Some reading…..

1. NAD Deficiency, Congenital Malformations and Niacin Supplementation.
Shi H, Enriquez A, Rapadas M, Martin EMMA. Wang R, Moreau J, Lim CK, Szot JO, Ip E, Hughes J, Sugimoto K, Humphreys D, McInerney-Leo AM, Leo PJ, Maghzal GJ, Halliday J, Smith J, Colley A, Mark PR, Collins F, Sillence DO, Winlaw DS, Ho J, Guillemin GJ, Brown MA, Kikuchi K, Thomas PQ, Stocker R, Giannoulatou E, Chapman G, Duncan EL, Sparrow DB, **Dunwoodie SL.**
The New England Journal of Medicine. 2017;377(6):544-552.

2. Metabolism and Congenital Malformations — NAD's Effects on Development
Matthew G. Vander Heiden,
The New England Journal of Medicine. 2017; 377(6):509-511

Sally Dunwoodie

s.dunwoodie@victorchang.edu.au

Summer Student Scholarships

Victor Chang
Cardiac Research Institute

Identifying genetic and environmental factors that disrupt embryogenesis

Sally L Dunwoodie

s.dunwoodie@victorchang.edu.au

Dr Victor Chang AC 1936-1991, Pioneering Cardiothoracic Surgeon and Humanitarian

Mesoderm Development Lecture

Gastrulation

Early Mesoderm Development

Notochord

Paraxial Mesoderm

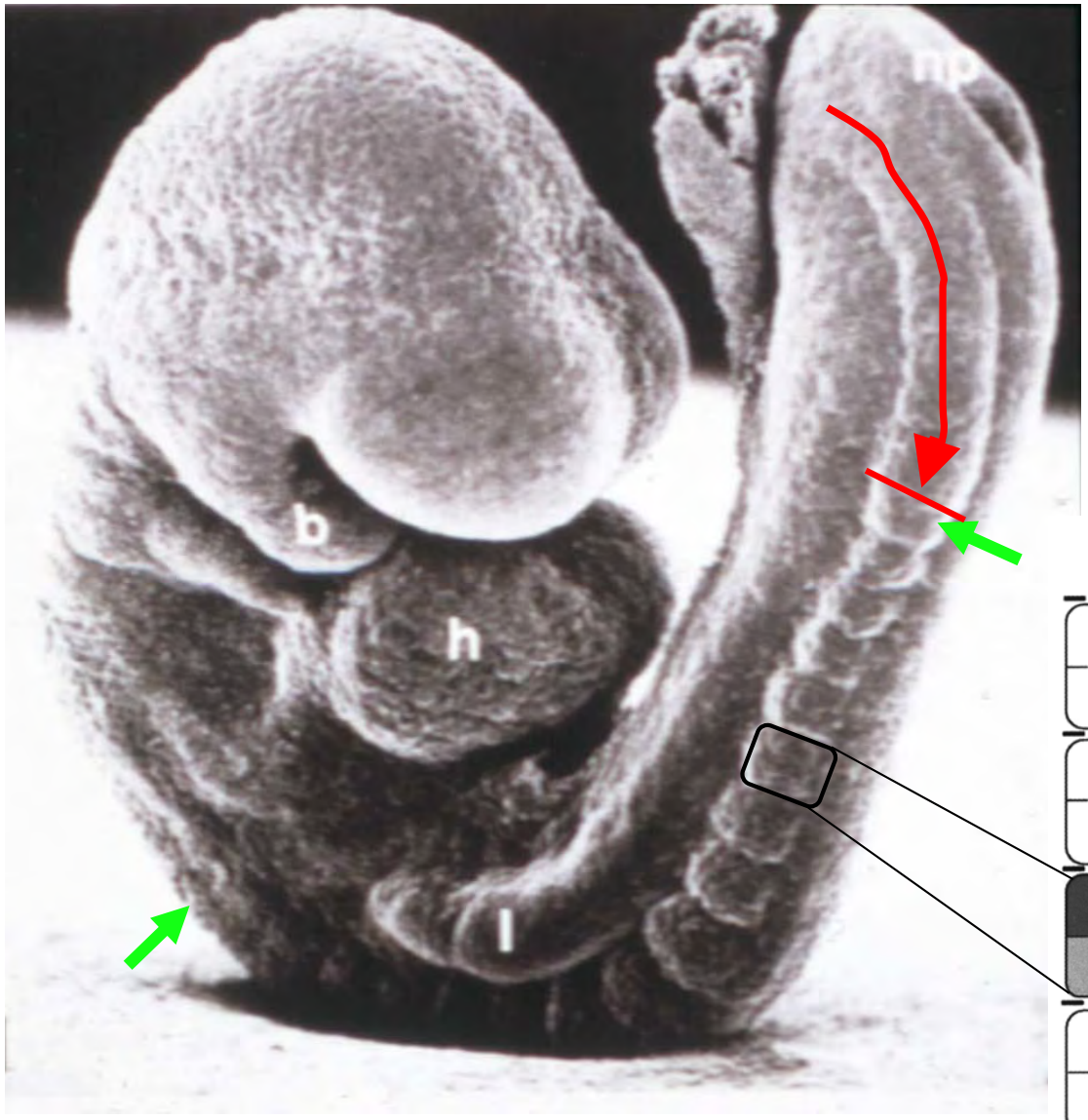
Intermediate Mesoderm

Lateral Plate Mesoderm

Early Heart Development

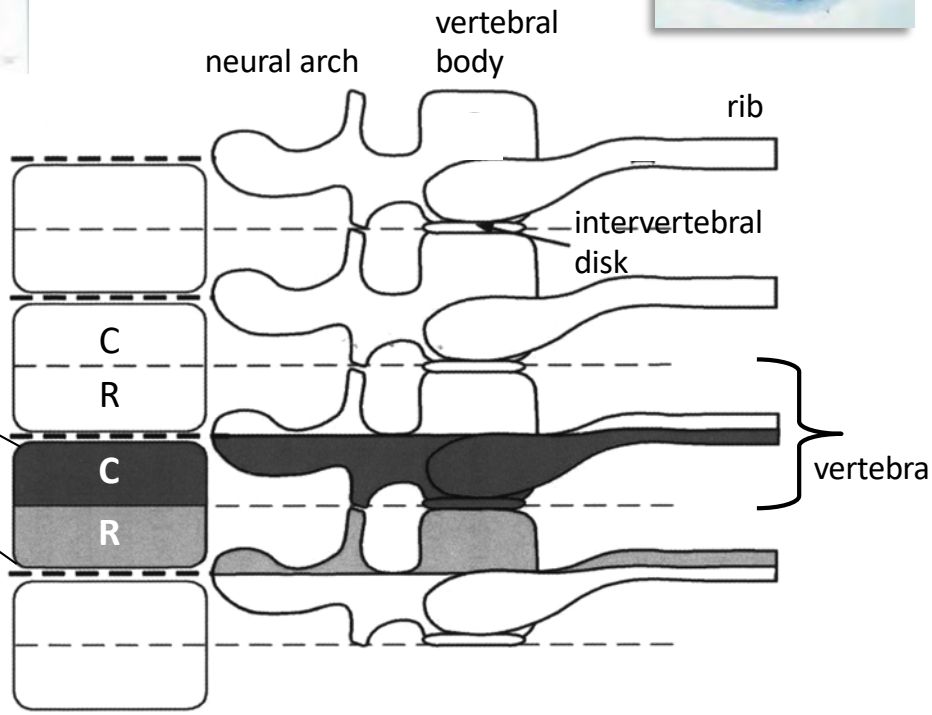
Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au

Somites give rise to the vertebral column



Notch
FGF
Wnt
Retinoic acid

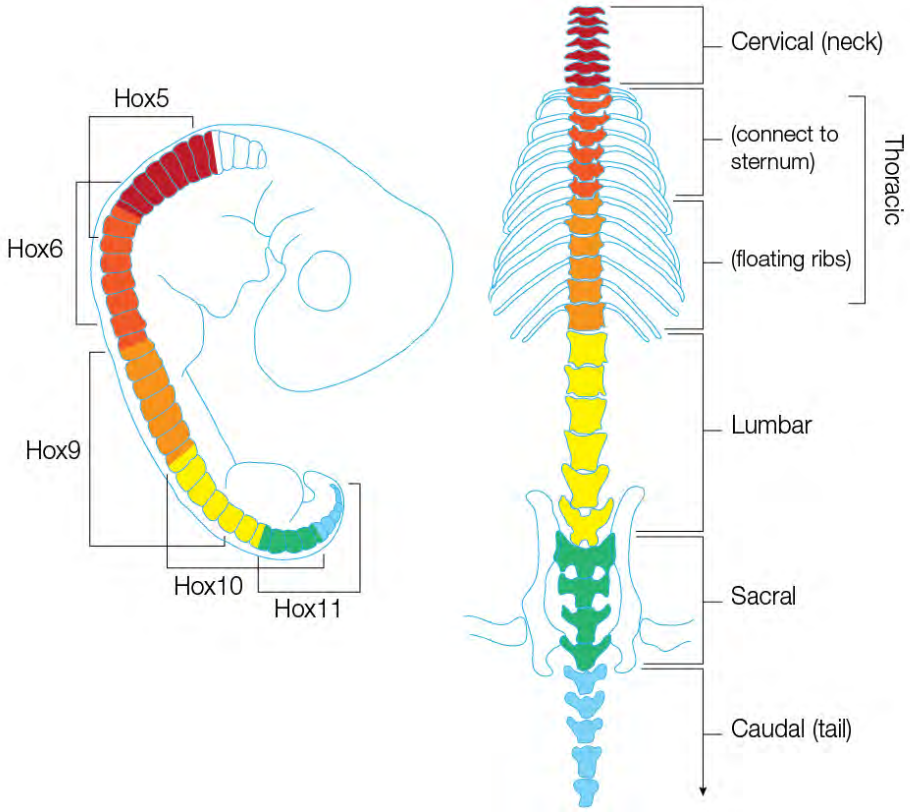
bone
muscle
ligament
tendon



2: Paraxial Mesoderm

AP patterning

Somite Derivative Specification depends on AP level/*Hox* code



7: cervical region

12: thoracic region

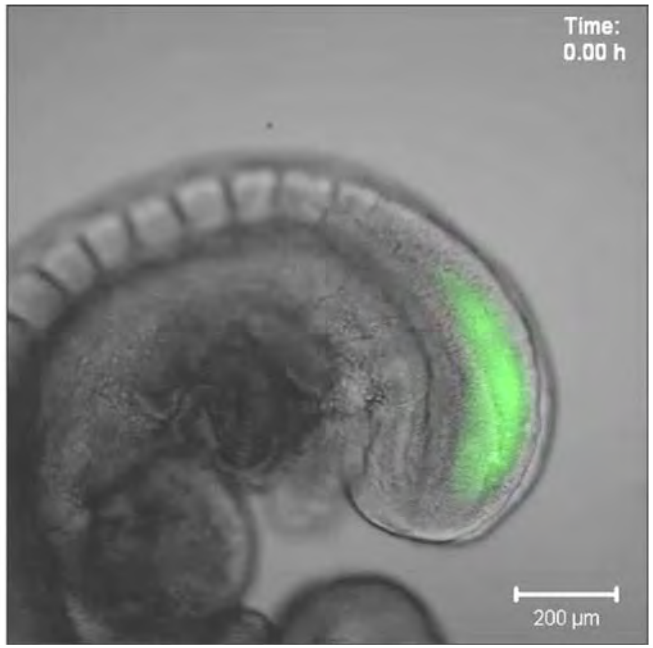
5: lumbar region

5: sacral region

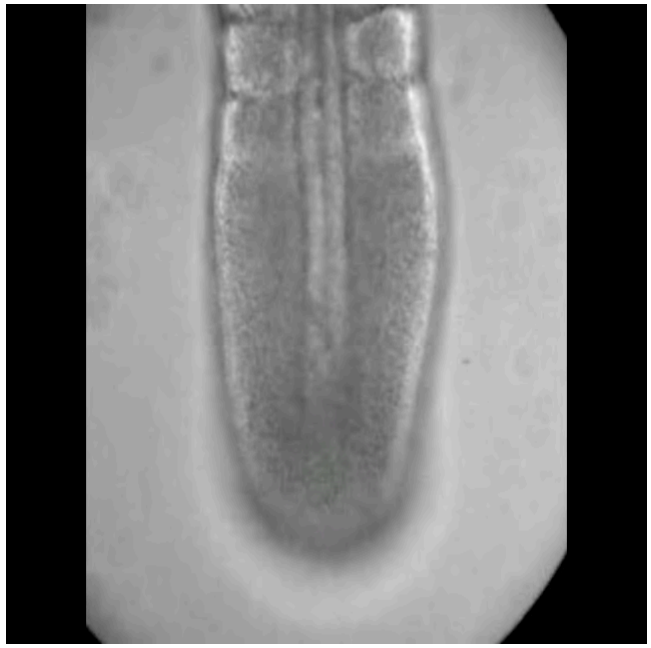
4: caudal region (tail)

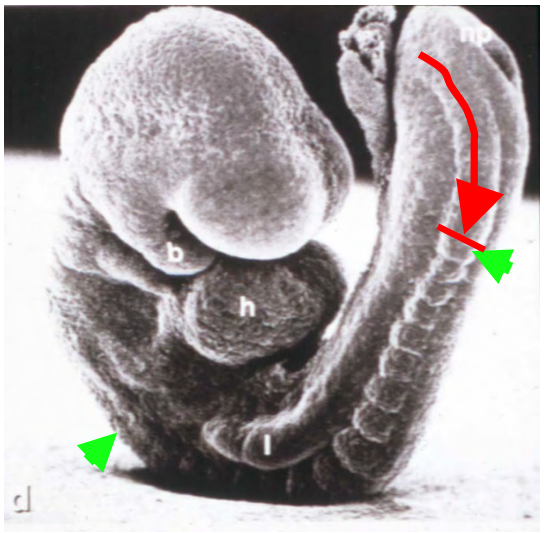
NOTCH1 target gene expression in the presomitic mesoderm

Lfng-GFP Aulehla *et al* Nat Cell Biol 2008

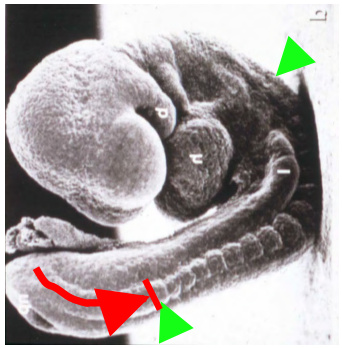


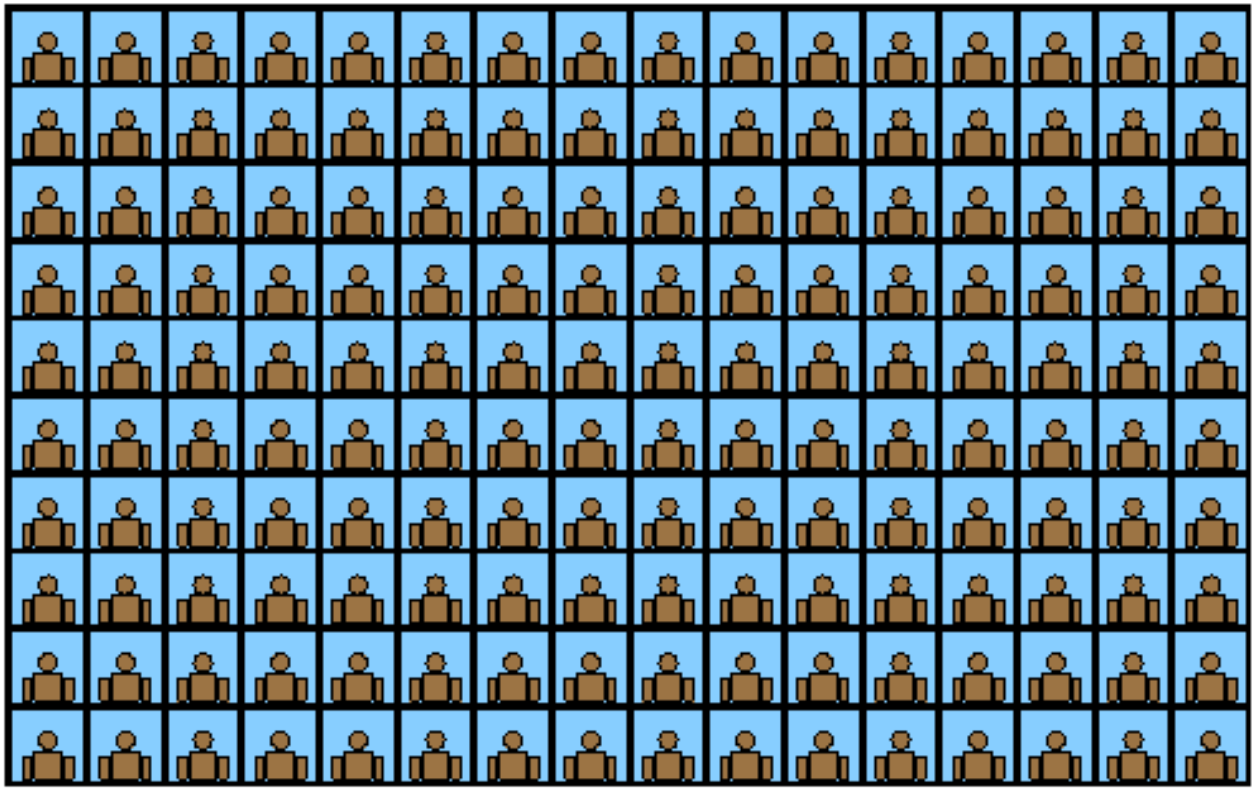
Hes7-Luc Kageyama *et al* WIREs Dev Biol 2012

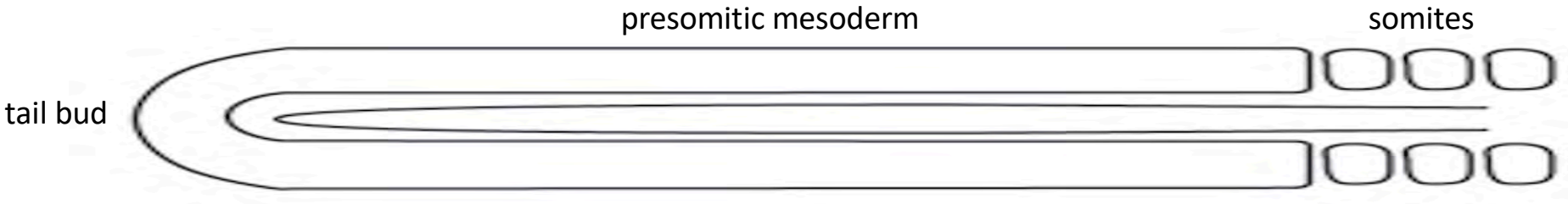




NOTCH1 activity in the presomitic mesoderm

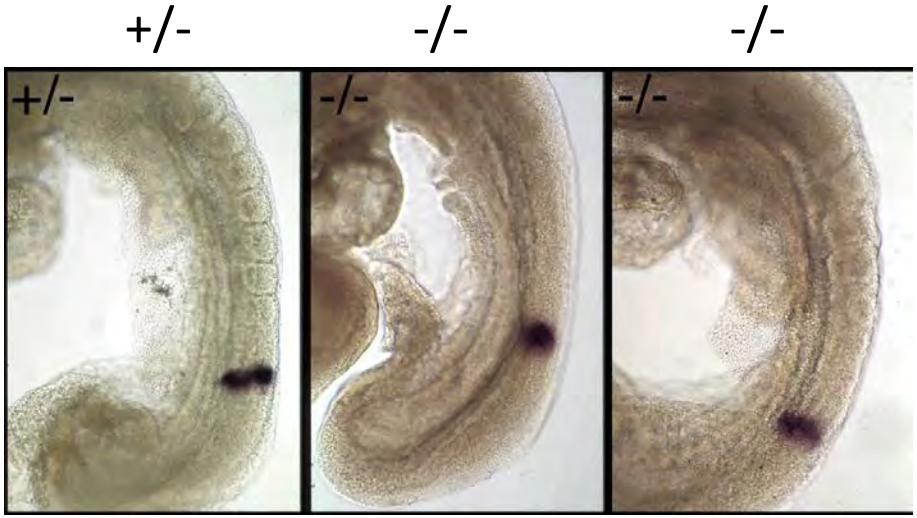


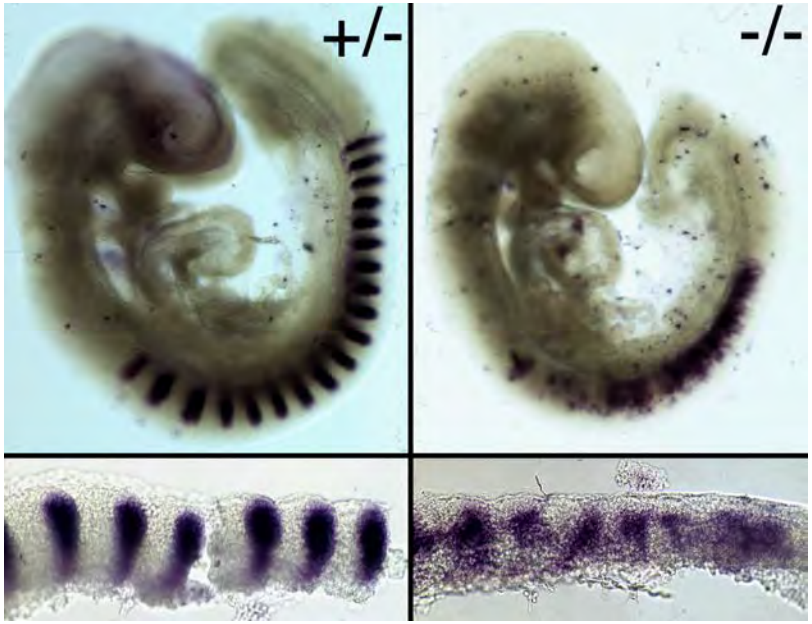


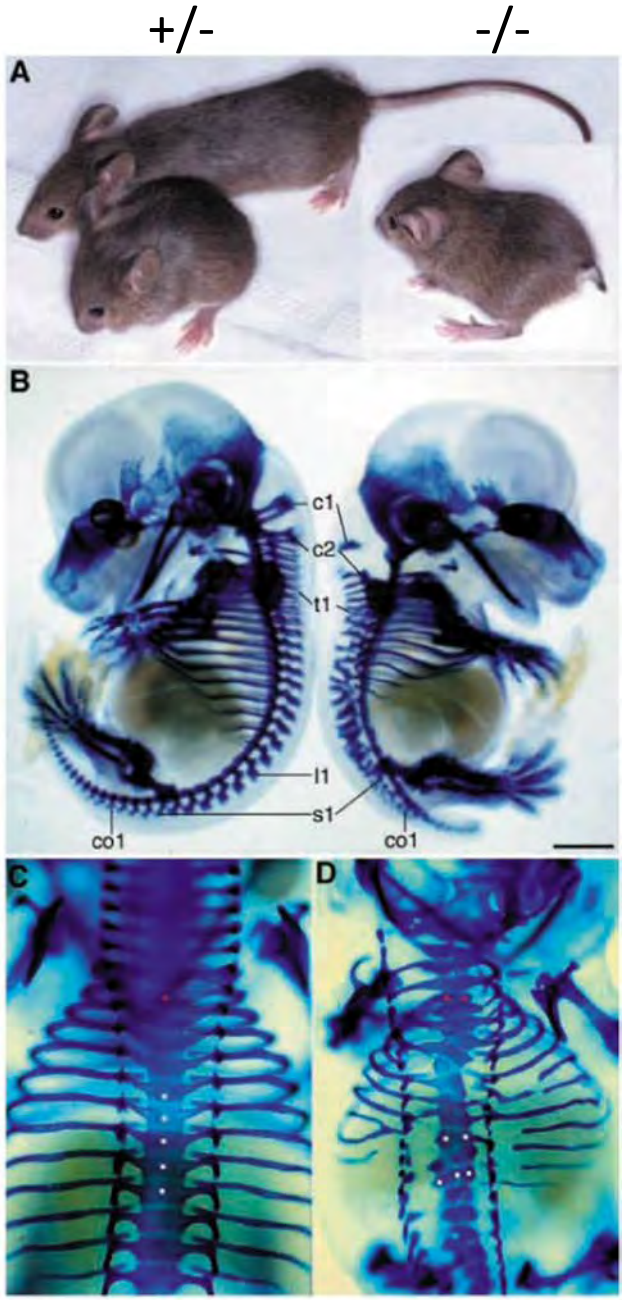


Farkas *et al* Nature 2002

Dll3 is required for formation of somites and vertebrae

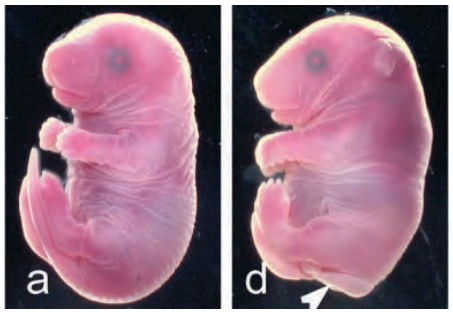




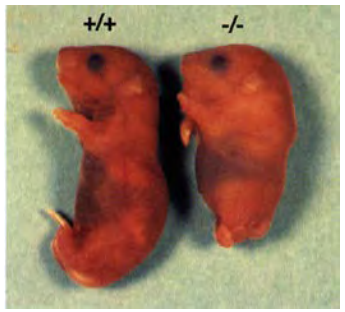


Dunwoodie *et al* Dev 2002

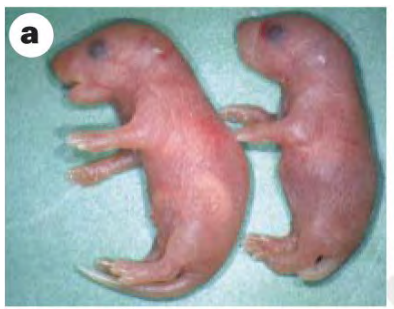
Genes required for somitogenesis in mouse



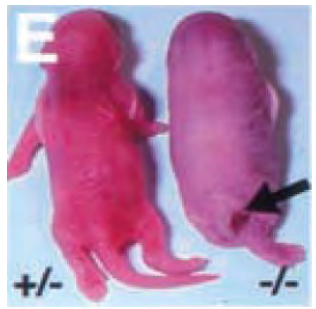
Dll3-/-



Mesp2-/-



Lfng-/-



Hes7-/-

Adam10
Aldh1a2
Aphla
Axin1
Cdh2
Cdh2
Cdh11
Cyp26a1
Dact1
Dll1
Dll3
Dkk1
Dvl2

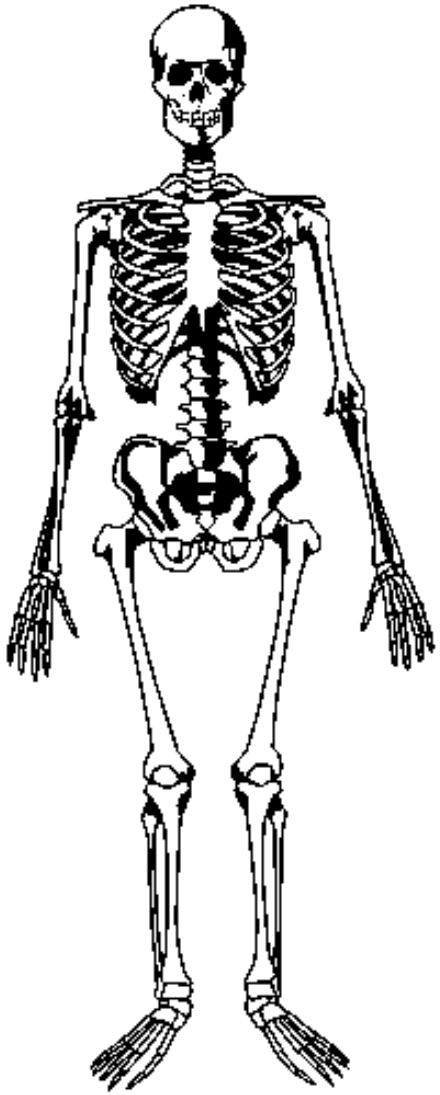
Efnb2
Epha1
Fn1
Fgf3
Fgf4/Fgf8
Fgfr1
Foxc1
Foxc2
Has2
Hes7
Itgav
Lef1
Lfng
Lrp6

Meox1
Meox2
Mesp2
Mib1
Msgn1
Ncstn
Notch1
Pax1
Pax3
Pofut1
Psen1
Rbpj
Rere

Ripply1
Ripply2
Sfrp1
Sfrp2
Sip1
Tbx6
Tbx18
Tcf15
Uncx
Wnt3a
Zic2
Zic3

Sparrow *et al* Mammalian Genome 2011

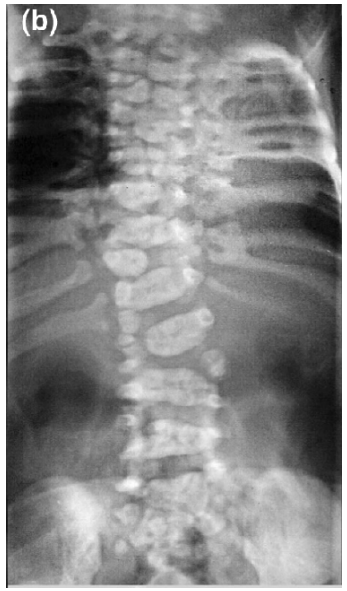
MOUSE
1-3 hours per somite
embryonic days 8-13
36 vertebrae + 26 in tail



HUMAN
4-6 hours per somite
embryonic days 20-30
33 vertebrae

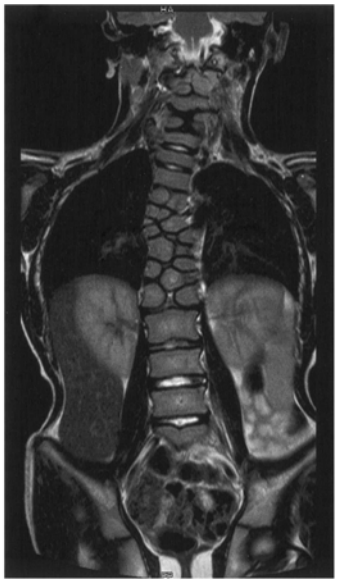
Spondylocostal dysostosis (SCD) is caused by mutation in Notch associated genes

SCD1 *DLL3*



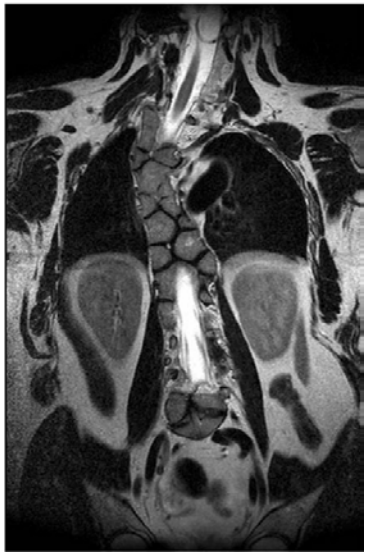
Bulman *et al* 2000
Turnpenny *et al* 2003

SCD2 *MESP2*



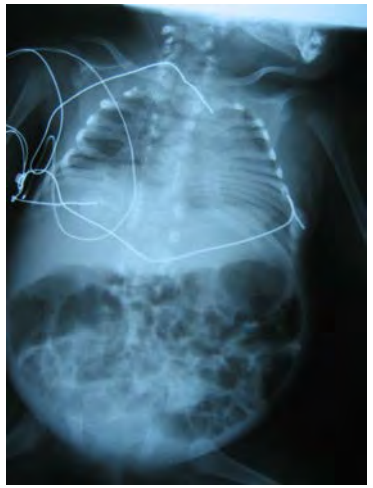
Whittock *et al* 2004

SCD3 *LFNG*



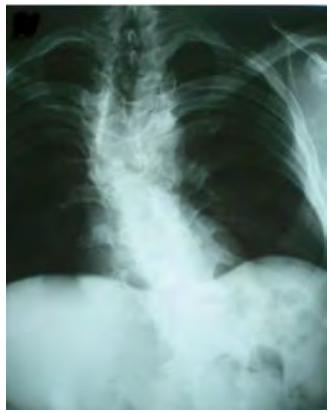
Sparrow *et al* 2006

SCD4 *HES7*



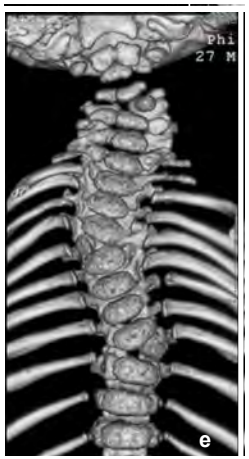
Sparrow *et al* 2008

SCD5 *TBX6* dominant

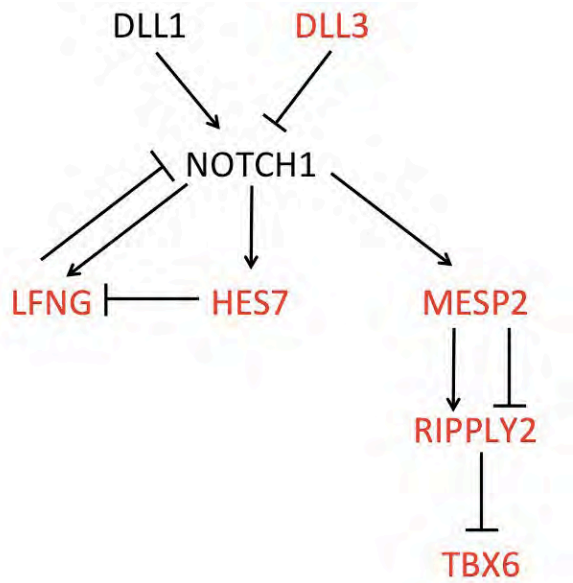


Sparrow *et al* 2013

SCD6 *RIPPLY2*



McInerney-Leo et al 2014

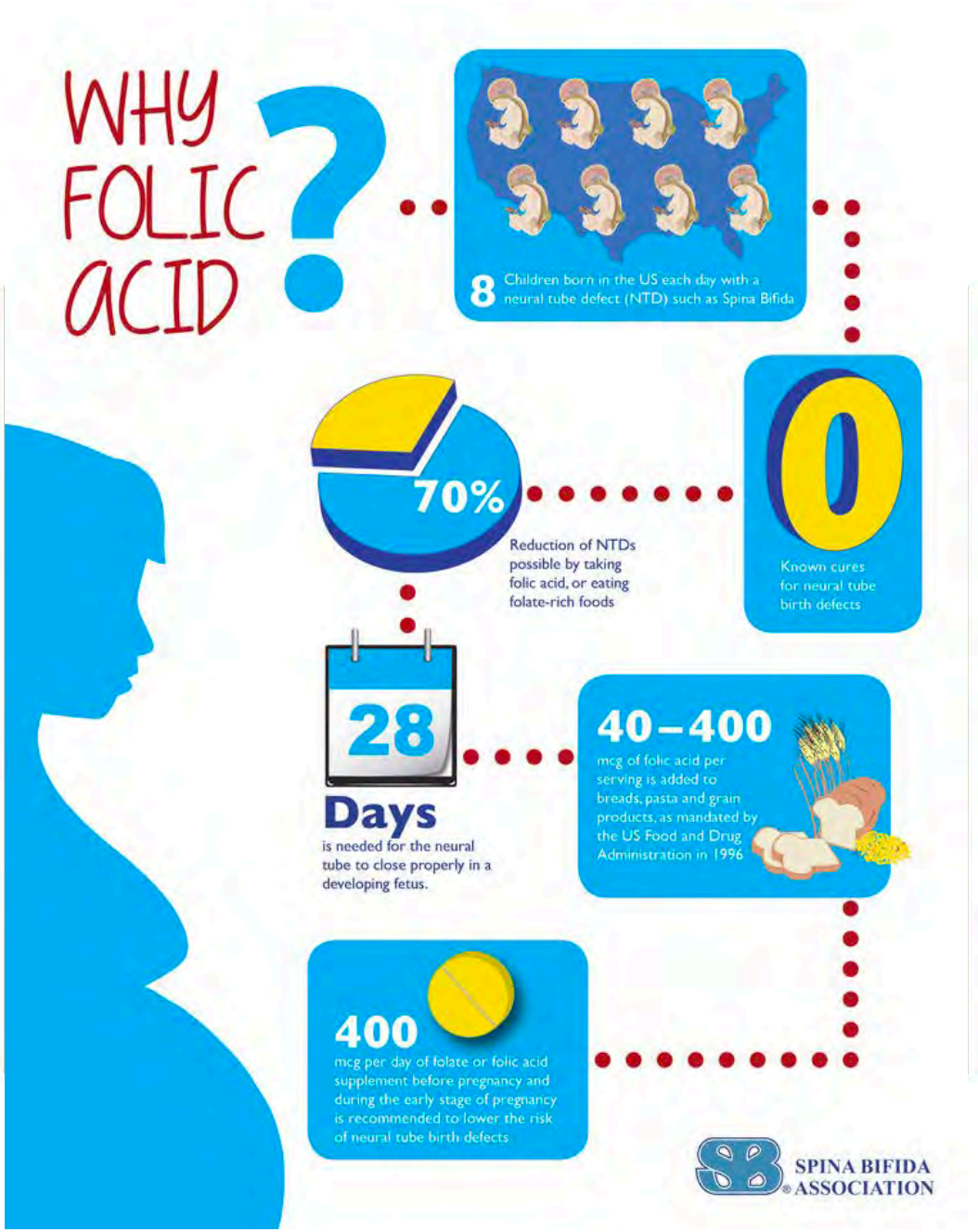


Complex birth defects

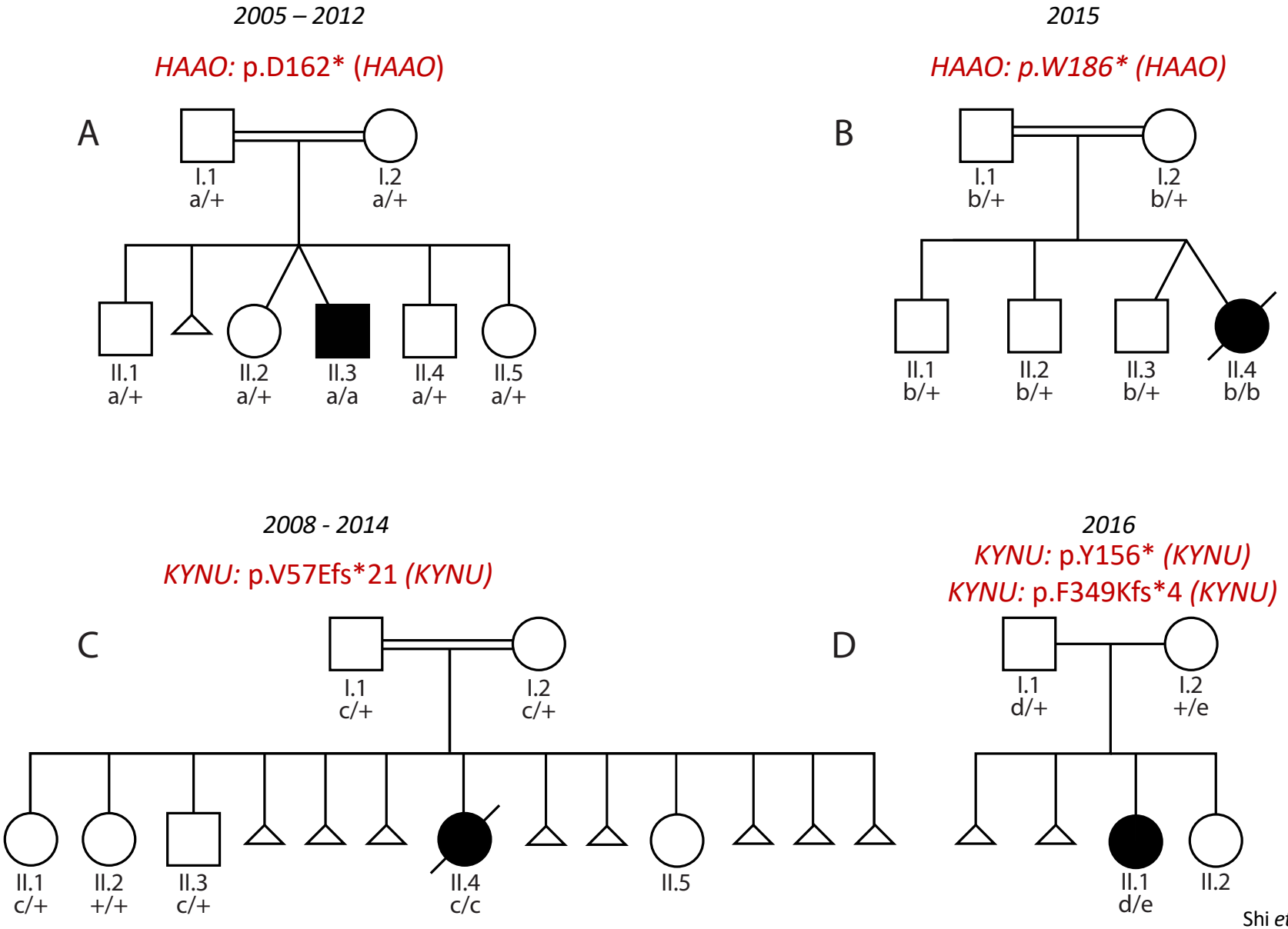
heart
vertebra
kidney

limb
digit
palate

Folic acid supplementation reduces the incidence of neural tube defects

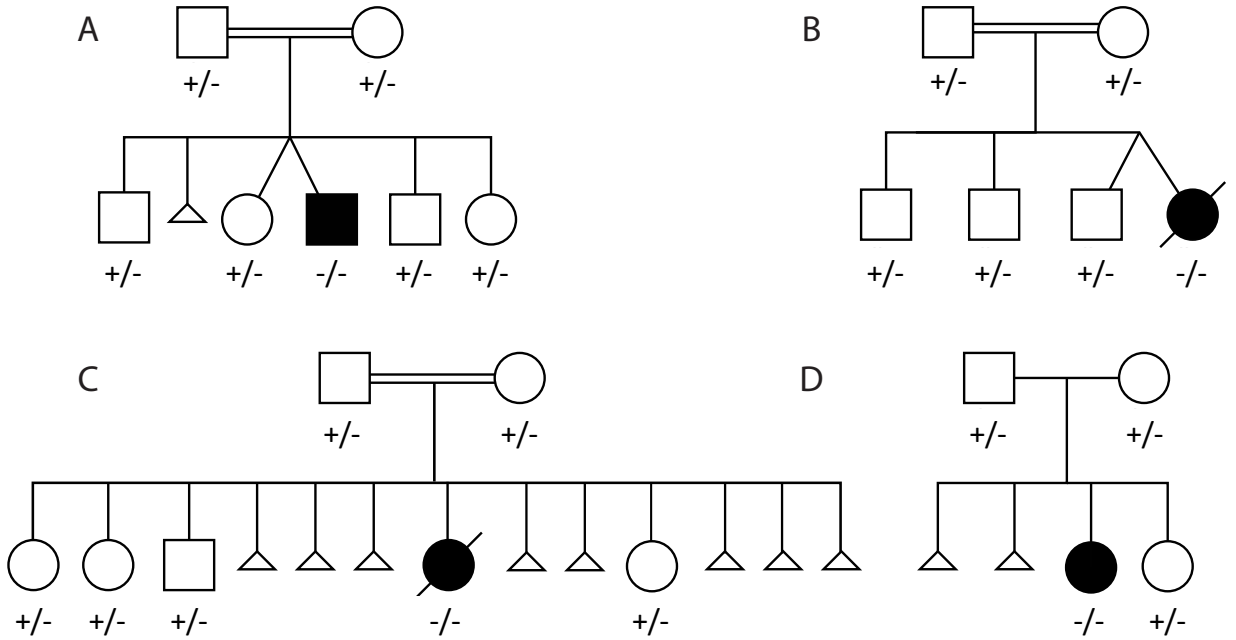


HAAO or *KYNU* variants in families with multiple congenital malformation and miscarriage



Shi *et al.* NEJM 2017

HAAO or *KYNU* variants in families with multiple congenital malformation and miscarriage

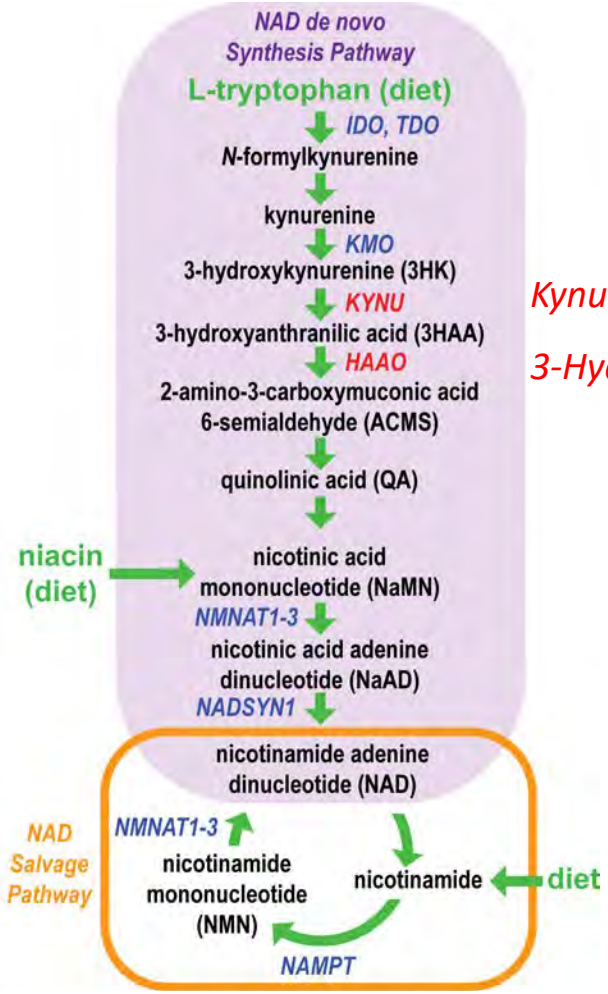


Patient	**A** *HAAO* ■	**B** *HAAO* ◪	**C** *KYNU* ◪	**D** *KYNU* ●
Vertebral segmentation anomalies	+			+
Cardiac	ASD			HLH
Renal	hypopl			solitary kidney, chronic disease
Limb	talipes			short long bones
Ear	SNHL,			-
Other features	short st laryngе malacia,			short stature, speech delay

heart
vertebra
kidney

limb
digit
palate

KYNU and HAAO required to synthesise NAD from tryptophan



Kynureninase

3-Hydroxyanthranilate 3,4-dioxygenase

NAD precursors

tryptophan 60:1

vitamin B3 1:1

niacin equivalents

Vitamin B3

niacin/nicotinic acid

nicotinamide

nicotinamide riboside

Building evidence of a gene or variant's role in disease

Does the variant disrupt protein function?

Does the variant affect protein function in patients?

Is the genes required for embryogenesis?

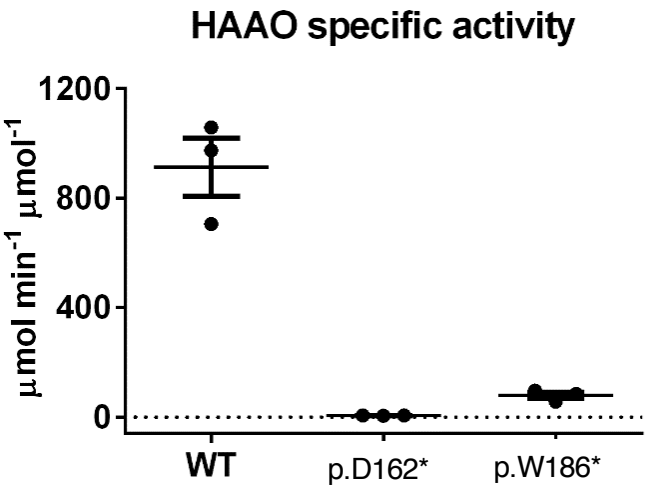
What alters penetrance and expressivity of the variant?

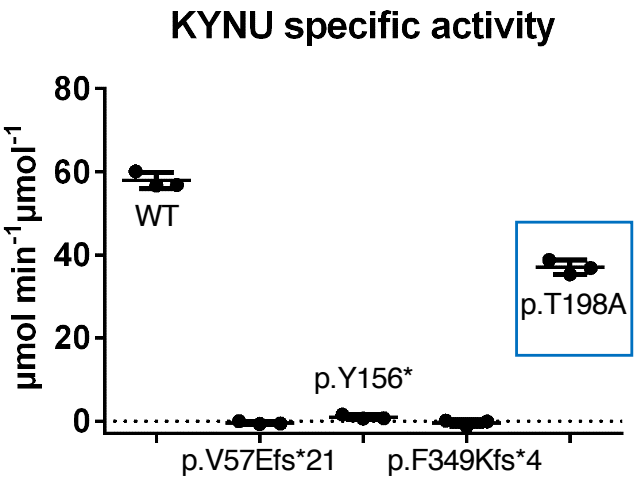
Are the variant enzymes active?

YES!

- quantifying enzymatic activity in vitro

Family	A	B	C	D
Gene	*HAAO*	*HAAO*	*KYNU*	*KYNU*
DNA variant(s)	c.483dupT homozygous	c.558G>A homozygous	c.170-1G>T homozygous	c.468T>A c.1045_1051 delTTTAAGC
Protein variant(s)	p.D162*	p.W186*	p.V57Efs*21	p.Y156* p.F349Kfs*4

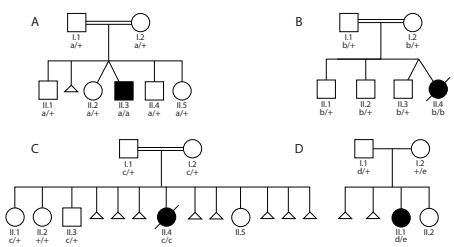




KYNU p.T198A that is associated with hydroxy-kynurenin-uria but not congenital malformation

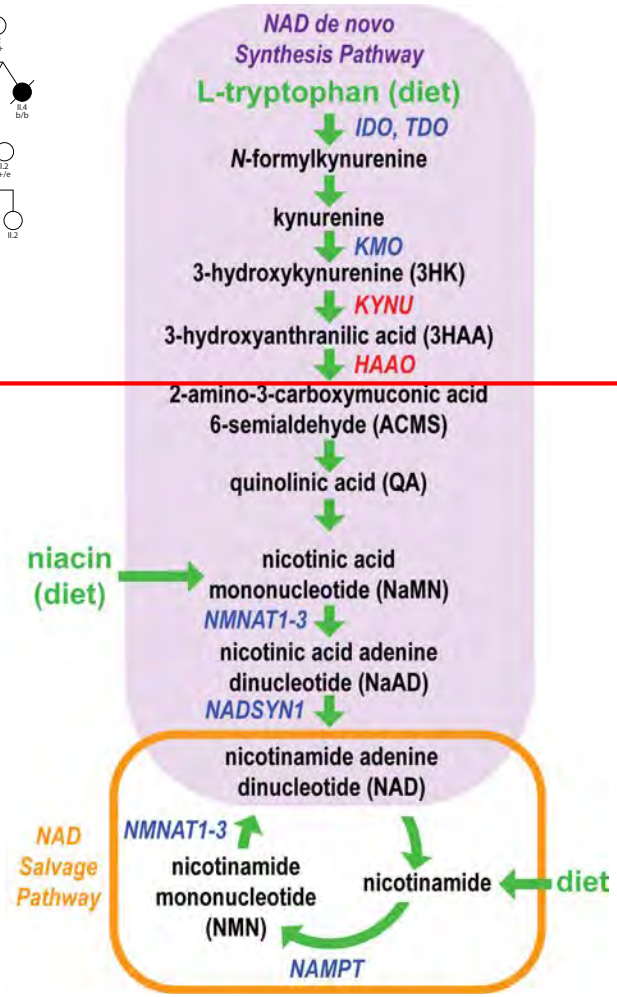
Do the variants affect enzyme function in patients? **YES!**

-quantifying metabolites in patients



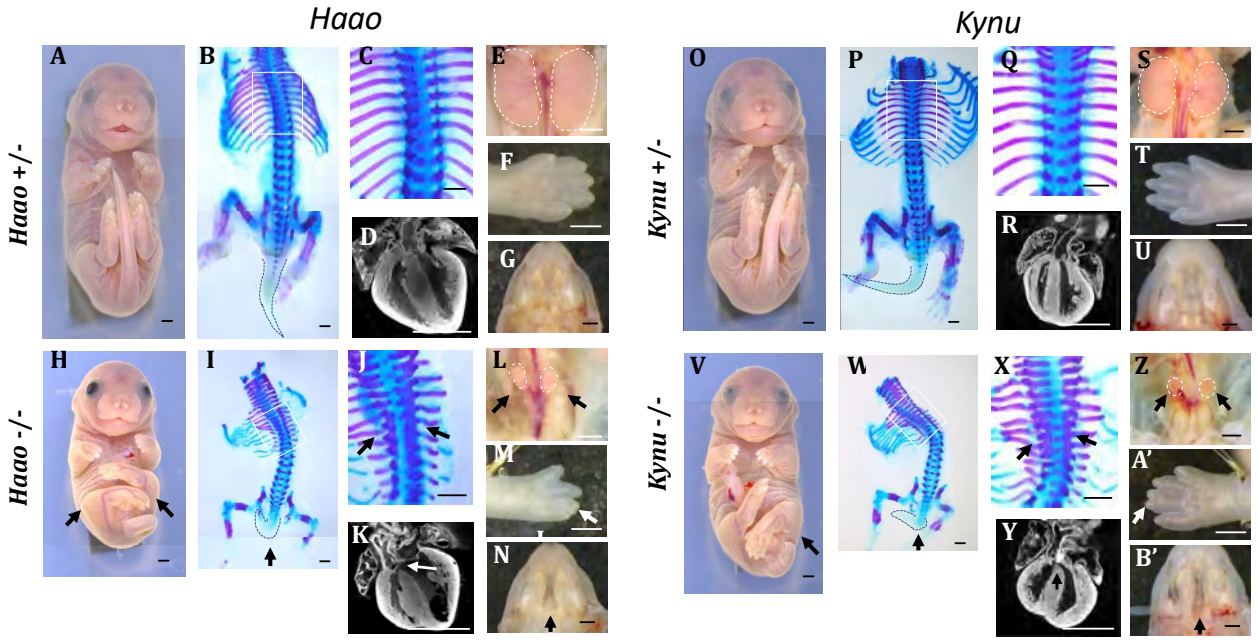
3HK / 3HAA:
64-385 x up

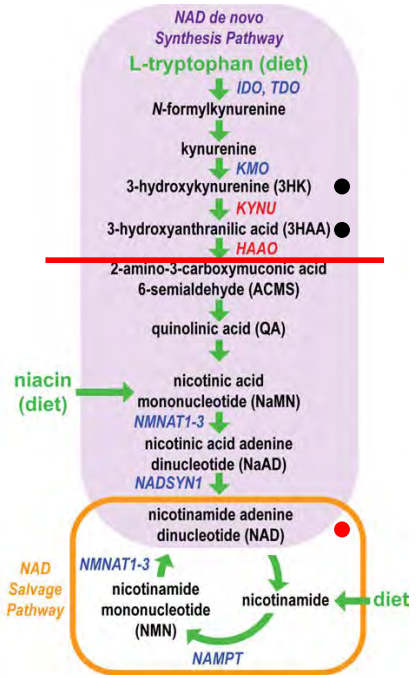
NAD:
3-9 x down



Is the genes required for embryogenesis? **YES**

-identifying a phenotype in mice





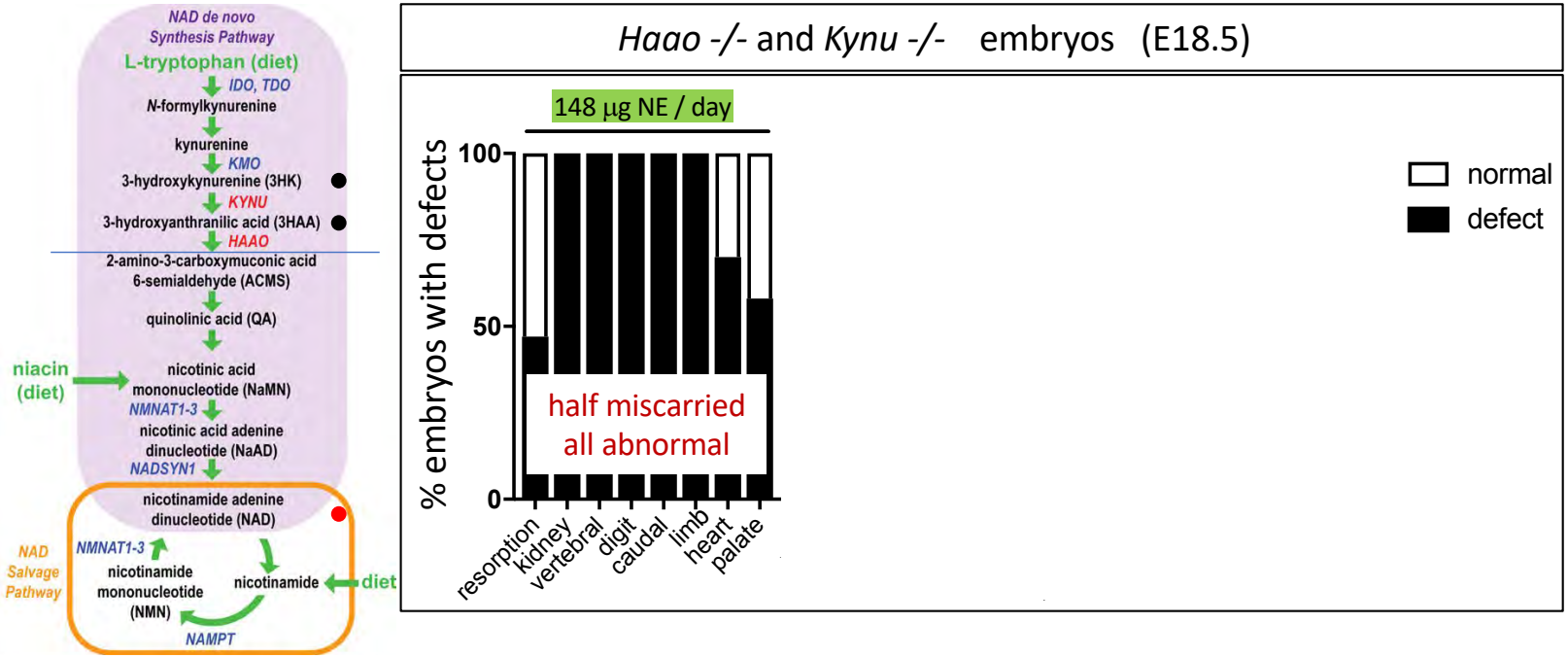
Defects: heart, vertebral, kidney, cleft palate, talipes, syndactyly, caudal agenesis

Increase in upstream metabolites
Decrease in downstream metabolites

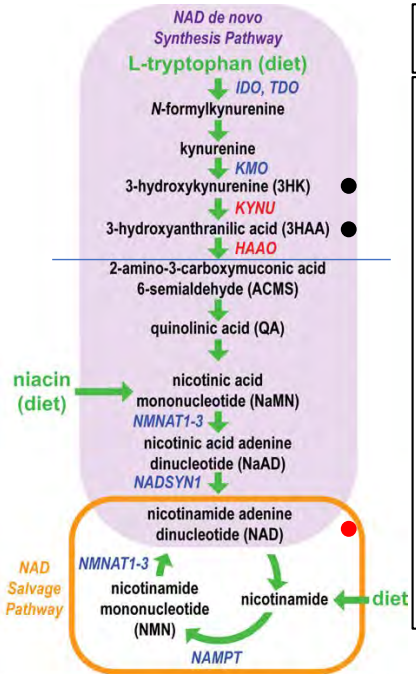
Shi *et al.* NEJM 2017

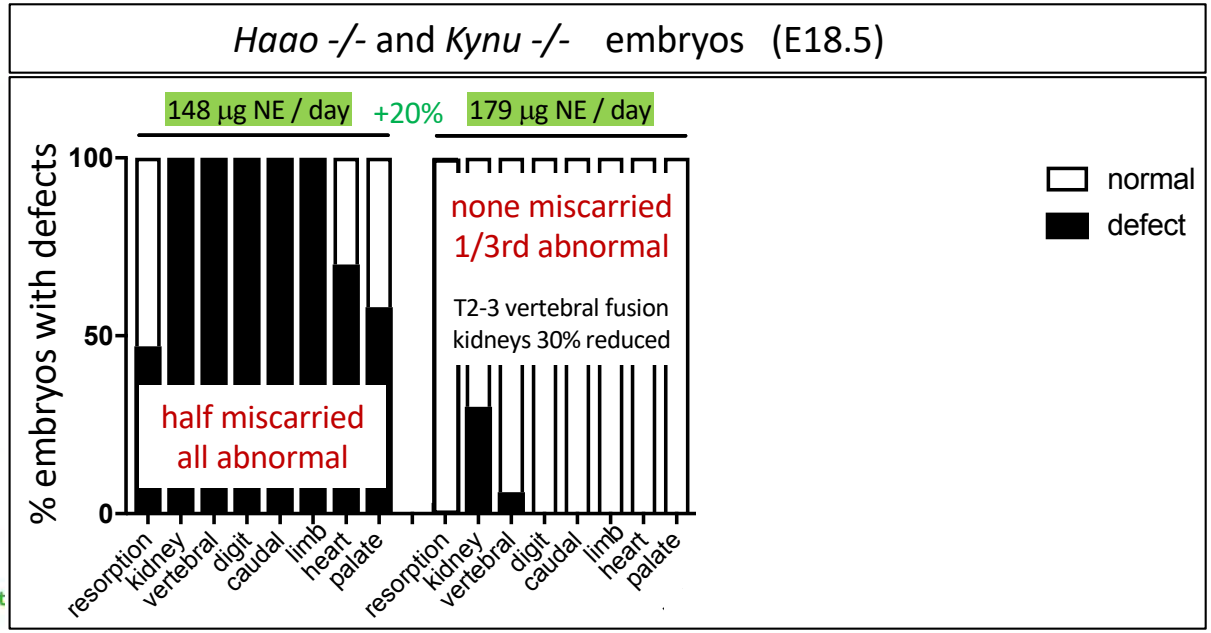
What alters the effect of (penetrance/expressivity) of the variant?

Niacin supplementation prevents NAD deficiency and defects

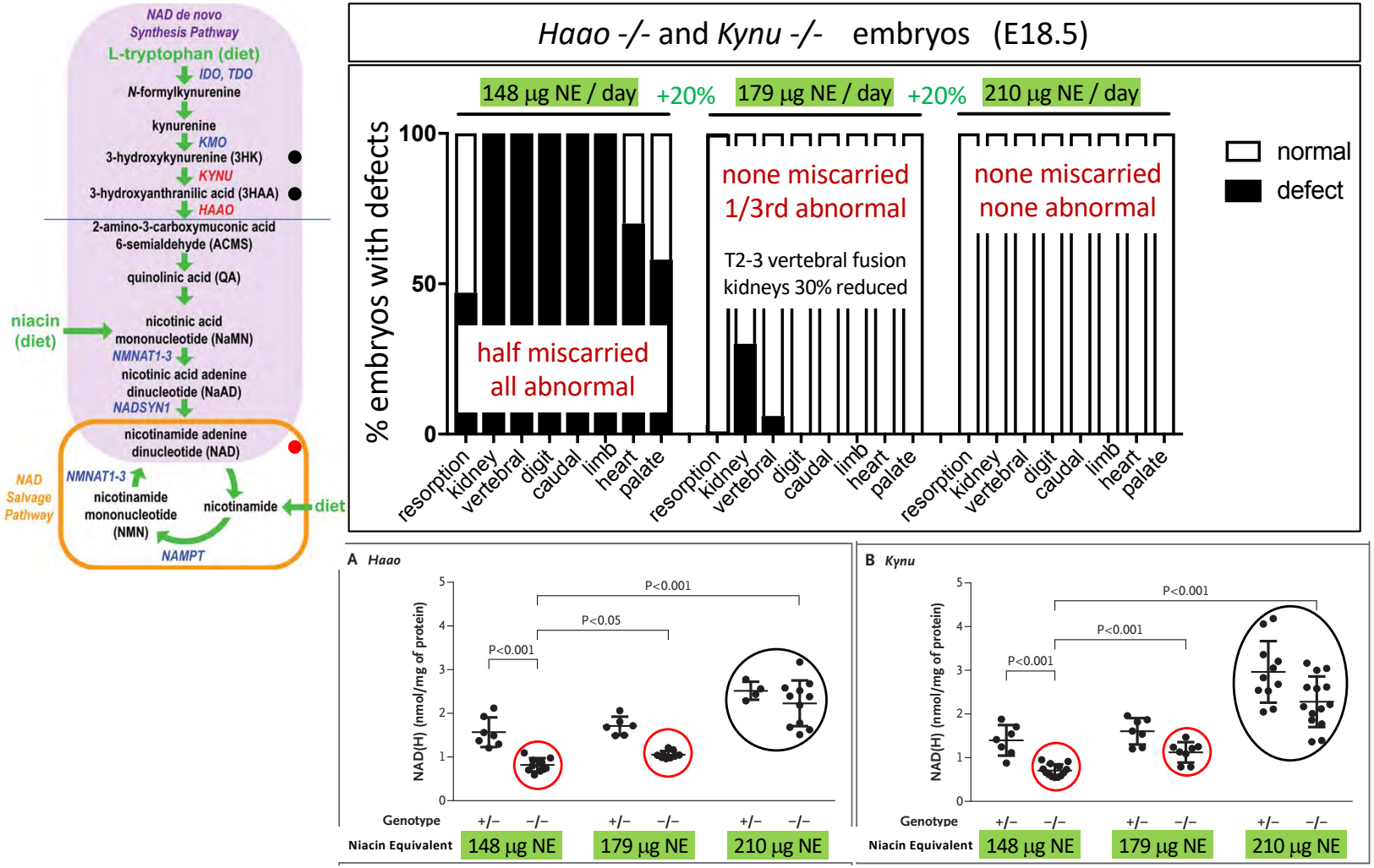


Niacin supplementation prevents NAD deficiency and defects



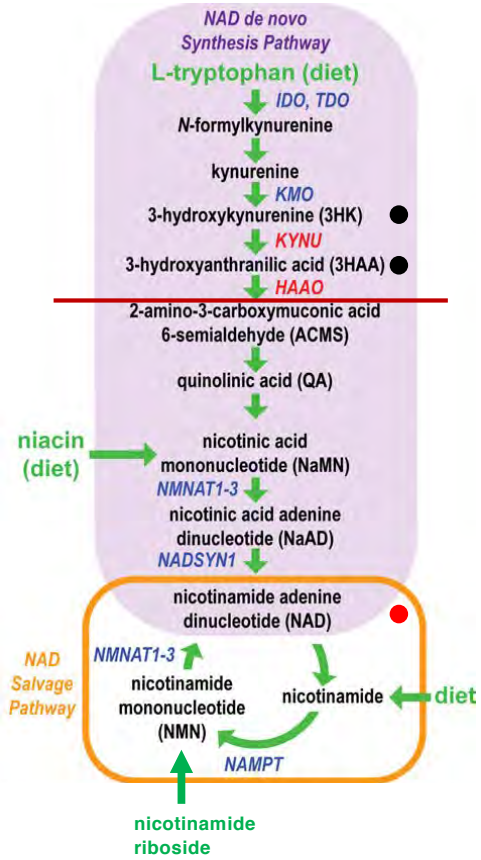


Niacin supplementation prevents NAD deficiency and defects



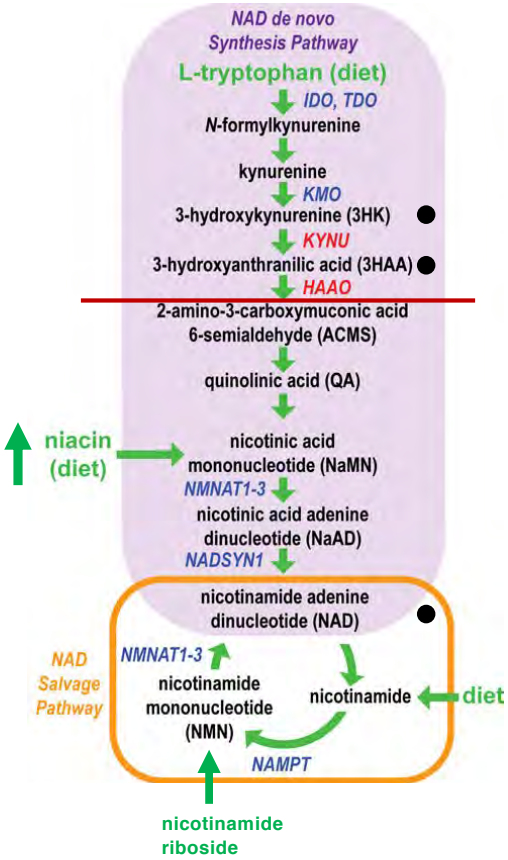
Loss of *KYNU* or *HAAO* causes NAD deficiency and defects

humans and mice



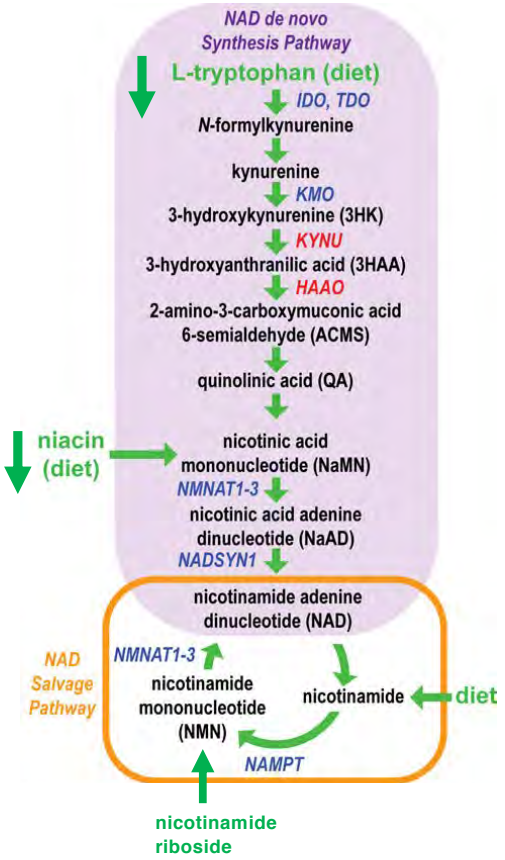
Vitamin B3 supplementation bypasses mutations prevents NAD deficiency and defects

mice



Can diet cause NAD deficiency?

mice



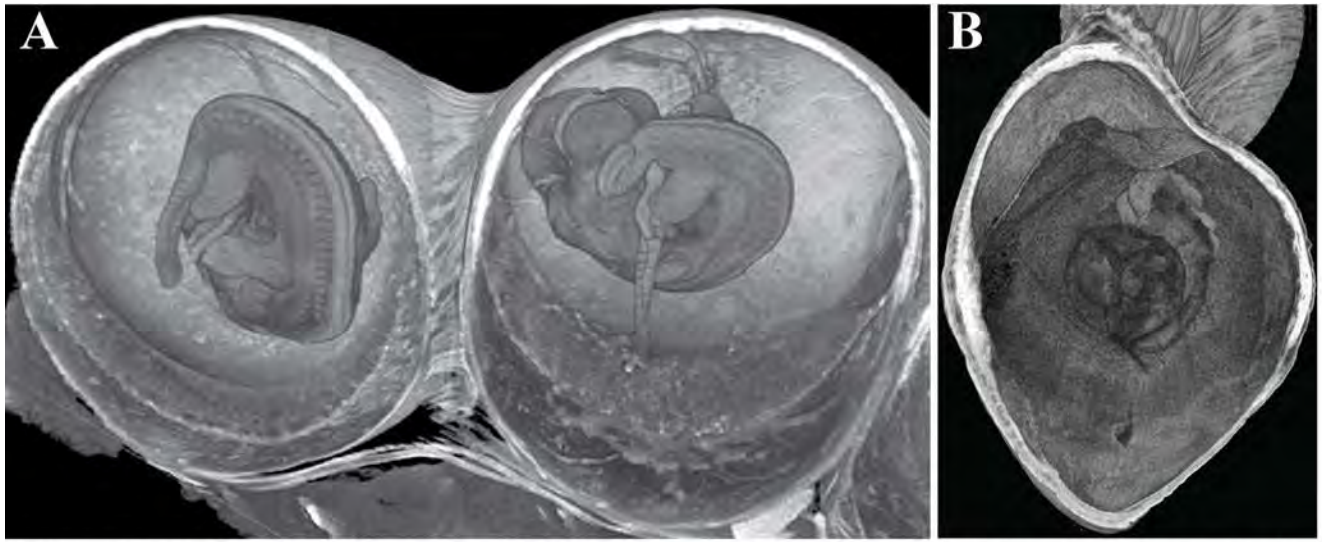
Shi *et al.* NEJM 2017

Methodologies used in this research

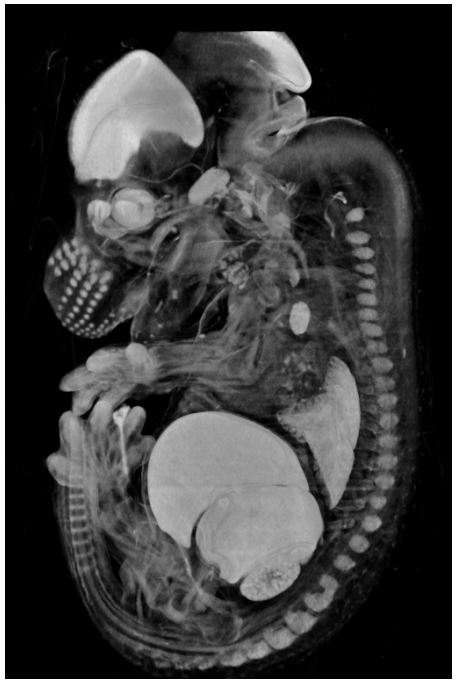
- genome sequencing (human)
- enzyme activity assays *(in vitro)*
- quantifying metabolites with LC-MS (human and mouse)
- generation of mutant mice (CRISPR-Cas9)
- mouse phenotyping (skeletal, heart, kidney, etc)
- whole mouse embryo phenotyping (microCT)

Using microCT to phenotype mouse embryos and whole litters

E11.5



E14.5



Gavin Chapman

s.dunwoodie@victorchang.edu.au

Victor Chang
Cardiac Research Institute

Dunwoodie Laboratory
Gavin Chapman
Justin Szot
Ella Martin
Joelene Major
Kavitha Iyer
Annabelle Enriquez
Dimuthu Alankarage
Hartmut Cuny
Jess Gereis
Debjani Das
Moran Almog
Duncan Sparrow
Hongjun Shi
Rosemary Kirk
Melissa Rapadas

Collaborators- clinical
David Sillence, Children's Hospital Westmead, Sydney
Janine Smith, Children's Hospital Westmead, Sydney
Alison Colley, Liverpool Hospital, Sydney
Paul Mark, Spectrum Health, Grand Rapids, Michigan

CHD Collaborators- clinical
David Winlaw, Children's Hospital Westmead, Sydney
Gillian Blue, Children's Hospital Westmead, Sydney
Nicholas Pachter, Genetic Services of WA, Perth
Katrina Harrison, Genetic Services of WA, Perth
Edwin Kirk, Sydney Children's Hospital, Sydney

Bioinformatics @ VCCRI
Eleni Giannoulatou
David Humphreys
Eddie Ip
Michael Troup

telethon

NSW GOVERNMENT Health

Australian Government
National Health and Medical Research Council

CHAIN REACTION
THE ULTIMATE CORPORATE BIKE CHALLENGE