Does paternal imprinting of *FOXF1* on 16q24.1 explain the maternal UPD(16) phenotype?

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Declaration of conflict of interest

I have no commercial disclosure

Maternal UPD(16)

- Trisomy 16 is the most common prenatal trisomy - occurs in >1% of clinically recognized pregnancies
- It typically results from maternal meiosis I nondisjunction and is lethal unless rescued early embryonically
- In 1/3 of cases, trisomy rescue leads to maternal UPD(16), usually accompanied by confined placental mosaicism with trisomy 16 cell line

Mat UPD(16) vs. Pat UPD(16)

Maternal UPD(16) second most common after UPD(15)	**IUGR, congenital heart defects, pulmonary hypoplasia, hemorrhages, tracheosophageal fistula, gut malrotation, renal agenesis, hypospadias, clinodactyly**
Paternal UPD16 very rare	**Relatively normal phenotype**

The differences in outcomes between mat and pat UPD(16) strongly indicate the presence of imprinted gene(s) on chromosome 16. However, no candidate gene(s) proposed.

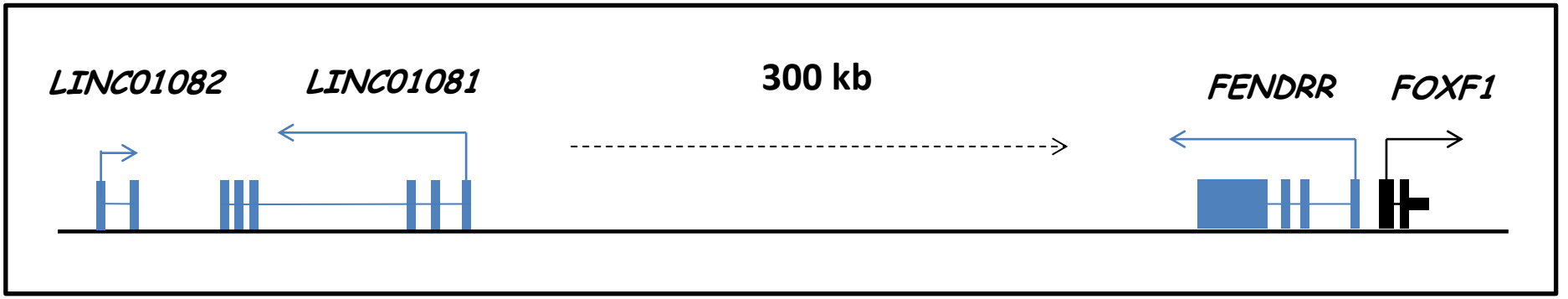
Yong et al. Am. J. Med. Genet. 2002;112,123-32

Alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV)

- Interestingly, most clinical features of mat UPD(16), except IUGR, are observed in children with a neonatally lethal lung developmental disorder - ACDMPV
- ACDMPV is caused by heterozygous point mutations or genomic deletions involving *FOXF1* on 16q24.1

Forkhead box F1 (*FOXF1*) on chr16q24.1

- Transcription factor with a winged-helix DNA binding domain
- Primarily expressed in lung, intestine, and placenta



- *Foxf1* $^{+/-}$ mice exhibit lung defects similar to those in patients with ACDMPV

Kalinichenko et al., *Dev Biol.* 2001

- *Fendrr* $^{-/-}$ mice exhibit similar lung & heart defects

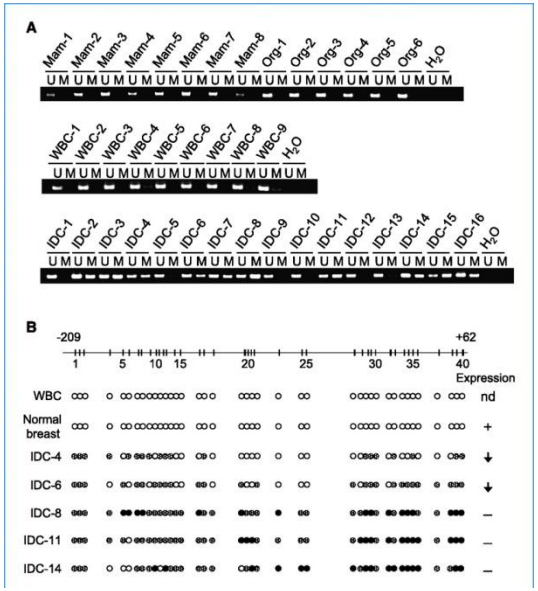
Grote et al., *Dev Cell* 2013
Sauvageau et al., *Elife* 2013

Paternal imprinting of the *FOXF1* locus

073712 (*PLEKHC1*)	14q22.1	P
183992	14q31.1	M
185469 (*RTL1*)	14q32.31	M
126290 (*HV2A*)	14q32.33	P
151802 (*Q9P168*)	15q13.1	P
005513 (*SOX8*)	16p13.3	P
172268 (*Q96S05*)	16p13.3	P
103449 (*SALL1*)	16q12.1	M
103005 (*C16orf57*)	16q13	M
102977 (*ACD*)	16q22.1	M
1032[illegible] (*FOXF1*)	16q24.1	M
183788 (*Q8N206*)	16q24.3	M
183518	17p13.3	M
167874 (*TMEM88*)	17p13.1	M
181977 (*PYY2*)	17q11.2	P
173917 (*HOXB2*)	17q21.32	M
120093 (*HOXB3*)	17q21.32	M
141378 (*YCE7*)	17q23.2	M
181428 (*Q8N8L1*)	17q25.3	P

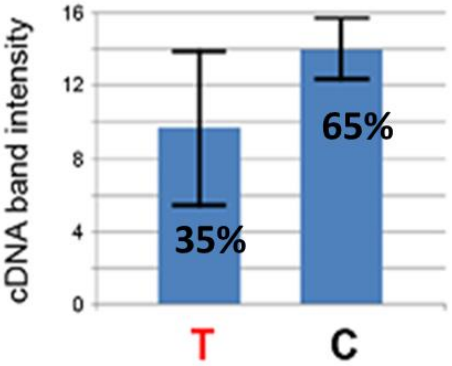
FOXF1 was computationally predicted to be paternally imprinted in humans

(Luedi et al., Genome Res 2007)



Epigenetic inactivation of *FOXF1* in primary invasive breast tumors

(Lo et al.,Cancer Res 2010)

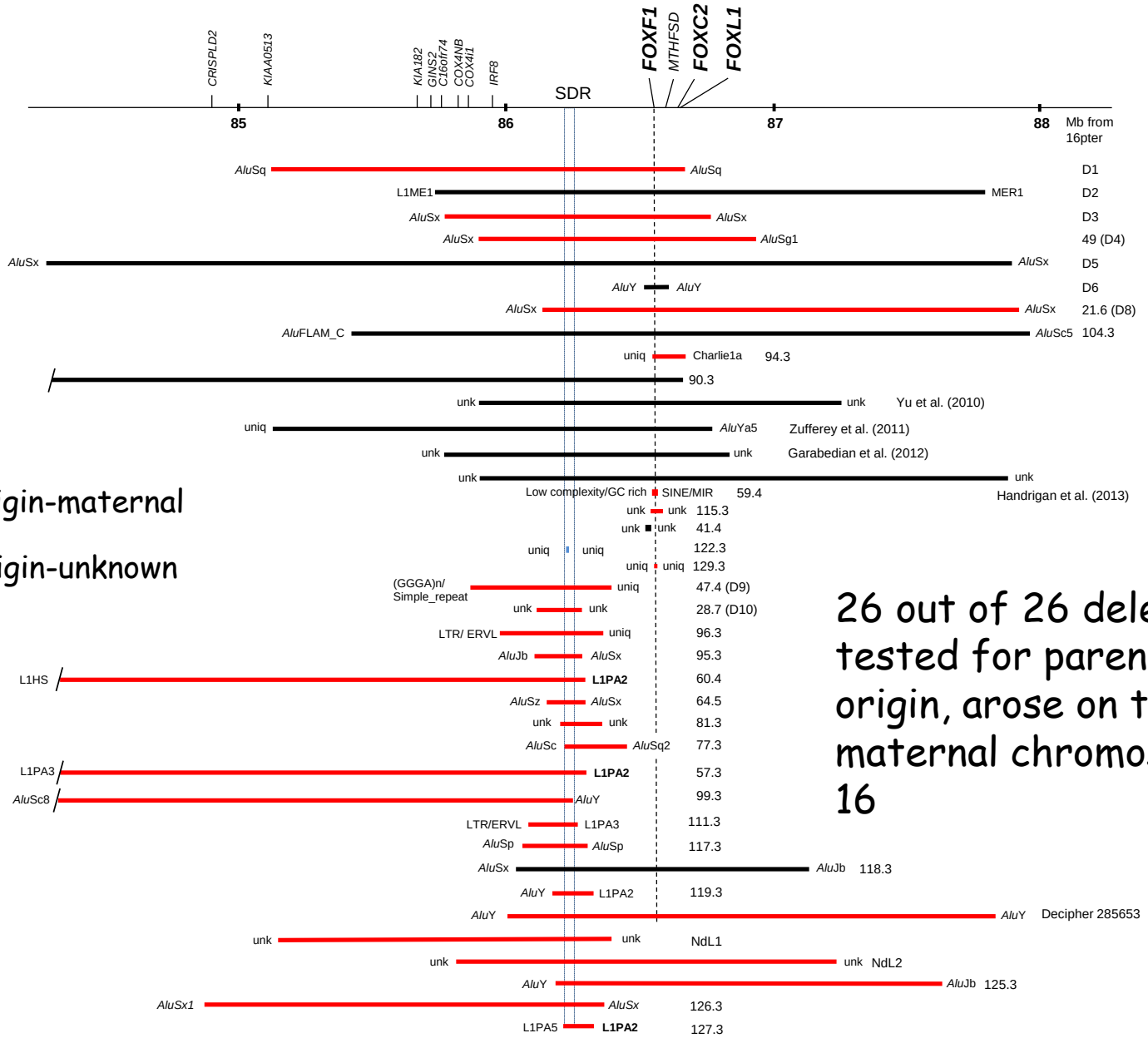


P=0.011

Differential allelic expression of *FOXF1* in newborn human lungs

(Szafranski et al., Genome Res 2013)

ACDMPV: maternal origin of *de novo* deletions in 16q24.1



Parental origin-maternal

Parental origin-unknown

26 out of 26 deletions tested for parental origin, arose on the maternal chromosome 16

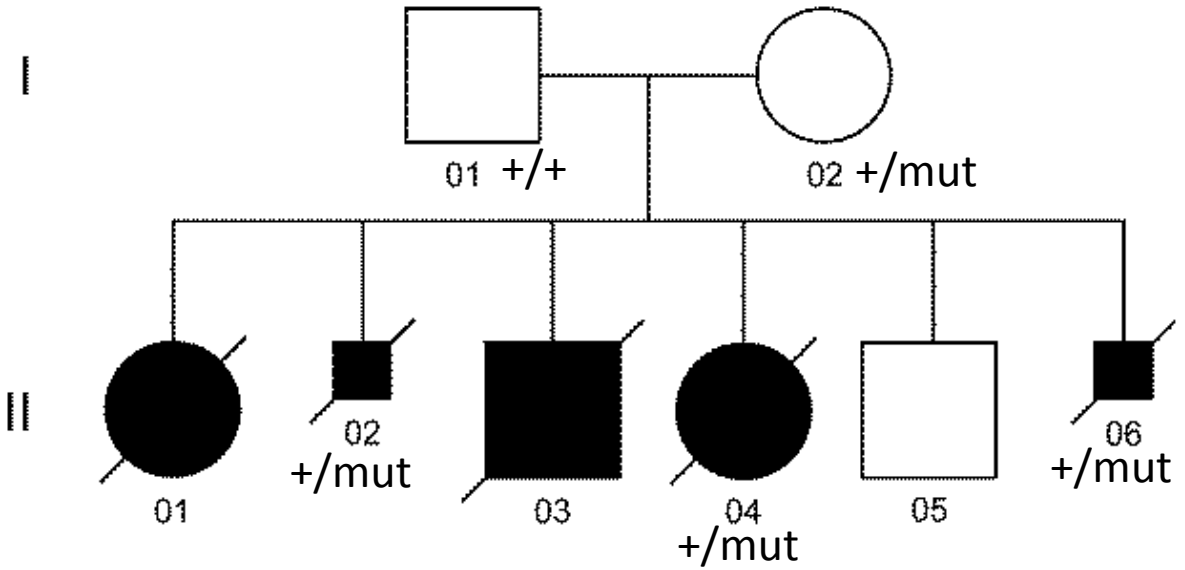
European Journal of Human Genetics (2012), 1–4

www.nature.com/ejhg

SHORT REPORT

A familial case of alveolar capillary dysplasia with misalignment of pulmonary veins supports paternal imprinting of *FOXF1* in human

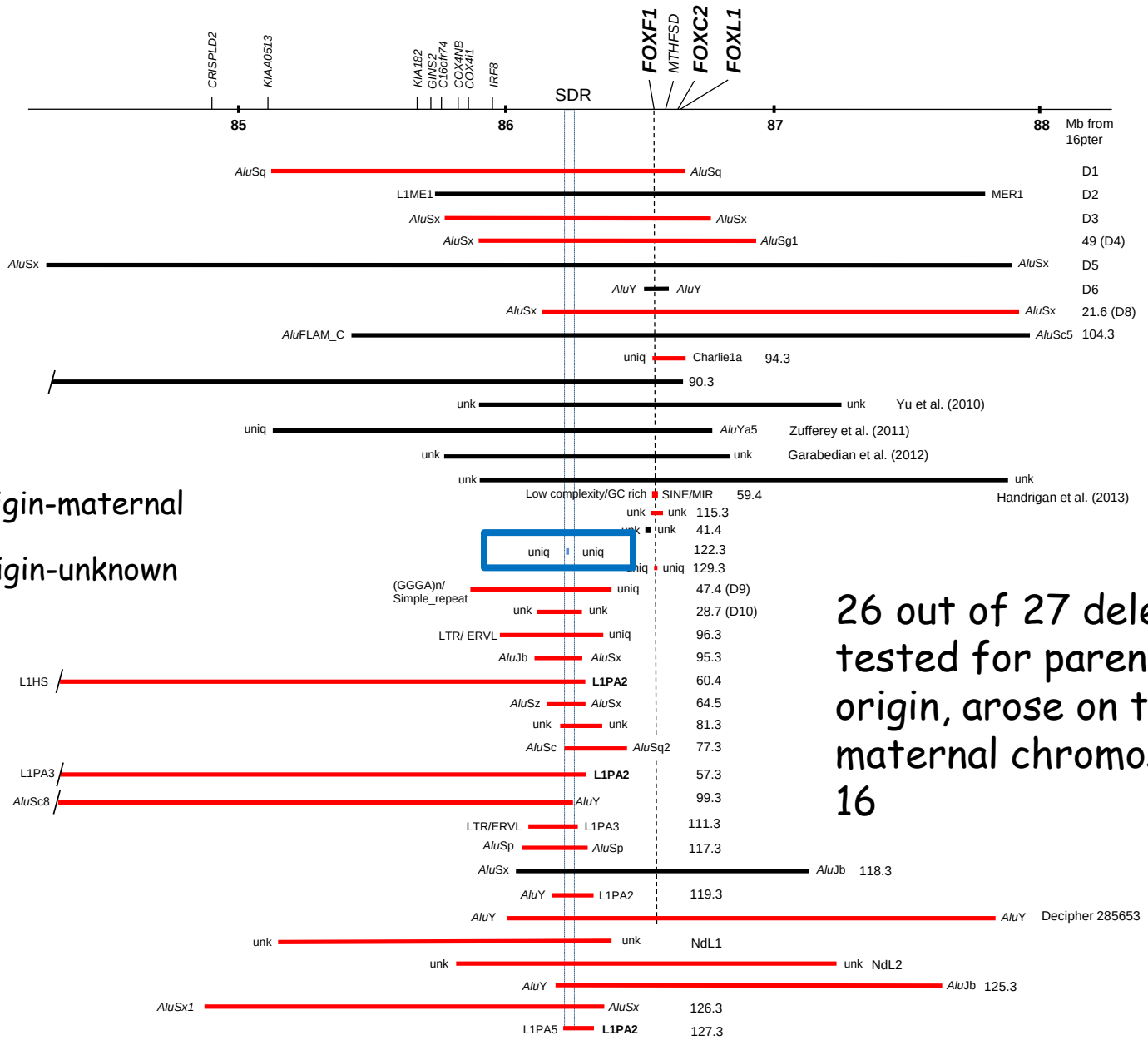
Partha Sen*[,1], Romana Gerychova[2], Petr Janku[2], Marta Jezova[3], Iveta Valaskova[4], Colby Navarro[1], Iris Silva[1], Claire Langston[5], Stephen Welty[1], John Belmont[1,6] and Pawel Stankiewicz[6]



c.416G>T

p.Arg139Leu

ACDMPV: maternal origin of *de novo* deletions in 16q24.1



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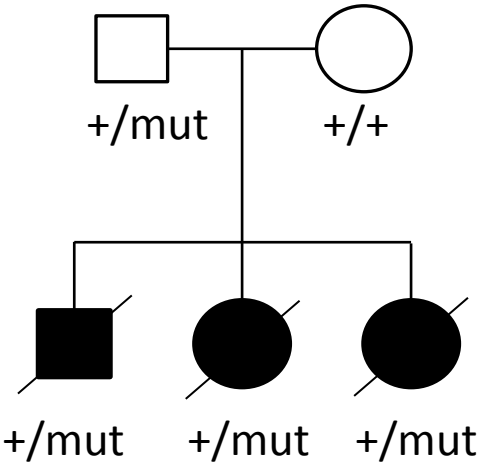
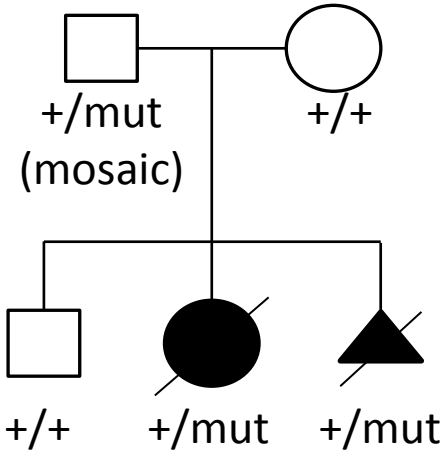
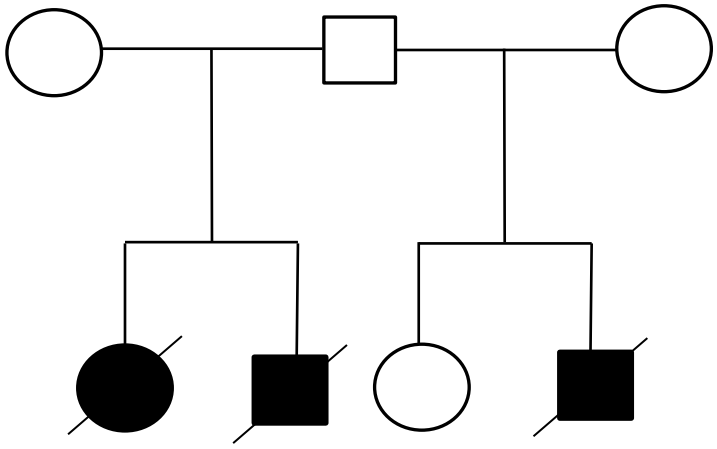
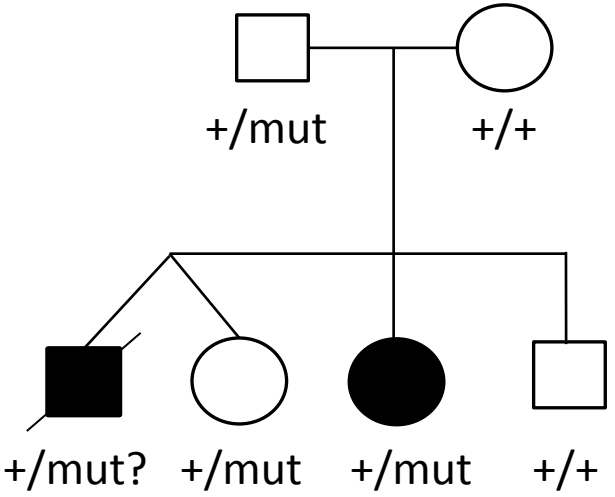
ACDMPV families with reportedly healthy carrier fathers transmitting *FOXF1* mutation to affected children

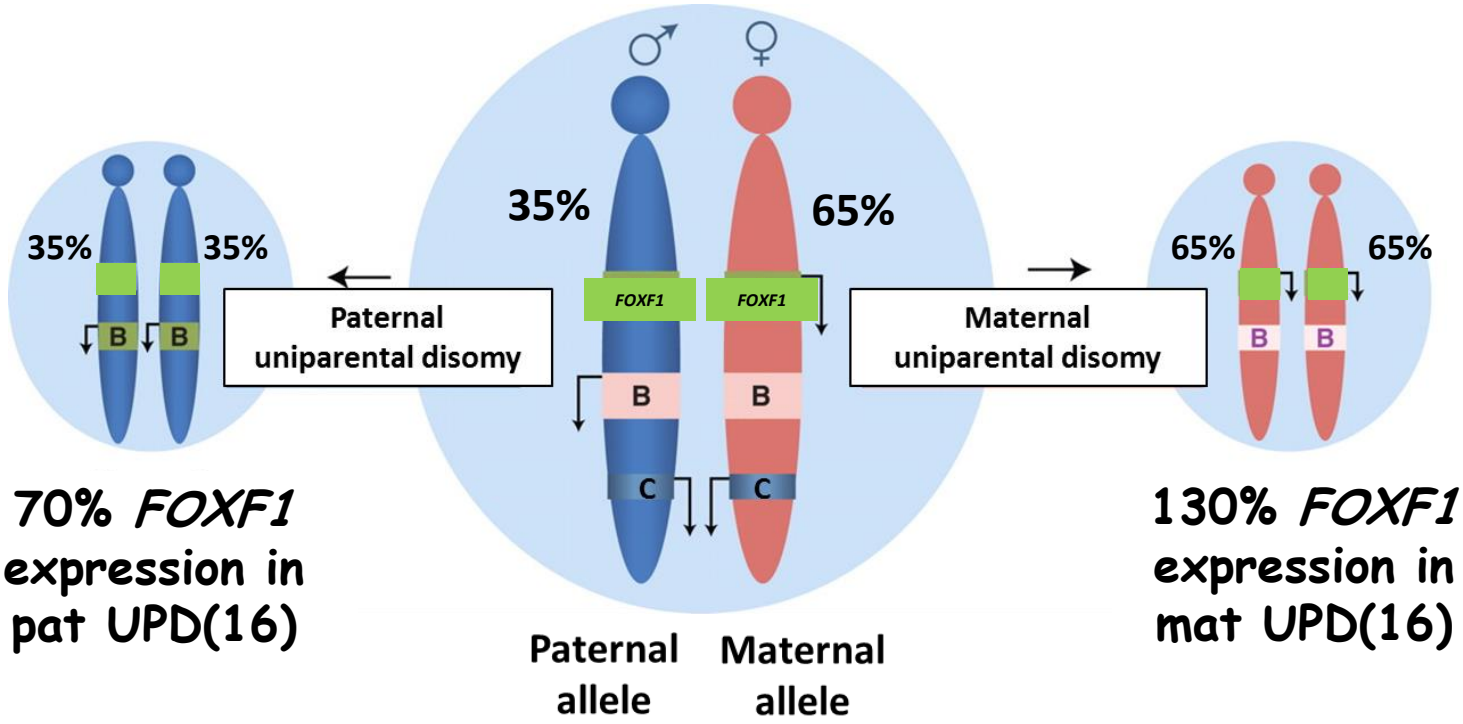








FOXF1 dosage mat vs. pat UPD(16)

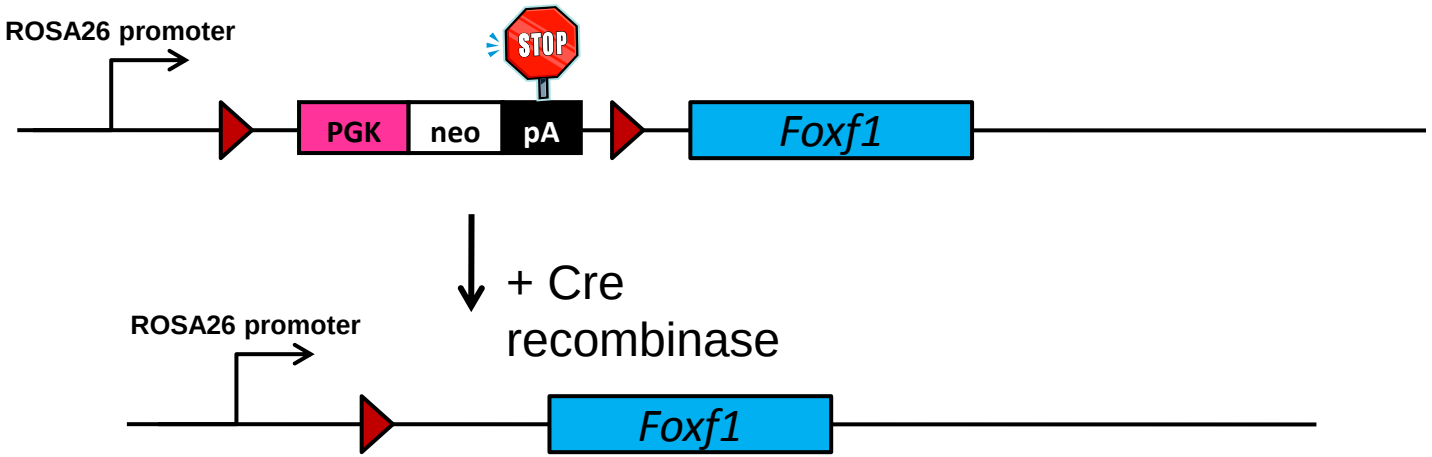


Modeling *FOXF1* overexpression

- In humans, 16q24.1 duplications involving *FOXF1* correlated with pyloric stenosis, mesenterium commune and aplasia of the appendix

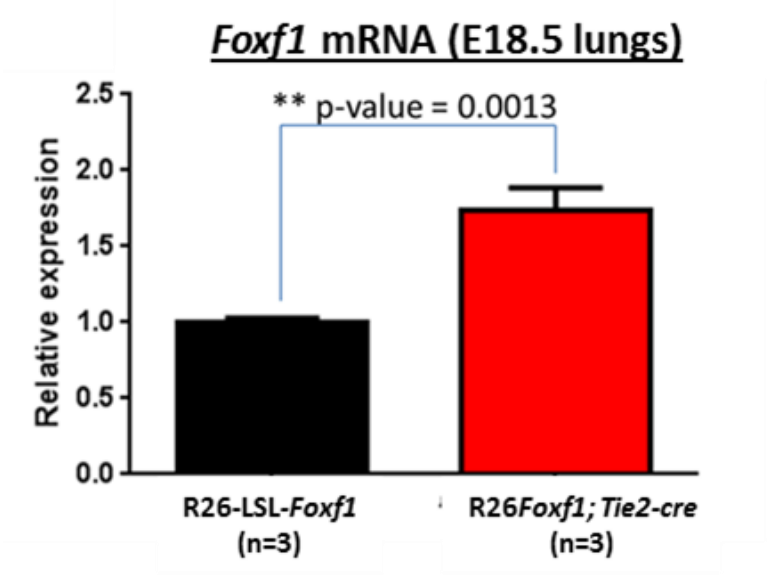
Dharmadhikari et al.
BMC Med Genet 2014;15:128

- To investigate potential effects of *Foxf1* overexpression in mice, we knocked in a Cre-inducible *Foxf1* allele at the ROSA26 locus



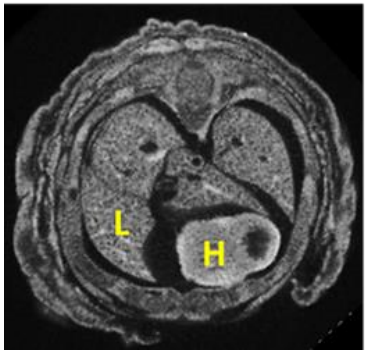
Tie2-cre mediated overexpression of *Foxf1* causes embryonic and perinatal lethality

Age	No. of litters	+/R26-LSL-*Foxf1*	+/R26*Foxf1*; +/*Tie2*-cre	Abnormal/Dead +/R26*Foxf1*; *Tie2*-cre	Total	p-value (χ^2 test)
E15.5	3	12/29(41%)	11/29(38%)	6/29(21%)	29	ns
E18.5	4	12/30(40%)	12/30(40%)	6/30(20%)	30	ns
P0.5	9	40/67(60%)	8/67(12%)	19/67(28%)	67	<0.0001

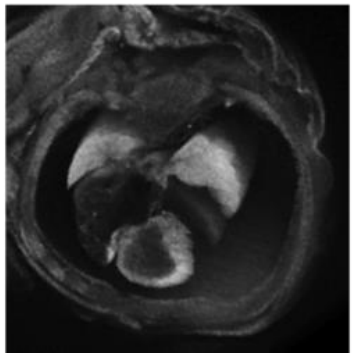


ROSA26*Foxf1*; *Tie2*-cre mice have hypoplastic lungs

Micro-Computed Tomography (micro-CT)

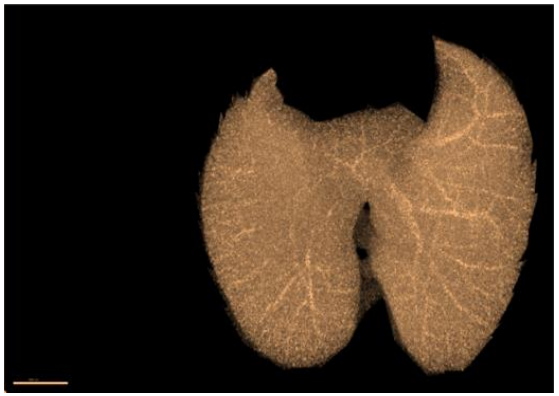


R26-LSL-*Foxf1*

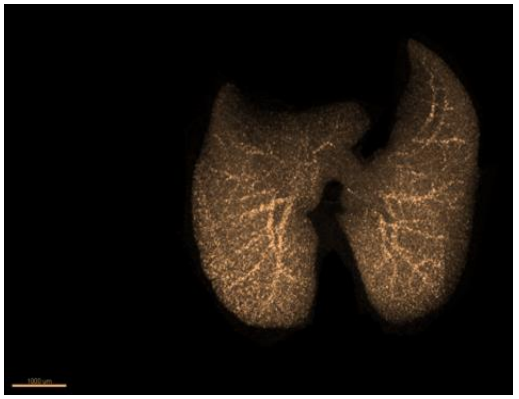


R26*Foxf1*; Tie2-cre

Lung specific micro-CT

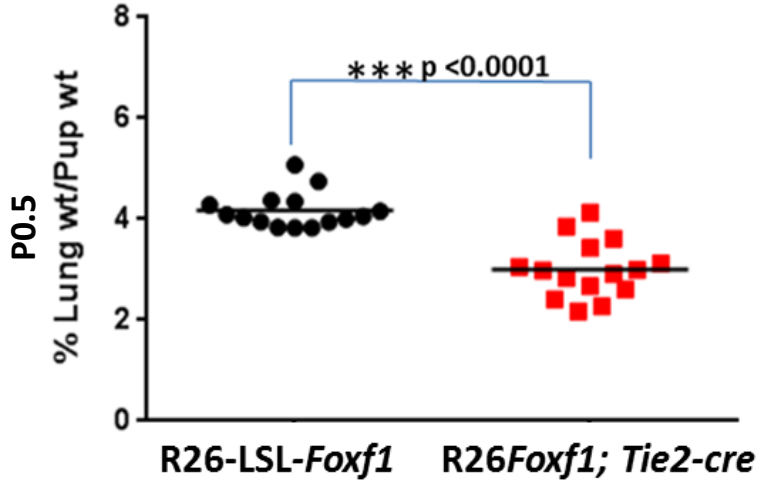


R26-LSL-*Foxf1*

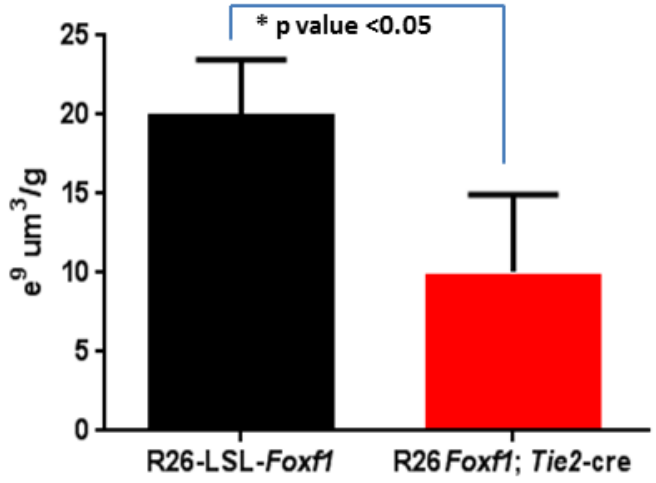


R26*Foxf1*; Tie2-cre

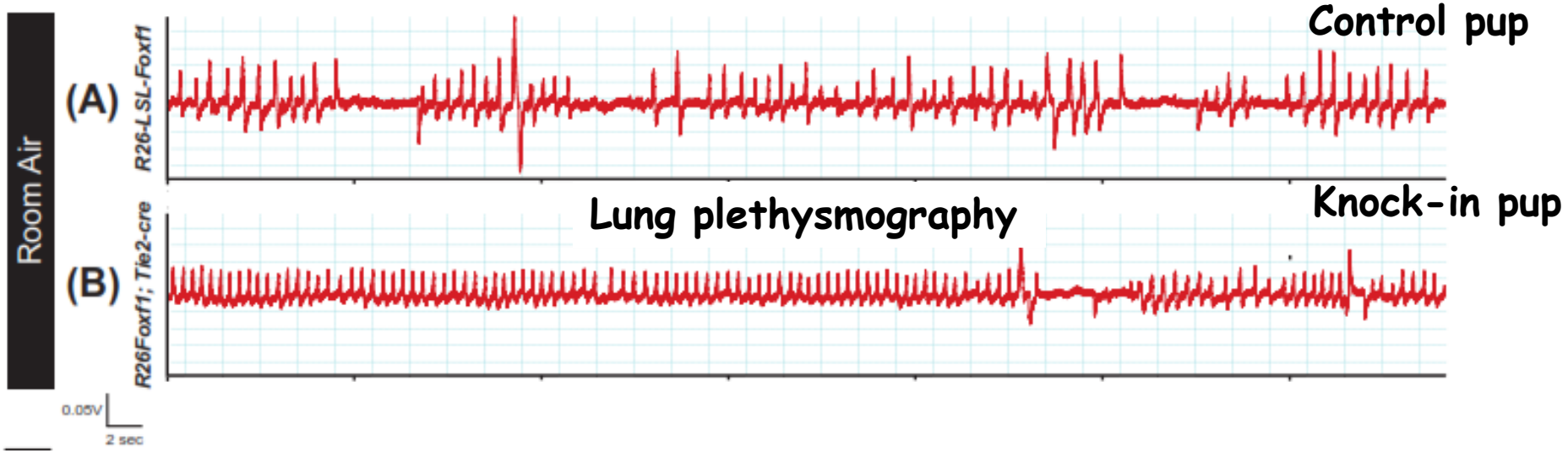
Lung weights

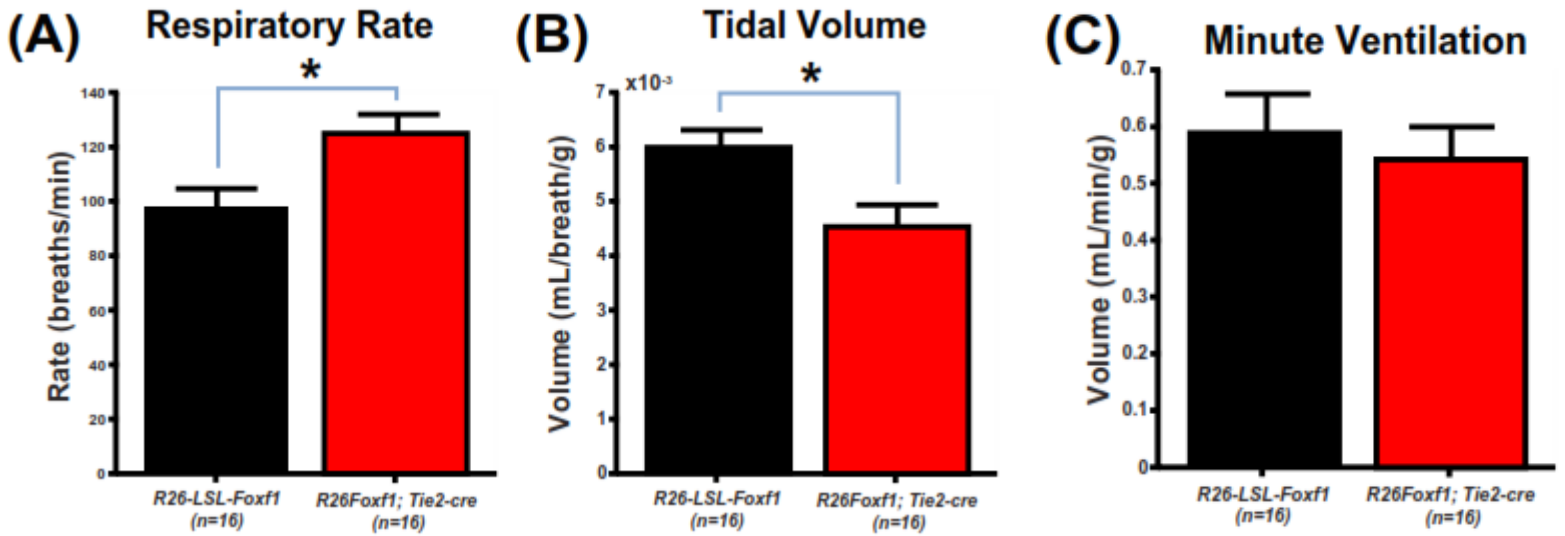


Lung Volumes

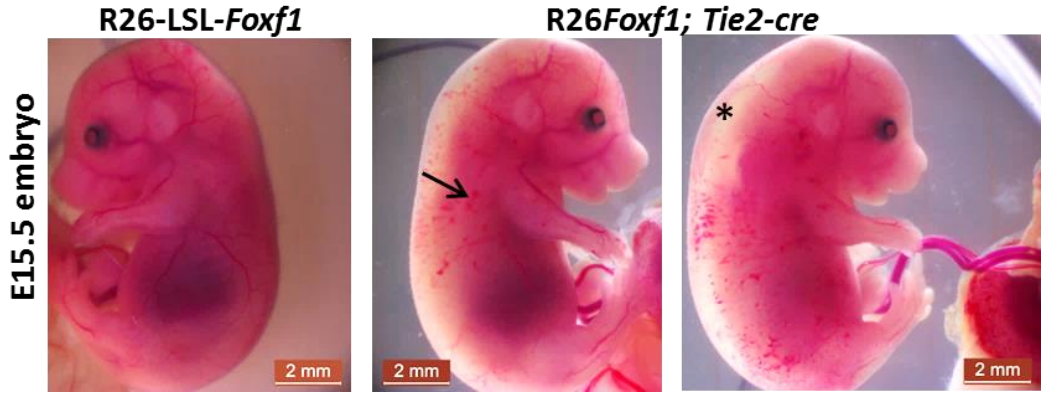


ROSA26*Foxf1*; *Tie2*-cre P0.5 pups exhibit respiratory defects

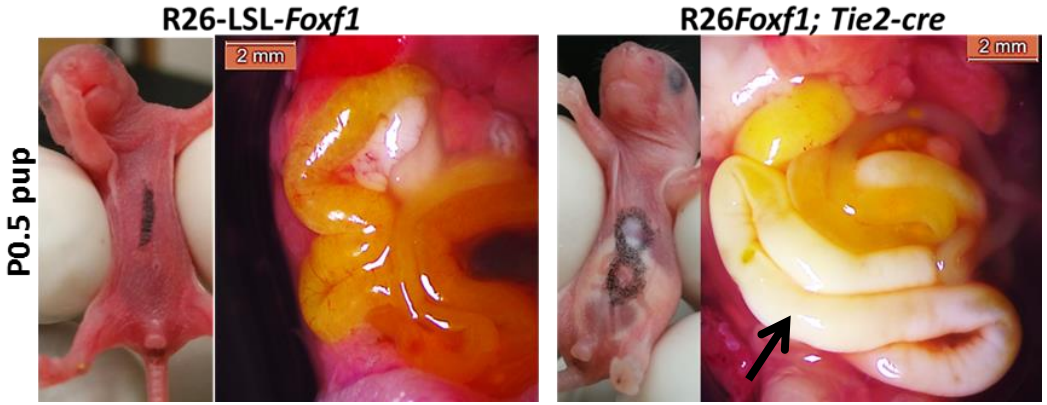




Tie2-cre mediated overexpression of *Foxf1* causes vascular & lymphatic defects

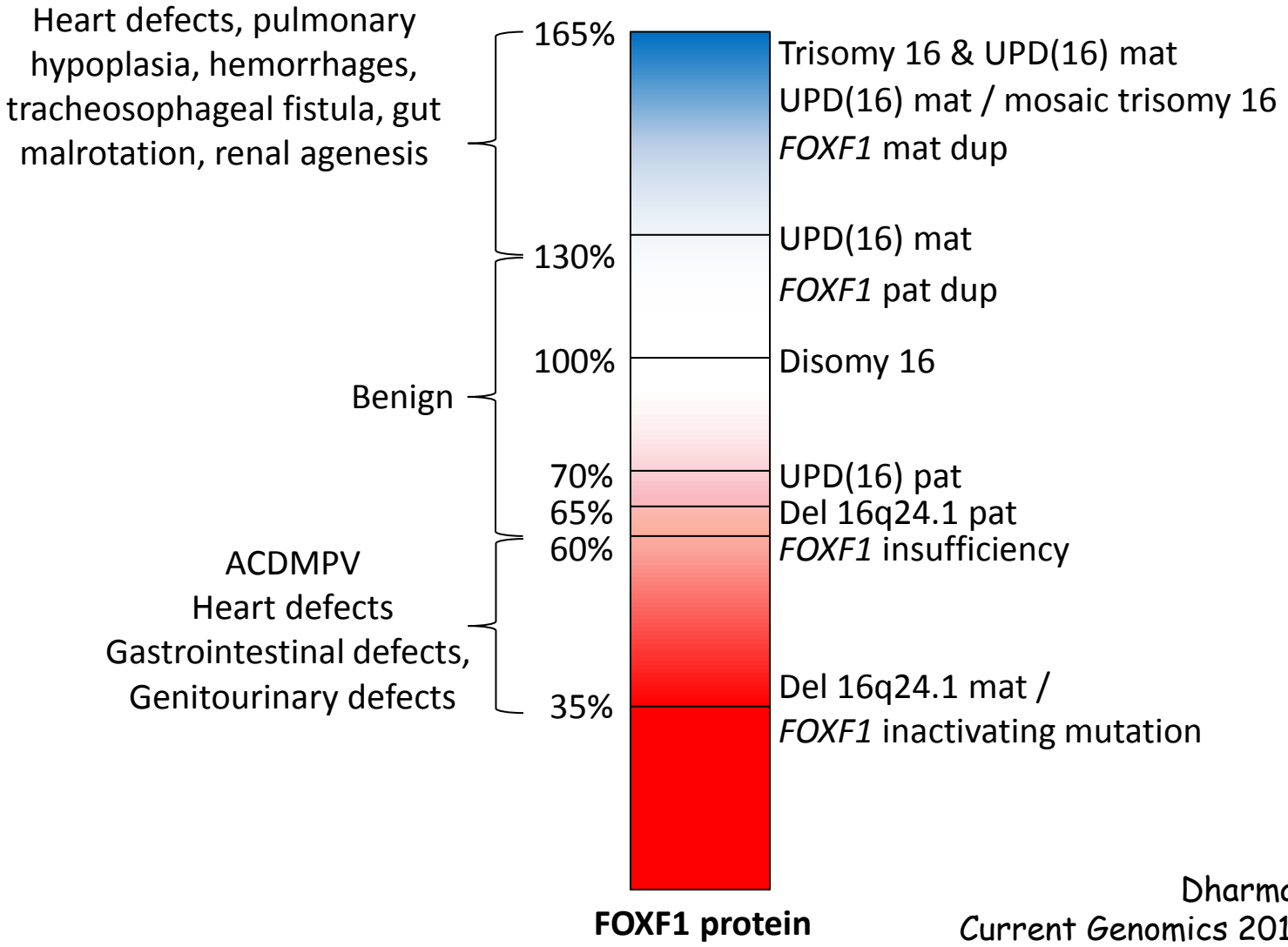


Subcutaneous hemorrhages and edema



Intestinal lymphatic defect

Correlation of predicted FOXF1 levels and associated phenotypes



Dharmadhikari et al.
Current Genomics 2015;16,107-116.

Conclusions

- *Foxf1* overexpression in mice recapitulates defects seen in patients with mat UPD(16)

- Paternal imprinting of *FOXF1* explains key phenotypic differences between mat vs. pat UPD(16)

- Our data aid the prenatal diagnosis and genetic counseling of families with *FOXF1* alterations

Acknowledgements

Baylor College of Medicine

Translational Biology & Molecular Medicine Graduate Program

Nijmegen, Netherlands
Nicole de Leeuw

Exeter, UK
Charles Shaw-Smith

Sheffield, UK
Oliver Quarrell

Jerusalem, Israel
Eitan Kerem
Joel Reiter

BCM Cores:
Mouse Embryonic Stem Cell core
Genetically Engineered Mouse Core
Optical Imaging and Vital Microscopy core

TBMM:
Mary Estes
Ignatia B. Van den Veyver

Funding Support:
NIH RO1HL101975
NORD