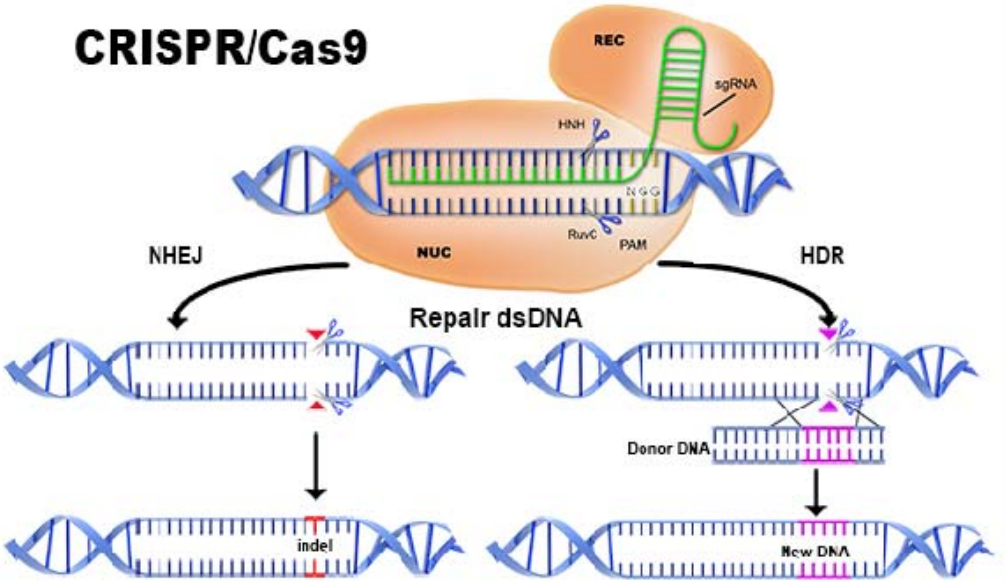
Modeling human disease variants in murine orthologs with CRISPR/Cas9

Jason Heaney, Ph.D.
Assistant Professor
Department of Molecular and Human Genetics
Academic Director, Mouse ES Cell Core
Baylor College of Medicine
Houston TX
heaney@bcm.edu

CRISPR/Cas9 genome editing at BCM



Null alleles
Indels
Interval (exon) deletions

Conditional alleles
Reporter alleles
Point mutation alleles

Image Credit: Advanced Analytical Technologies, Inc.

BCM Core activity to date

- 273 null alleles
- 58 conditional knockout alleles using short and long ssODNs
- 79 point mutation and other knock-in allele types using short and long ssODNs

Human genomic discovery programs

Centers for Mendelian Genomics

Workflow for precision modeling

Human Genomic Discovery Programs

- Candidate gene discovery (WGS, WES, RNA-Seq)
  - Gene
  - Variant/mutation
- Variant classification
  - Loss of function
  - Hypermorphic
  - Hypomorphic
  - Dominant/Recessive

BCM-KOMP2

- Mouse model production
  - Null allele
  - Variant knock-in allele
  - Conditional null or variant allele
- Phenotyping
  - Broad
  - Targeted
- Validation & functionalization of discoveries
- Pre-clinical models

OTUD6B

Biallelic Variants in *OTUD6B* Cause an Intellectual Disability Syndrome Associated with Seizures and Dysmorphic Features

Teresa Santiago-Sim,[1,2,27] Lindsay C. Burrage,[1,3,27] Frédéric Ebstein,[4] Mari J. Tokita,[1,2] Marcus Miller,[1,2] Weimin Bi,[1,2] Alicia A. Braxton,[1,2] Jill A. Rosenfeld,[1] Maher Shahrour,[5] Andrea Lehmann,[4] Benjamin Cogné,[6] Sébastien Küry,[6] Thomas Besnard,[6] Bertrand Isidor,[6,7] Stéphane Bézieau,[6] Isabelle Hazart,[8] Honey Nagakura,[9] LaDonna L. Immken,[9] Rebecca O. Littlejohn,[10] Elizabeth Roeder,[1,10] EuroEPINOMICS RES Consortium Autosomal Recessive working group, S. Hande Caglayan,[11] Bulent Kara,[12] Katia Hardies,[13,14] Sarah Weckhuysen,[13,14,15] Patrick May,[16] Johannes R. Lemke,[17] Orly Elpeleg,[18] Bassam Abu-Libdeh,[5] Kiely N. James,[19] Jennifer L. Silhavy,[19] Mahmoud Y. Issa,[20] Maha S. Zaki,[20] Joseph G. Gleeson,[19] John R. Seavitt,[1] Mary E. Dickinson,[1,21] M. Cecilia Ljungberg,[22,23] Sara Wells,[24] Sara J. Johnson,[24] Lydia Teboul,[24] Christine M. Eng,[1,2] Yaping Yang,[1,2] Peter-Michael Kloetzel,[4,25] Jason D. Heaney,[1,26,28,*] and Magdalena A. Walkiewicz[1,2,28,*]

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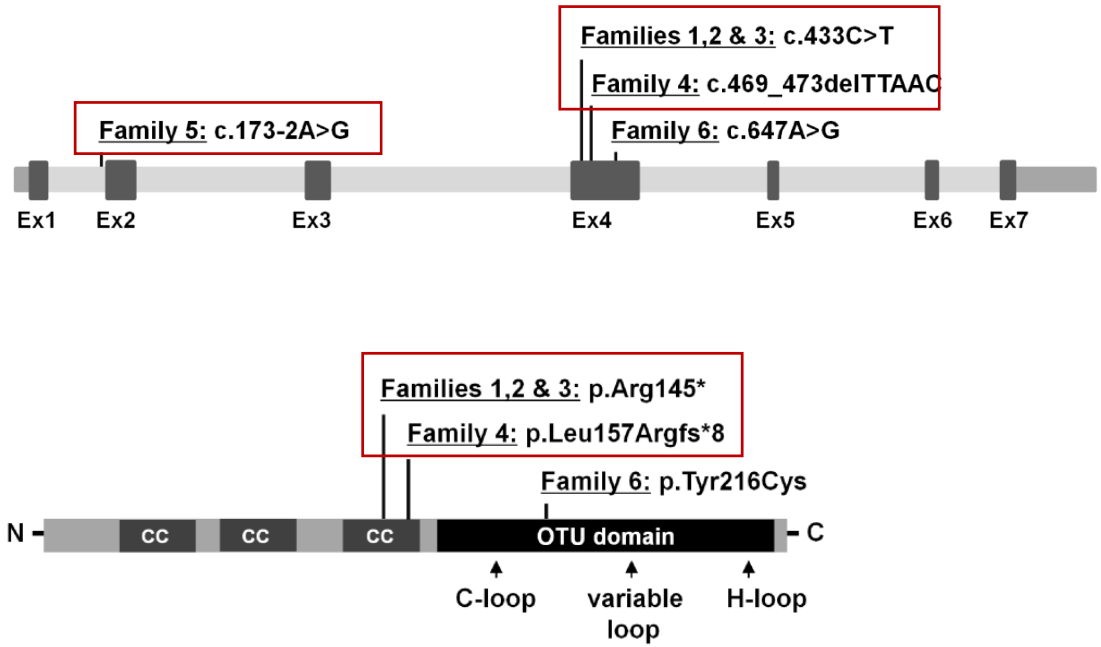
DEPARTMENT OF MOLECULAR & HUMAN GENETICS

Pathogenic variants cause nonsense mediated decay or suppress protein function

- Member of the ovarian tumor domain(OTU)-containing subfamily of deubiquitinating enzyme

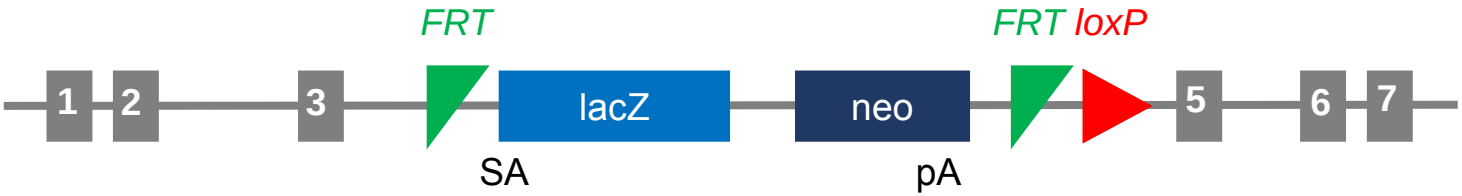
- Biallelic loss-of-function:
  - ***growth retardation***
  - ***congenital heart defects***
  - severe intellectual disability
  - dysmorphic features
  - structural brain abnormalities
  - seizures
  - hypotonia
  - feeding difficulties
  - digit anomalies

- Homozygous missense:
  - mild intellectual disability
  - seizures
  - dysmorphic features



Otud6b knockout mice are sub-viable

***Otud6b*^{tm1b} LacZ reporter knockout allele**

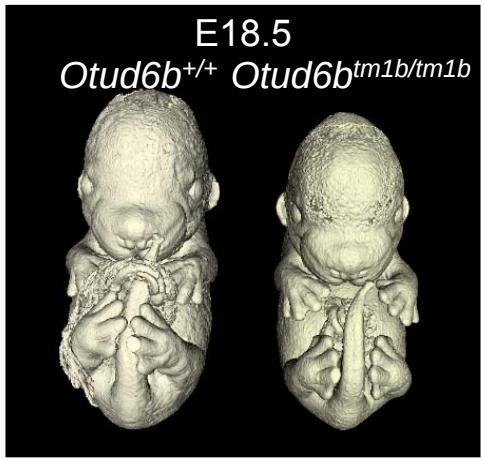


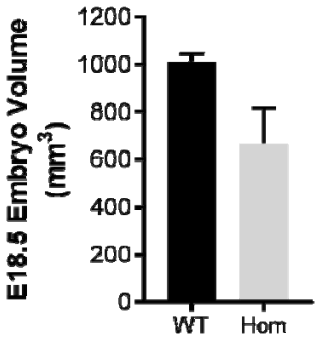
***Otud6b* genotypes observed one day after birth.**

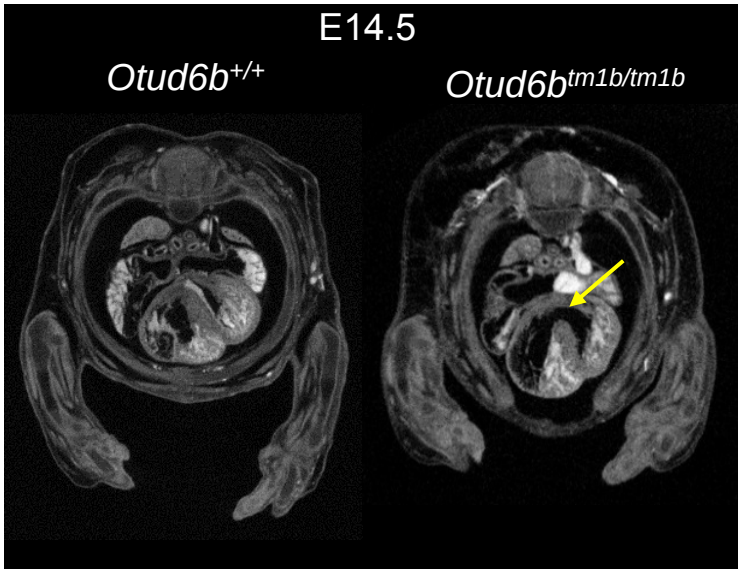
Genotype	No. observed*	No. expected	Test score (χ^2, *P* value)†
Otud6b$^{+/+}$	32	32	
Otud6b$^{tm1b/+}$	63	64	28.14, < 1E-5
Otud6b$^{tm1b/tm1b}$	2	32	

***** Assumes *Otud6b*$^{+/+}$ mice were observed at the expected frequency and a 1:2:1 segregation ratio. † Chi-square goodness-of-fit tests (2 D.F.).

Otud6b deficiency reduces embryo volume and causes ventricular septal defects (VSDs)







- Out of 12 human subjects :
  - 7 had IUGR
  - 7 had failure to thrive
  - 8 had small stature

- Out of 12 human subjects :
  - 4 with congenital heart defects
  - 2 with a VSD

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Variant knock-ins: Challenges for allele design and phenotyping

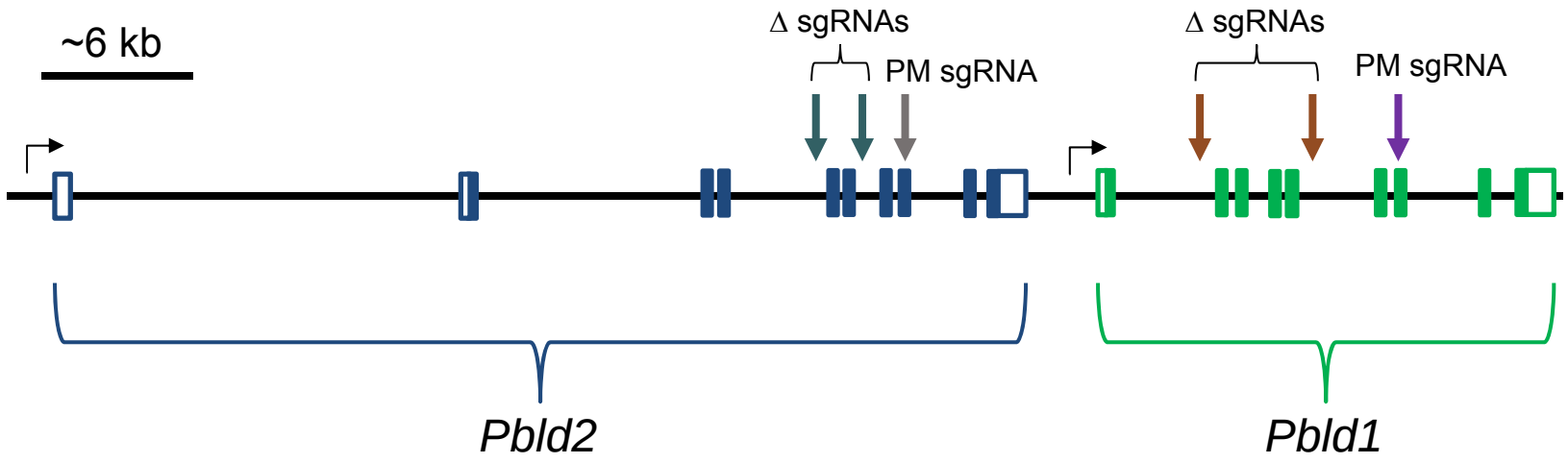
- Is there an orthologous gene in mouse
- Is the sequence conserved in mouse
- Will the phenotype be lethal in mouse
- Is there gene duplication (paralogs) in one species but not the other
- Does the mutation affect fertility or fecundity

PBLD

- 5 year old with phenotypes consistent with a connective tissue disorder
- Homozygosity for a candidate variant, p.F213I, affecting a conserved residue in *PBLD* (Phenazine biosynthesis-like protein domain-containing)
- Function to suppress TGFβ signaling by altering downstream phosphorylation targets like Smad3
- Given the important role of TGFβ in regulating and causing connective tissue phenotypes, it is hypothesize that this variant is pathogenic and causes increased TGFβ activity

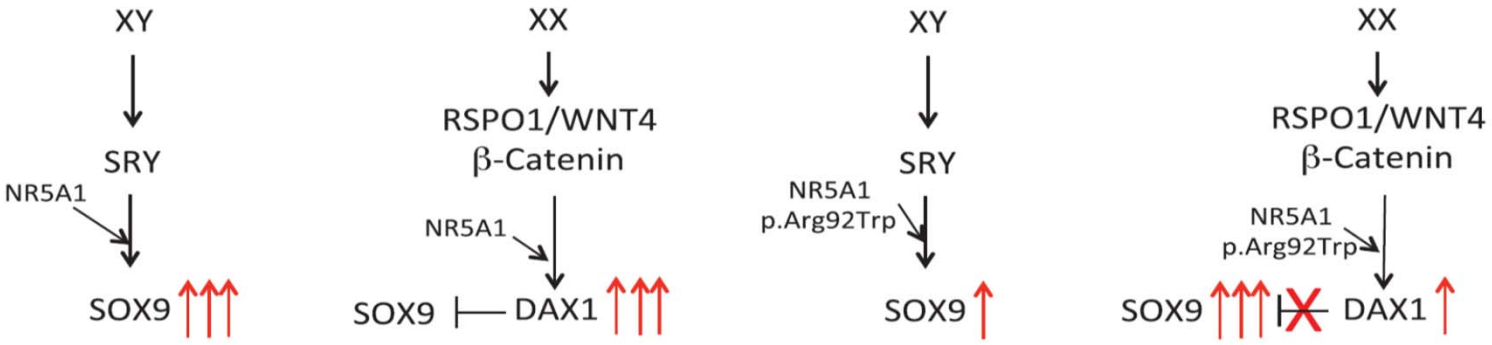
Pbld1 & *Pbld2* locus on mouse chromosome 10

- *Pbld1* and *Pbld2* in mice
  - Duplication on Chr10 – 3 kb apart in the same transcriptional orientation
  - 93% coding sequence identity; 91% amino acid sequence identity
  - 84% and 81% amino acid sequence identity to human *PBLD*
  - F213 is conserved
- Requires simultaneous or sequential gene editing
  - *Pbld1*$^{F213I/F213I}$, *Pbld2*$^{-/-}$
  - *Pbld1*$^{-/-}$, *Pbld2*$^{F213I/F213I}$
  - *Pbld1*$^{F213I/F213I}$, *Pbld2*$^{F213I/F213I}$



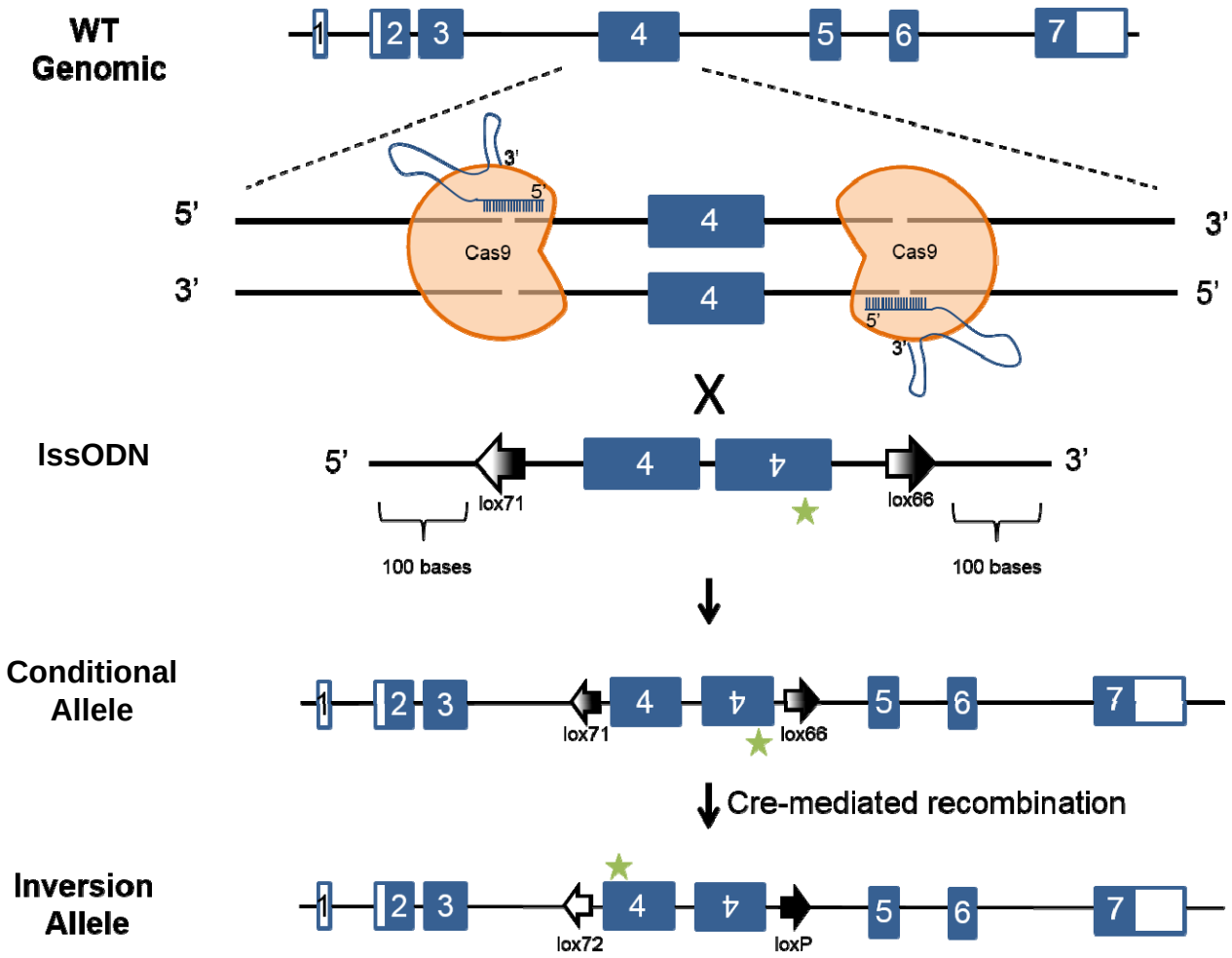
NR5A1

- Heterozygous missense mutation (p.R92W) in the accessory DNA-binding region of Nuclear receptor subfamily 5 group A member 1 (Steroidogenic factor-1)
- Variable degrees of testis development in 46,XX individuals from 4 families
- 46,XY sibling identifying as female with labial fusion, mild clitoral enlargement had a left dysgenic testis & right ovotestis
- *In vitro* assays predict a decreased transcriptional co-activation function



Bashamboo et al., *Hum Mol Genet*, 2016

Nr5a1 inversion allele



Conditional point mutation: Zhang et al., Nucleic Acid Res, 2002

Summary

- BCM-KOMP2 is collaborating with Human Genomic Discovery Programs at BCM to create and characterize mouse models of human disease relevant mutations
- Traditional null alleles remain a valuable resource for precision disease modeling
- Variant knock-in alleles in mice present unique challenges that require close collaboration between genomic discovery and animal modeling teams

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Mary E. Dickinson

M. Cecilia Ljungberg

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National Institutes of Health

Undiagnosed Diseases Network

Centers for Mendelian Genomics

No phenotypic abnormalities observed in *Otud6b* heterozygous mice

- LacZ staining revealed *Otud6b* expression in several adult tissues including:
  - Heart
  - Spinal cord, peripheral nervous system, brain
  - Skeletal muscle and cartilage
  - Large intestine and stomach
- Consistent with the multiple organ systems affected in the described subjects including

Otud6b$^{tm1b/+}$ Brain