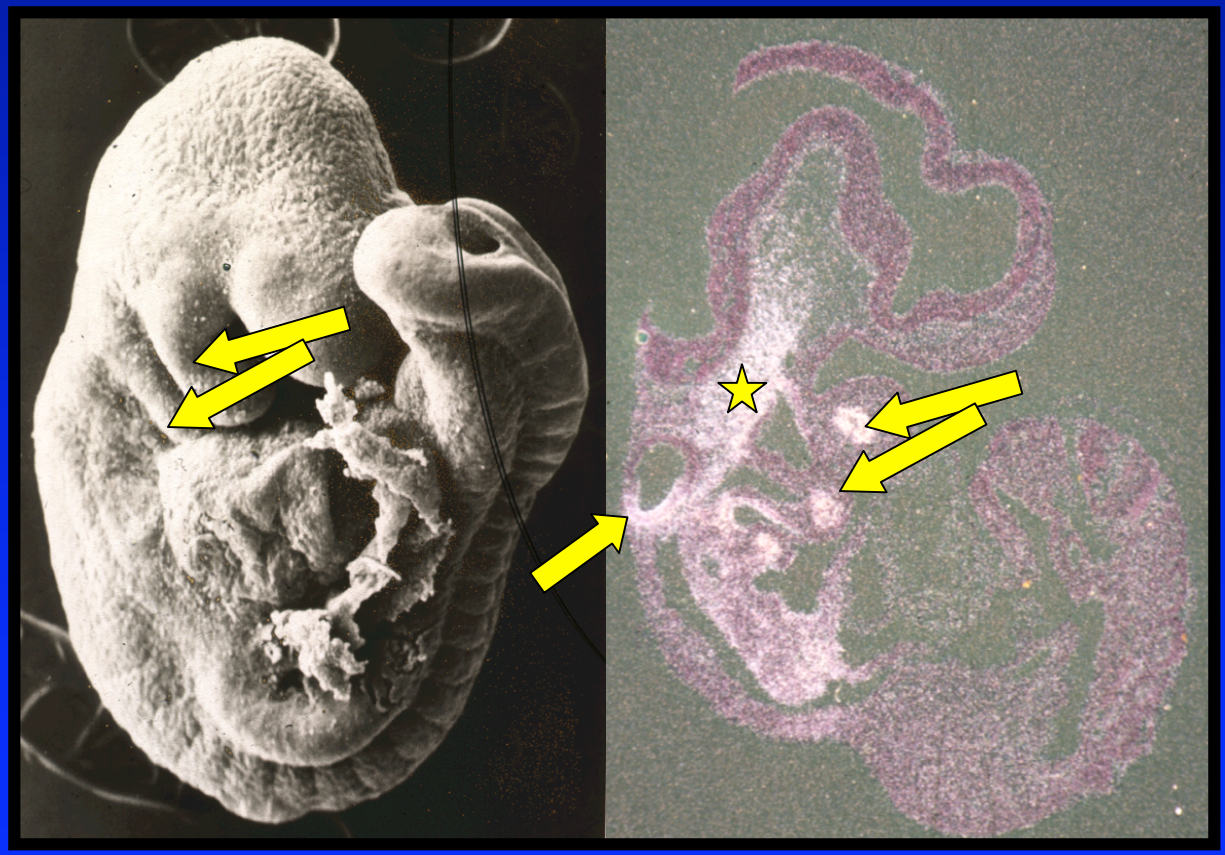
Mouse

- *Tbx2* – heart, limbs
- *Tbx3* – limbs, mammary, yolk sac
- *Tbx4* – hindlimb, allantois
- *Tbx5* – heart, forelimb
- *Tbx6* – paraxial mesoderm
- *T* – posterior mesoderm, notochord
- *Tbr1* – brain
- *Eomes* – trophectoderm, mesoderm
- *Tbet* – T cells
- *Tbx15* – D/V patterning
- *Tbx18* – somite patterning
- *Tbx20* – heart patterning

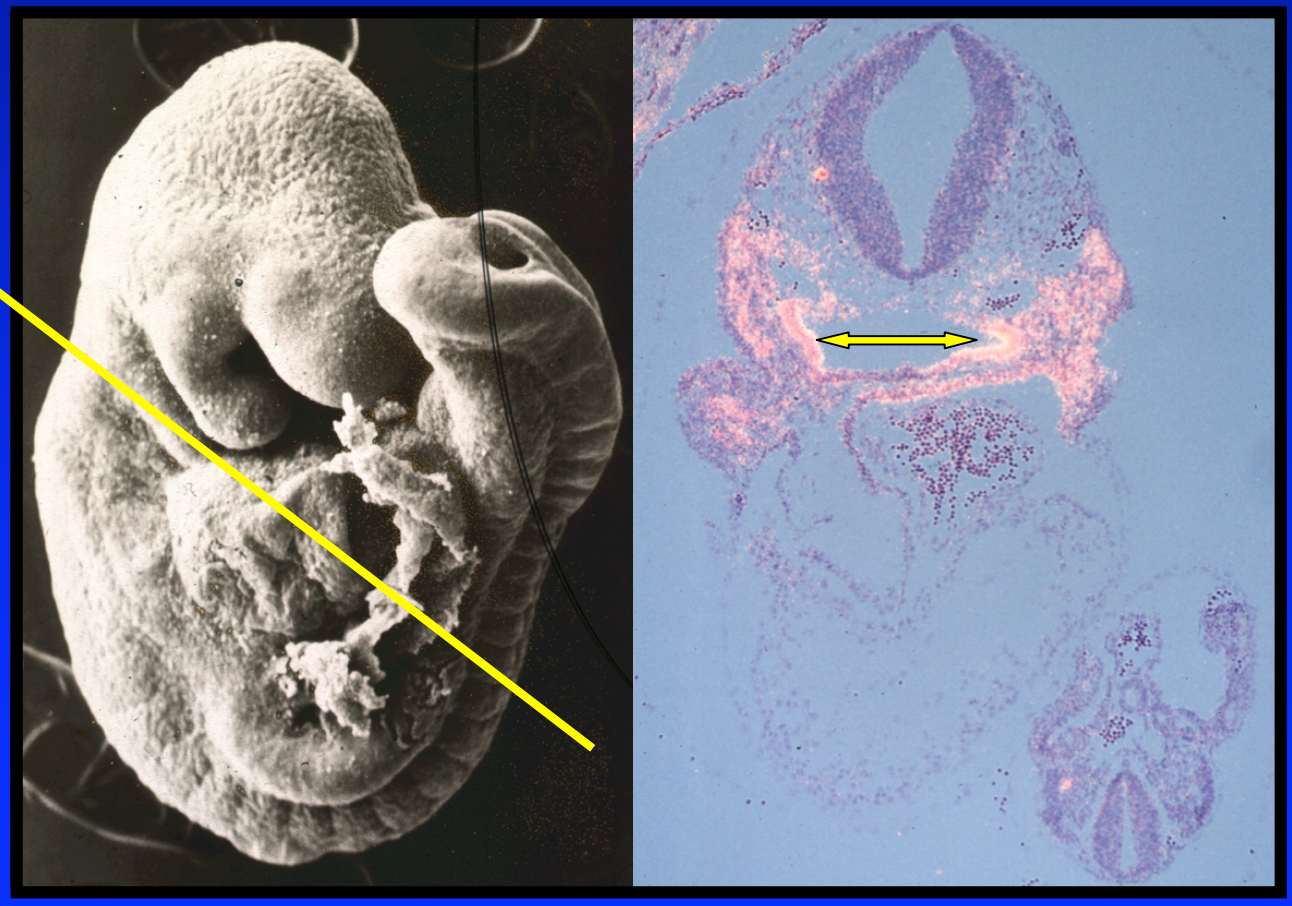
Chromosomal Locations of T-box Genes



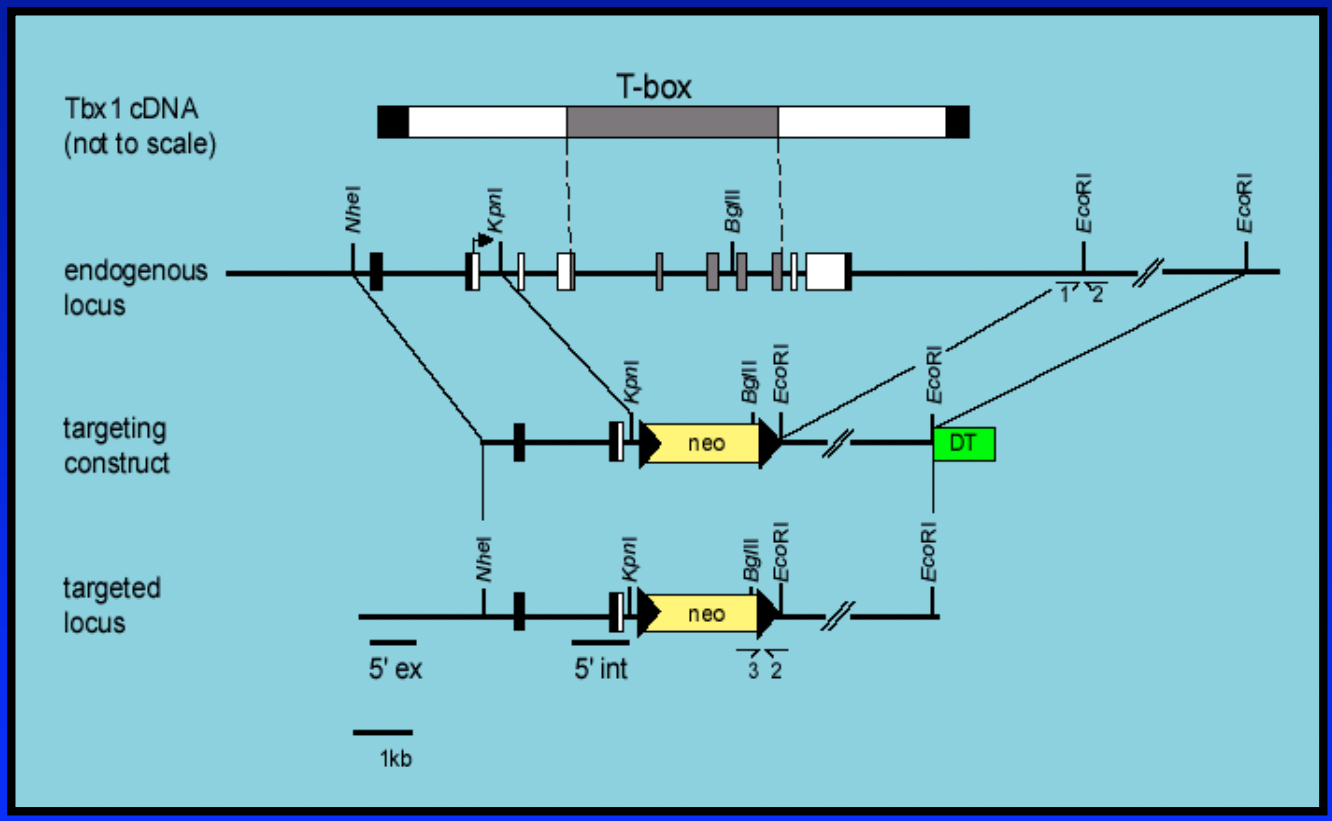
Tbx1 Expression at midgestation



Tbx1 Expression at midgestation



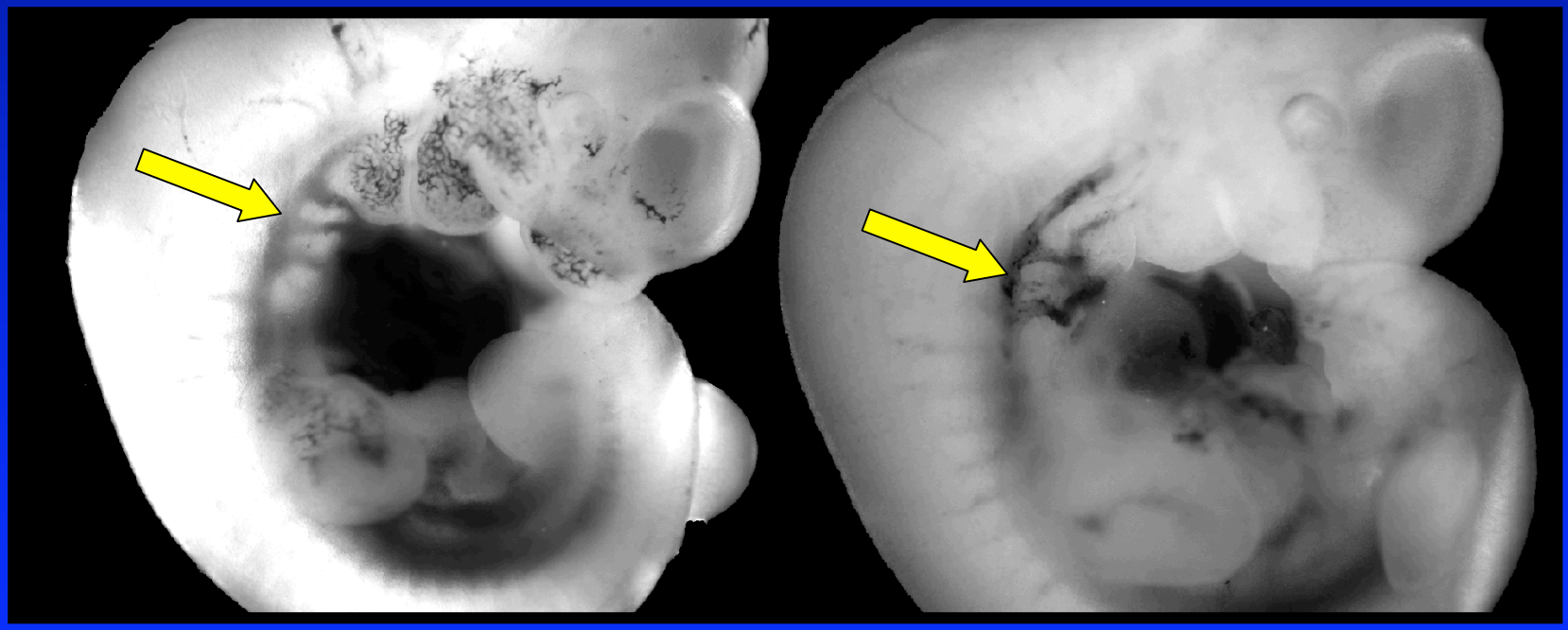
Tbx1 Targeted Mutagenesis



- Heterozygotes are viable and fertile
- Homozygotes die at birth

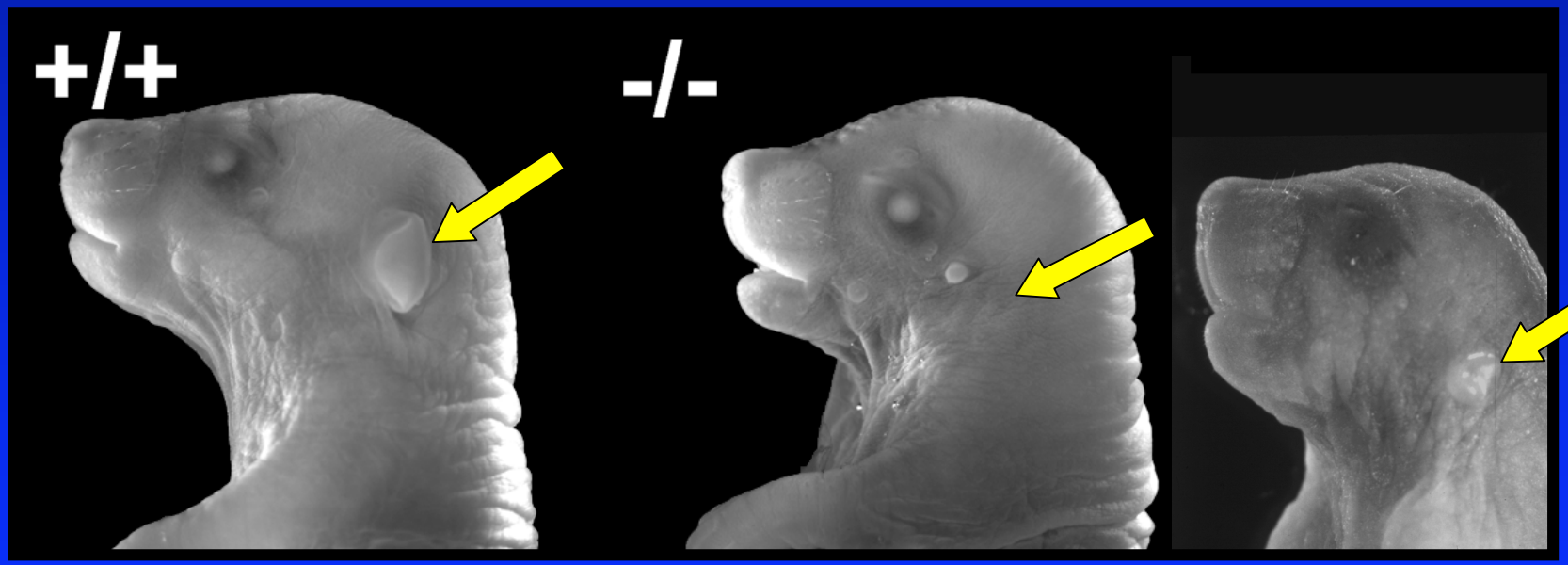
Aortic Arch Artery Defect in *Tbx1* Heterozygous Embryos

+/+ **+/-**



E11.5

Ear Defect in *Tbx1* Homozygous Mutant Newborns

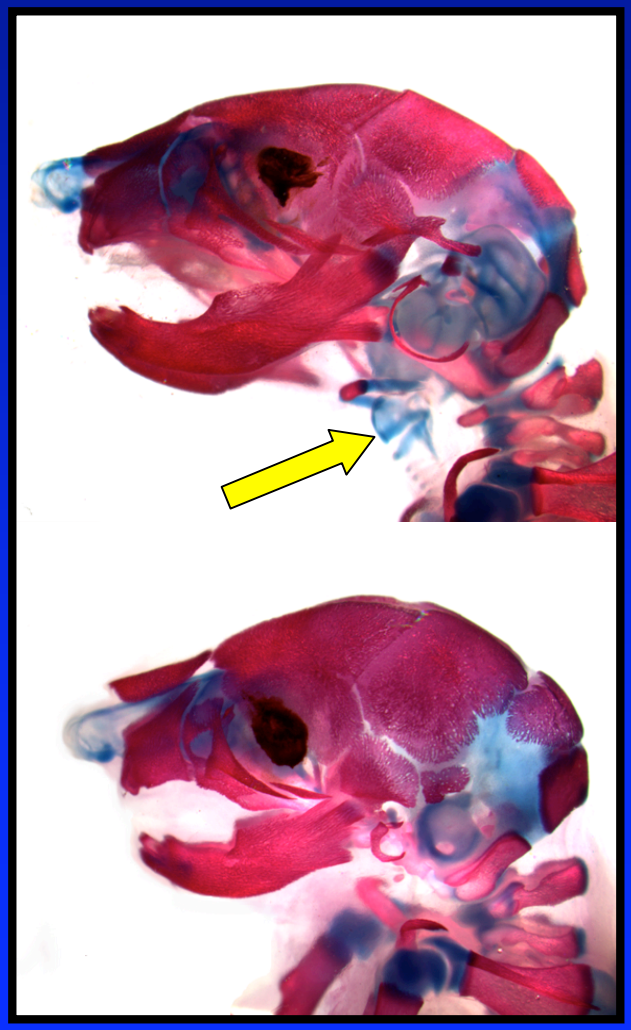


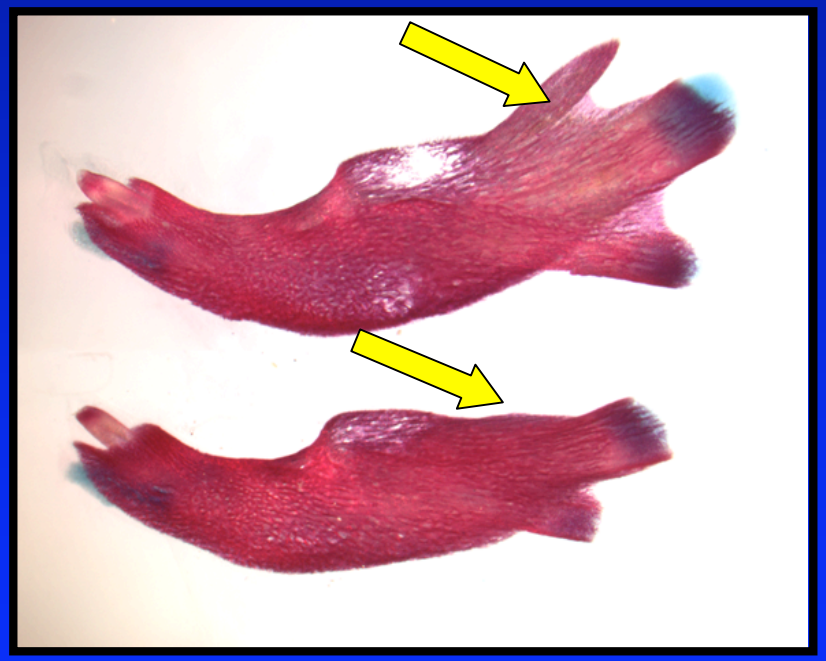
neonates

Craniofacial Abnormalities in *Tbx1* Mutants

+/+

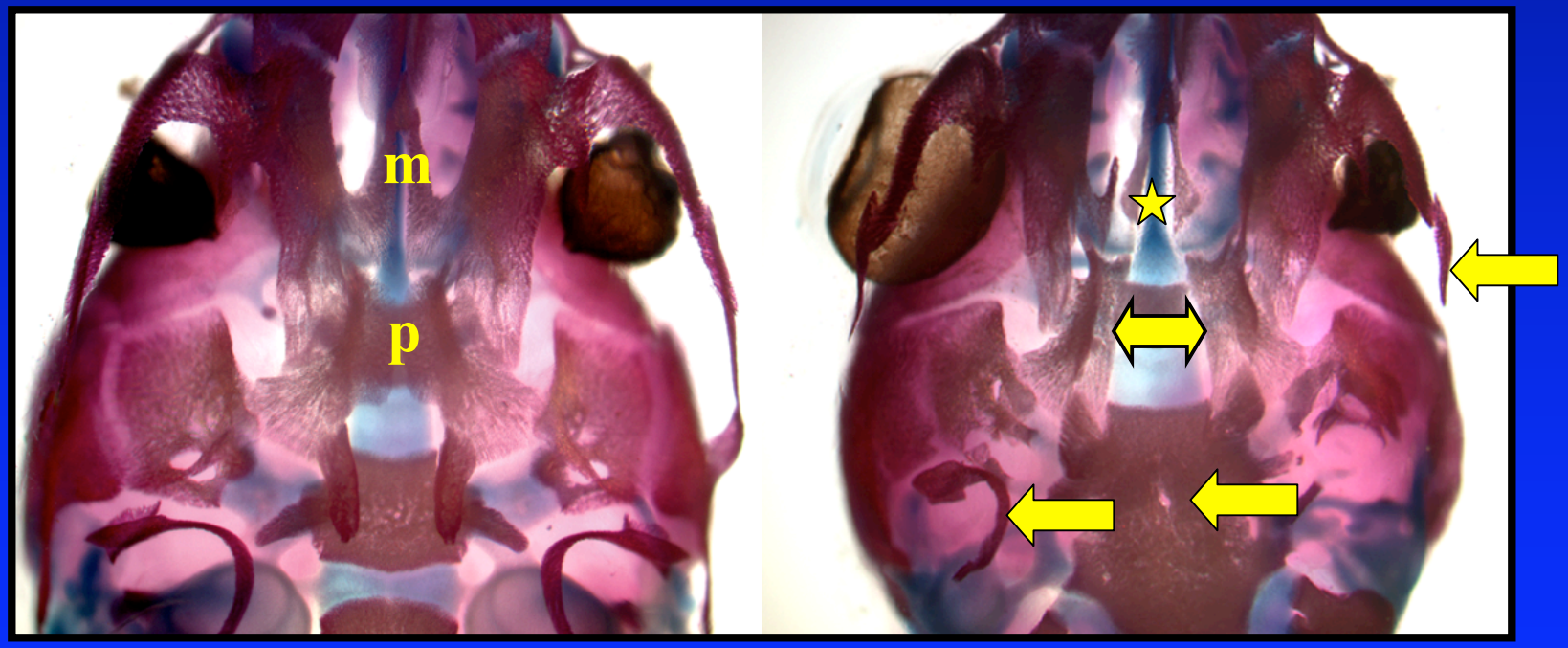
-/-



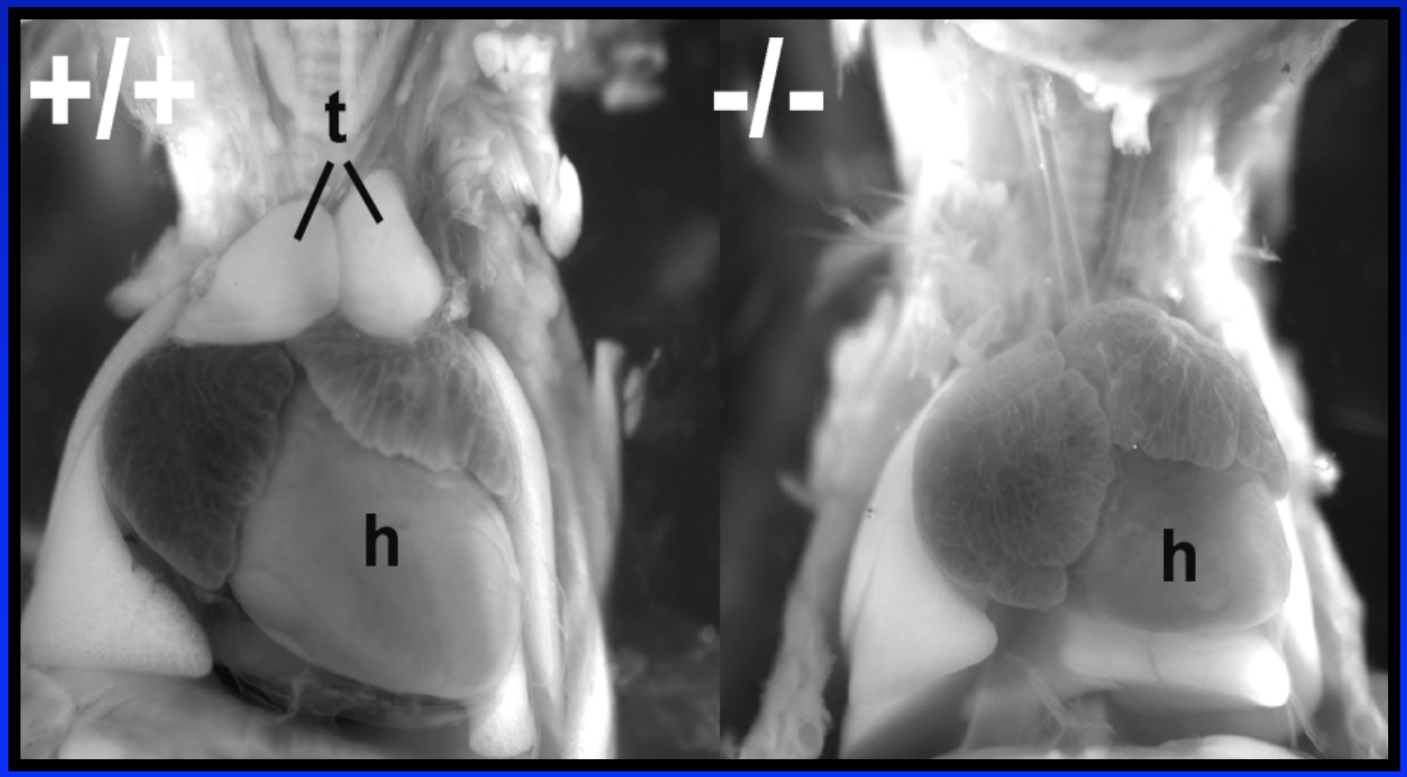


Cleft Palate in *Tbx1* Mutant Mice

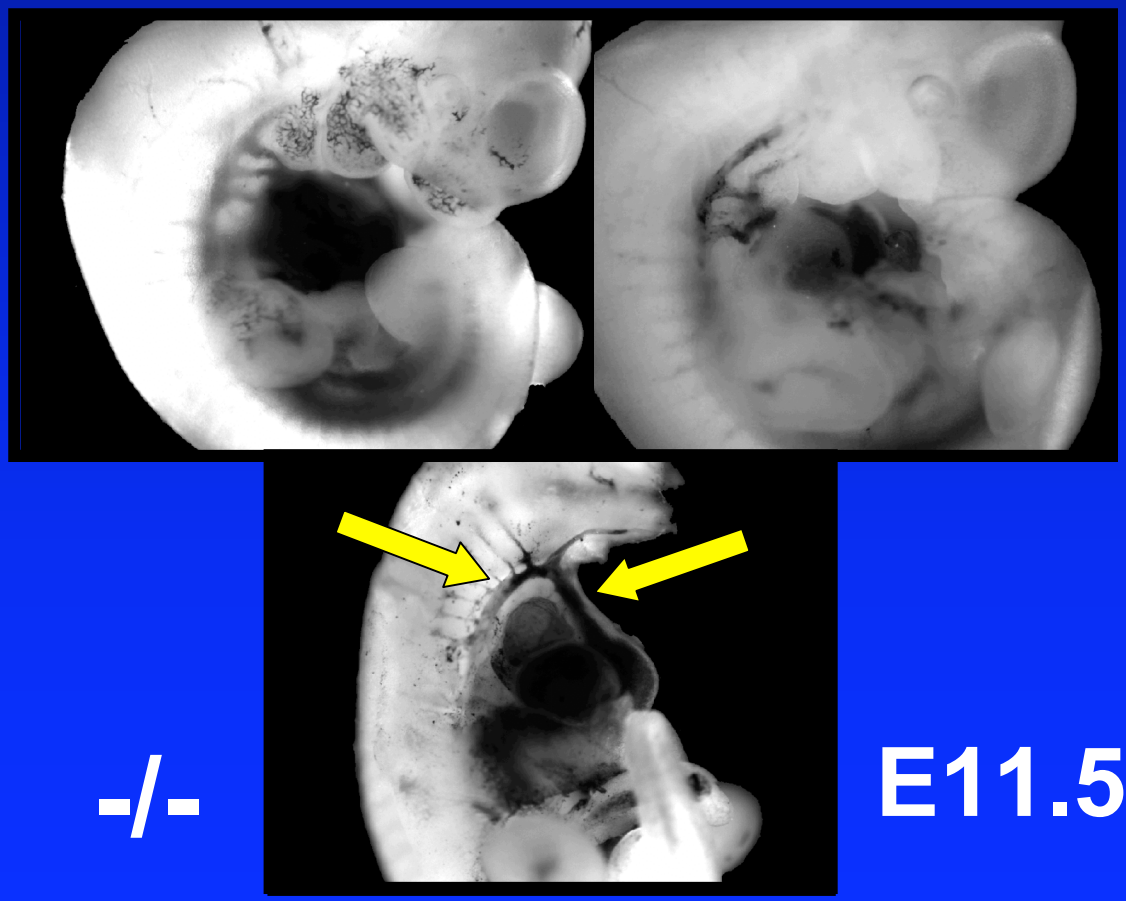
+/+ **-/-**



Glandular and Heart Abnormalities in *Tbx1* Mutant Mice

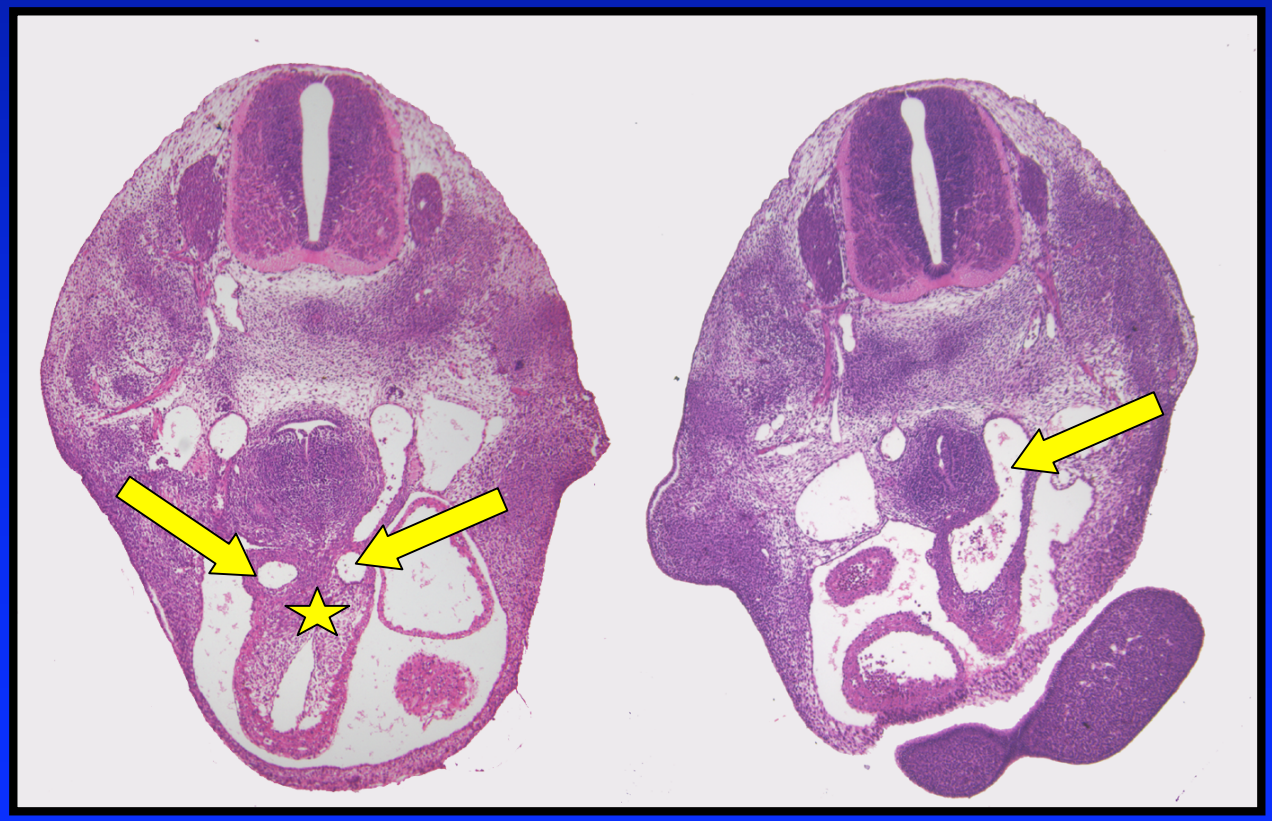


Aortic Arch Abnormalities



Cardiovascular Defects in *Tbx1* Mutant Mice

+/+ -/-



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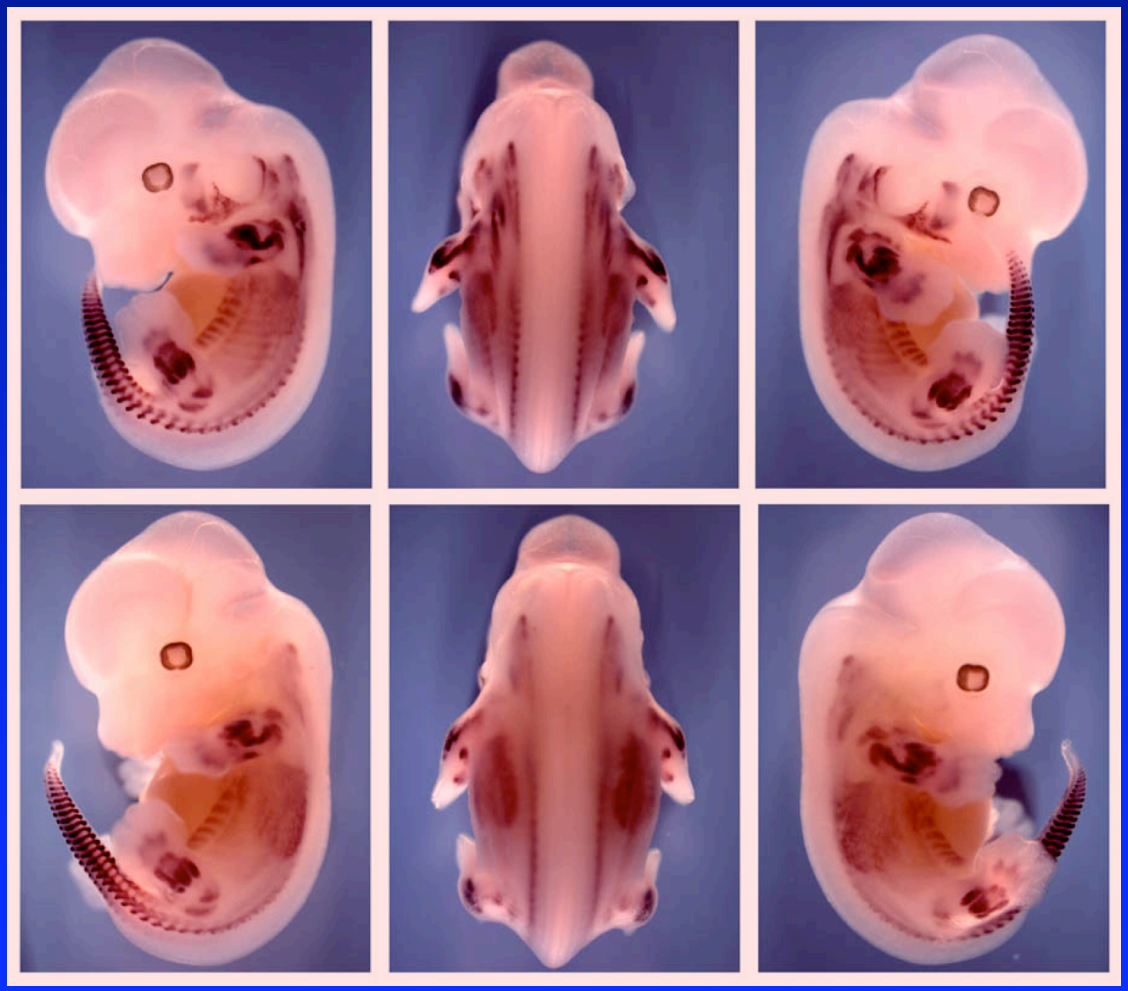
Failure of branchiomeric myogenesis in *Tbx1*$^{-/-}$ embryos



Tbx1$^{+/-}$

Tbx1$^{-/-}$

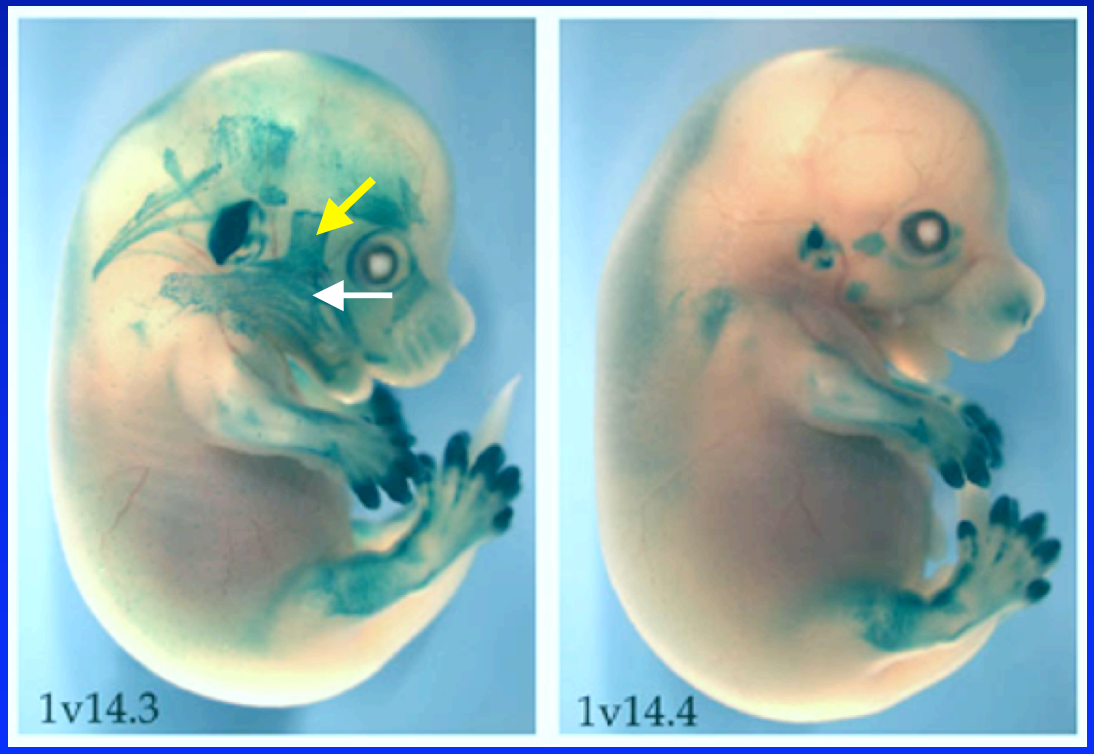
MyoD

E12.5

Craniofacial (branchiomeric) musculature not specified in *Tbx1* mutant mice

E14.5

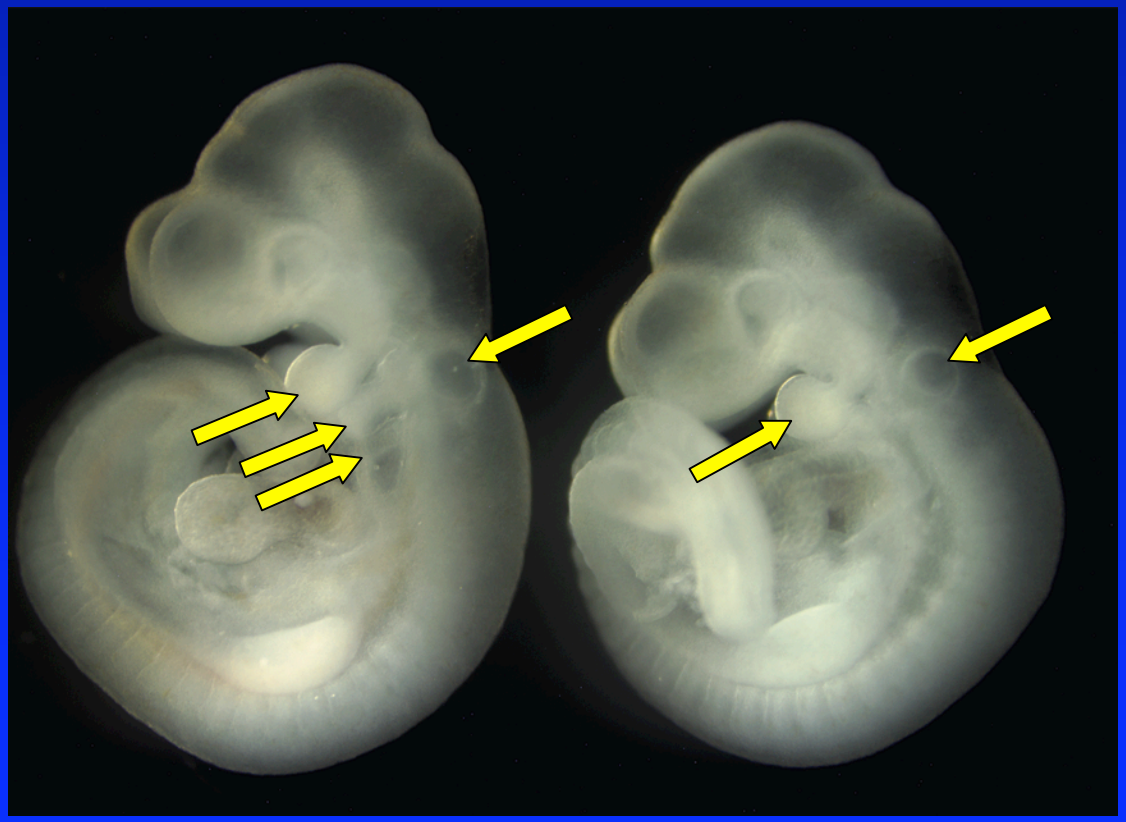
FGF10
enhancer trap



***Tbx1*$^{+/-}$** ***Tbx1*$^{-/-}$**

Otic Vesicle, Pharyngeal Arch and Pouch Defects

+/+ -/-

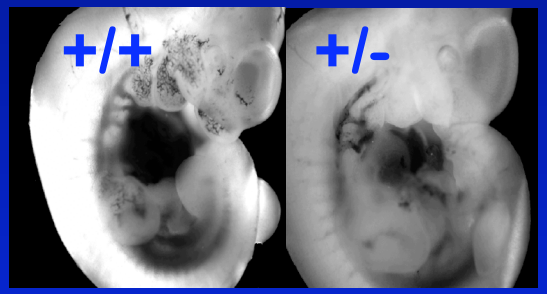


E9.5

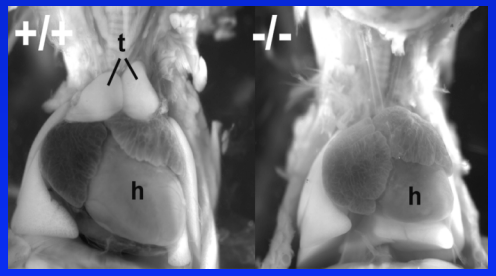
Major Embryonic Derivatives of *Tbx1* Expressing Tissues

- Otic vesicle – inner ear
- Pharyngeal arches – craniofacial bones, neck cartilage, ears, musculature
- Pharyngeal pouches – thymus, parathyroid
- Pharyngeal arch arteries – aorta
- (Neural crest – cardiac septum)

Tbx1 mutant mice have many features of DiGeorge syndrome

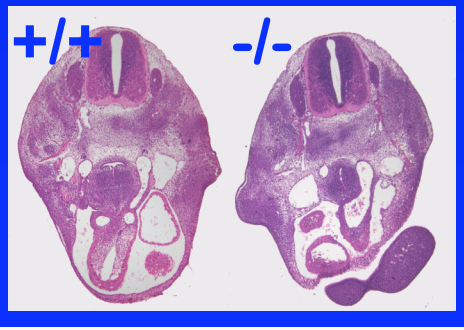


Abnormal aortic arches

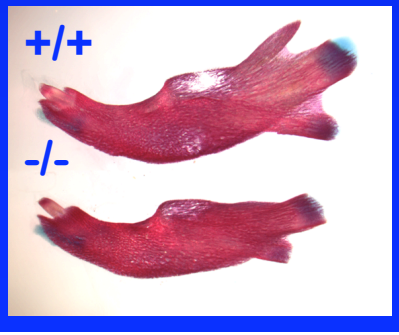


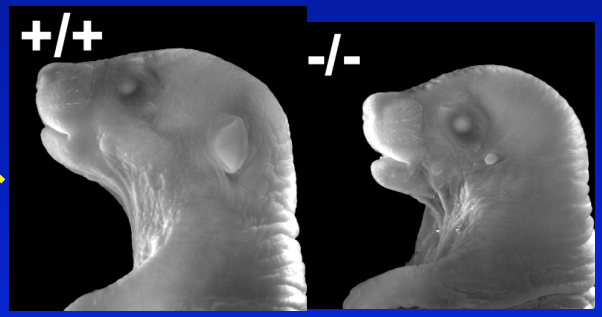
Hypoplastic thymus

Septal defects

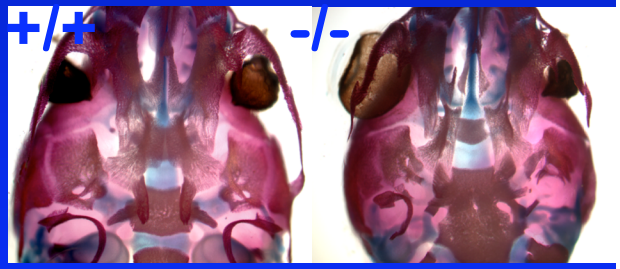


Micrognathia





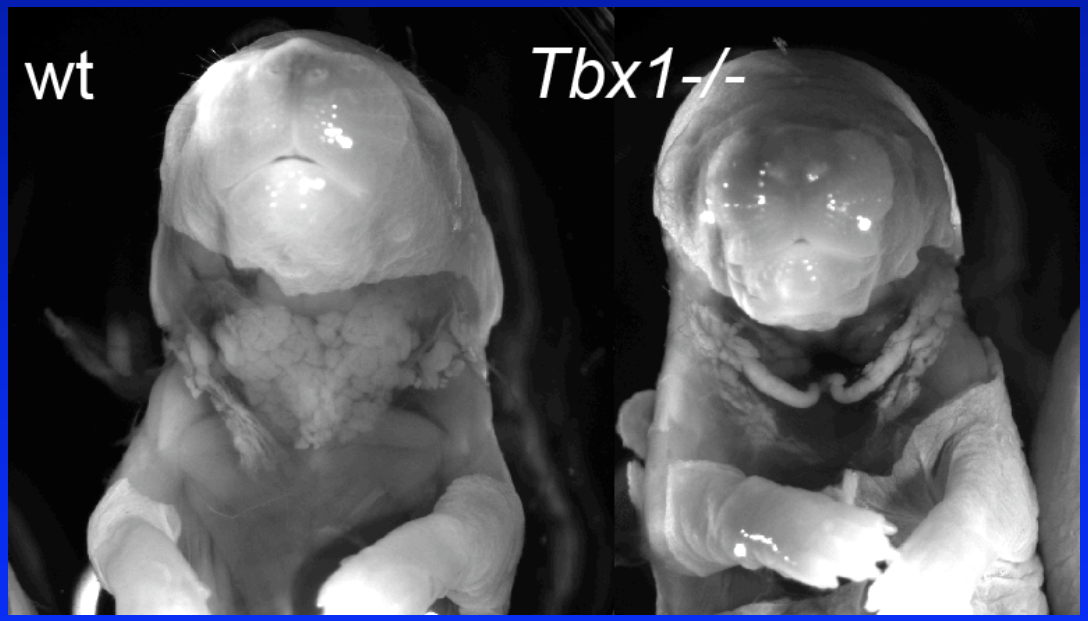
Low set, abnormal ears



Cleft palate

DGS/VCFS	*Tbx1* Mutant
• Cardiac outflow tract & septal defects	• Cardiac outflow tract & septal defects
• Thymus & parathryoid hypoplasia	• Thymus & parathryoid hypoplasia
• Laryngeotrachial anomalies	• Laryngeotrachial anomalies
• Craniofacial anomalies	• Craniofacial anomalies
• Cleft palate	• Cleft palate
• Micrognathia	• Micrognathia
• Low-set, abnormal ears	• Low-set, abnormal ears
• Muscle hypotonia	• Myogenesis defects

Salivary gland defects in *Tbx1* mutant mice



sublingual and submaxillary salivary glands missing or reduced

Is *TBX1* the only gene involved in DiGeorge syndrome?

Issues to be resolved

- Mouse haploinsufficiency does not recapitulate DGS
- Not all DGS deletions include *TBX1*
- Most non-deletion DGS patients do not have *TBX1* mutations
- However, several non-deletion pedigrees do have *TBX1* mutations

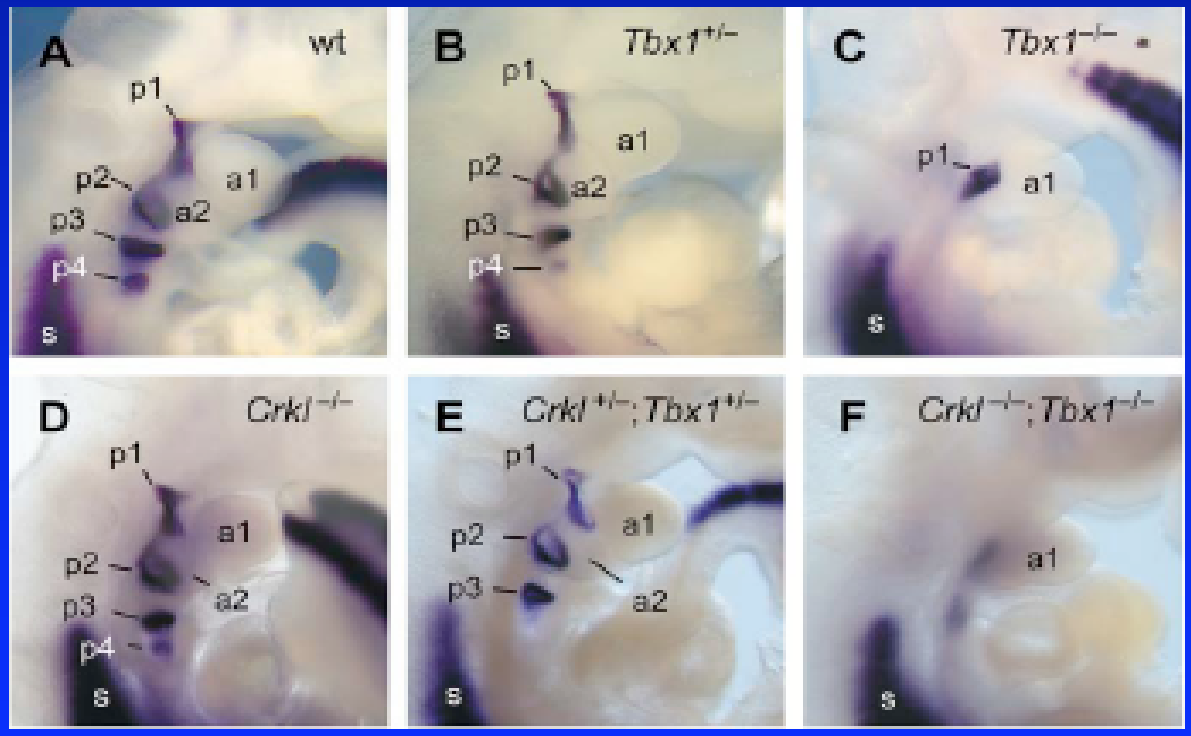
Is *TBX1* the only gene involved in DiGeorge syndrome?

- Deletion of regulatory elements?
- Linked modifier loci?
- Chromatin conformational effects?
- Linked genes affecting the same tissues?
- Exacerbation of haploinsufficiency?

Genetic interactions with linked genes

- *Crkl* gene in 22q11
- Sometimes deleted in DGS
- Expressed in neural crest
- Exacerbated cardiac phenotype in *Crkl*; Tbx1 compound mutants

Genetic interaction *Crkl*



pharyngeal pouch development as visualized by *Pax1* in situ hybridization

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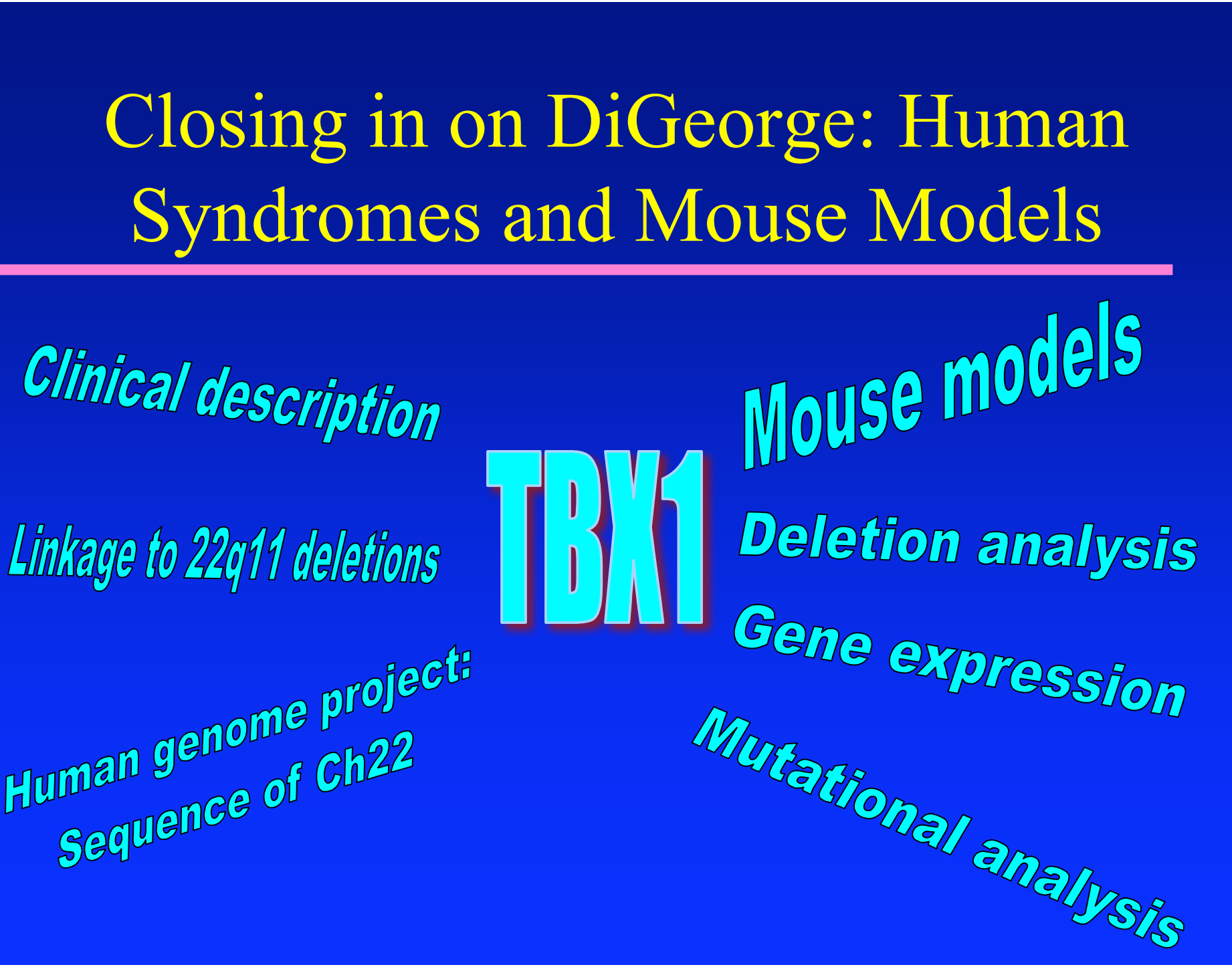

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