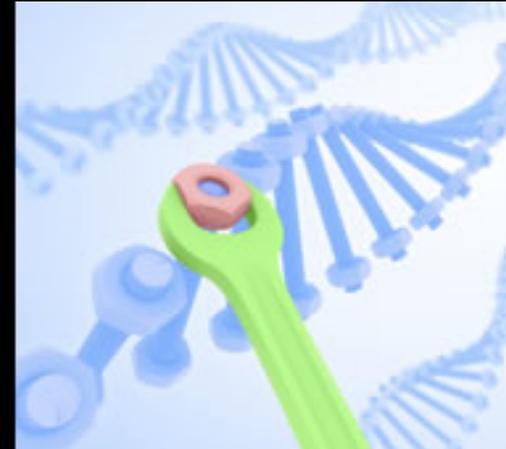


DSHS Grand Rounds

Oct. 29

Using Modern Genetic Tools to Understand Clinical Unknowns

Presenter: Richard Finnell, PhD,
Director, Genomic Research, Dell
Children's Medical Center



Logistics

Registration for free continuing education (CE) hours or certificate of attendance through TRAIN at:

<https://tx.train.org>

Streamlined registration
for individuals not requesting CE hours
or a certificate of attendance

1. webinar: <http://extra.dshs.state.tx.us/grandrounds/webinar-noCE.htm>
2. live audience: sign in at the door

For registration questions, please contact Laura Wells, MPH at
CE.Service@dshs.state.tx.us

Logistics (cont.)

Slides and recorded webinar available at:

<http://extra.dshs.state.tx.us/grandrounds>

Questions?

There will be a question and answer period at the end of the presentation. Remote sites can send in questions throughout the presentation by using the GoToWebinar chat box or email GrandRounds@dshs.state.tx.us.

For those in the auditorium, please come to the microphone to ask your question.

For technical difficulties, please contact:

GoToWebinar 1-800-263-6317(toll free) or 1-805-617-7000

Disclosure to the Learner

Requirement of Learner

Participants requesting continuing education contact hours or a certificate of attendance must register in TRAIN, attend the entire session, and complete the online evaluation within two weeks of the presentation.

Commercial Support

This educational activity received no commercial support.

Disclosure of Financial Conflict of Interest

The speaker and planning committee members have not disclosed any relevant financial relationships.

Off Label Use

There will be no discussion of off-label use during this presentation.

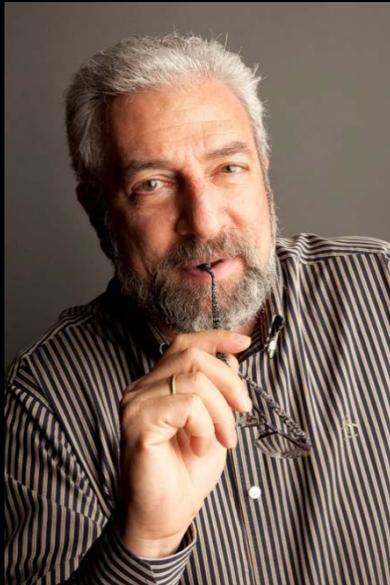
Non-Endorsement Statement

Accredited status does not imply endorsement by Department of State Health Services - Continuing Education Services, Texas Medical Association, or American Nurses Credentialing Center of any commercial products displayed in conjunction with an activity.



David Lakey, MD
DSHS Commissioner
is pleased to introduce our
DSHS Grand Rounds speaker

Using Modern Genetic Tools to Understand Clinical Unknowns



Richard Finnell, PhD, Director
Genomic Research,
Dell Children's Medical Center

Learning Objectives

Participants will be able to:

- Describe the basic concepts of next generation DNA sequencing methods.
- Explore how these methods are applied to population-wide studies as well as to clinical unknowns.
- Examine how a novel clinical diagnosis based on whole exome DNA sequencing can lead to therapeutic strategies for intervention and prevention.

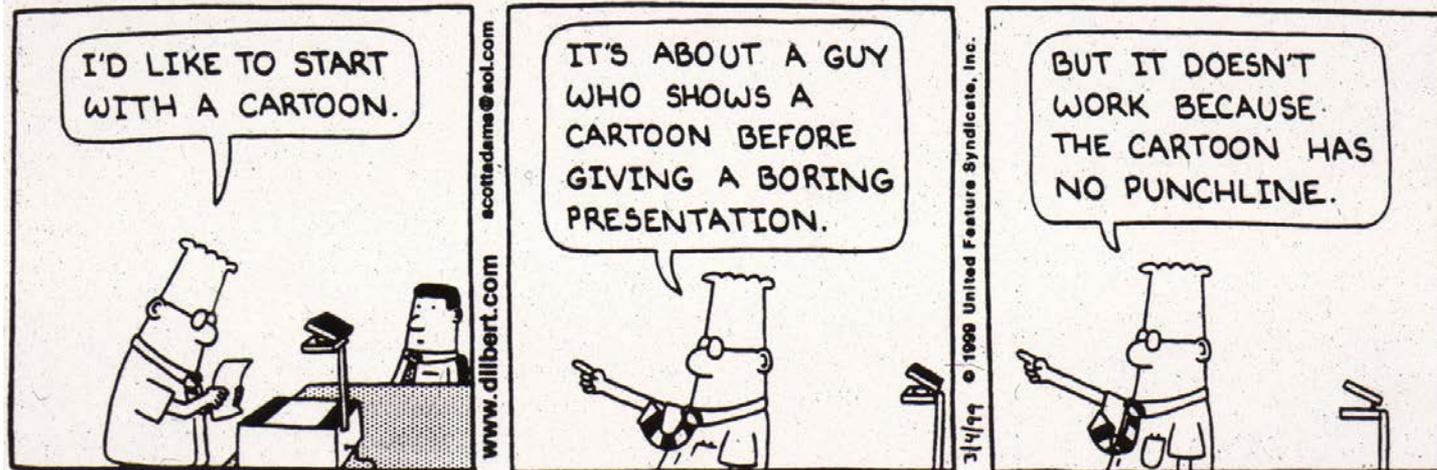


Using Modern Genetic Tools to Understand Clinical Unknowns

Richard H. Finnell

Dell Pediatric Research Institute
The University of Texas at Austin

rfinnell@austin.utexas.edu

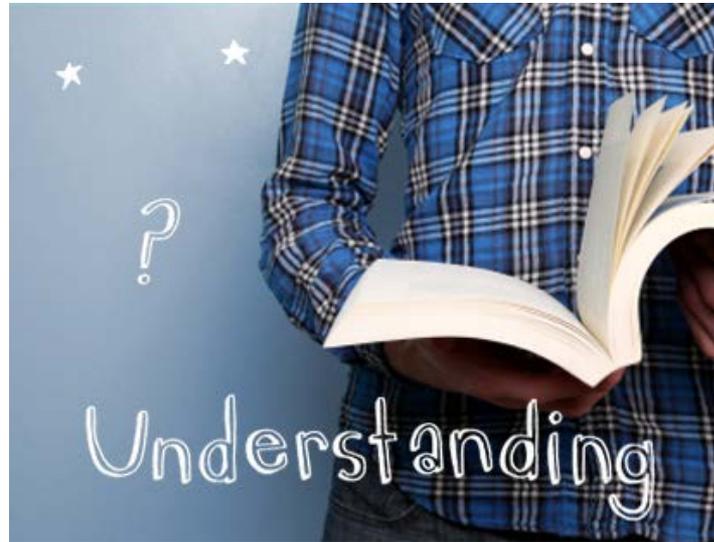




Prevention

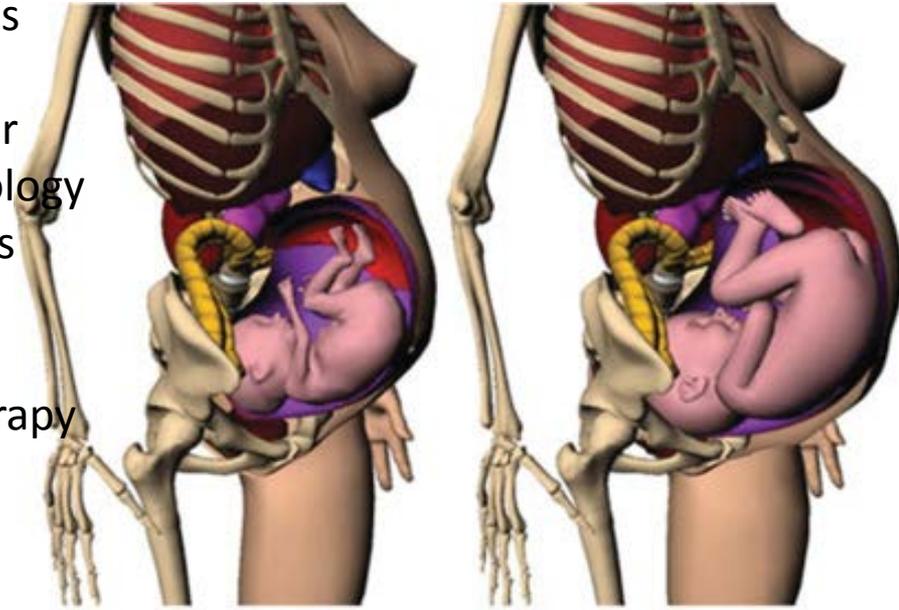


PREVENTION SERVICES



Treatment

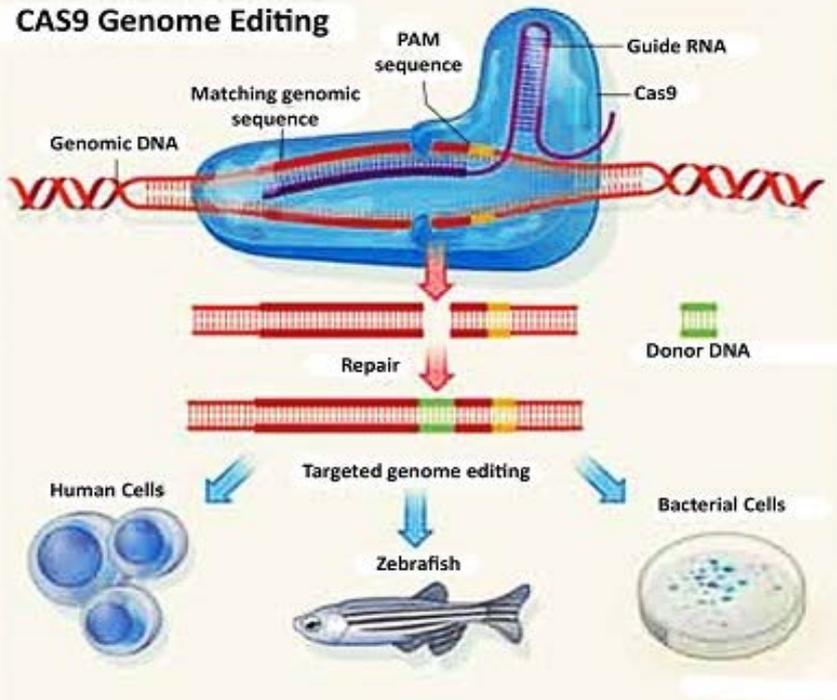
- Human Studies
 - iPS cells
 - Molecular Epidemiology
- Mouse Models
 - Genome Editing
- Stem Cell Therapy
 - Human
 - Mouse
 - Sheep





Modern Genetic Tools Include

CAS9 Genome Editing



Genome Editing



Next Generation DNA Sequencing



Adverse Health Effects of Abnormal Folate Transport and Metabolism



Neural Tube Defects



Cerebral Folate Deficiency Syndrome



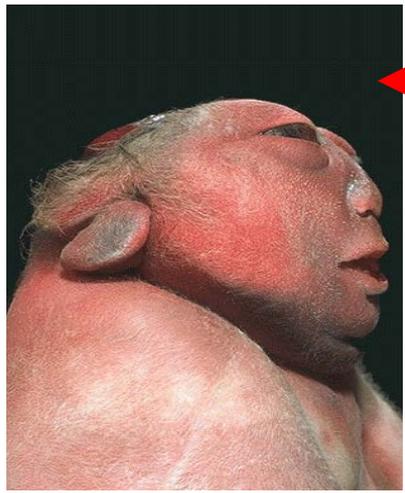
Hereditary Folate Malabsorption Syndrome

Neural Tube Defects

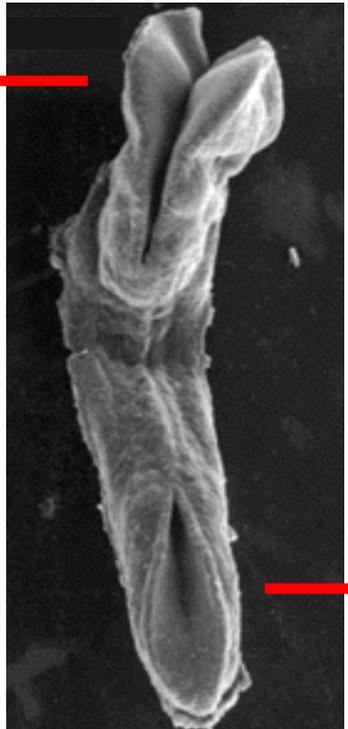


枕骨裂脑露畸形

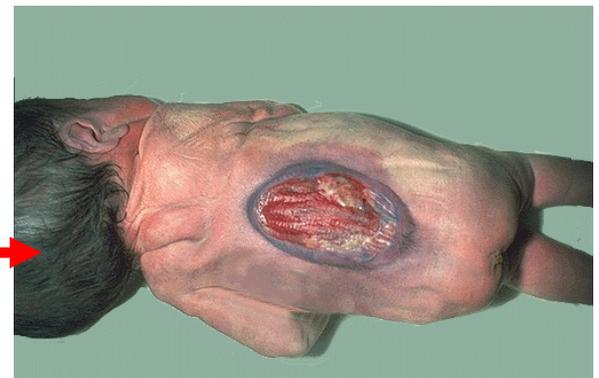
neural plate neural folds neural tube



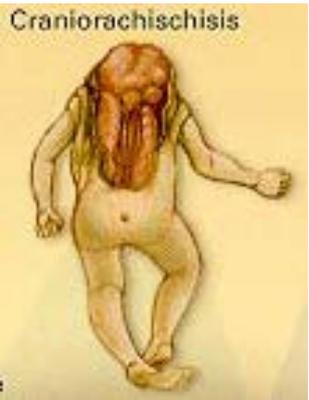
Anencephaly 无脑儿



Spina Bifida 脊柱裂



颅脊柱裂





- ◎ Result in lifelong disability
 - Problems with bladder, bowel, and sexual function
 - Learning and developmental problems
 - Orthopedic problems
- ◎ Some NTDs are **folate** preventable



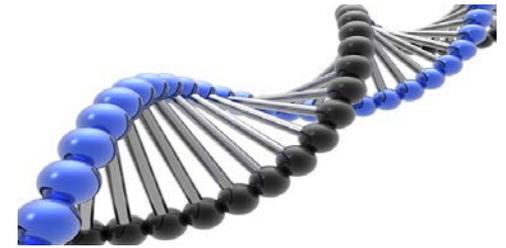


Strong genetic component:

- Twin concordance data
- Varying prevalences amongst ethnicities

BUT non-Mendelian inheritance

- a couple with 1 affected child have a 1-in-20 risk of having another
- with 2 or more affected children they have a 1-in-10 risk



Multiple gene (& gene-environment) effects are needed to produce NTDs

Over 250 gene mutations are now linked to NTDs in mouse models

Folic acid supplementation can reduce NTD occurrence by 70%

- How, when hundreds of genes are potential targets causing NTDs?



Environmental Modifier of Neural Tube Defect Risk

HUGE PUBLIC HEALTH VICTORY !





It All Started with Spinach

- 1940s-Start with 4 tons of spinach, lots of chemistry graduate students, and a steam kettle and filter press in the attic of Welch Hall at the Univ. of Texas

- Esmond Snell credited with the isolation of vitamin B9, which they named folic acid



Spinacia oleracea

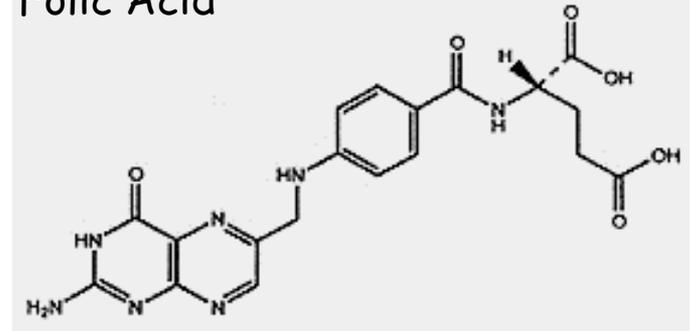




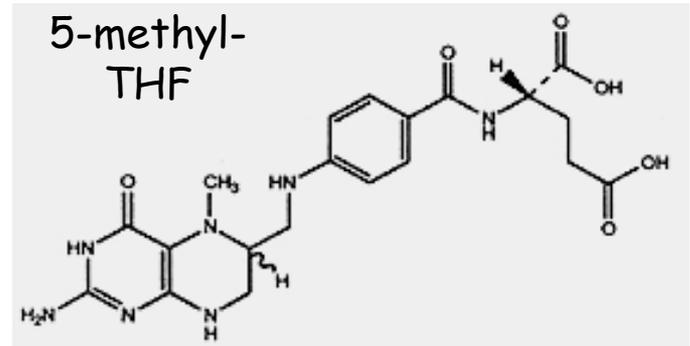
Folates and NTD Risk

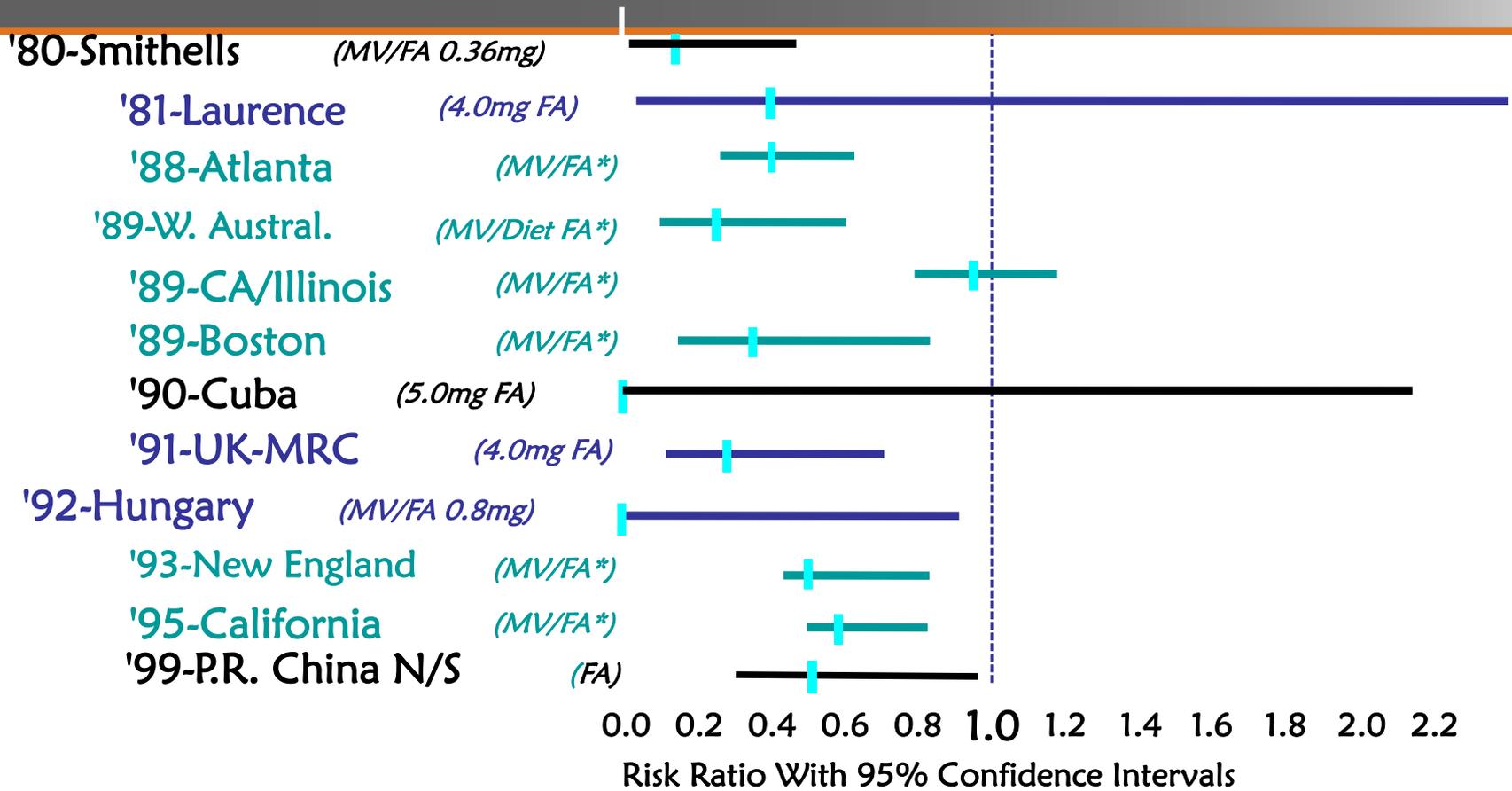
- Folic acid (pteroylglutamic acid) is an essential vitamin
- *In vivo*:
 - Reduced to bioactive tetrahydrofolates (THF)
 - Polyglutamated
- Biologic role: coenzyme in one-carbon metabolism
 - Synthesis of nucleic acids, amino acids, neurotransmitters
 - Methylation
 - 5MTHF is involved in >100 different methyl transfer reactions

Folic Acid



5-methyl-THF





Randomized trials

Non-randomized trials

Observational studies

* All observational studies researched lower dosage folic acid (FA) (0.1 - 1.0mg)



3 potential approaches for delivering folic acid

- improvement of dietary habits
- fortification of the U.S. food supply
- use of dietary supplements

Pre-prenatal care.

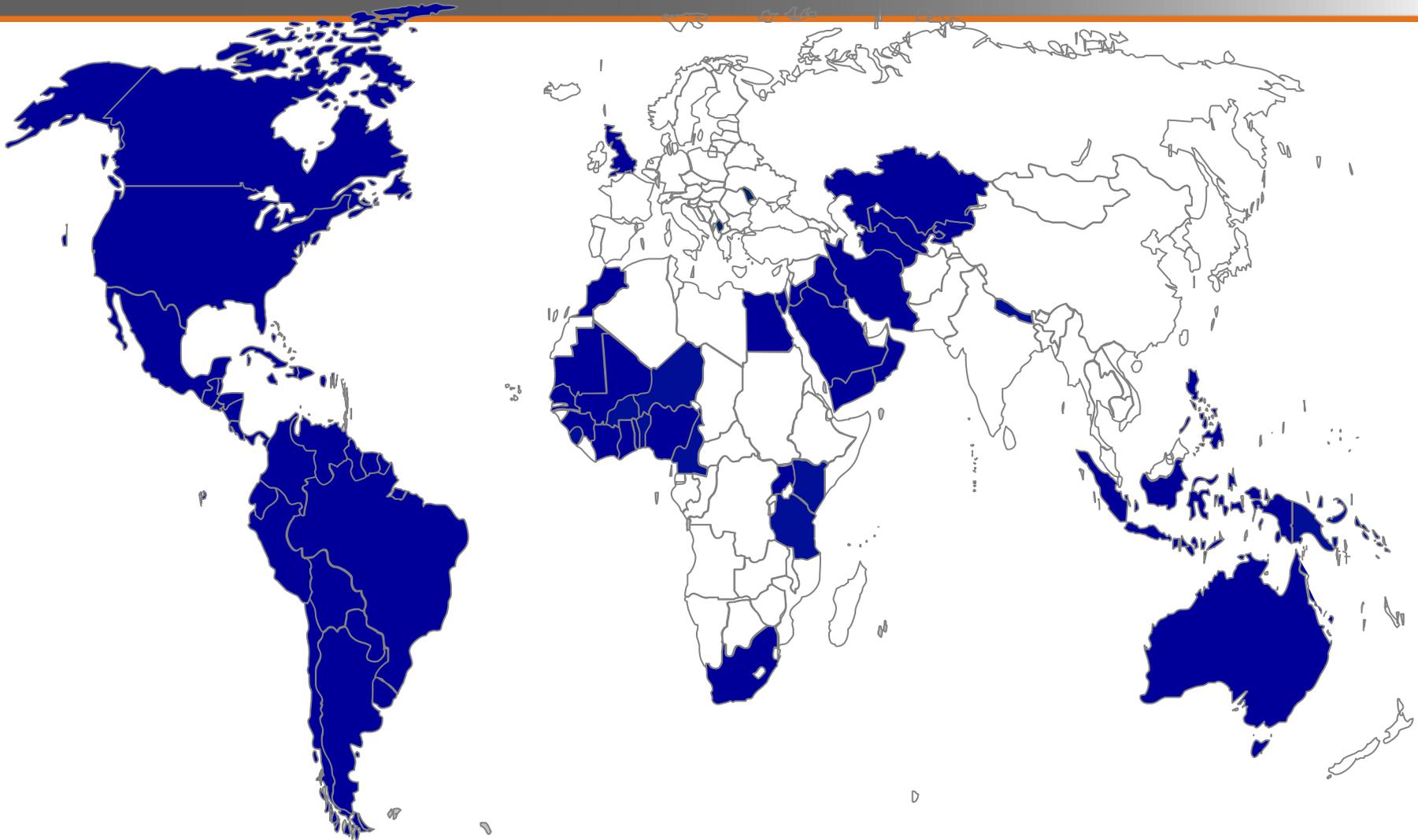
ONE A DAY
Women's
Formula

One-A-Day® Women's Formula
reduces the risk of certain birth defects.

You already know how taking care of yourself during pregnancy affects your baby's health, too. But now scientific studies confirm that by taking recommended daily amounts of folic acid throughout your childbearing years, you may actually reduce your baby's risk of birth defects of the brain and spinal cord. That's why One-A-Day Women's Formula is so important to all women of childbearing age. Women's Formula has the recommended 4 mg of folic acid you need to help lower the risk of these birth defects*. It also gives you more of the calcium you need for stronger bones, as well as extra iron.

ONE A DAY
Formula

We've got the one for you.



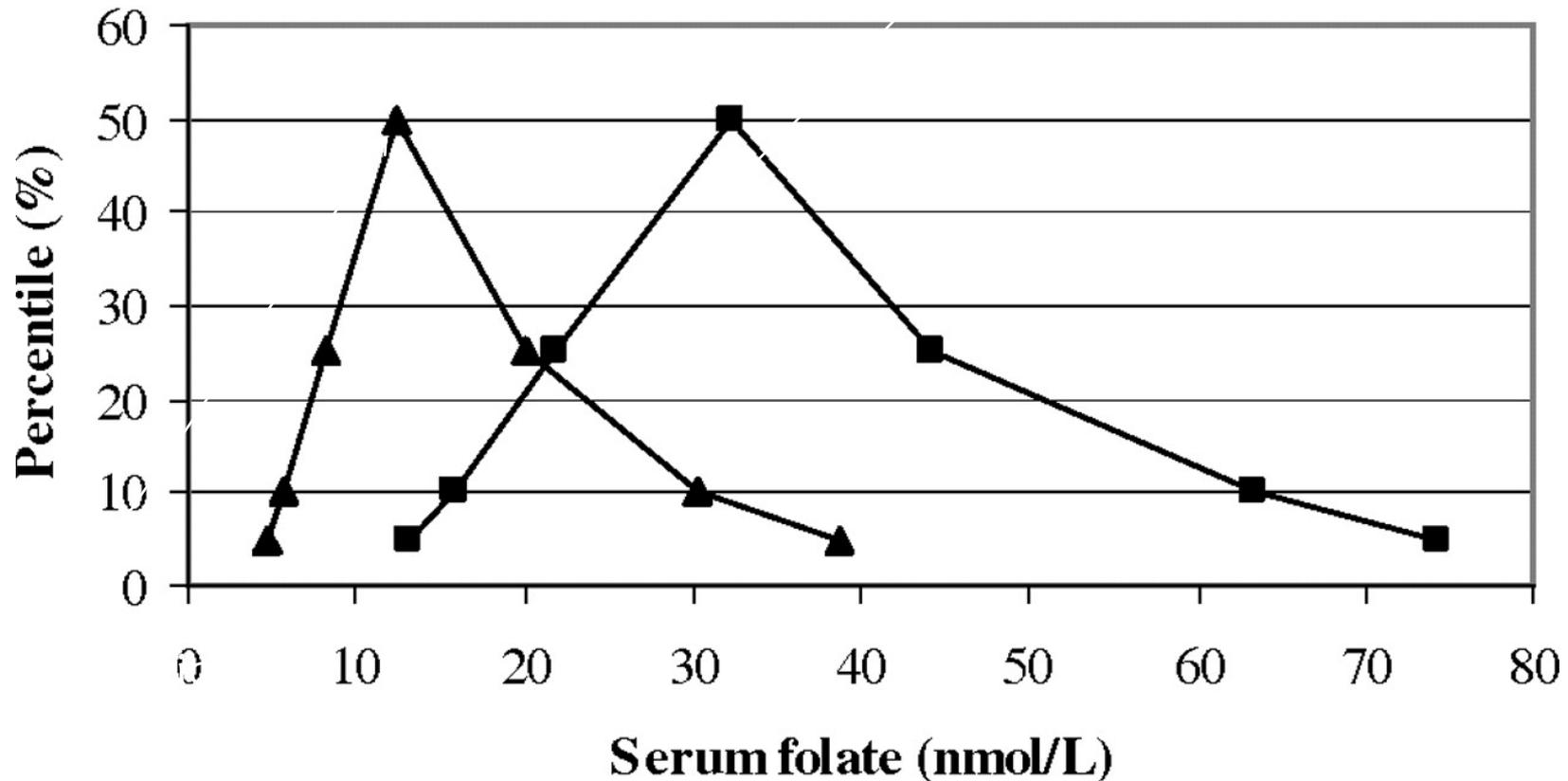
77 countries require fortification of wheat flour, maize flour, and/or rice

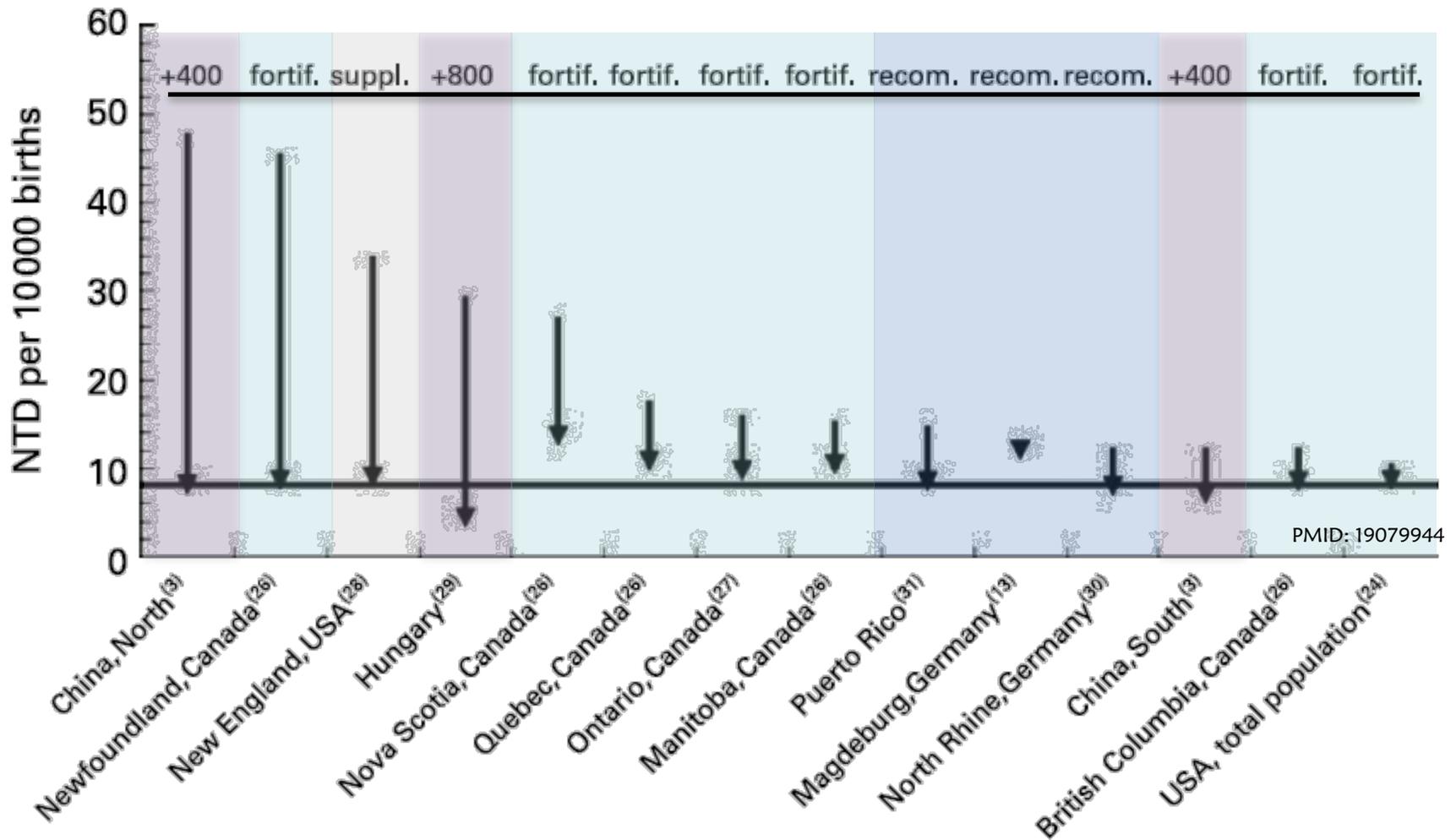
June 2013. Source: Flour Fortification Initiative. http://www.ffinetwork.org/global_progress/index.php

To request data, e-mail info@ffinetwork.org



1999-2000 
1988-1994 
Serum folate

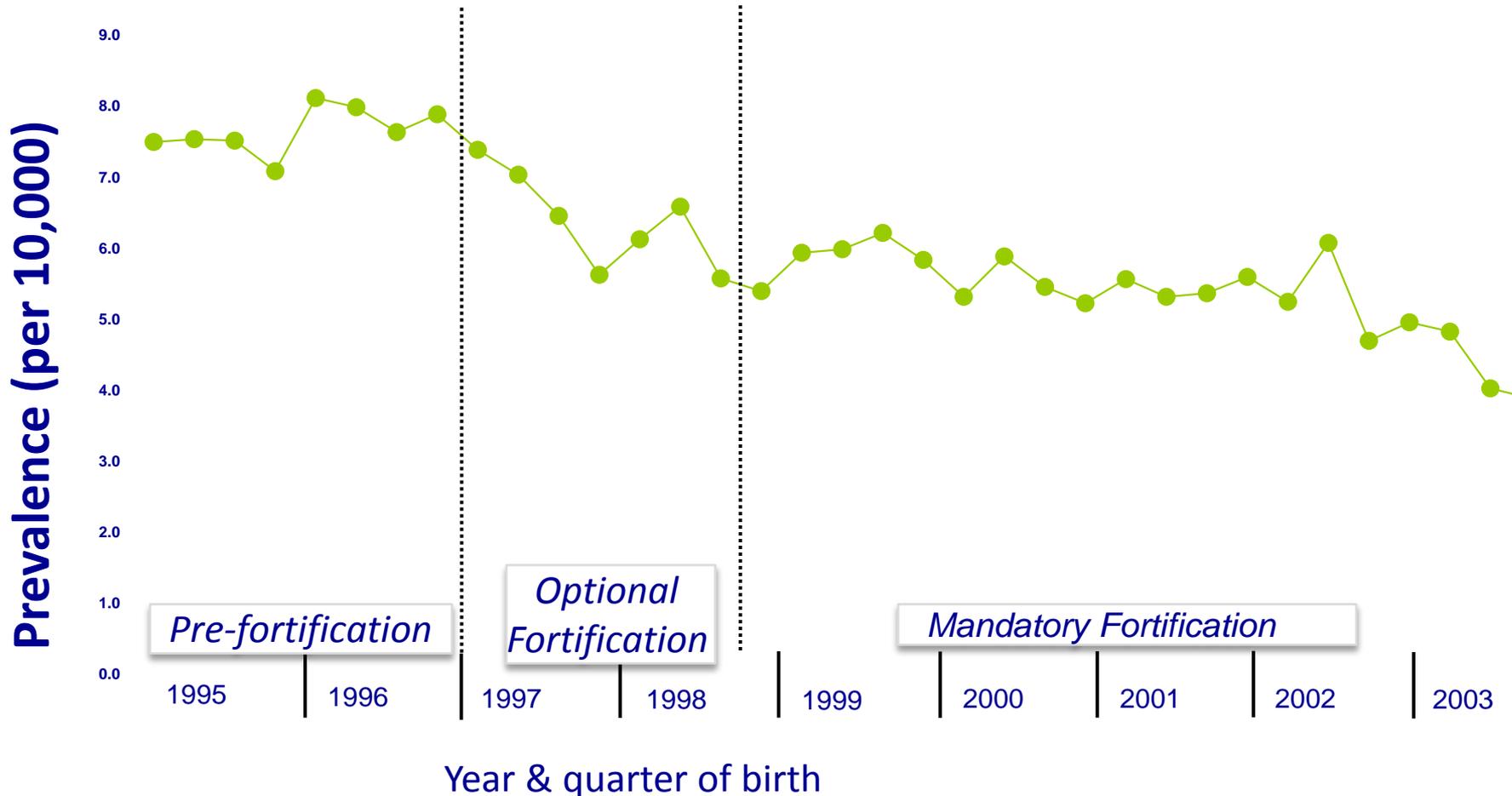




Largest reduction in risk for those with higher baseline prevalences of NTDs



Prevalence of Neural Tube Defects, Surveillance Programs, National Birth Defects Prevention Network

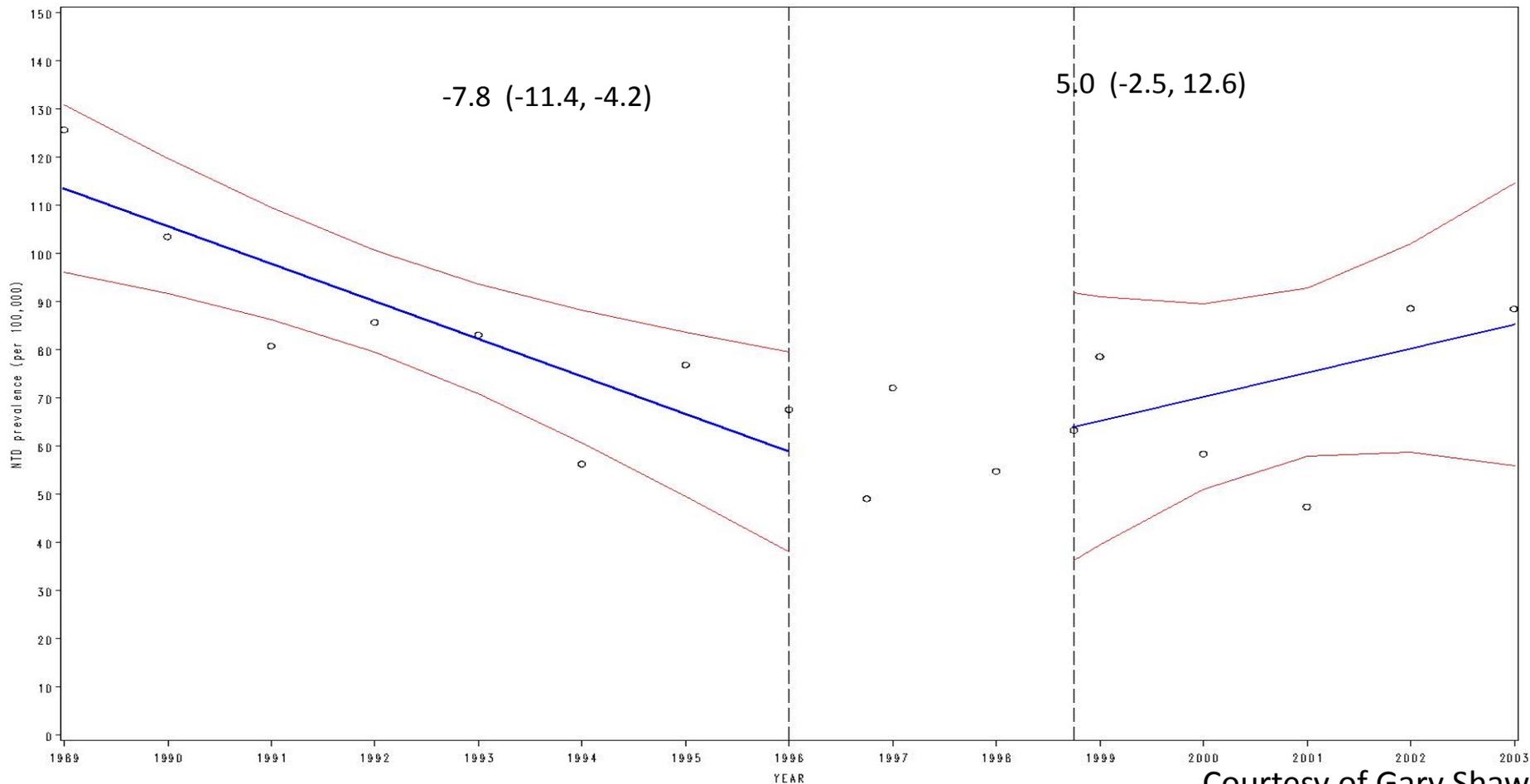




Annual NTD Prevalences in Central California, 1989-2003

Difference in slopes = 12.8 (4.4, 21.2)

Figure 1 — NTD prevalences



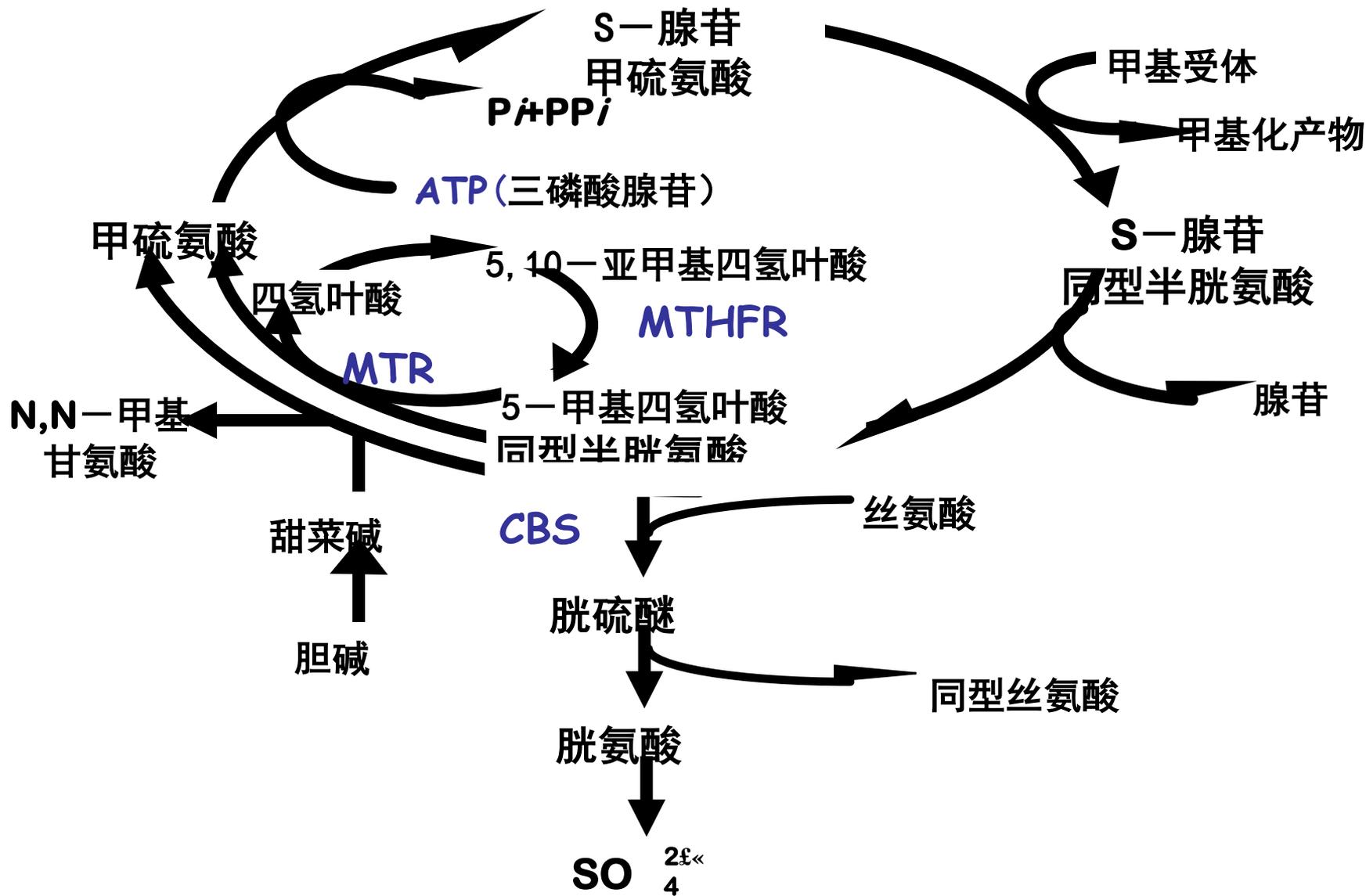


图1 同型半胱氨酸代谢
 CBS: 胱硫醚 β 合成酶
 MTR: 蛋氨酸合成酶
 MTHFR: 亚甲基四氢叶酸还原酶



Modelo de ratón knockout para un gen transportador de ácido fólico.

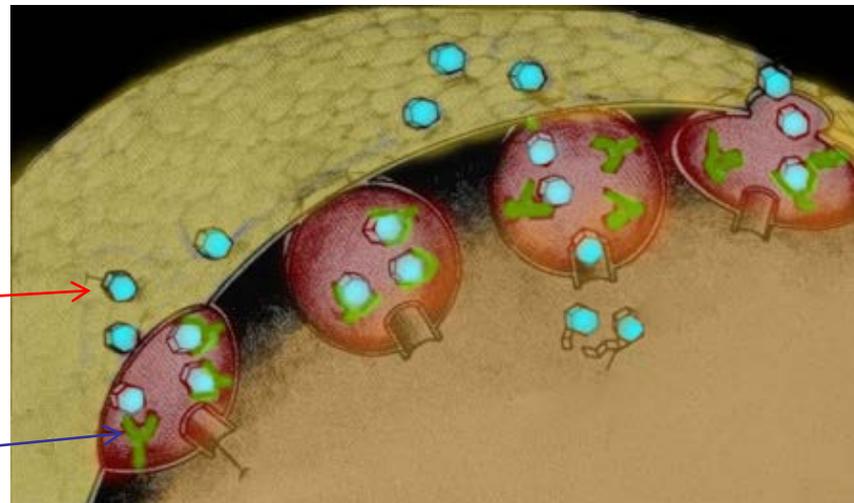
Receptor de folatos

Folr1

Folr2, Folr4

RFC1

PCFT



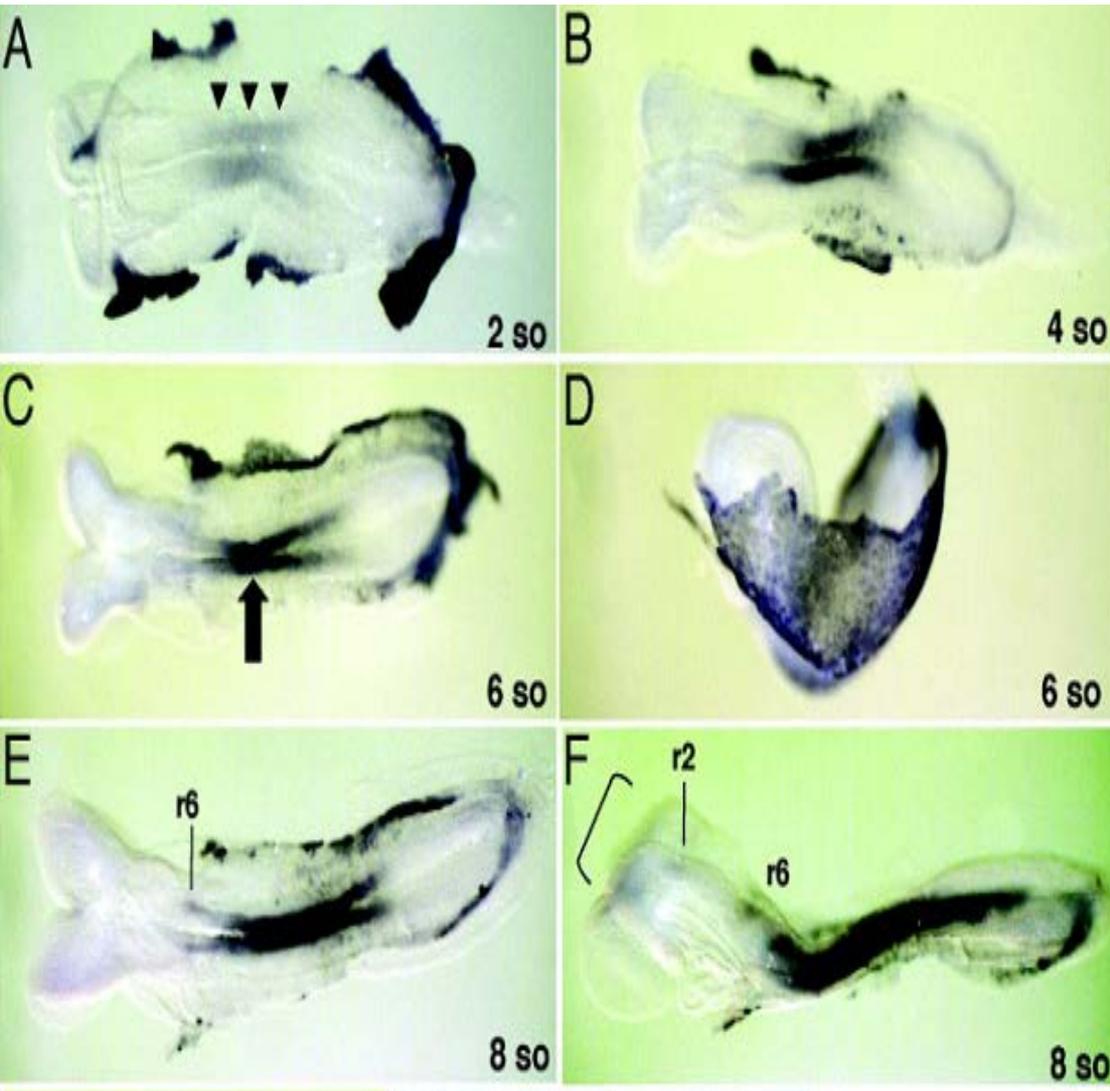
Ácido Fólico

(Folr1) Receptor de Folatos





Folate Receptor Expression Patterns

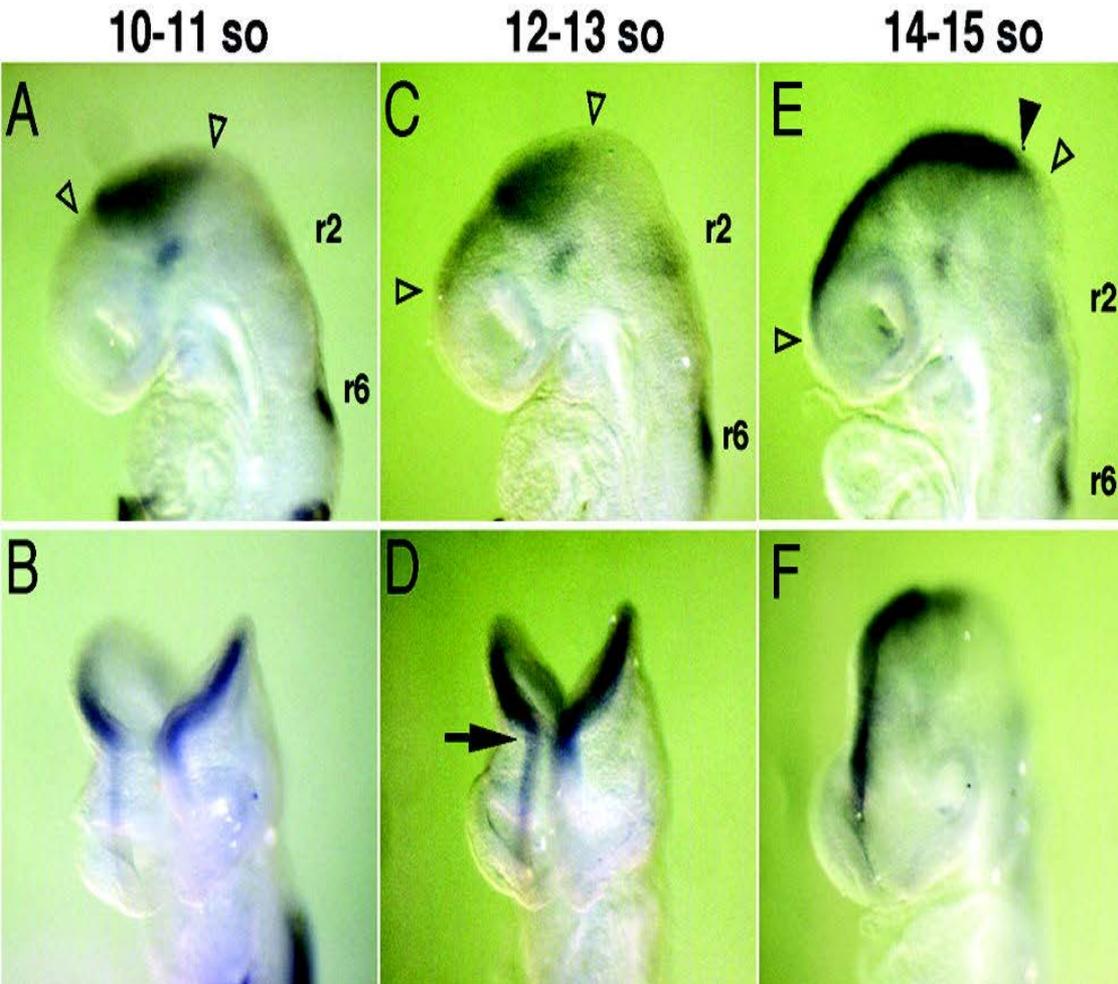


- FOLR1 mostly in epithelial cells
- FOLR3 is specific to the hematopoietic system
- FOLR2 and FOLR4 are found in diverse tissues
- FOLR4 found on regulatory T-cells

Shown: Folr1 Expression
in Early Mouse
Embryos (Saitou et al.
2003)



Folate Receptor Expression Patterns

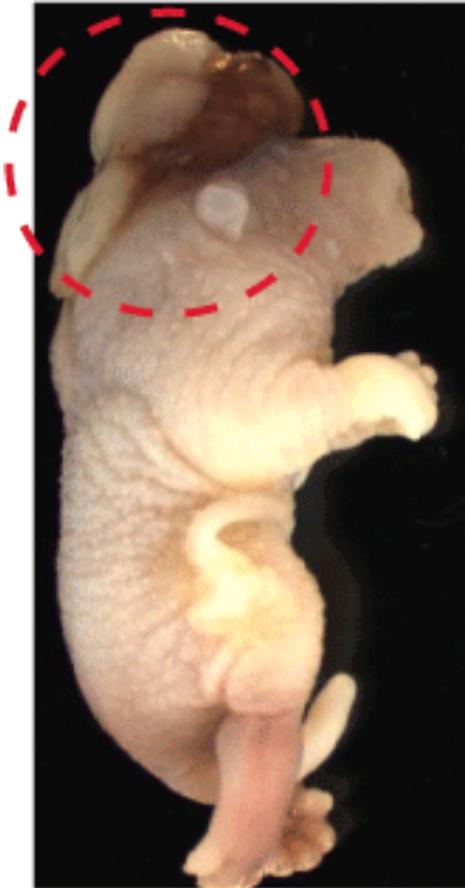


Shown: Folr1 Expression in Early Mouse Embryos
(Saito et al. 2003)

- Folr1 is expressed in distinct and specific tissue distributions during development
- Expression in neural tube represents a direct link to NTDs thru a cell-autonomous function of the gene
- Neural tube cells and supporting adjacent cells are thought to be involved in NTDs

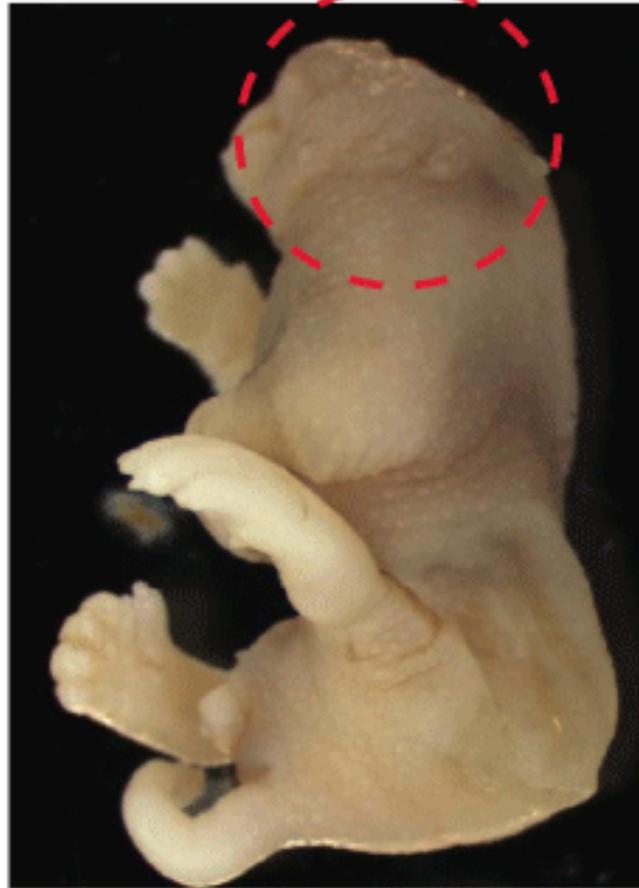


Exencephaly



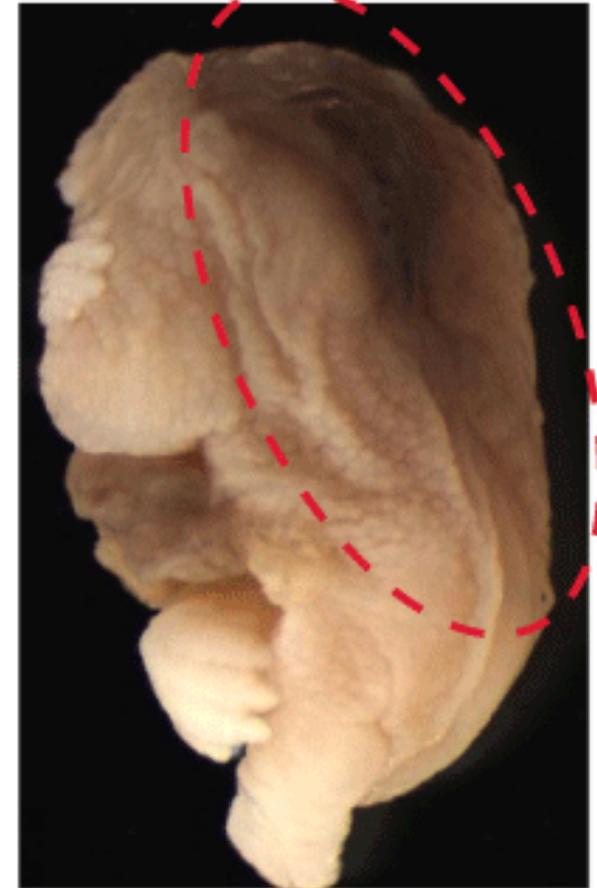
无脑儿

Iniencephaly

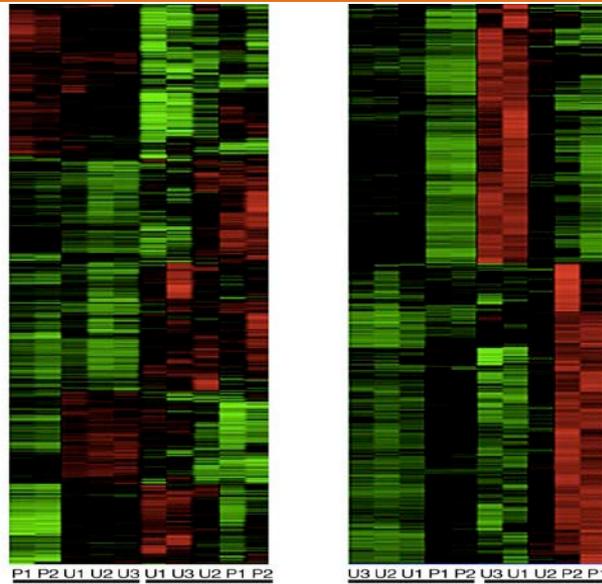


枕骨裂脑露畸形

Craniorachischisis



颅脊柱裂

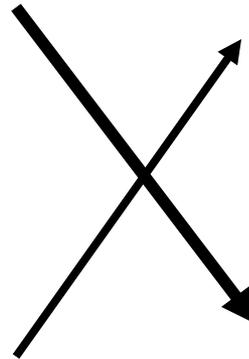


The Wnt signaling pathway appears to be strikingly sensitive to the individual folate metabolic status and is a target of methyl-chromatin remodeling





From Mouse Models to Human Studies



Wellcome Images





Folate receptor- α (FOLR1) transports folate into cells

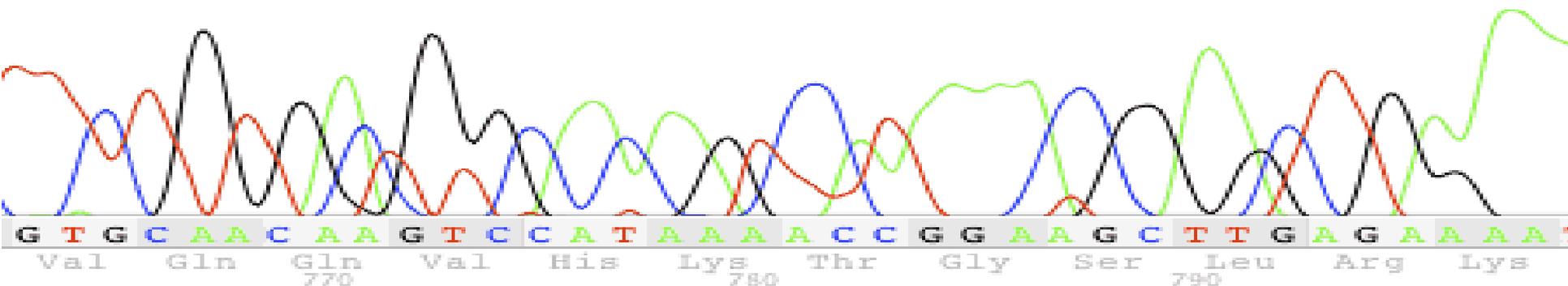
- High affinity for folate.
- 7 exons
- 6.70 kb
- Chromosome 11
- 971 bp in coding region



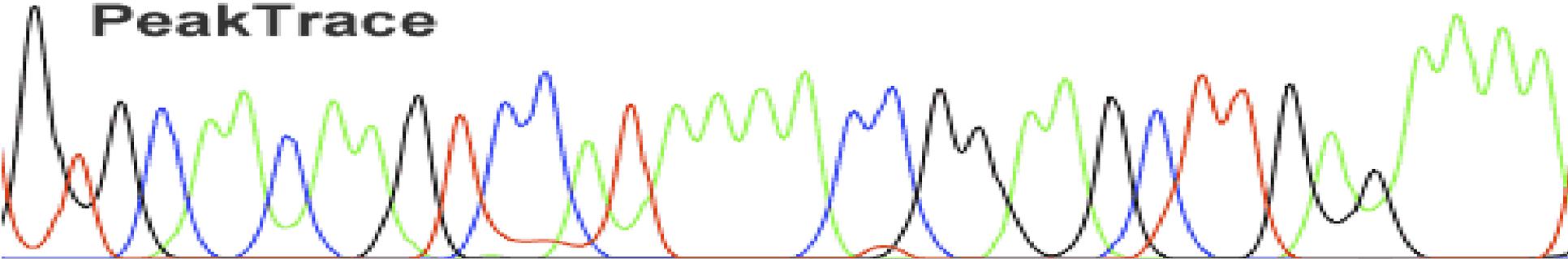
- No Sequence Variants in 971 bp of Coding Region of the Gene

C T C T G G T G A T G **N** G C A C A G T C C A T A A A A C G G A A G C T G G A G A A
Phe Trp *** --- Ala Gln Ser Ile Lys Arg Lys Leu Glu Lys
730 740 750 760

ABI

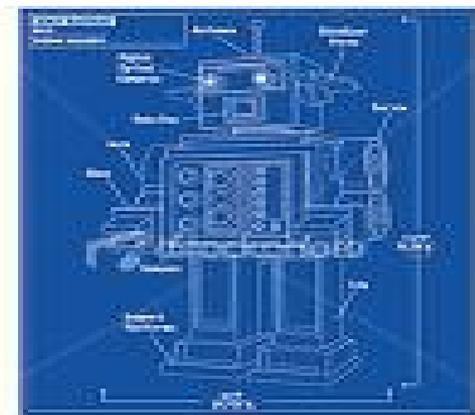
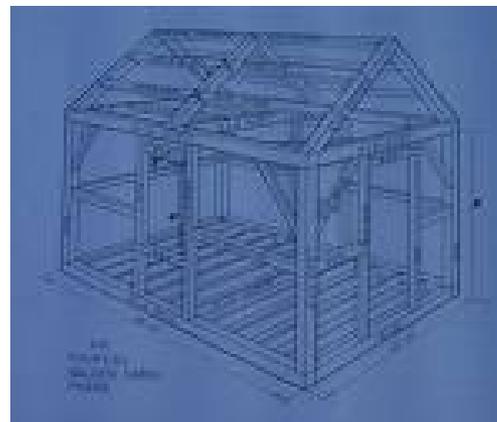
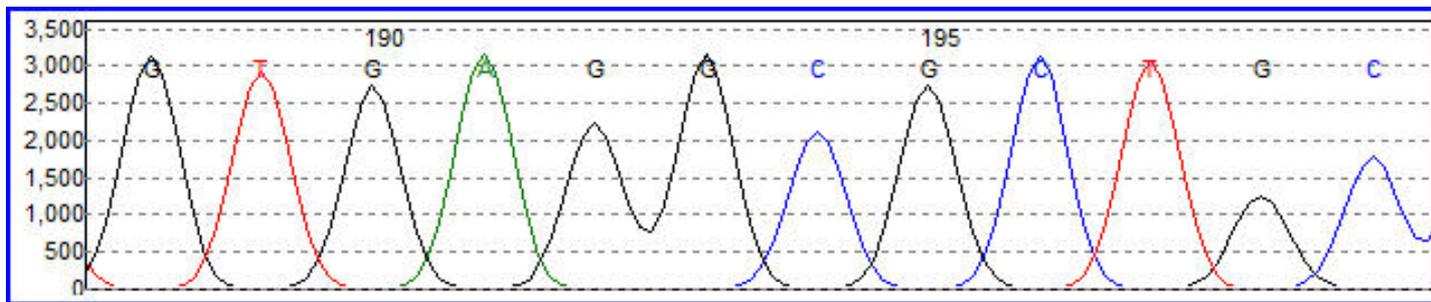


PeakTrace





Rather than a defective gene with an incorrect sequence, what if there was something biological that was preventing the gene from working properly?





The **NEW ENGLAND**
JOURNAL of **MEDICINE**

Autoantibodies against folate receptors in women with a pregnancy complicated by a neural-tube defect.

Rothenberg SP, daCosta MP, **Sequeira JM**, Cracco J,
Roberts JL, Weedon J and **Quadros EV**

N Engl J Med. 2004 Jan 8;350(2):134-42

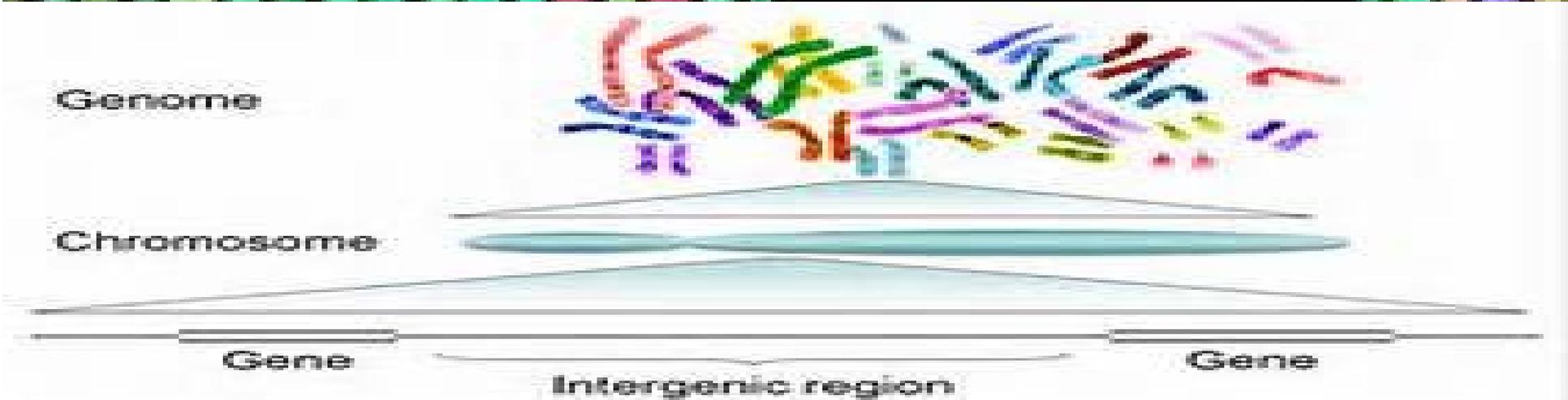


Finnell Laboratory

vs.

Quadros Laboratory







A Consideration of the Evidence That Genetic Defects in Planar Cell Polarity Contribute to the Etiology of Human Neural Tube Defects

Diana M. Juriloff[†] and Muriel J. Harris

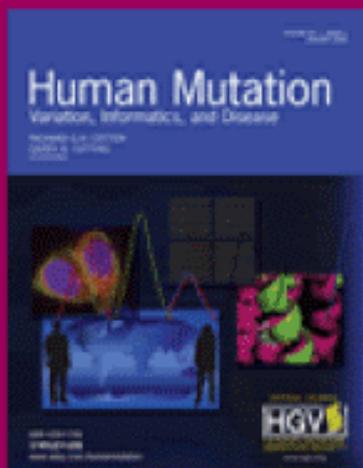


Identification of Novel CELSR1 Mutations in Spina Bifida

Yunping Lei¹, Huiping Zhu¹, Wei Yang³, M. Elizabeth Ross⁴, Gary M. Shaw³, Richard H. Finnell^{1,2*}

Mutations in Planar Cell Polarity Gene *SCRIB* Are Associated with Spina Bifida

Yunping Lei¹, Huiping Zhu¹, Cody Duhon¹, Wei Yang³, M. Elizabeth Ross⁴, Gary M. Shaw³, Richard H. Finnell^{1,2*}



Mutations in the COPII Vesicle Component Gene *SEC24B* are Associated with Human Neural Tube Defects

Xue-Yan Yang,^{1†} Xiang-Yu Zhou,^{1†} Qing Qing Wang,^{2†} Hong Li,³ Ying Chen,³ Yun-Ping Lei,¹ Xiao-Hang Ma,¹ Pan Kong,¹ Yan Shi,¹ Li Jin,¹ Ting Zhang,^{4*} and Hong-Yan Wang^{1,5**}



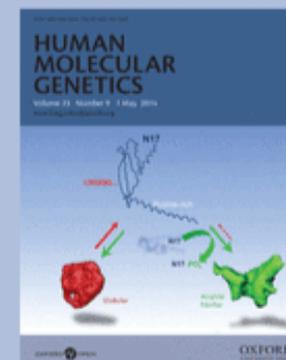
Identification of Novel Rare Mutations of *DACT1* in Human Neural Tube Defects

Yan Shi,^{1,6†} Yi Ding,^{2†} Yun-Ping Lei,^{1†} Xue-Yan Yang,¹ Guo-Ming Xie,² Jun Wen,² Chun-Quan Cai,³ Hong Li,⁴ Ying Chen,⁴ Ting Zhang,⁵ Bai-Lin Wu,^{6,7} Li Jin,^{1,6} Ye-Guang Chen,^{2*} and Hong-Yan Wang^{1,6,8*}

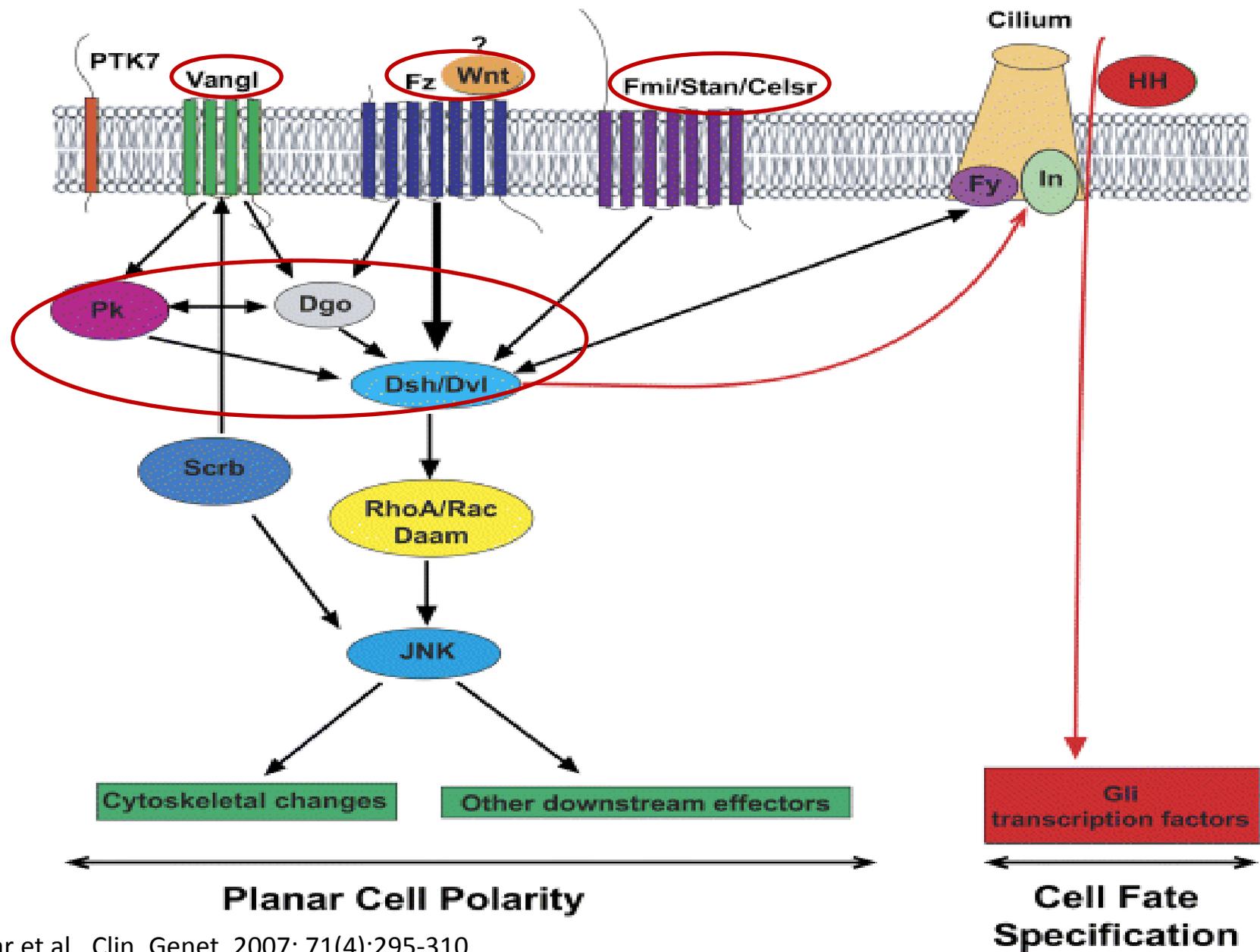


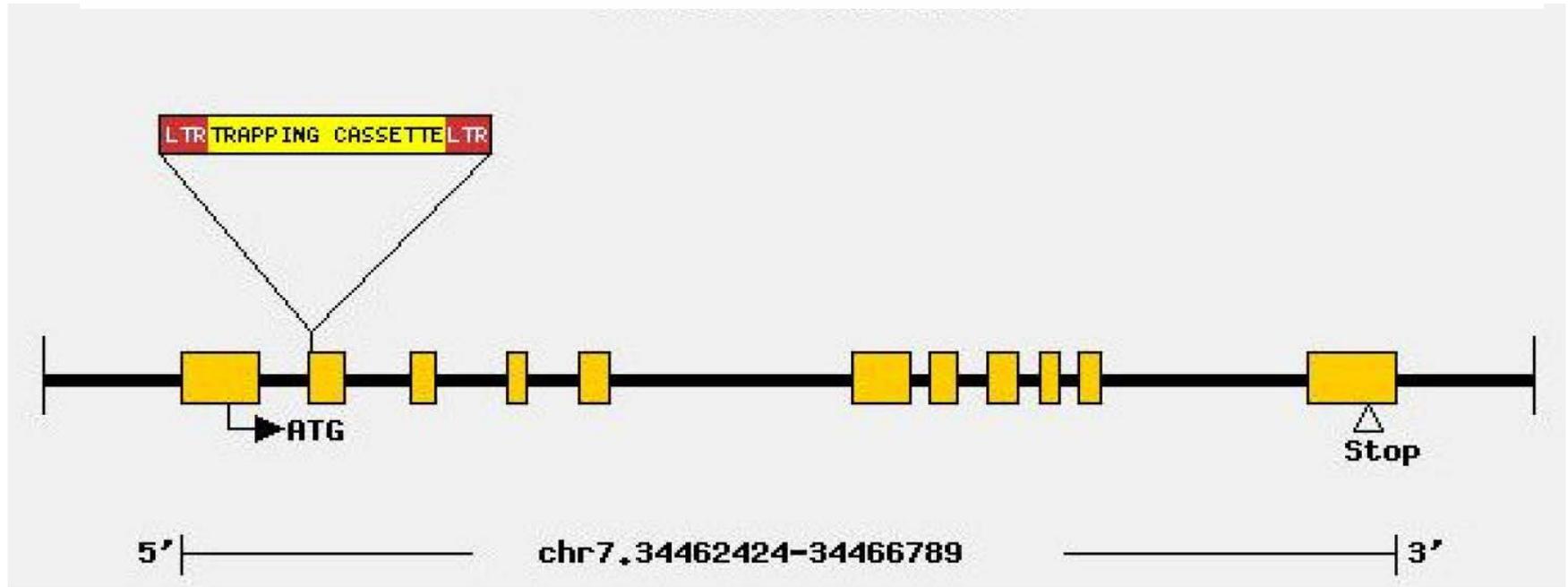
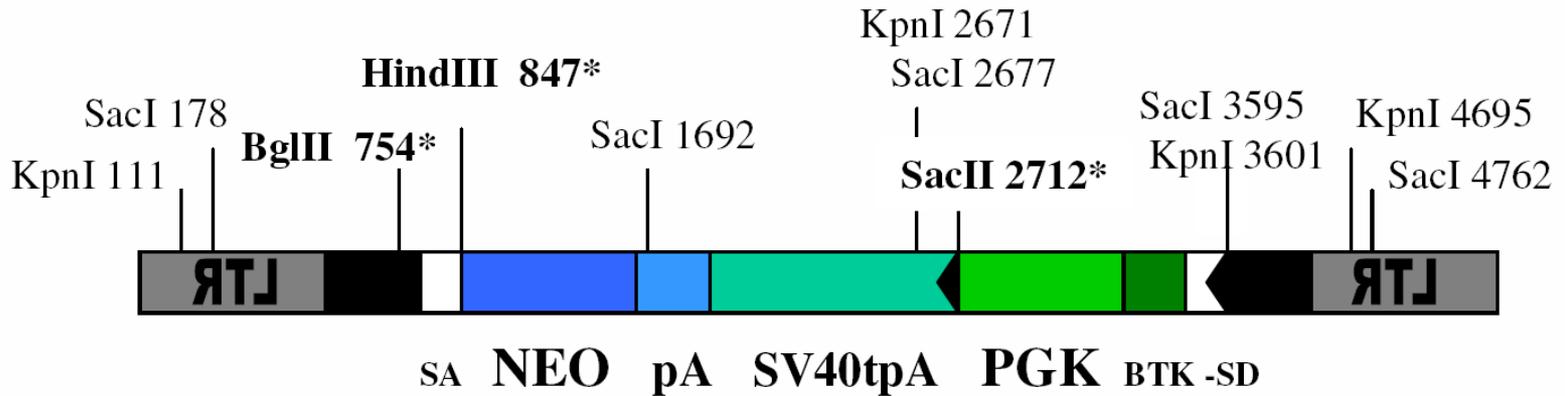
Mutations in the planar cell polarity gene, *Fuzzy*, are associated with neural tube defects in humans

Jung Hwa Seo¹, Yulia Zilber¹, Sima Babayeva¹, JiaJia Liu¹, Paulina Kyriakopoulos¹, Patrizia De Marco², Elisa Merello², Valeria Capra², Philippe Gros³ and Elena Torban^{1,*}



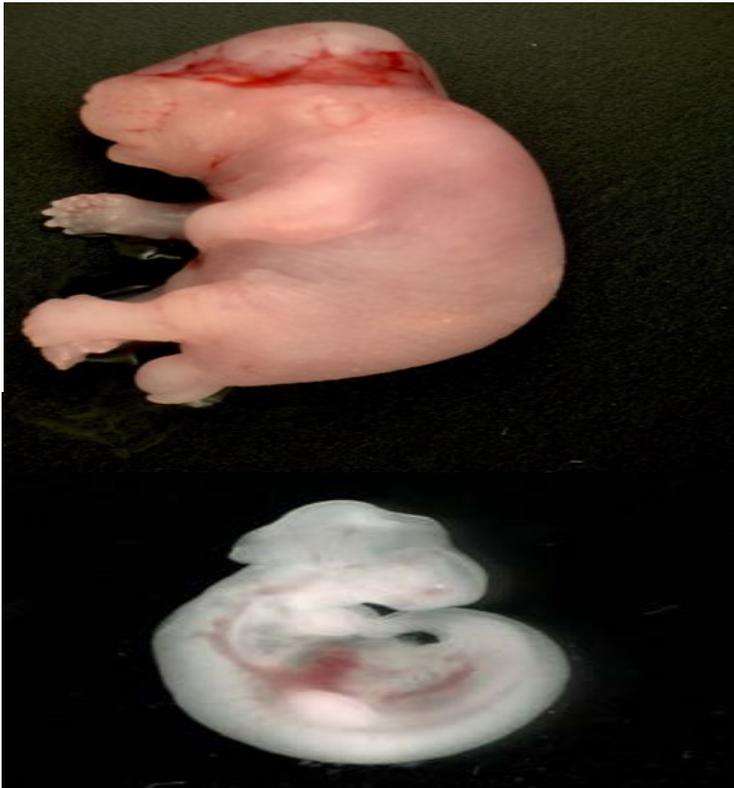
PCP Pathway Linkage to Hh Signaling

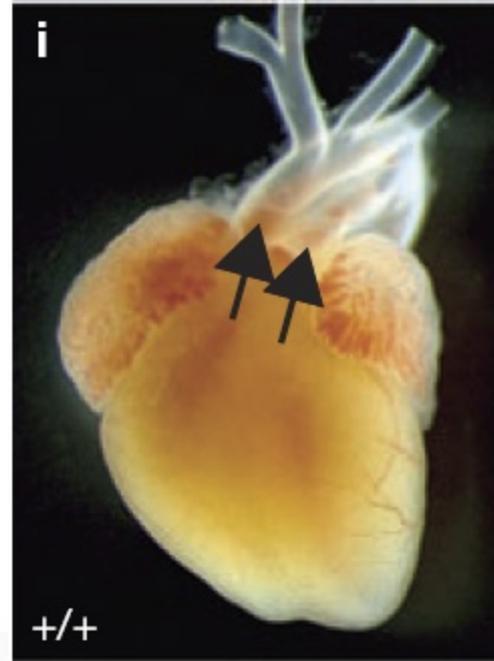
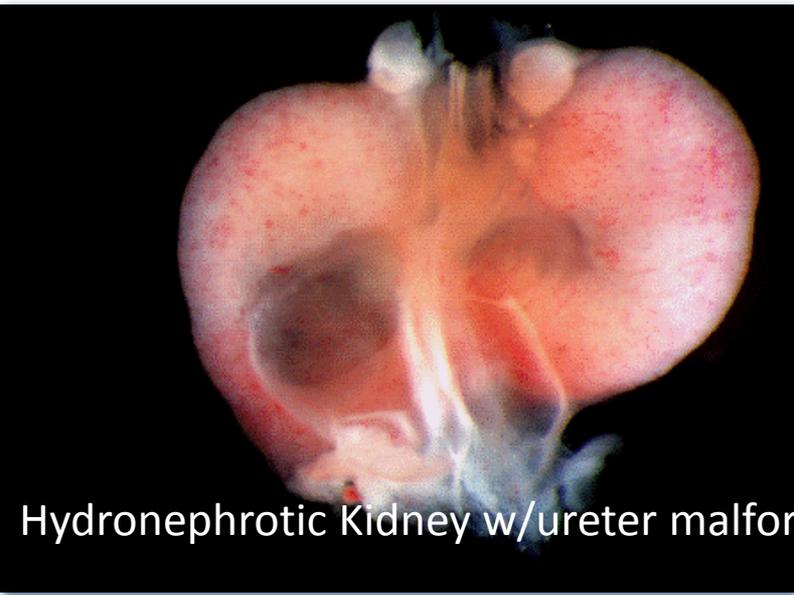




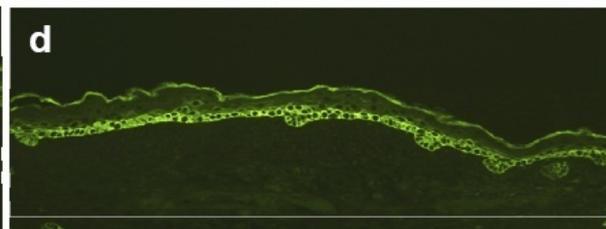
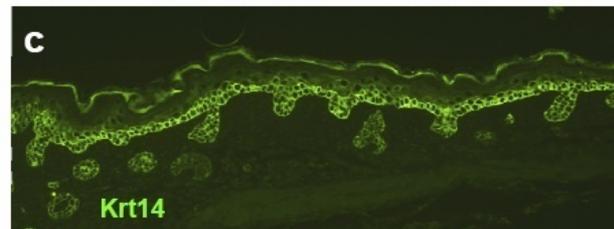
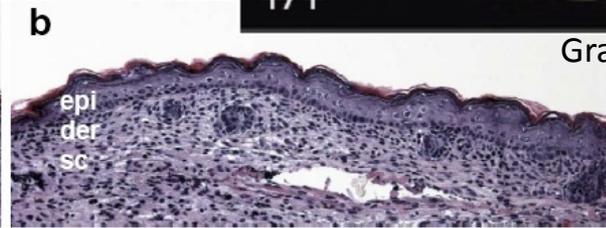
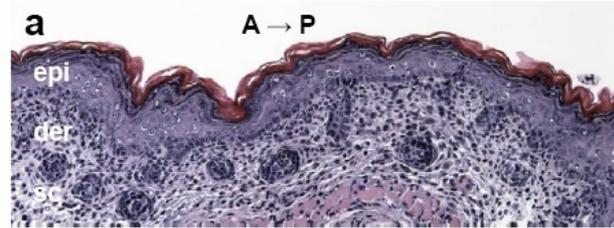


Anterior and Posterior NTDs in Nullizygotes $Fuz^{Gt1(neo)}$





Gray et al. Nature Cell Biol. 11:1225-34, 2009



Fuz wildtype

Fuz nullizygotes

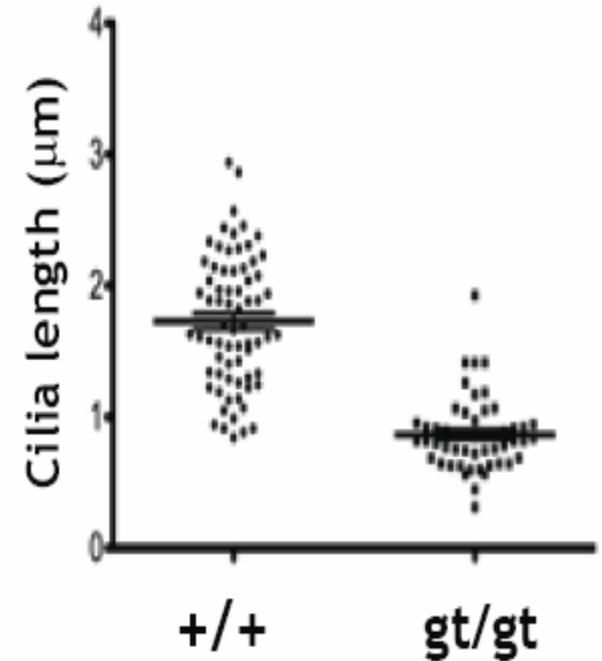
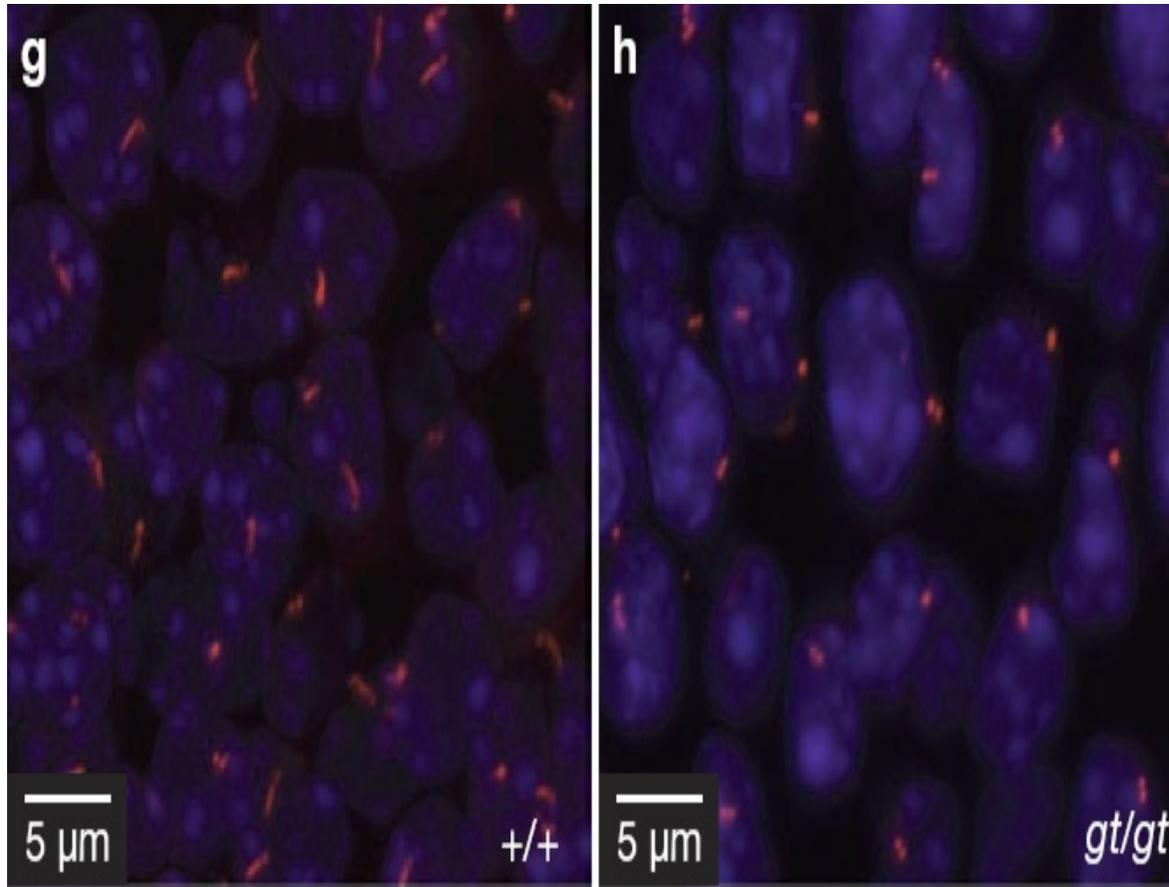
Hair follicles on dorsal aspect of E15 fetuses

Krt14-keratin immunostaining

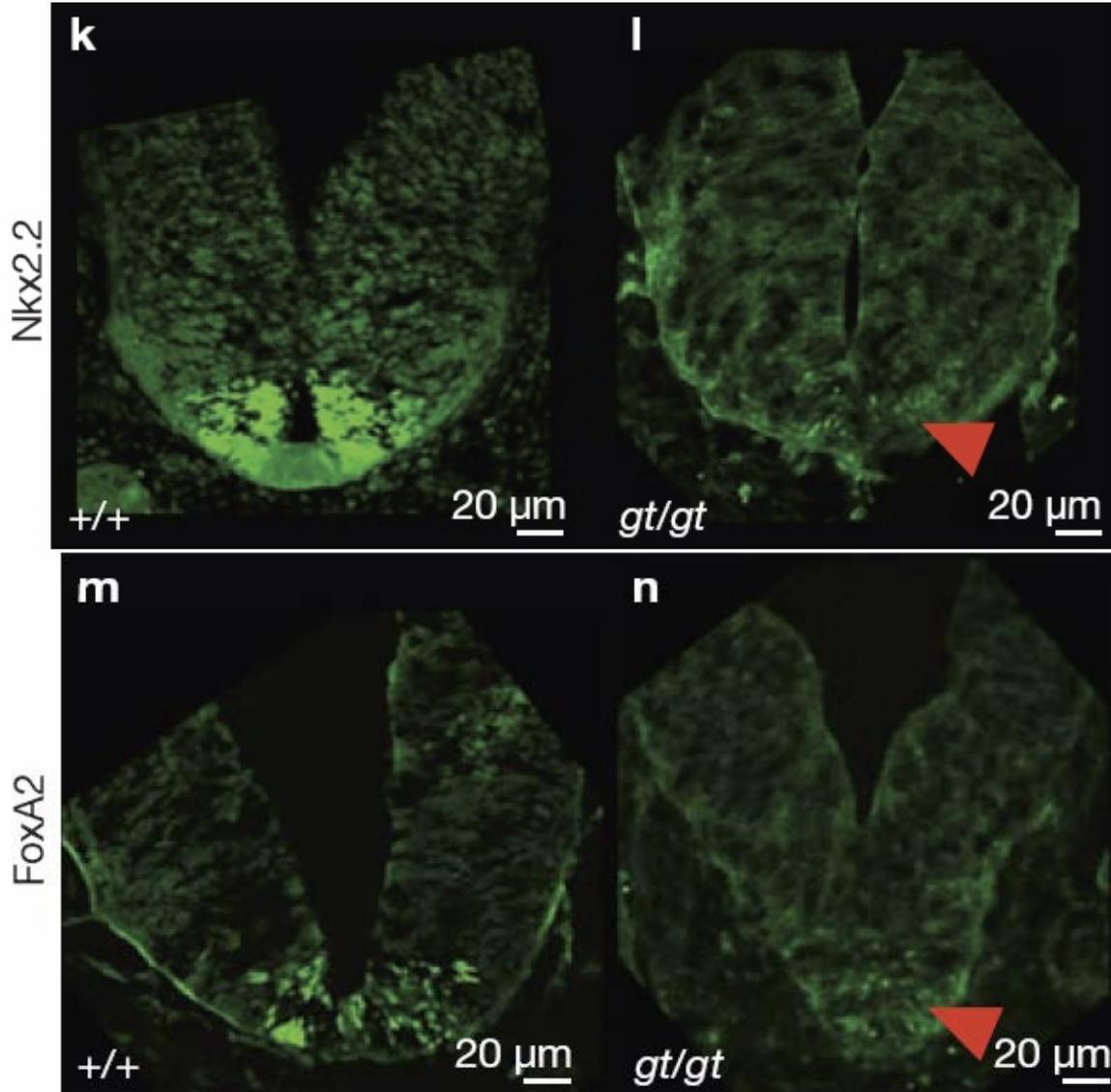
D.Dai et al, J. Inv. Derm. 2010 Oct 21. [Epub]PMID: 20962855



Diminished Cilia in Meckels Cartilage in Fuz Mutants



Acetylated Tubulin (red) DAPI (blue)

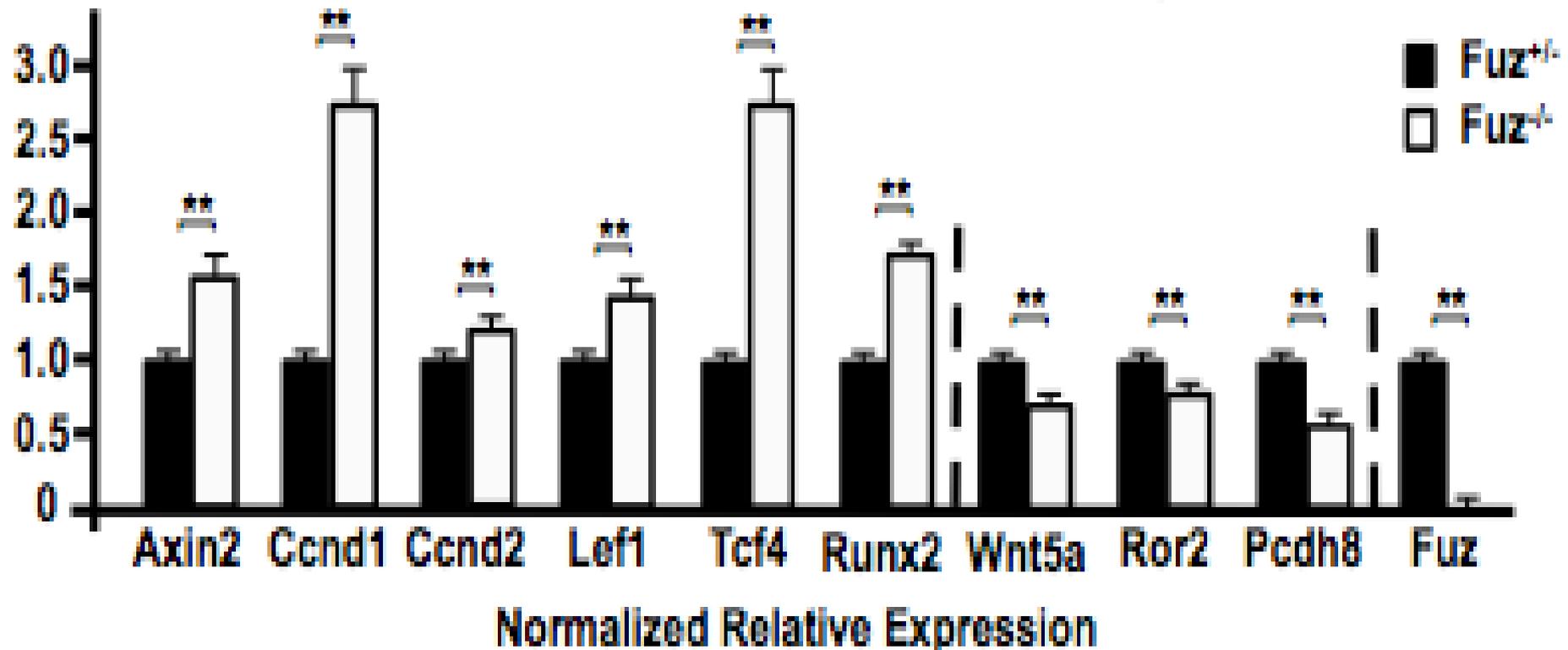




F.

Canonical Wnt Target Genes

Non-canonical Wnt
Pathway Genes





- Fuz, a PCP effector protein, appears to be required for normal embryogenesis
- Fuz mutant mice resemble mice with ciliogenesis defects such as BBS and other ciliopathies
- As cilium transduce Hh signals, Fuz mice lacking cilia also fail to express Hh target genes, Gli1 and Ptch1
- Coordination of vesicle trafficking might be a unifying mechanism by which PCP signaling controls a range of diverse cellular behaviors during embryonic development
- Folate supplementation fails to rescue the normal phenotype in Fuz nullizygotes



Whole Genome Sequencing of Patients with Spina Bifida from 3 Cohorts



73 NTD
patients

- 50 Qatari
- 23 US

50 Controls

Working
towards 200-
400 NTD cases

China Study
400 NTD Trios





Mapping

Illumina Provides

fastq

bwa, ssaha2, bowtie

sam

SamTools view, sort, rmdup

sorted, filtered bam

Variant calling

Illumina Provides

dindel, samtools

indel calls

GATK UnifiedGenotyper

raw SNP calls

GATK VariantFiltration

filtered by quality, depth, SNP clusters, homopolymer runs, strand bias

filtered SNP calls

Annotations

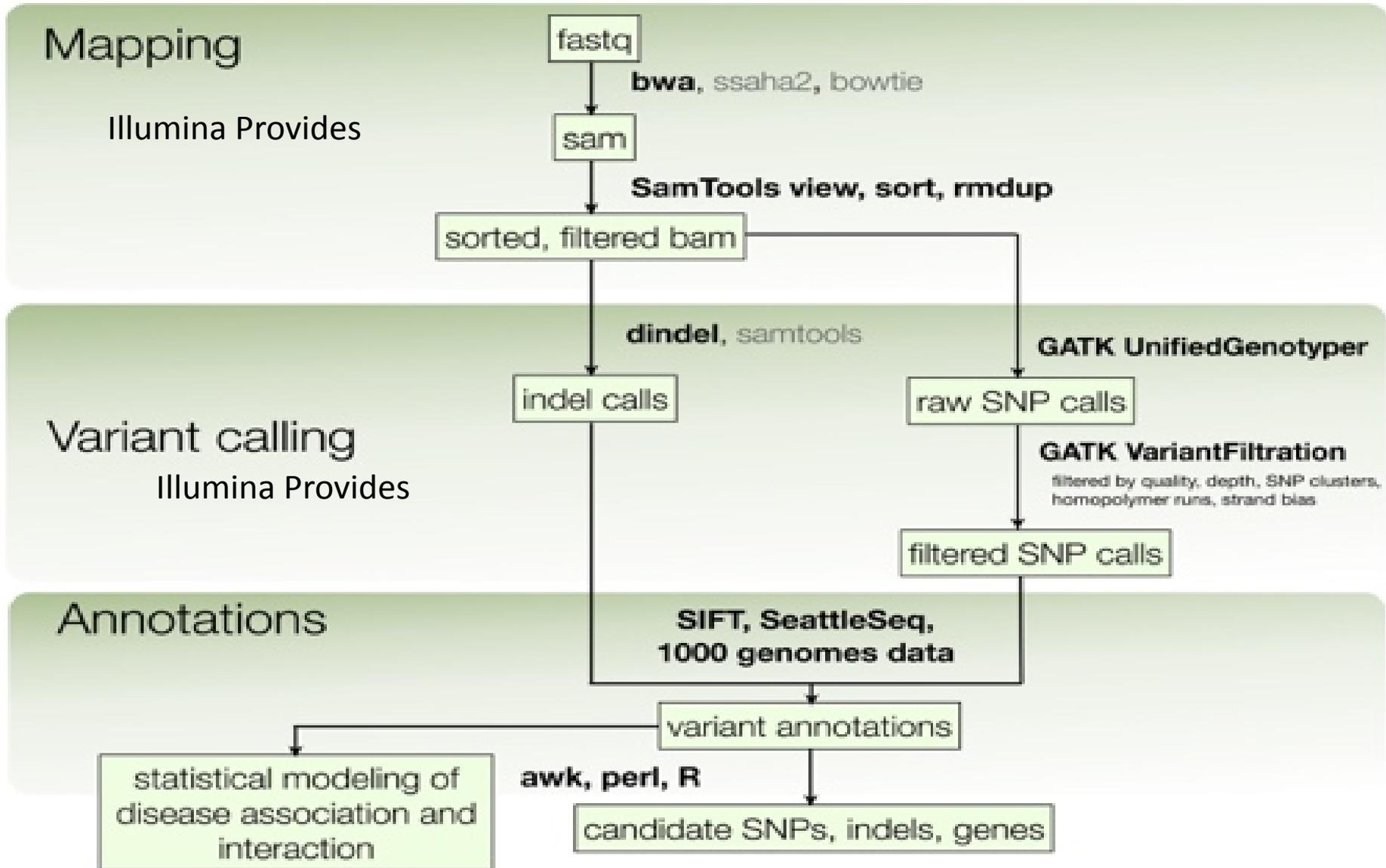
**SIFT, SeattleSeq,
1000 genomes data**

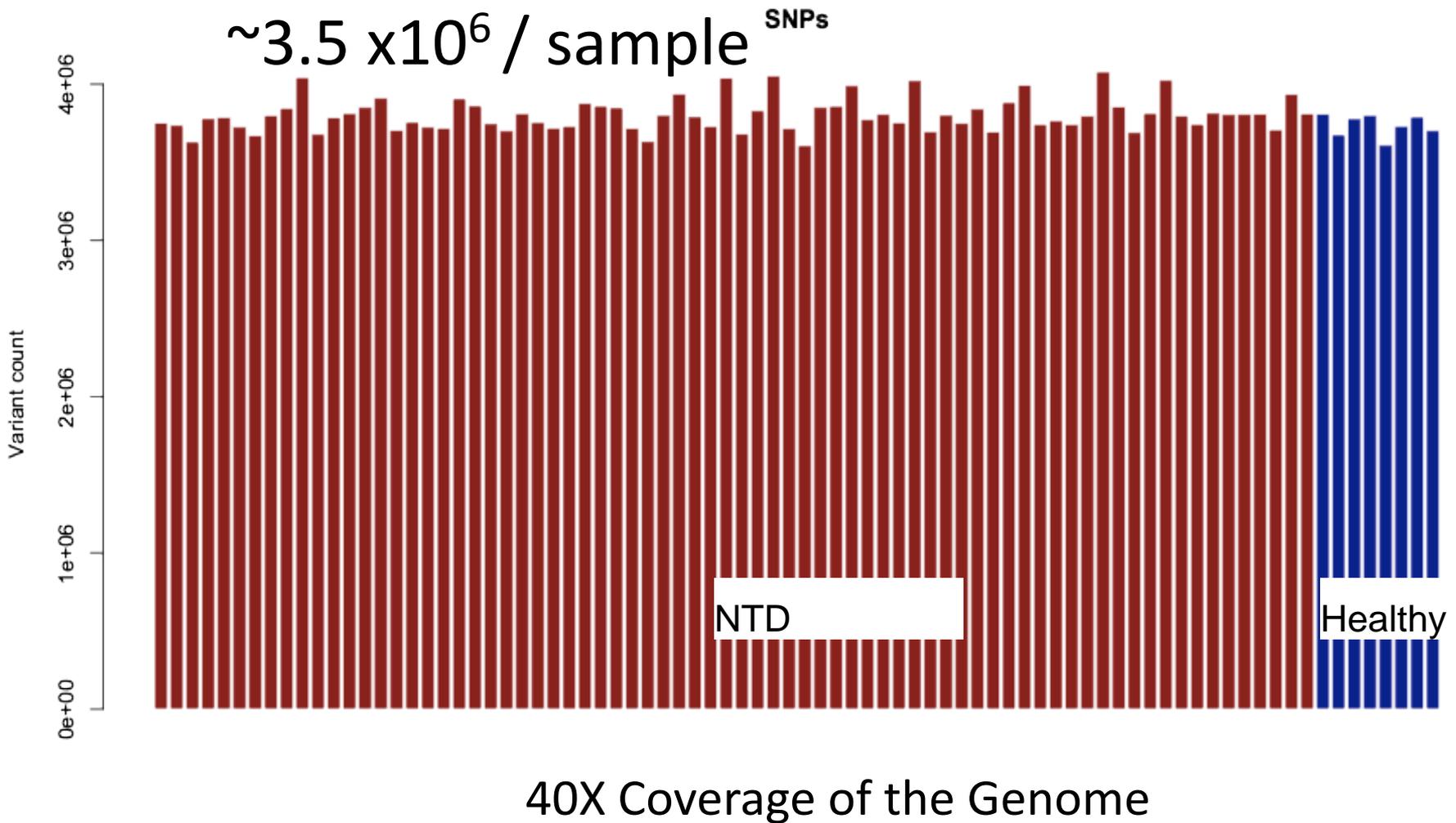
variant annotations

statistical modeling of
disease association and
interaction

awk, perl, R

candidate SNPs, indels, genes







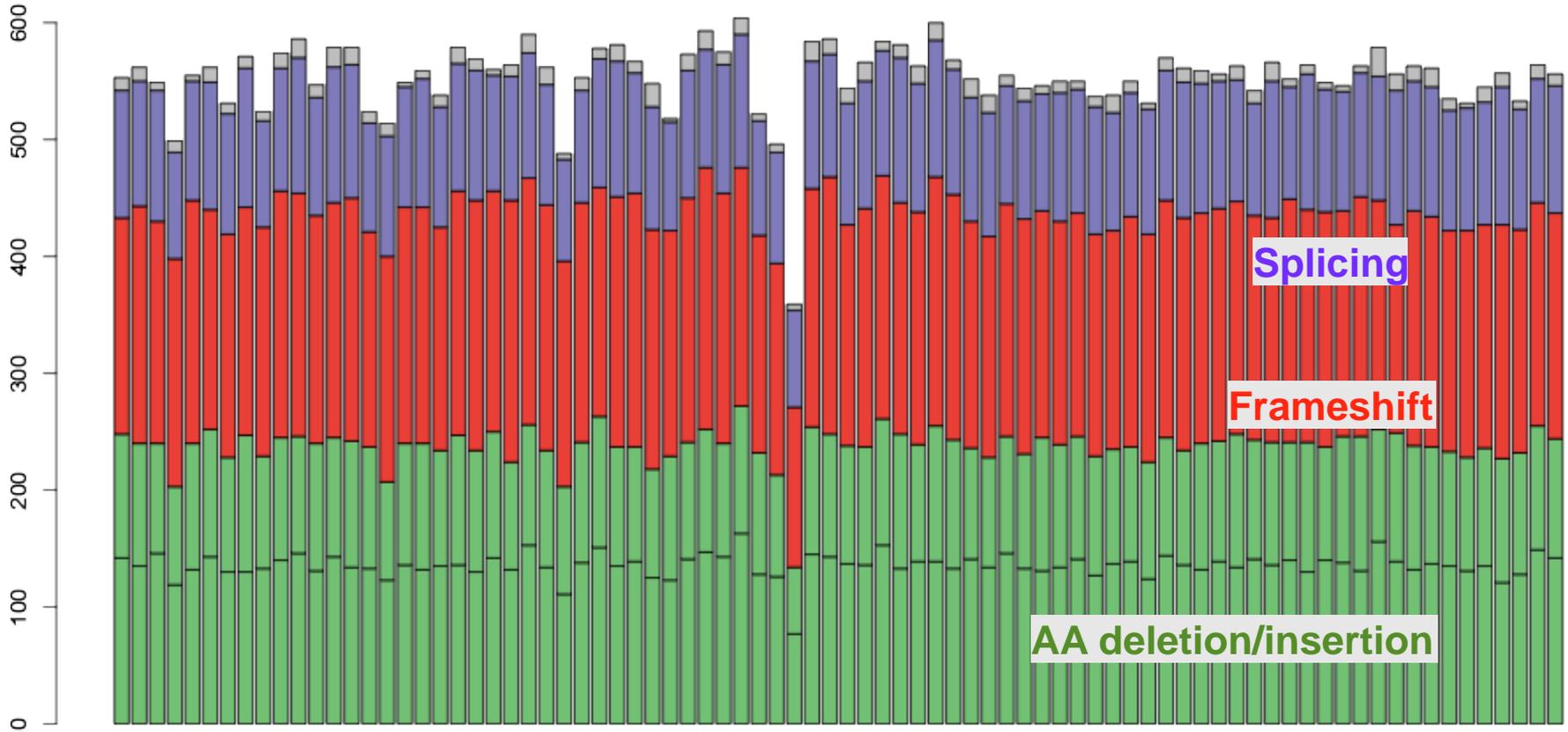
~700,000 / sample

Indels





Exonic Indels





- Indels leading to frameshift protein changes in at least one known transcript
- Not found in healthy parents
- Found in at least 15 NTD cases

OR9Q1

PABPC3

HERC2

LGALS9B

ANKRD36

GNRH2

PPP4R2,EBLN2

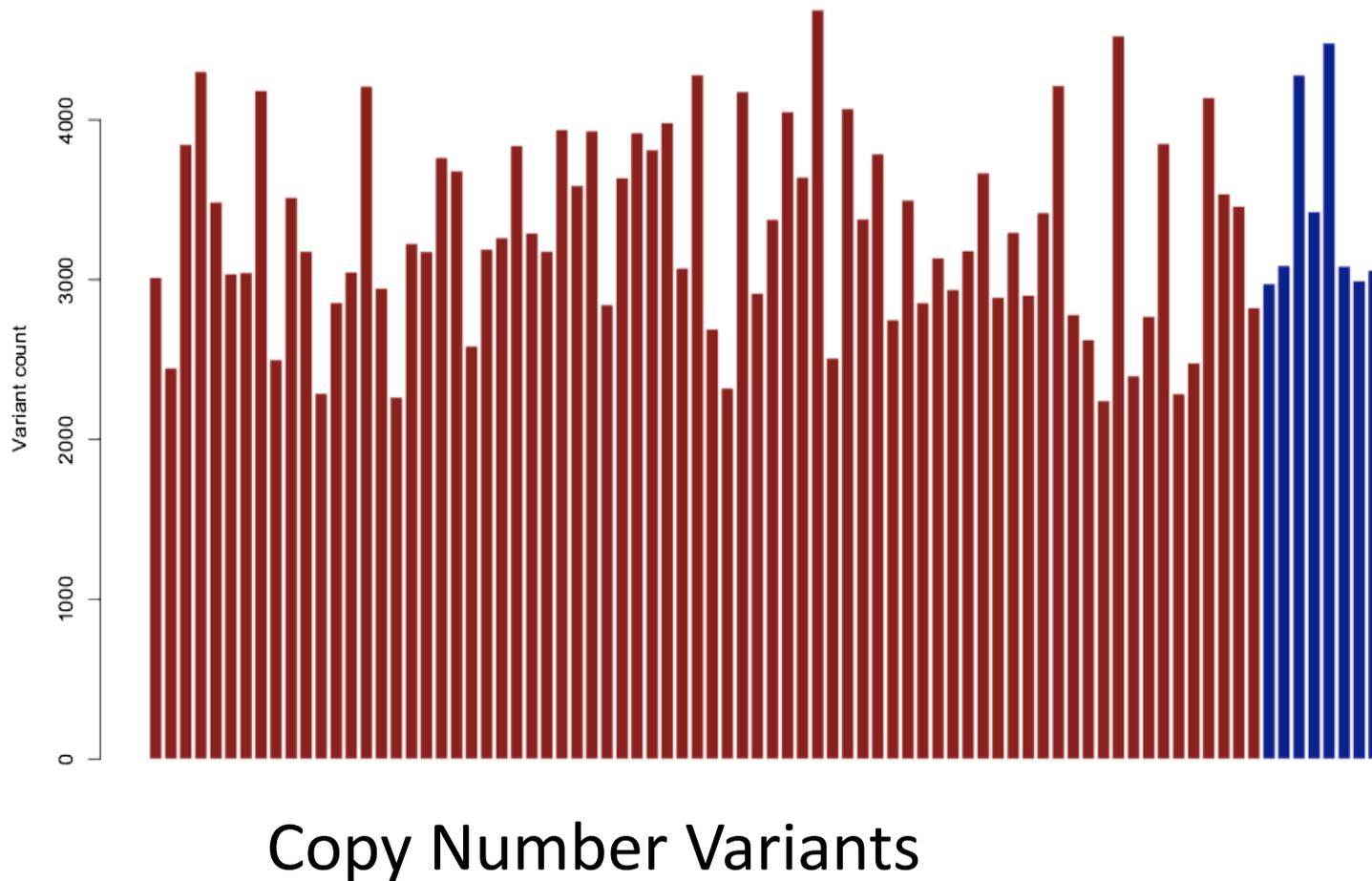
DDIT4L

OR1J2

ANKRD20A4



~2,500-4,000 / sample SVs





- SNPs leading to premature stop codon
- Not found in healthy parents and in the general population (using data from U Washington Exome database, with data from ~6000 individuals)
- 350 novel nonsense SNPs overall, most (314) singletons
- On average, 4.7 novel nonsense SNPs per individual
- 337 genes have at least one nonsense SNP
- Network enrichment analysis using IPA

SNPs found in 3 or more NTD cases:

<i>NBPF10</i>	<i>SYS1-DBNDD2</i>
<i>TP53TG5</i>	<i>CCBL2</i>
<i>KIAA1644</i>	<i>RBMXL1</i>
<i>ZNF193</i>	<i>ASCC1</i>
<i>WASH1</i>	<i>OR4C3</i>
<i>C9orf144B</i>	<i>ZNF816-ZNF321P</i>
<i>ANKRD20A3</i>	<i>ZNF816</i>
<i>ANKRD20A1</i>	<i>FLJ22184</i>
<i>FAM104B</i>	<i>SYS1</i>



“NTDs are caused
by a little bit of
this and a little bit
of that”

Clarke Fraser
09/12/09



F. Clarke Fraser



Adverse Health Effects of Abnormal Folate Transport and Metabolism



Neural Tube Defects



Cerebral Folate Deficiency Syndrome



Hereditary Folate Malabsorption Syndrome



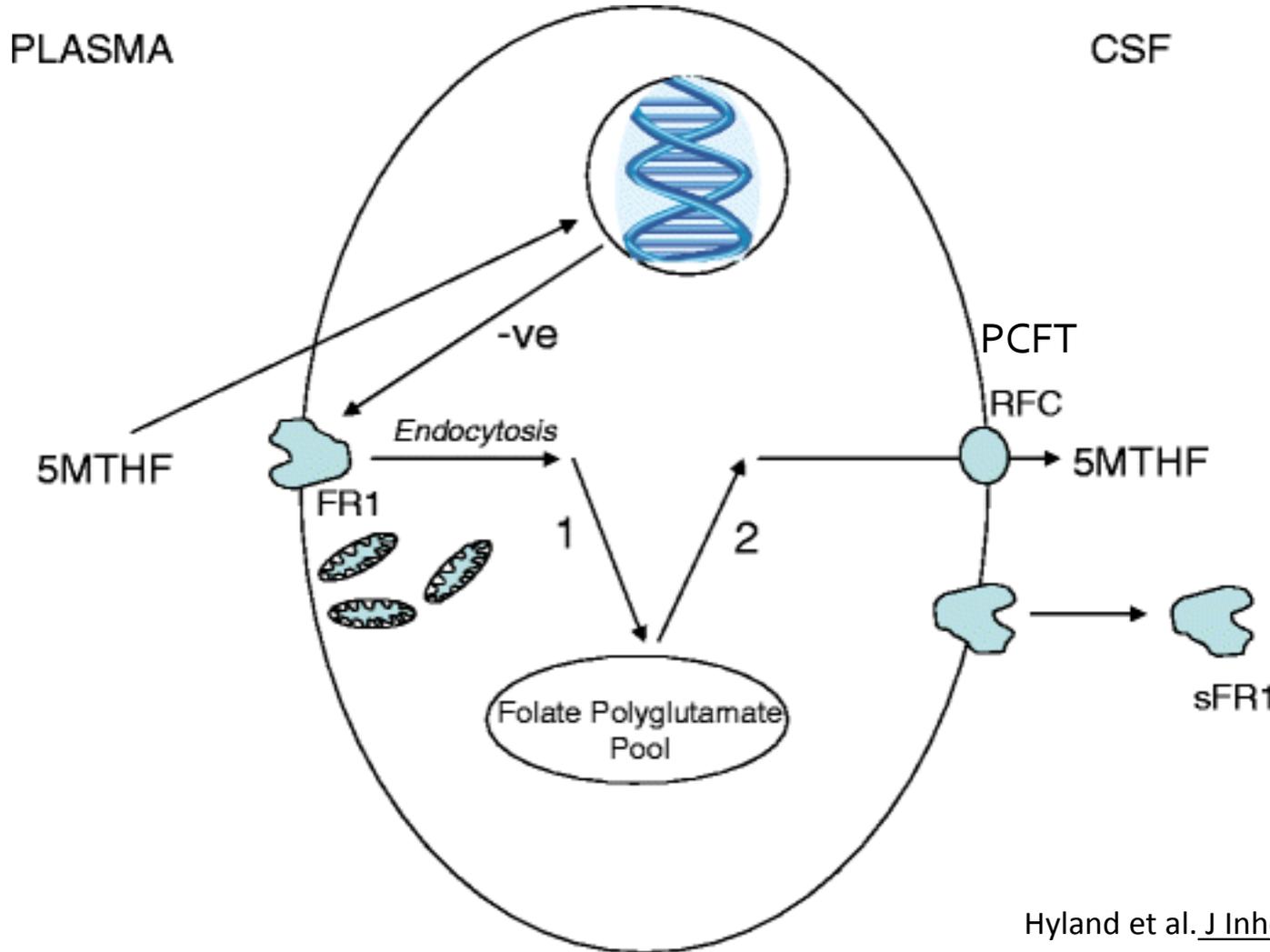
	<u>Low CSF 5MTHF^a</u>	<u>Very low CSF 5MTHF^b</u>	<u>Total</u>
Rett syndrome	4	2	6
Autism	3	1	4
Mitochondrial disorders	1	1	2
Joubert syndrome	1	0	1
Friedreich ataxia	0	1	1
Aicardi–Goutières syndrome	0	1	1
GTP cyclohydrolase 1 Def.	1	0	1
Oculomotoric apraxia	1	0	1
3-Phosphoglycerate dehydrogenase deficiency	1	0	1
Pontocerebellar hypoplasia	0	1	1
Arthrogryposis	0	1	1
Schizophrenia	0	1	1
Myoadenylate deaminase deficiency	0	1	1
		Total	22

a-3rd std. dev. < 5MTHF < -2nd std. dev.

b-5MTHF < -3rd std. dev.



Folate Transport into CSF



- 5MTHF transport into the CNS occurs at the choroid plexus epithelial cells where the FR1 receptor is anchored

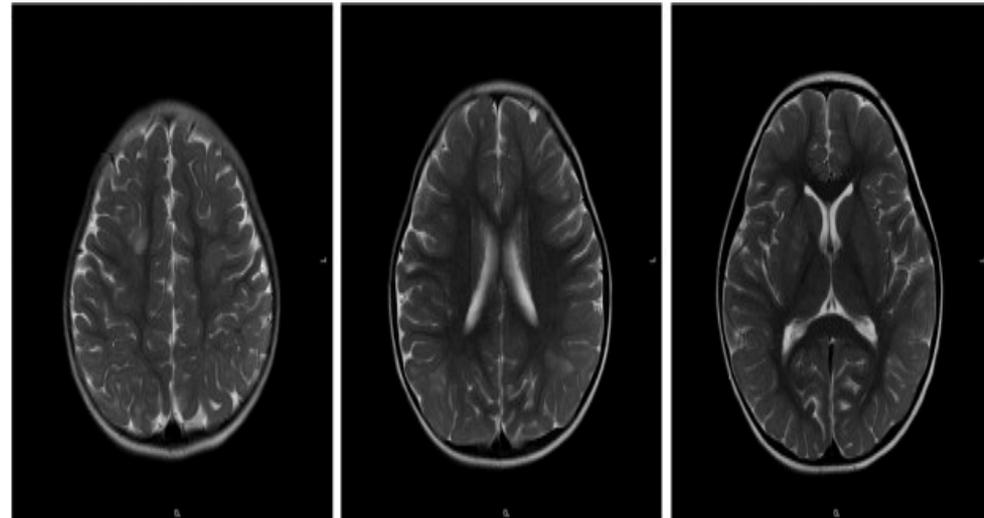
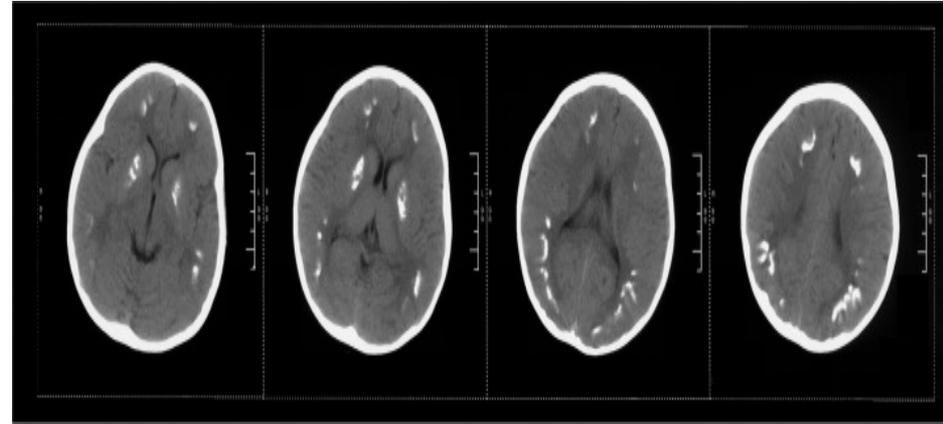
- 5MTHF can enter the cerebral spinal fluid (CSF) via the RFC. PCFT is also involved in getting blood across the choroid plexus barrier

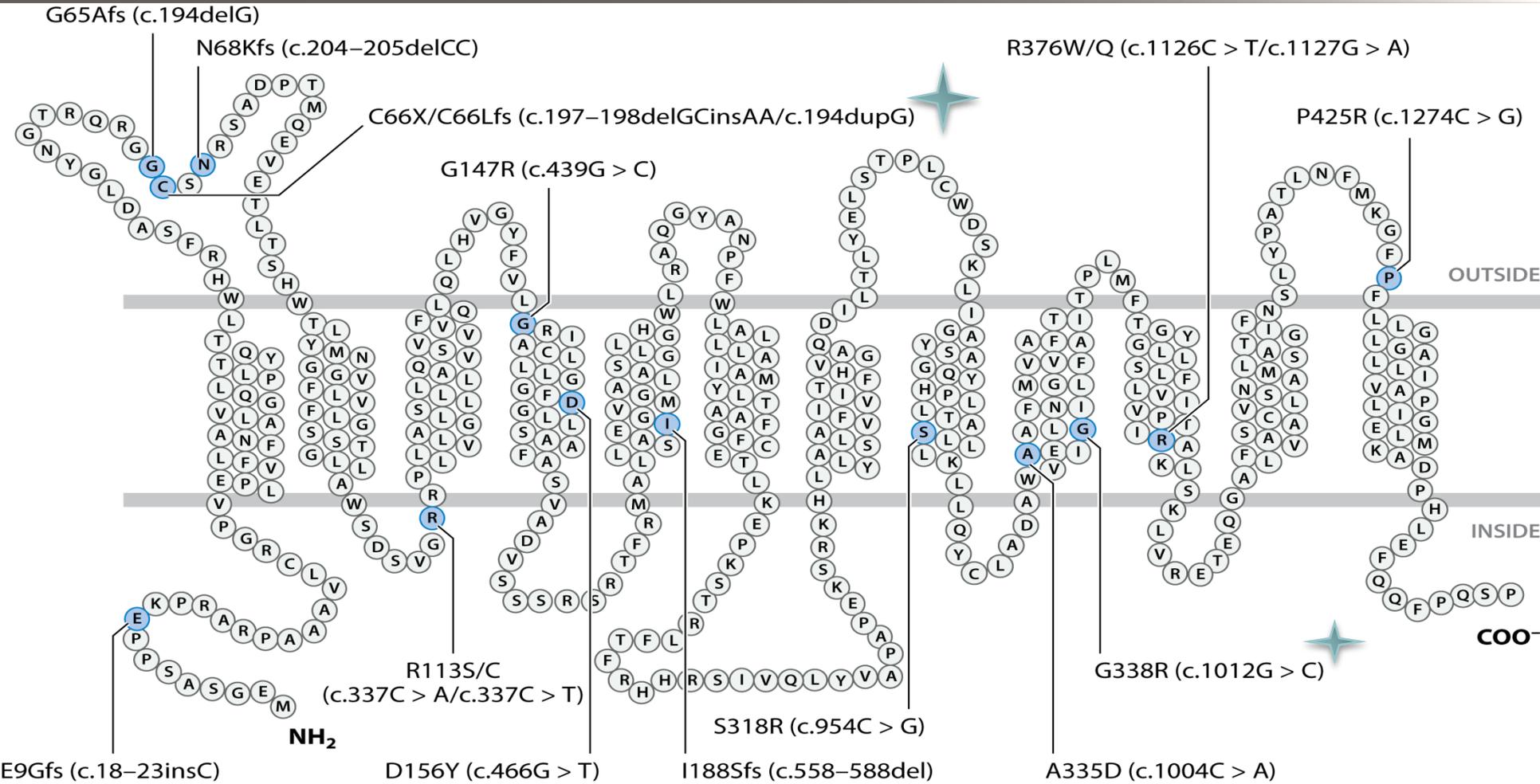
- Some FR1 can be cleaved from the plasma membrane and can then be detected in the CSF as soluble FR1 (sFR1).



Hereditary Folate Malabsorption Syndrome

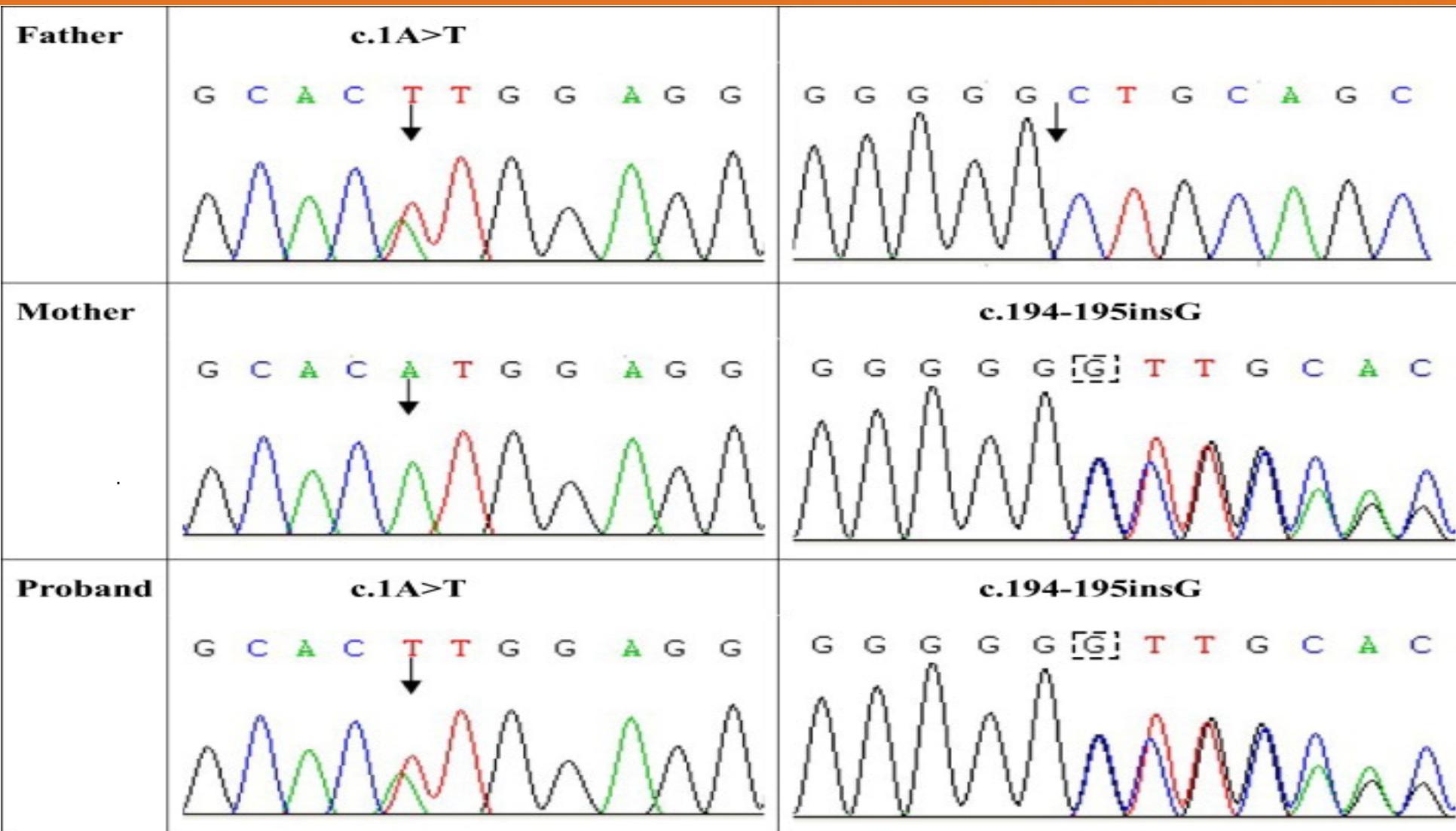
- Autosomal Recessive Disorder
- Variants in the Proton Coupled Folate Transporter (SLC46A1) Gene
- Low CSF and Serum 5MeTHF Concentrations
- Megaloblastic Anemia in First Few Months of Life
- Limb Tremors and Seizures Often Follow
- Intracranial Calcification
- 25 Patients with HFM syndrome have been described
- Folinic Calcium (180mg/day) Treatment Partially Normalizes Values to Low End of Normal Range





AR Visentin M, et al. 2014.
Annu. Rev. Physiol. 76:251–74

- 16 Known Variants in the PCFT Gene
- Poor Correlation Between Genotypes and Phenotypes





	Patient	Father	Mother	Reference range	Units
Plasma folate	4.49	4.69	23.47	>6.8	nmol/L
Plasma total hcy	28.11	27.51	12.49	5–15	μmol/L
Plasma cobalamin 571		127	190	133–675	μmol/L
Urine total hcy	46.76			<20	μmol/L
CSF folate	0	N.D	N.D	>20	nmol/L
CSF 5-MeTHF	0	N.D	N.D	48 – 210	nmol/L
SLC46A1					
Mutation 1	c.1A>T	c.1A>T			
Mutation 2	c.194-195insG			c.194-195insG	



- Very low concentrations of 5-methyl-tetrahydrofolate (5MTHF) in cerebrospinal fluid (CSF)
- Near normal folate levels in plasma and red blood cells
- Delayed development, ataxia, dyskinesias, spasticity, speech difficulties and epilepsy



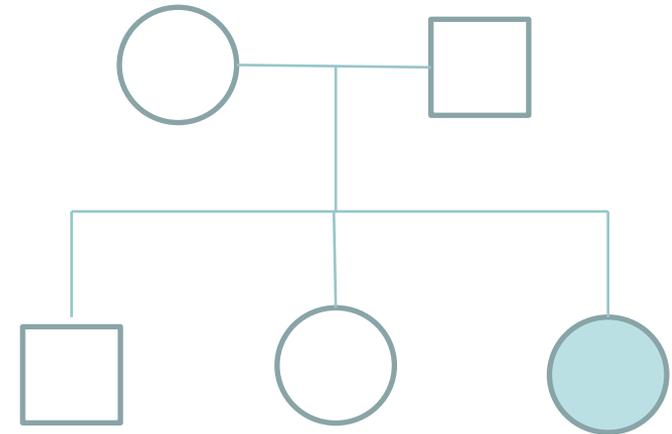


- Developmentally normal at 24 mos with delayed speech
- Diagnosed with ALL @ 26 months, treated with anti-folates for 2 yrs
- CFD symptoms occurred around 3 yrs- severe dev.delay including choreo-athetosis in arms, severe ataxia, uncontrolled seizures
- Mitochondrial function normal
- Folinic acid response varied yrs 7-10
- No mutation detected in *FOLR1* or *PCFT* (Sanger Seq)
- Homozygous CC for *SLC19A1* (RFC1) A80G





- Exome Sequencing:
 - Agilent SureSelect Kit v4.0 to build libraries
 - Covers all exons plus the 3'UTR included
 - Performed on an Illumina HiSeq 2000
 - Average 50 X coverage
- Exome Sequencing Data analyzed by
 - NextGENe (softgenetics)
- Bioinformatic Approaches:
 - Novel, missense mutations that could affect function
 - Recessive inheritance model
 - *de novo* mutations only in Patient #1



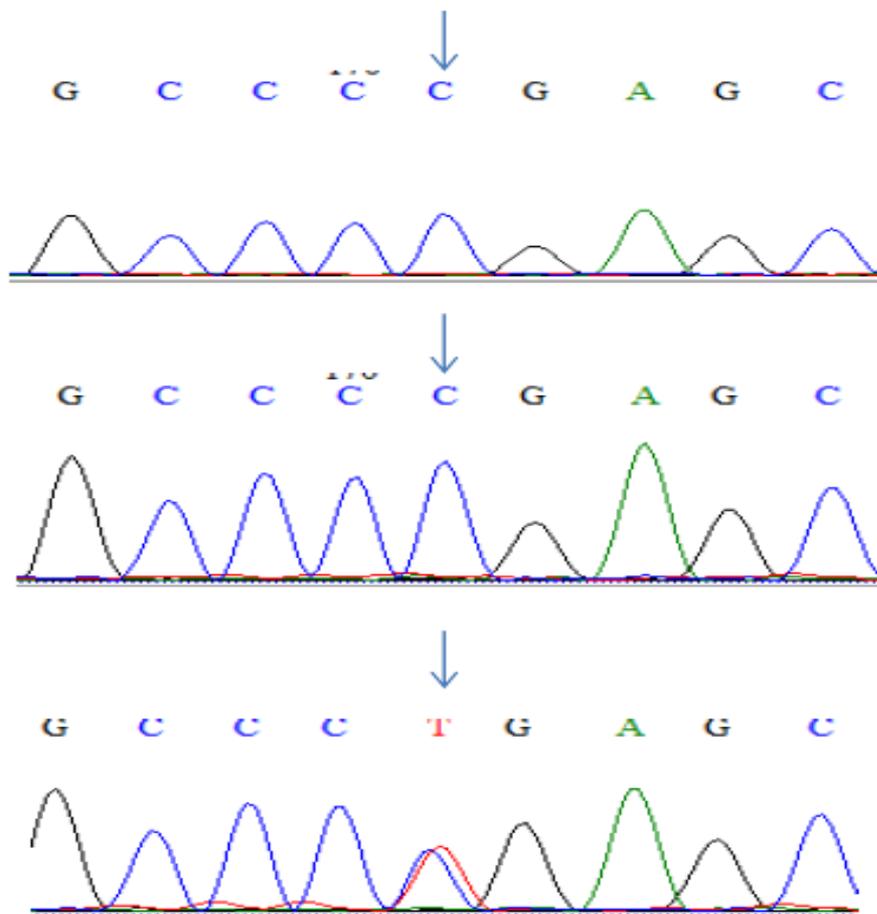


Exome Sequencing: Novel Missense Mutations in Patient #1

Chr Position	Gene	CDS	Chr	Mutation Call	AminoAcid Change	Sanger Sequencing	2nd exome sequencing (50x)
17526572 8	SCRN3	3	2	G>GT	118E>XE	excluded	not detected
88536460	DSPP	4	4	insTAGTGACAG;C>CT	FS		not detected
15331119 9	MTRF1L	7	6	C>AC	325R>IR	excluded	not detected
30918549	LYZL2	1	10	G>CG	29A>GA	excluded	not detected
76982017	VDAC2	7	10	G>GT	213G>WG	excluded	not detected
35550432	FAM177A1	5	14	delT	FS		not detected
2694677	SMCHD1	8	18	G>AG	342W>XW		not detected
42793165	CIC	7	19	C>CT	353R>RX	confirmed	detected, de novo
43243102	PSG3	2	19	G>GT	68Y>XY		not detected
22292180	ZNF645	1	X	C>CT	358H>HY		not detected



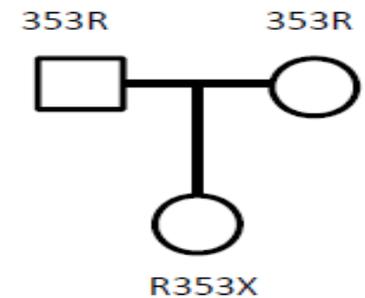
CIC c.1057C>T (p.R353X) Sanger sequencing result



Father

Mother

CFD child



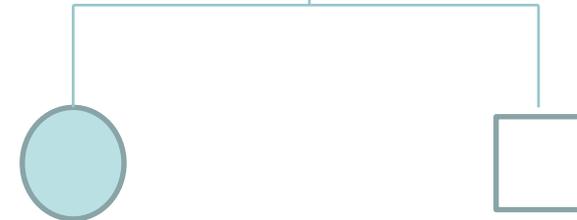


Gene	SLC19A1(RFC1)	CIC
SNP	c.80A>G (p.H27R)	c.1057C>T (p.R353X)
rs#	rs1051266	NA
Father	A/G	C/C
Proband Sibling 1	A/G	C/C
Patient #1	G/G*	C/T (de novo)
Proband Sibling 2	A/G	C/C
Mother	A/G	C/C

*Note: In women, but not in men, SLC19A1 c.80G>A (rs1051266) polymorphism explained 5% of the variation in red blood cell (RBC) folate levels (P=0.02). Relative to women with the SLC19A1 c.80GG genotype, women with the GA and AA genotypes had higher RBC folate concentrations. (Ann Hum Genet. 2009 73(Pt 5): 484–491)

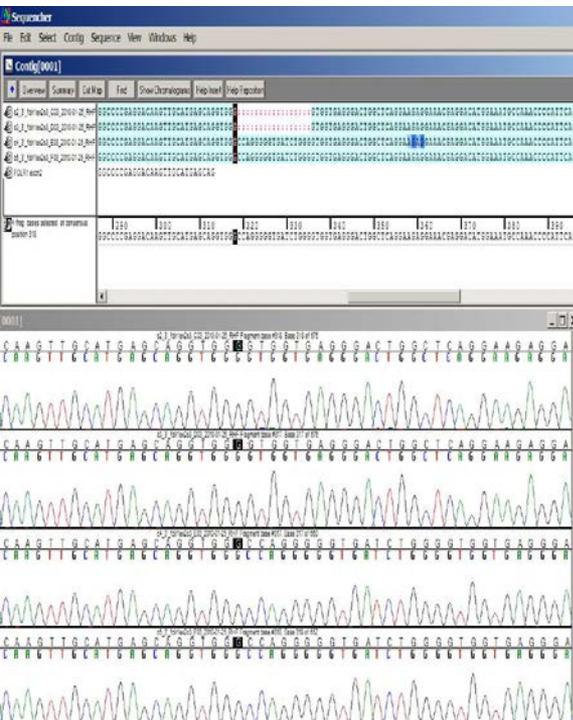


- FOLR1 autoantibody non-informative
- A 9-bp deletion detected in *FOLR1* , but also in father (Sanger Seq)
- Homozygous for MTHFS variant R34L



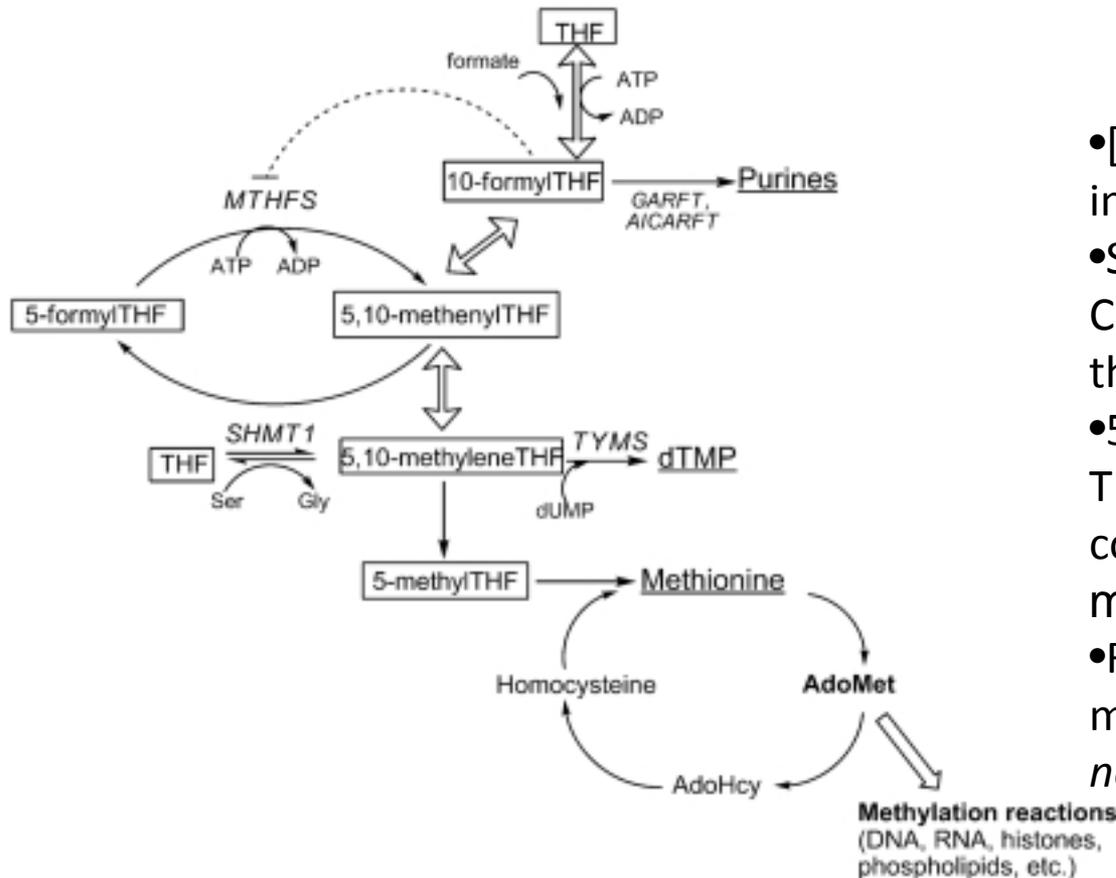
Exome Sequencing (Low Coverage):
MTHFS mutation : Recessive Inheritance
Mechanism unclear

CIC
Compound heterozygote:
IVS1360+32 G>AG and *IVS3796 15 C>CT*
Very close to exon/intron boundary-splice site
Parents each has one of the two alleles





Folate-dependent one-carbon metabolism in the cytoplasm



- [5-CHO-THF] are regulated by a cycle involving SHMT and MTHFS
- SHMT catalyzes the formation of 5-CHO-THF from 5,10-methenylTHF through a secondary catalytic activity
- 5-CHO-THF is mobilized back into the THF cofactor pool via MTHFS which converts 5-CHO-THF into 5,10-methenylTHF
- Role of MTHFS is not fully established- may be to provide 10-CHO-THF to the *de novo* purine synthesis pathway



Variants that could potentially explain Patient #2's folate deficiency

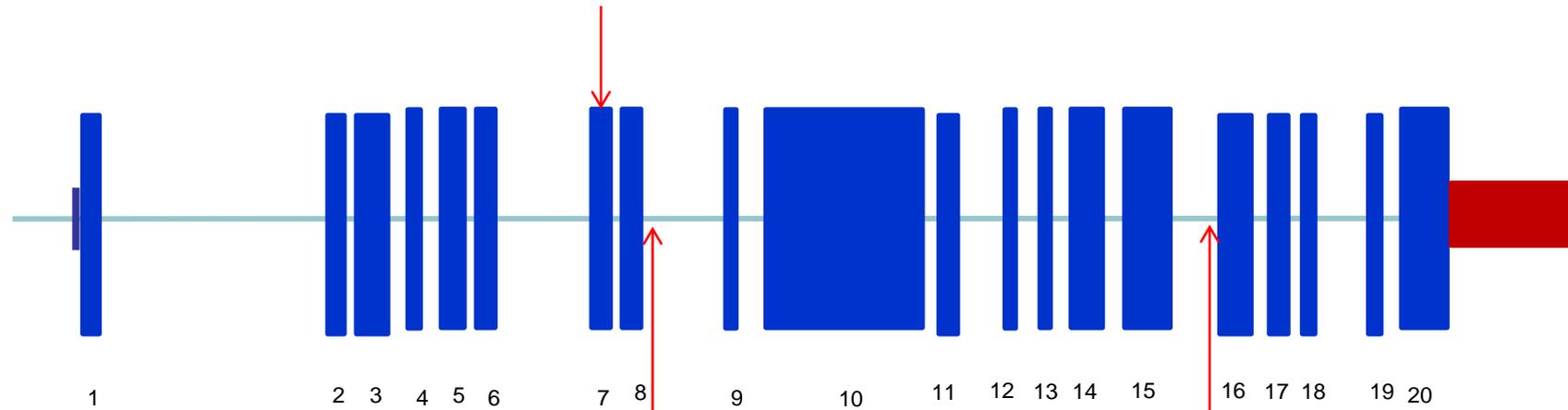
Gene	MTHFS	CIC	CIC	FOLR1
SNP	c.101G>T (p.R34L)	1360+32 G>A	3796-15C>T	9-bp deletion
rs#	NA	NA	NA	
Father	G/T	G/A	C/C	Het
Sibling	G/G	G/G	C/C	--
CFD patient#2	T/T	G/A	C/T	Het
Mother	G/T	G/G	C/T	--



**Patient #1
mutation:**

**c.1057C>T
(p.R353X)**

NM_015125



**Patient #2
mutation:**

c.1360+32 G>A

c.3796-15 C>T



The Human Gene Compendium

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WEIZMANN INSTITUTE OF SCIENCE



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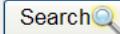
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Analyses:

keyword(s)

CIC



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CIC Gene
protein-coding **GIFTS: 61**
GCID: GC19P042772

capicua homolog (Drosophila)

(Previous name: capicua (Drosophila) homolog)

CIC: Approved symbol from the [HUGO Gene Nomenclature Committee \(HGNC\) database](#)

M Antibodies/cDNA/RNAi
Proteins & Enzymes
Assays & Kits/Pathways

SA Biosciences Gene Network
QIAGEN A QIAGEN Company TFBS
PCR Arrays Primers: ChIP / RT²

[Biological research products](#)
for CIC

ORIGENE Proteins
Antibodies
Assays/ Genes /shRNA/Primers

GenScript Proteins
Antibodies
Assays / Cell Lines / Clones

Jump to Section...

Aliases & Descriptions
for CIC gene

(According to ¹HGNC, ²Entrez Gene,

³UniProtKB/Swiss-Prot, ⁴UniProtKB/TrEMBL, ⁵OMIM, ⁶GeneLoc, ⁷Ensembl, ⁸DME, ⁹miRBase, and/or ¹⁰fRNAdb)

[About This Section](#)

Aliases & Descriptions

capicua homolog (Drosophila)^{1,2}

KIAA0306^{1,3,5}

capicua (Drosophila) homolog¹

protein capicua homolog²

External Ids: HGNC: 14214¹ Entrez Gene: 23152² Ensembl: ENSG00000079432⁷ OMIM: 612082⁵ UniProtKB: Q96RK0³

[Export aliases for CIC gene to outside databases](#)

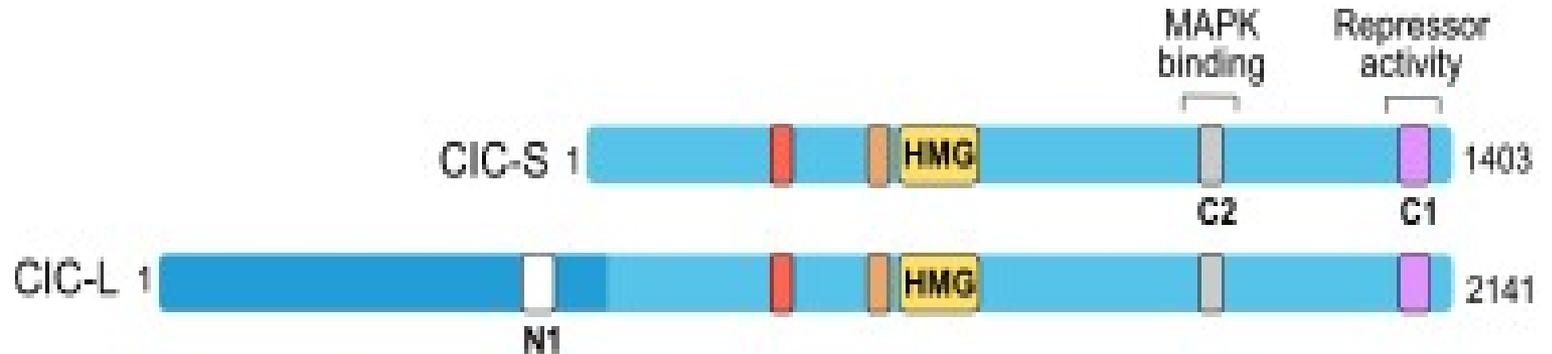
Previous GC identifiers: GC19P043435 GC19P043180 GC19P047454 GC19P047480 GC19P042788 GC19P039219



- Critical to early development of entire *Drosophila* embryo
- Human: [19q13.2](#) 1608 aa in length
- HMG (high motility group; 200-268 aa)-box protein which is a transcriptional repressor/DNA Binding
- There are at least two isoforms (short [CIC-S] and long [CIC-L])
- Binds octomer sequence **T(G/C)AATG(A/G)A**
- C1 motif, function unclear



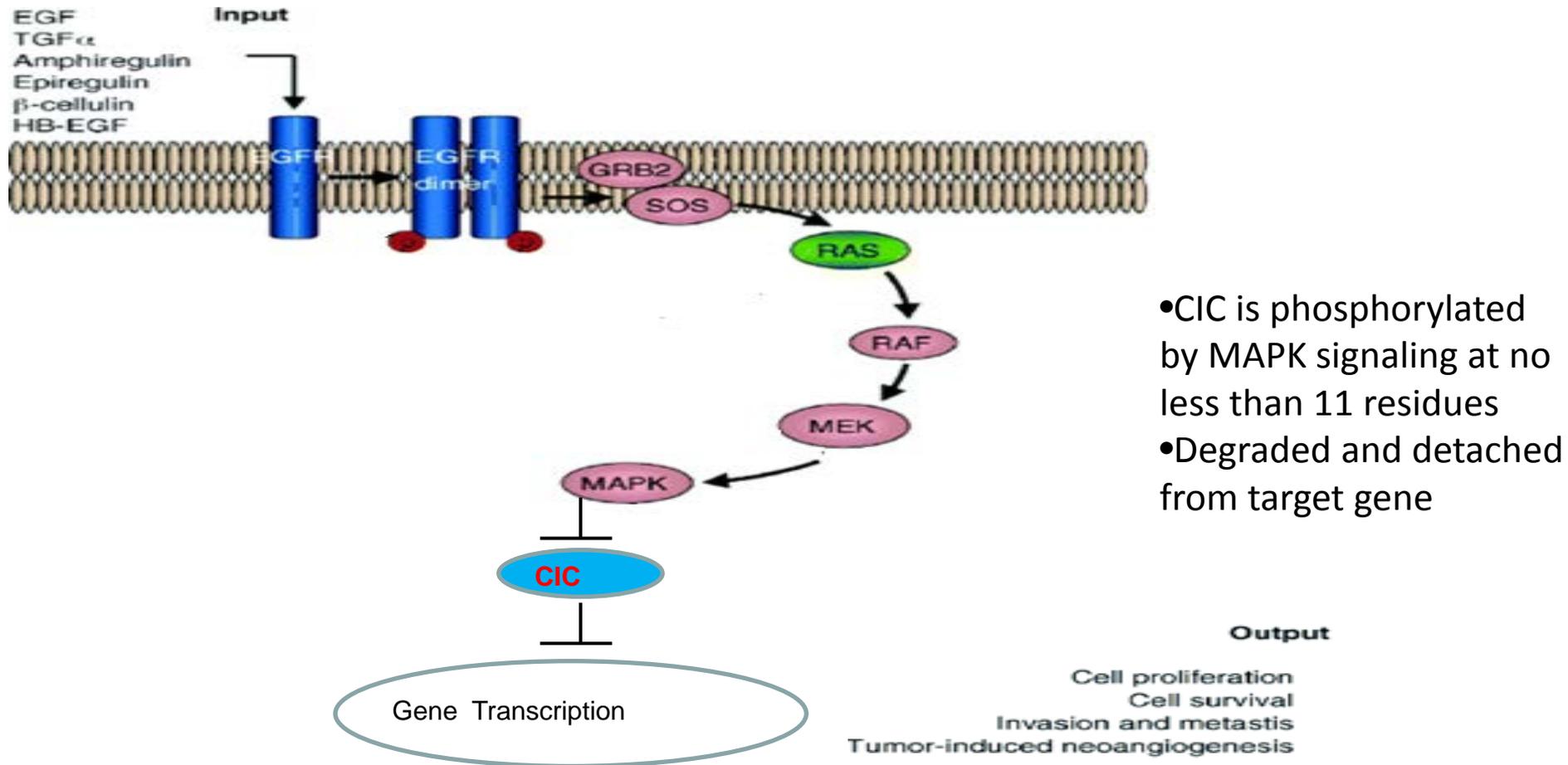
Drosophila



Human

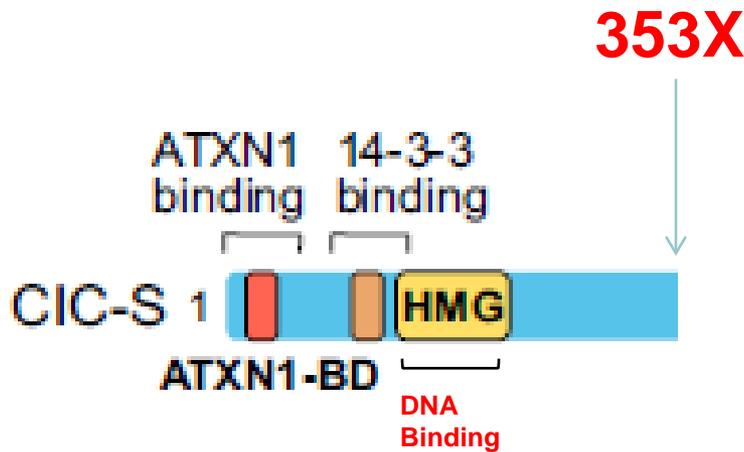


- 28-46 aa: Interacts with Ataxin-1 (ATXN1)
Mutations cause Spinocerebellar Ataxia type 1
Downregulating CIC can rescue SCA knockout mice





- 353R>RX:
 - truncated protein, 353aa
 - HMG box remains
- IVS1360+32G>AG
 - Intron 8, ~453aa
- IVS3796-15C>CT
 - Intron 14, ~1265aa
- p.G580C
 - Predicted to be damaging
- p.A878A(rs10410185) Known SNP in public database
- p.I1511I (rs1052023) Known SNP in public database



Mutated Protein (CIC p.R353X) lost these domains:

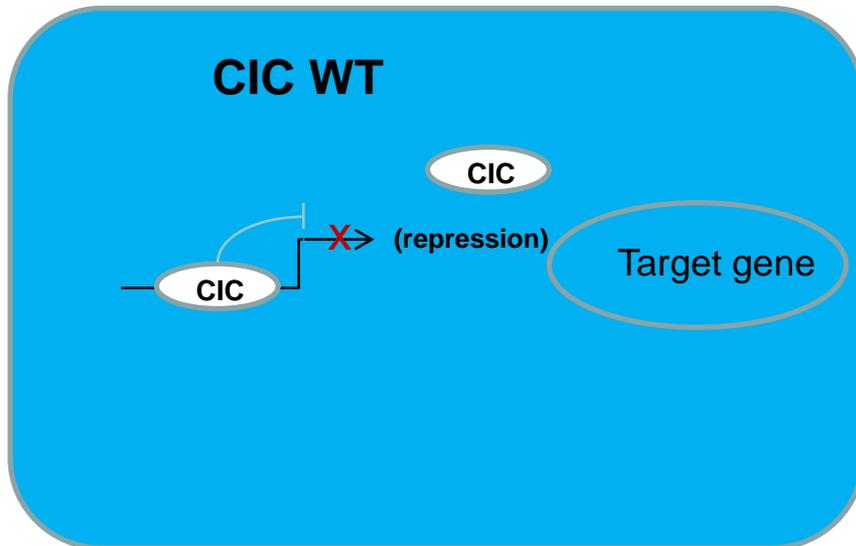
1. C1:Repressor activity domain
2. C2: MAPK docking motif
3. NLS1: Nuclear localization signal

CIC HMG-Box recognizes octameric T(G/C)AATG(A/G)A

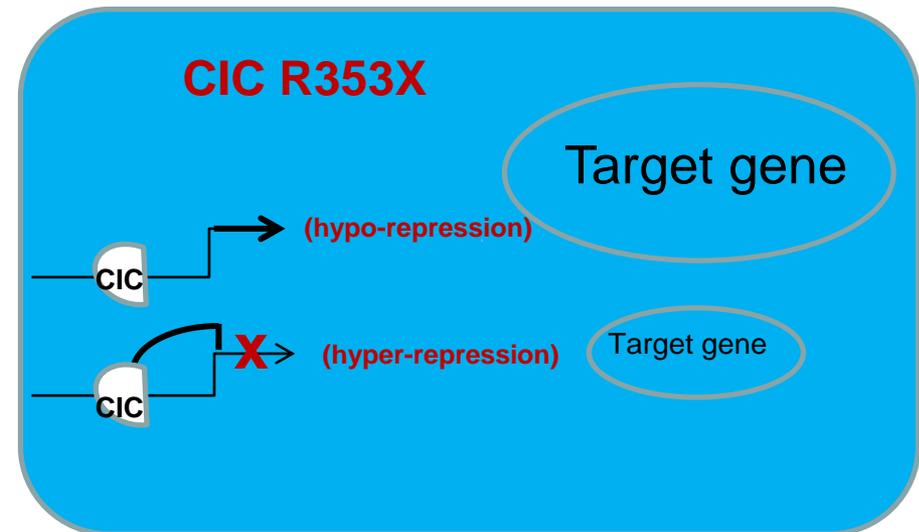


Two possible effects of R353X mutation

1. Hypo-repression (due to loss of C1 repressor activity domain)-target genes over-express
2. Hyper-repression (due to loss of MAPK binding domain)-target gene under-express



Transcriptional Repression



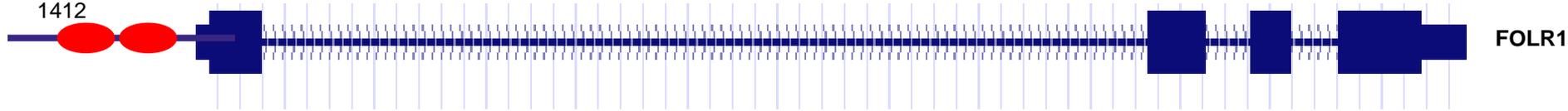
Loss of C1 repressor activity, decreased ability to repress target gene, which enables that gene to be over-expressed

- Loss of MAPK binding domain, CIC won't be phosphorylated and detached appropriately, therefore causes hyper-repression and further inhibits target gene expression



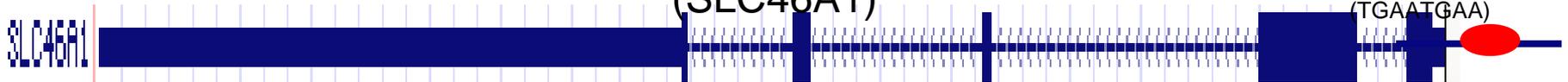
CIC Binding site:
TGAATGAA
-1442~-1435; & :-1419~-
1412

FOLR1



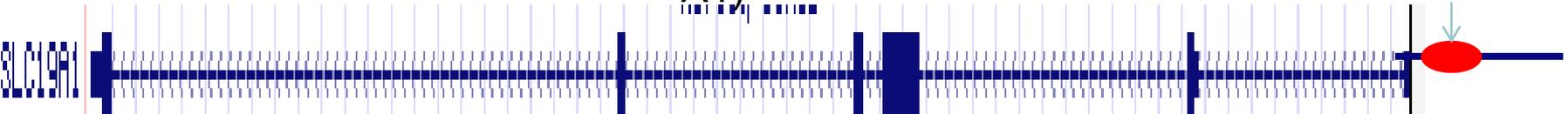
PCFT (SLC46A1)

CIC Binding site:
-1243~-1236
(TGAATGAA)



RFC1(SLC19 A1)

CIC Binding site:
-1926~-1919 TCAATGGA



All folate transport genes have CIC binding motifs in their regulatory region, while Folr1 has two binding sites in its promoter region



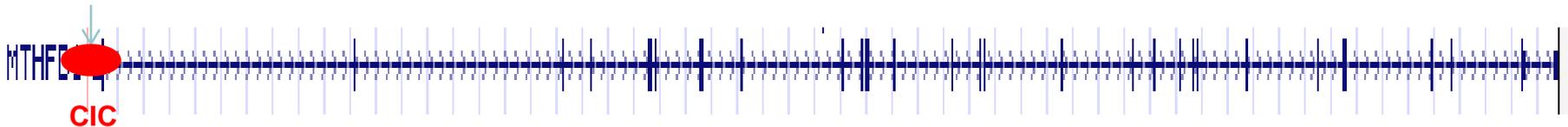
DHFR

CIC binding site
2479~2486:
TGAATGAA



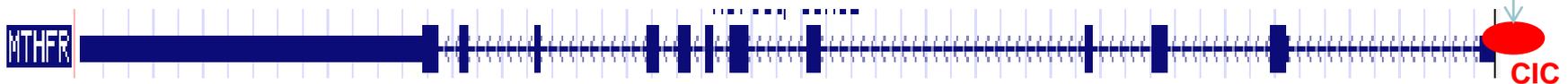
MTHFD1

CIC binding site
-2019~-2012:
TGAATGAA



MTHFR

CIC binding site
-1668~-1661:
TGAATGGA



Folate Enzymes have CIC Binding Motifs



Selected Folate/B12 Pathway Genes with CIC Binding Sites in Promoter

Gene Symbol	Gene Name	*Multiple CIC Binding Sites 19/49 have at least 1
SLC46A1 (PCFT)	proton-coupled folate transporter	
SLC19A1 (RFC1)	reduced folate carrier	
FOLR1*	folate receptor alpha (adult)	
FOLR2	folate receptor beta (fetal)	
FOLR3	folate receptor 3 (gamma)	
FOLR4	folate receptor 4 (delta)	
MTHFS	5,10-methenyltetrahydrofolate synthetase (5-formyltetrahydrofolate cycloligase)	
MTHFR	5,10-methylenetetrahydrofolate reductase	
DHFR*	dihydrofolate reductase	
TYMS	thymidylate synthetase	
MTRR*	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	
MTR*	5-methyltetrahydrofolate-homocysteine methyltransferase	
SHMT1	serine hydroxymethyltransferase 1 (soluble)	
SHMT2	serine hydroxymethyltransferase 2 (mitochondrial)	
CTH	cystathionase (cystathionine gamma-lysase)	
MTHFD1*	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1	
MTHFD1L	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-Like	
MTHFD2*	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2	
MTHFD2L	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2-Like	
PCMT1	protein-L-isoaspartate (D-aspartate) O-methyltransferase	



Selected Folate/B12 Pathway Genes with CIC Binding Sites in Promoter

TCN1	transcobalamin 1
TCN2	transcobalamin 2
GCH1	GTP cyclohydrolase 1
FOLH1	folate hydrolase (prostate-specific membrane antigen) 1
FTCD	formimidoyltransferase cyclodeaminase
SLC25A32	mitochondrial folate carrier
AHCY	adenosylhomocysteinase
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase?IMP cyclohydrolase
CD320	transcobalamin receptor
CUBN	cubilin (intrinsic factor-cobalamin receptor)
DMGDH	dimethylglycine dehydrogenase
DNMT1	DNA(cytosine-1-)-methyltransferase 1
DNMT3A	DNA(cytosine-5-)-methyltransferase 3 alpha
DNMT3B	DNA(cytosine-5-)-methyltransferase 3 beta
FPGS	folylpolyglutamate synthase
GART	phosphoribosylglycinamide formyltransferase
GGH	gamma-glutamyl hydrolase
GIF	gastric intrinsic factor (vitamin B synthesis)
GNMT	glycine N-methyltransferase
MAT1A	methionine adenosyltransferase 1, alpha
MAT2B	methionine adenosyltransferase 1, beta
MAT2A	methionine adenosyltransferase 2, alpha
MMAB	methylmalonic aciduria (cobalamin deficiency) cb1B type
MTFMT	mitochondrial methionyl-tRNA formyltransferase
SARDH	sarcosine dehydrogenase



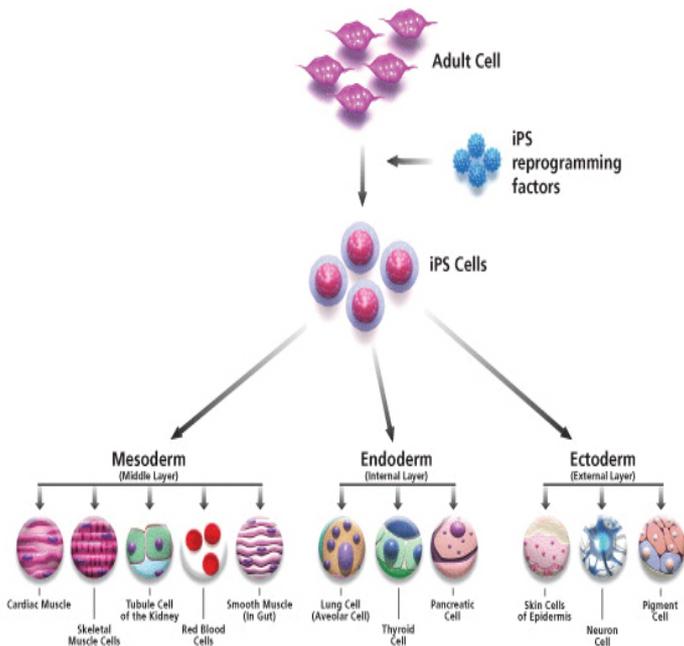
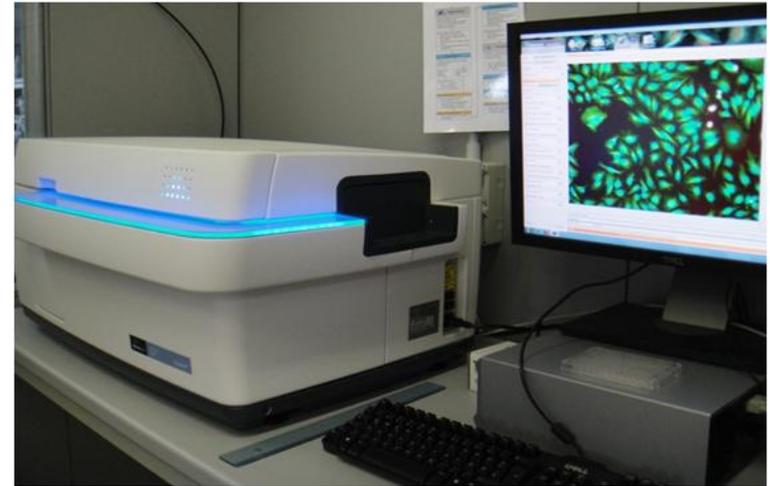
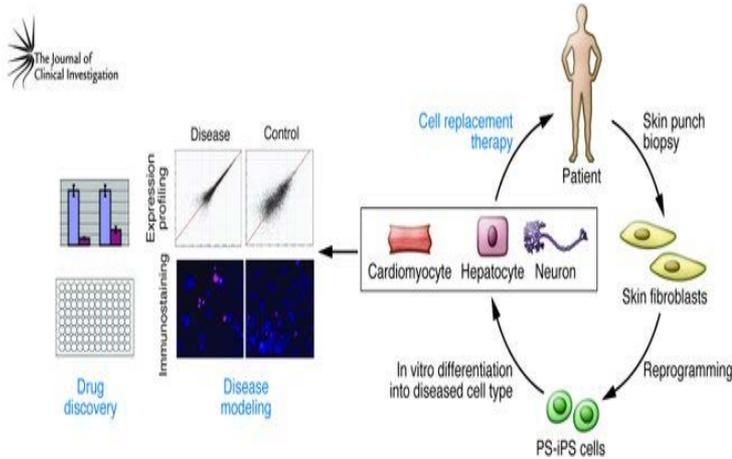
Treating Kids With Folate Transport Defects



TREATMENT



- Understand normal CIC functions
- Determine the effects of CIC R353X mutation in patient IPS cell lines

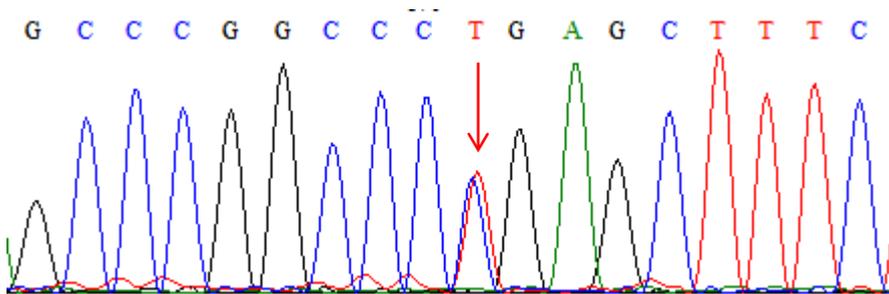


- Optimal matrix study – test a variety of extracellular matrices to determine if there is a difference in interactions
- Growth factor study – test dose response to growth factors for signal transduction/differentiation
- Likely find that cells from NTDs will have decreased activation to matrix/growth factors
- Seek factors to stimulate repair

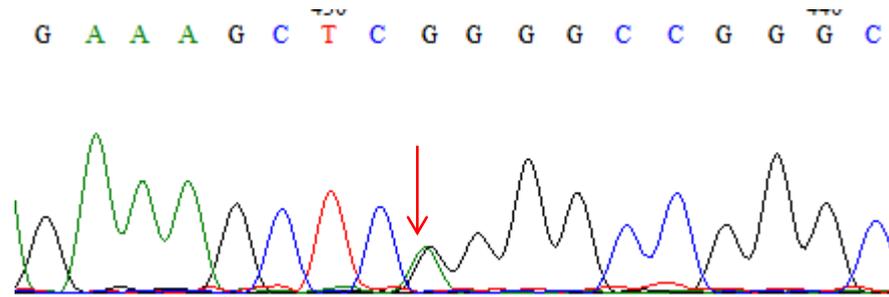


IPS cell DNA sequencing

Forward

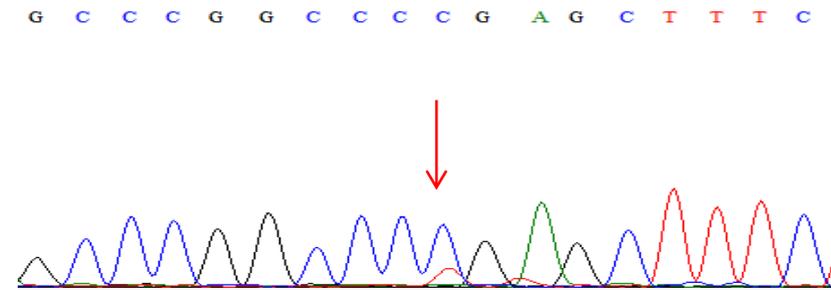


Reverse

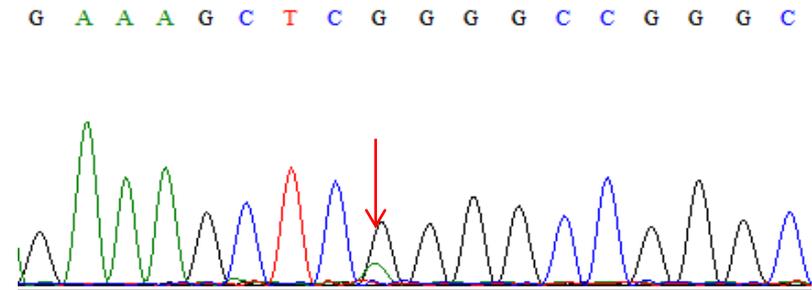


IPS cell cDNA sequencing

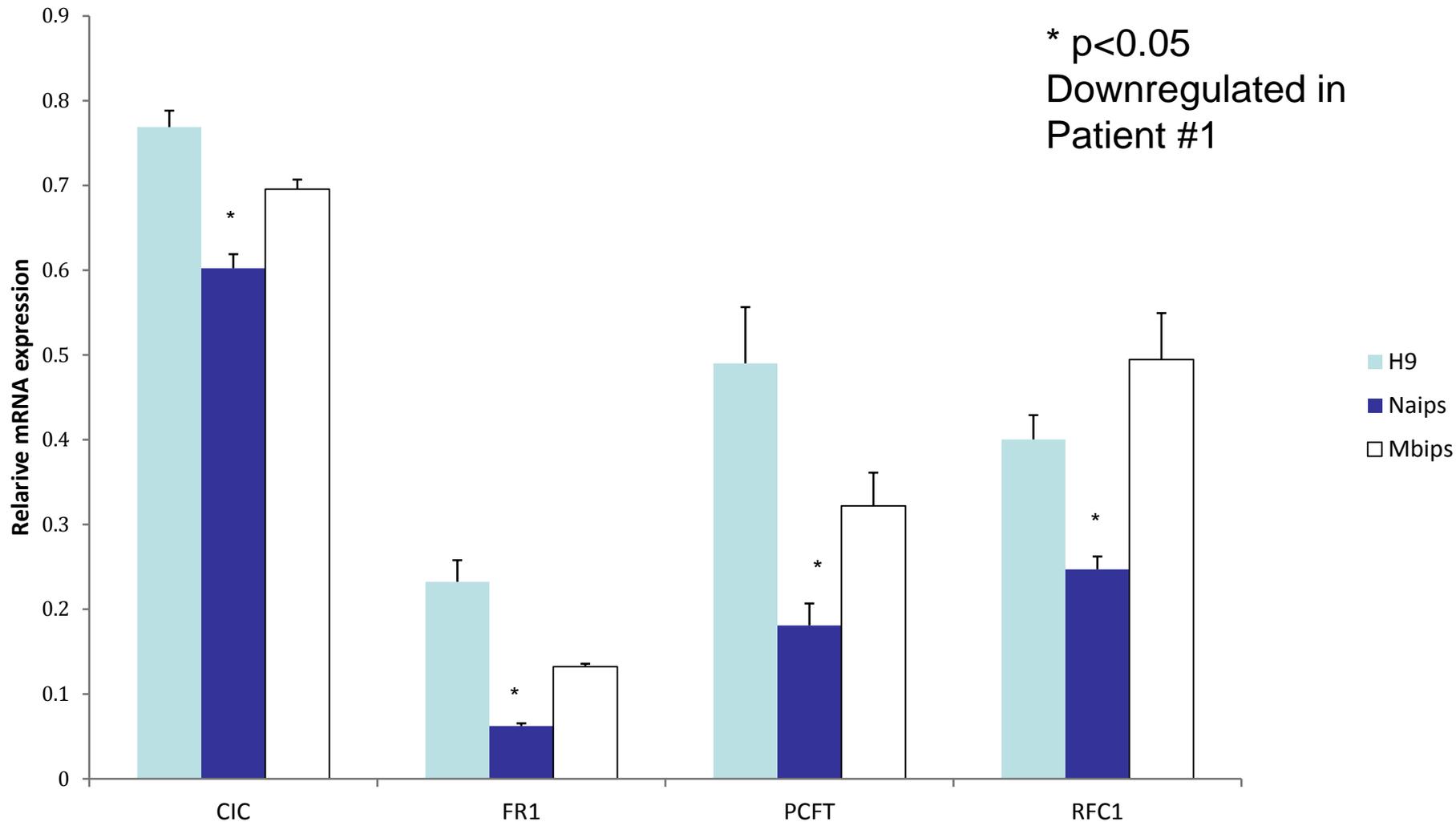
Forward

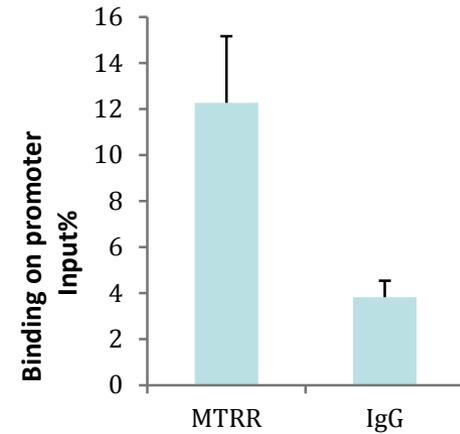
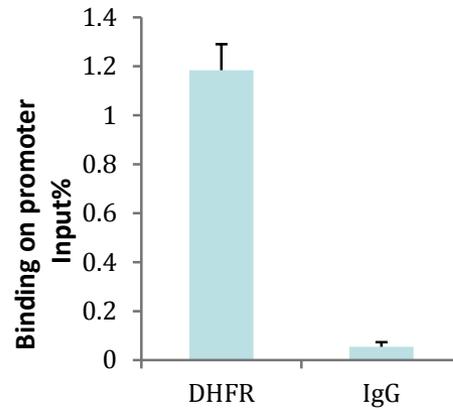
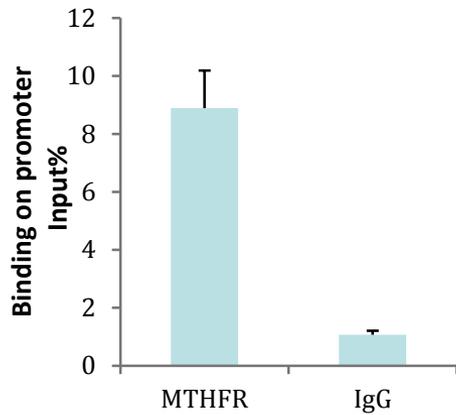
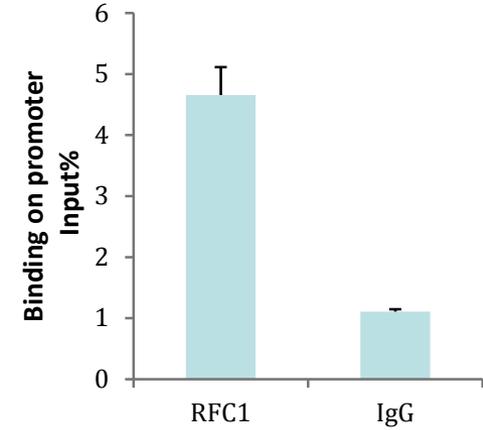
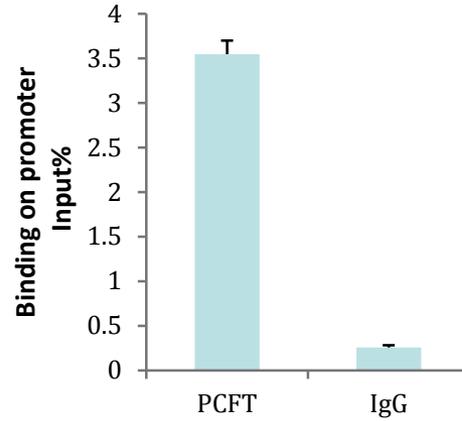
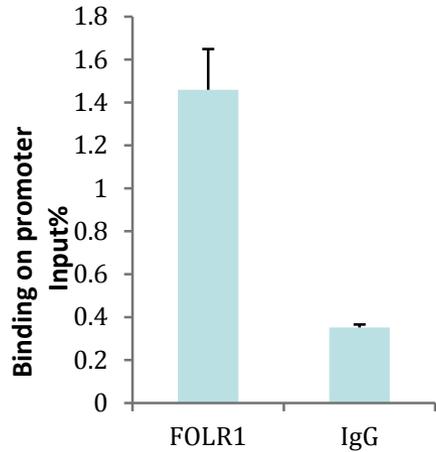


Reverse



cDNA CIC R353X sequencing demonstrated that the amount of mutant mRNA is less than the wild type mRNA







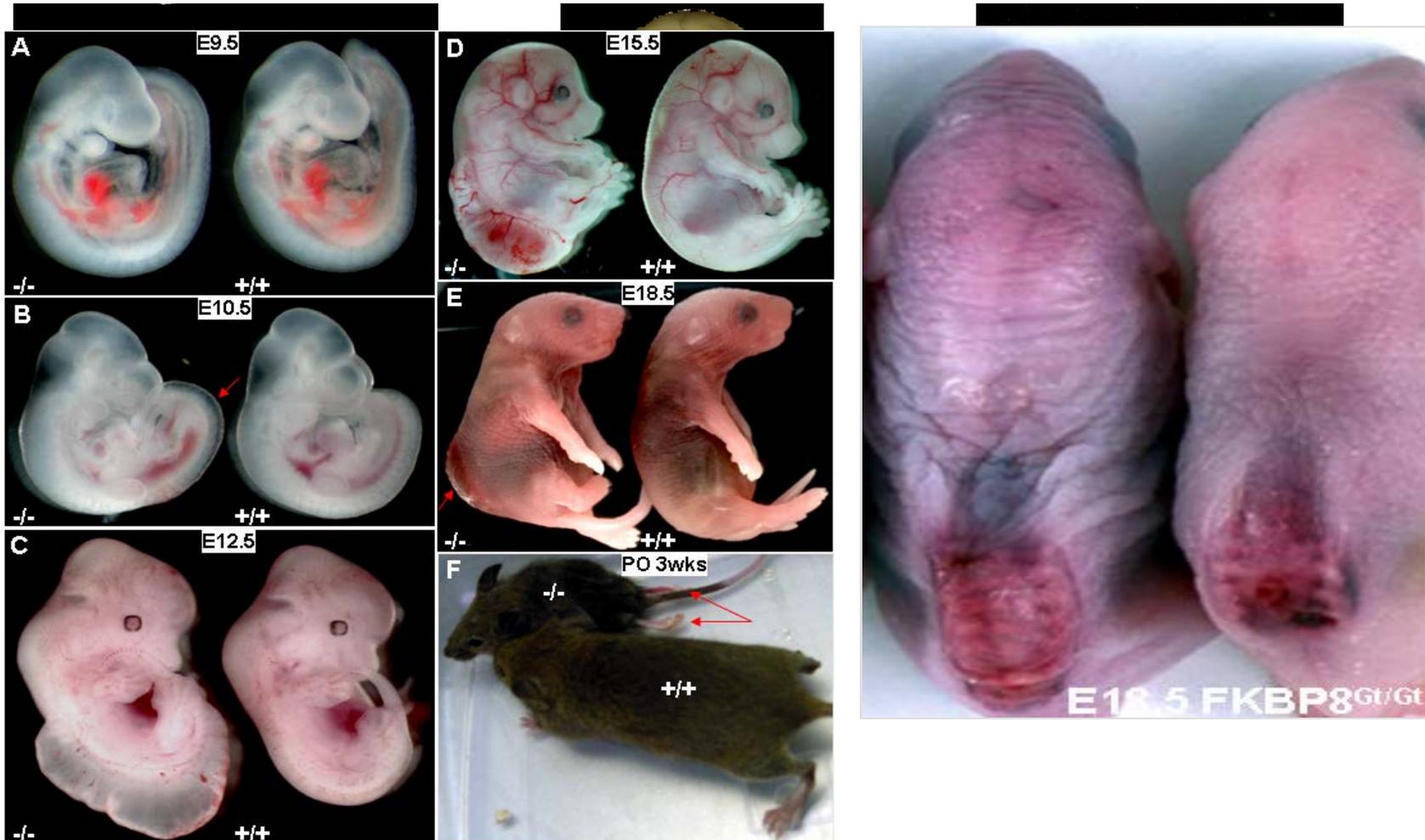
Use neuronal precursors to promote neural connectivity in damaged regions

Target neurogenic bladder and associated pathologies with scaffolding proteins and stem cells

Or manipulate one carbon metabolic pathway to increase availability of carbon units for transport into damaged regions



100% Penetrant Spina Bifida





- Sheep herd producing spina bifida lambs at 15% prevalence rate

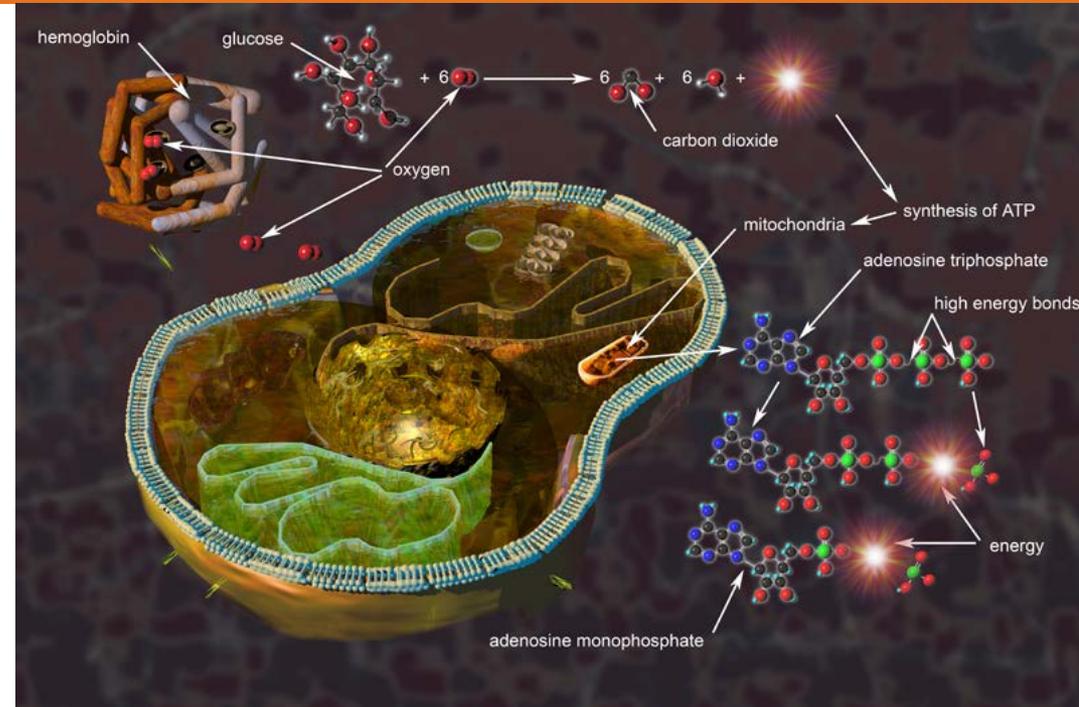
- Defects can be detected early by sonography



- Represent next phases of developing actual translatable treatments/repairs NOT just management of lesions



- 30 years after the recognition of folic acid's benefit to developing embryos
- - We know almost nothing about the cell biology or the molecular mechanisms of folate action in the embryo





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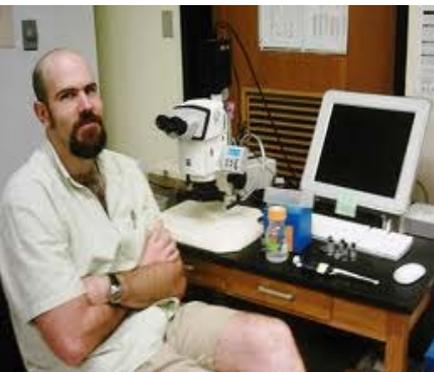
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Research Support

National Institutes of Health

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Questions and Answers



Evelyn Delgado
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For those in the auditorium, please come to the microphone to ask your question.

Our Next Grand Rounds

Nov. 5

The Community Guide: An Evidence-Based Public Health Resource

**Presenter: Anil Thota, MBBS, MPH,
Coordinating Scientist and Senior
Service Fellow, Office of Public Health
Scientific Services, Centers for Disease
Control and Prevention**

