Dr. Arthur Beaudet

Hosted By:
U.S. Department of Health and Human Services
National Institutes of Health

EPIGENETICS

- The study of changes in gene function that are stable and heritable (or potentially heritable as in terminally differentiated neurons) and do not entail a change in DNA sequence.

EPIGENETICS AS THE FONT OF DNA SEQUENCE (LETTERS)

CAGT	*CAGT*	$C^{M}GATC^{M}GATC^{M}GAT$
CAGT	***CAGT***	$\dot{C}GATC\dot{G}ATC\dot{G}AT$
~~**CAGT**~~	CAGT	
cagt	**•CAGT**	
CAGT	**•CAGT**	
CAGT		
CAGT		

Epi-genetics

on top of genetics

GENOMIC IMPRINTING

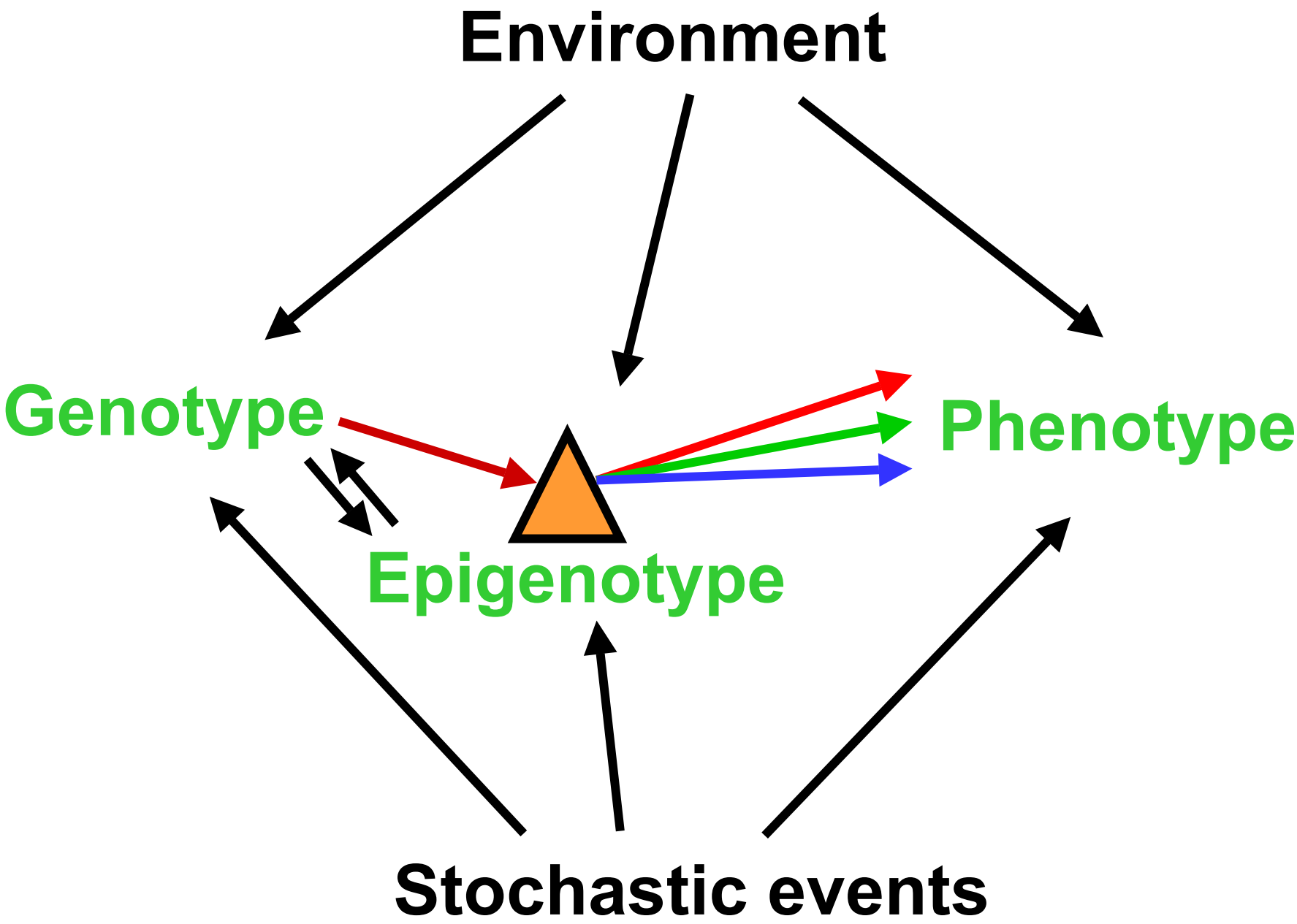
- An epigenetic phenomenon in which the activity of a gene is reversibly modified depending on the sex of the parent that transmits it. This leads to unequal expression from the maternal and paternal alleles of a diploid locus.
- Well described in plants and mammals, but not in egg-laying vertebrates.

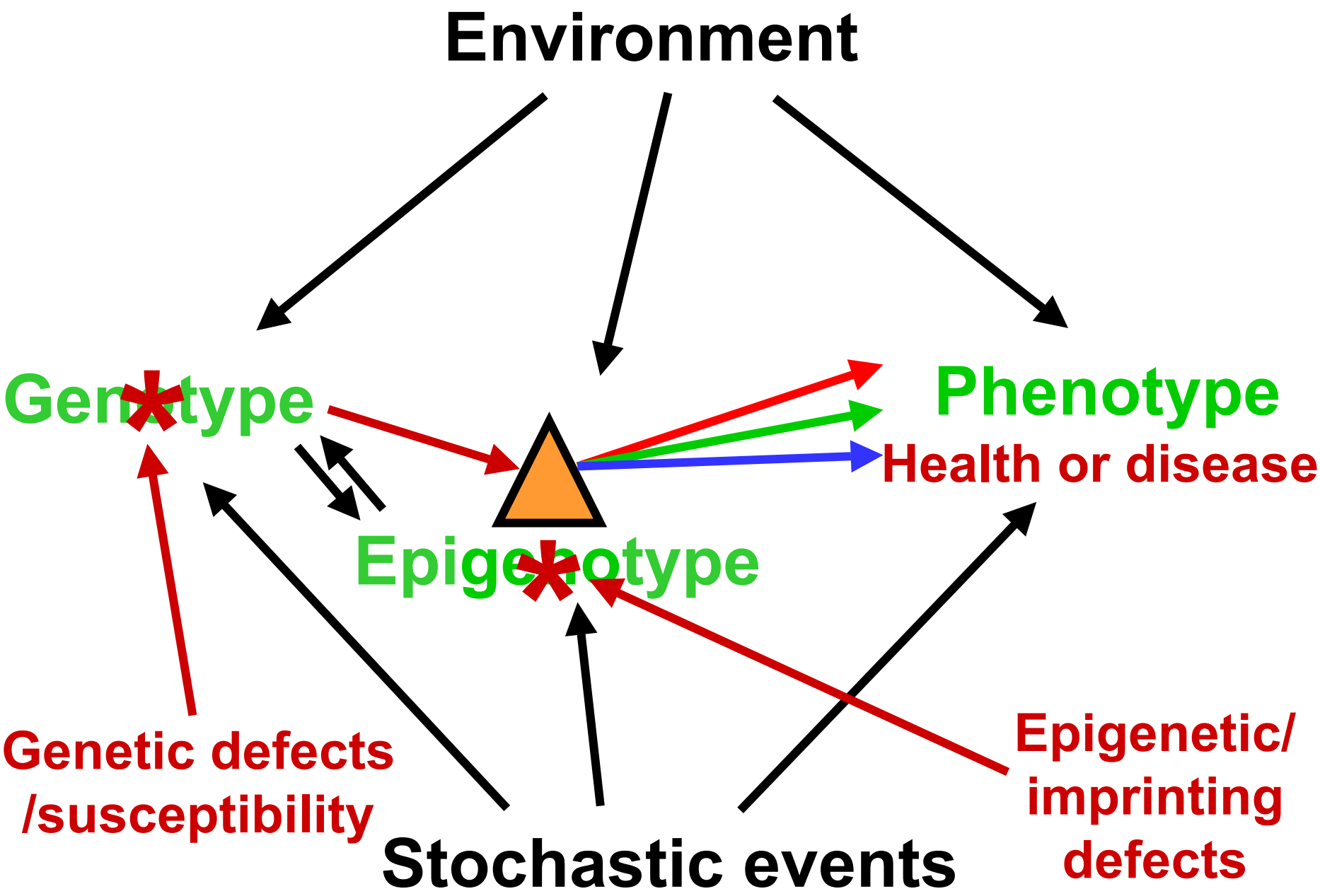
EPIGENETICS GENERALLY

- Any change in the “font.”
- All genes involved.
- Makes a brain cell different from a liver cell

GENOMIC IMPRINTING

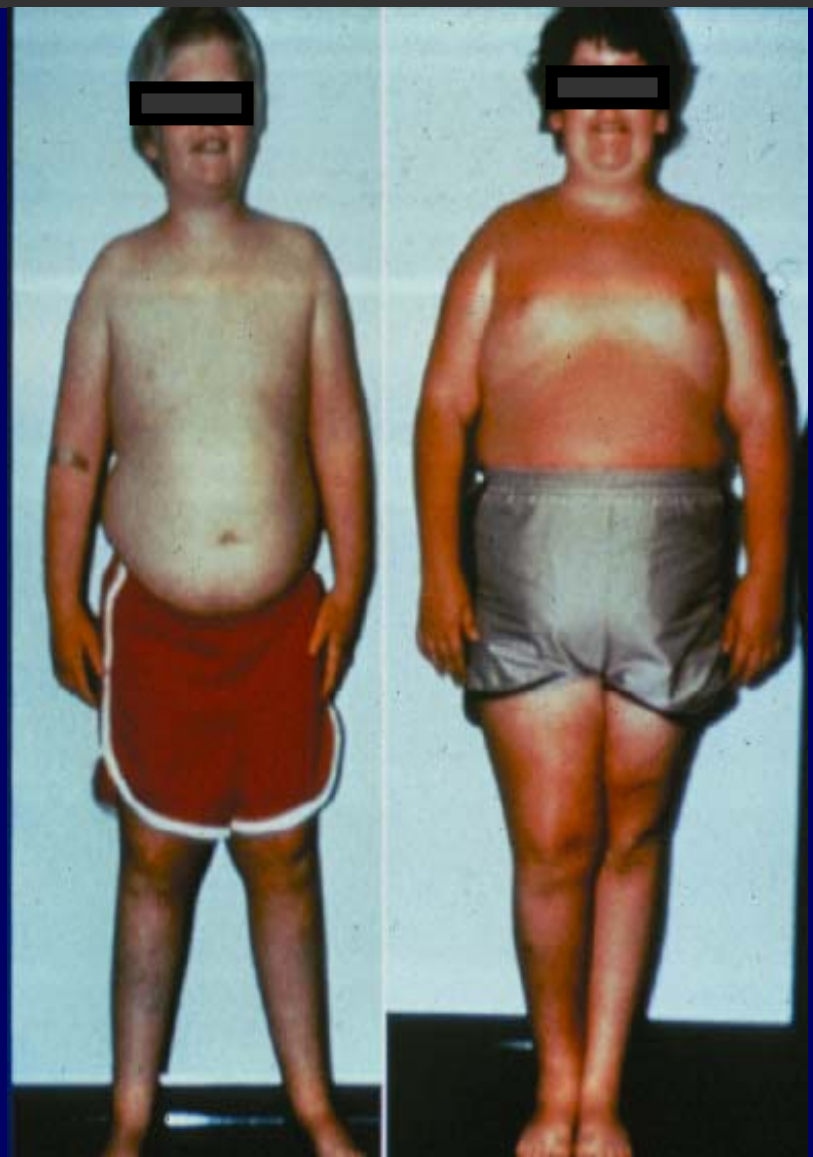
- Mom’s on & Dad’s off or vice versa
- Only a few genes involved.
- Mule vs hinney.


Environment
Genotype
Phenotype
Epigenotype
Stochastic events


Environment
Genotype
Phenotype
Health or disease
Epigenotype
Genetic defects
/susceptibility
Stochastic events
Epigenetic/
imprinting
defects

PRADER-WILLI SYNDROME

- Infantile hypotonia & feeding problems
- Hyperphagia & obesity
- Moderate MR
- Gonadal hypoplasia
- Short stature

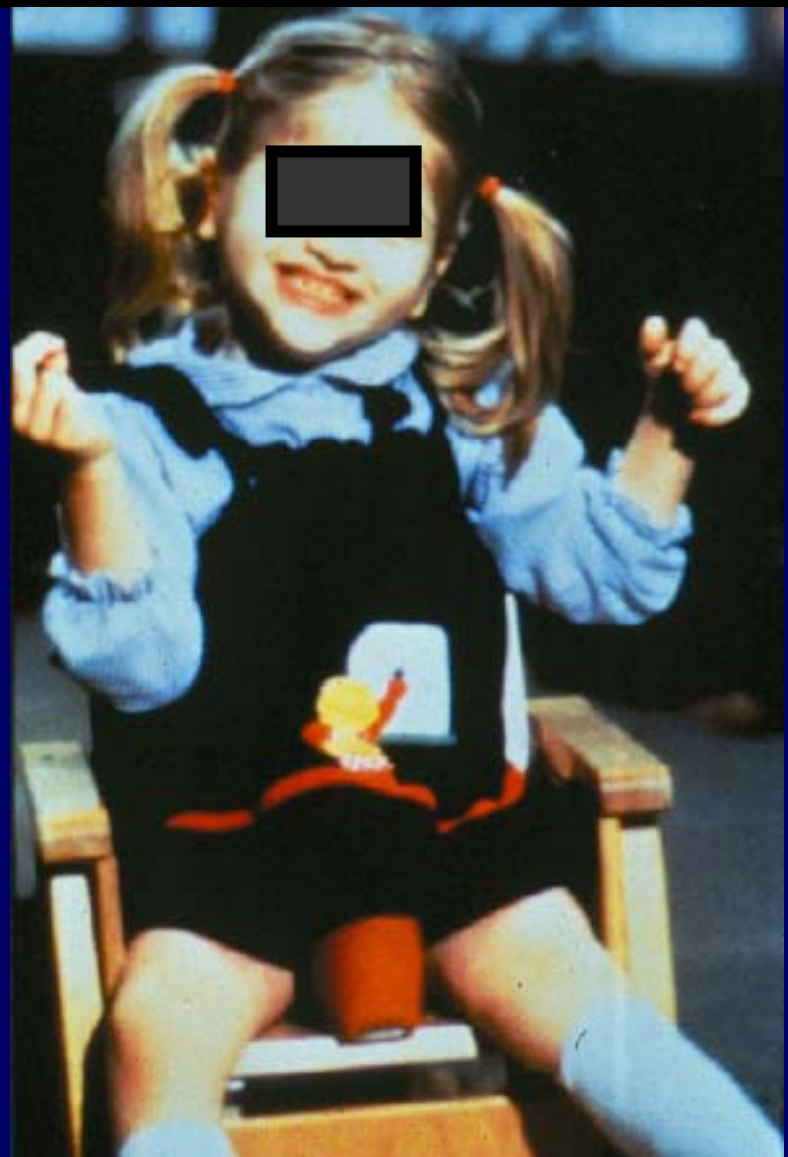


ANGELMAN SYNDROME

Severe learning def.
Absent speech
Happy disposition
Seizures
Ataxia / tremor
Microcephaly
Prominent mandible

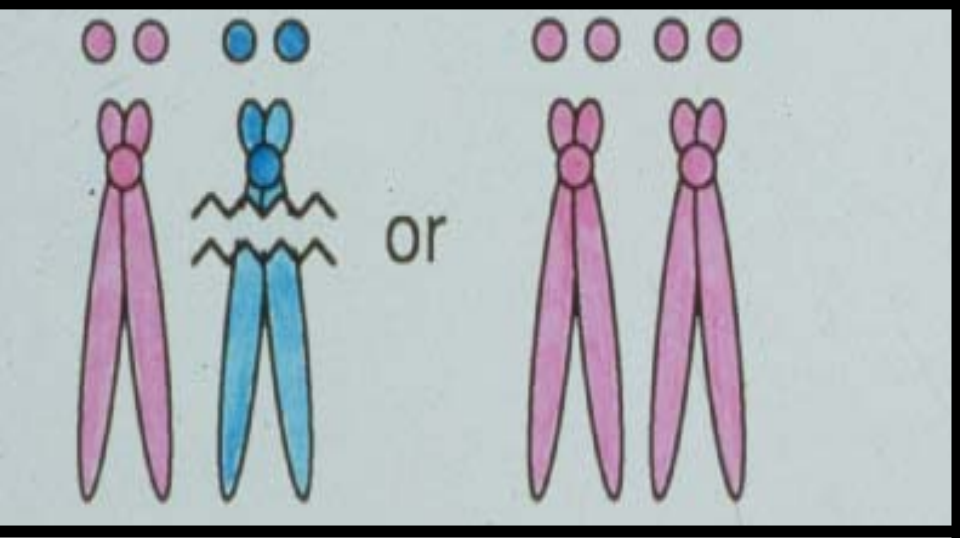
Behavior = anti-autism and like autism

Learning = like autism



Prader-Willi syndrome

Genetic | Epigenetic

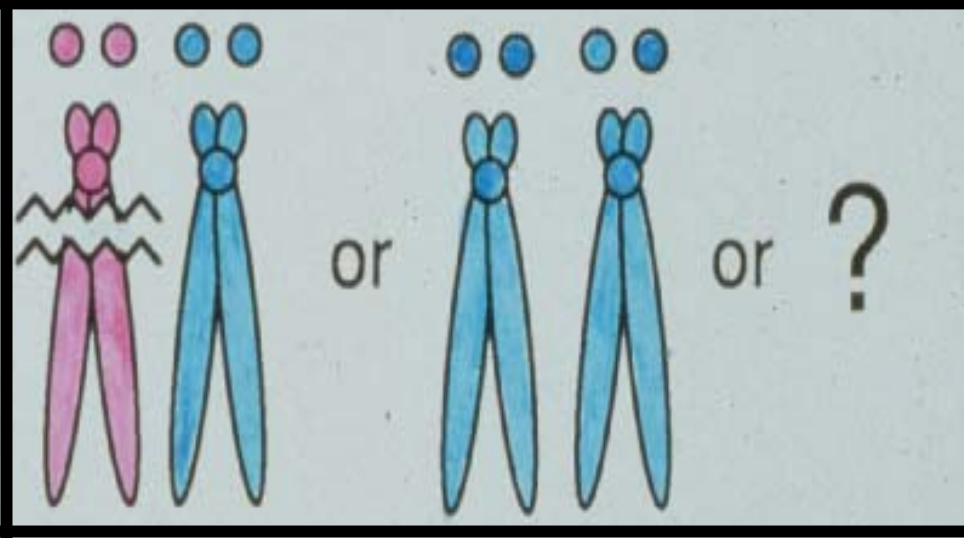


70% | 30%

Paternal deficiency 15q11-q13

Angelman syndrome

Genetic | Epigenetic



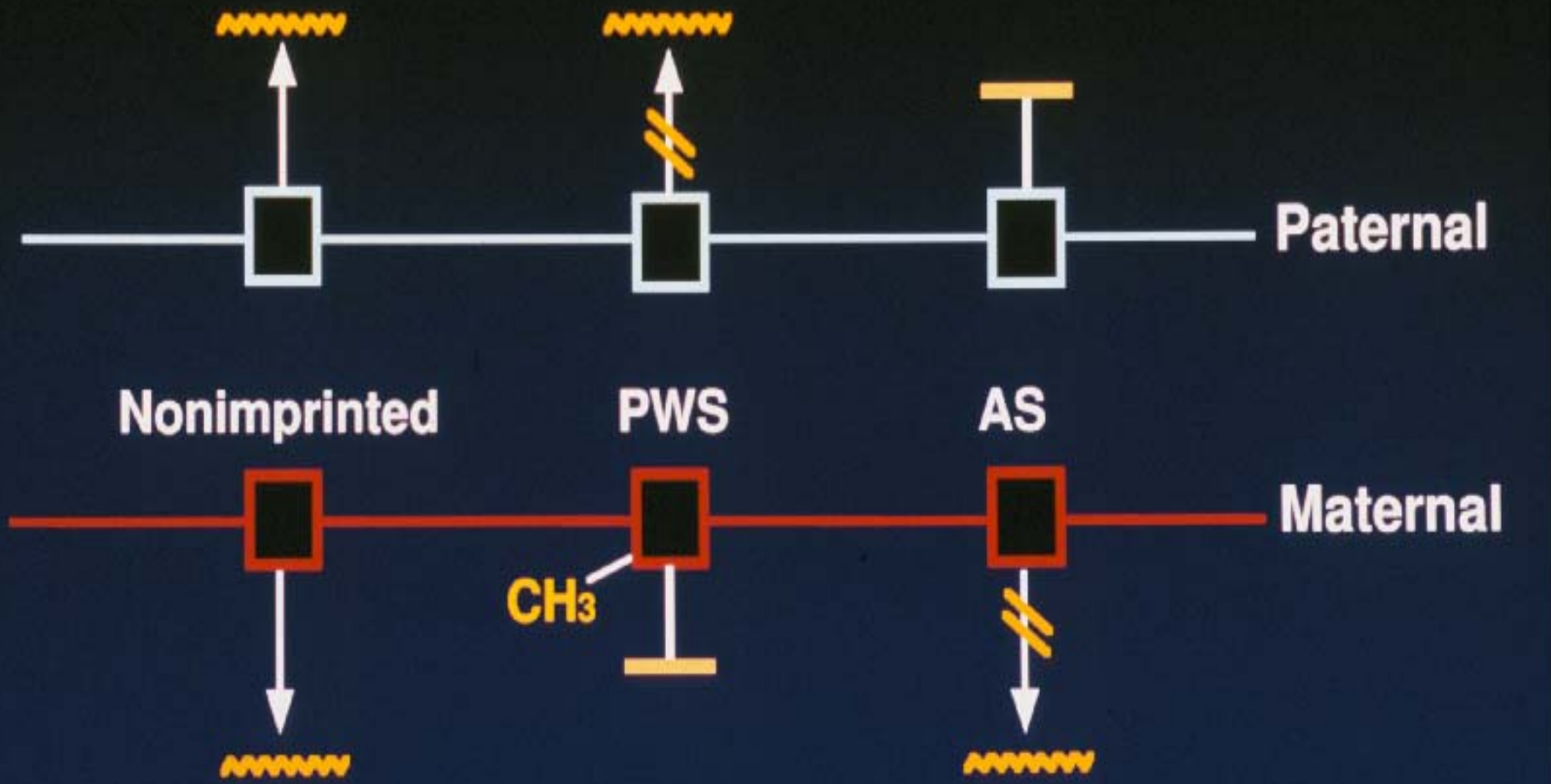
70% | Rare

Maternal deficiency 15q11-q13

DISEASE DEFINITIONS

- **Genetic disease** – an aberration in nucleotide sequence causing a disease phenotype
- **Epigenetic disease** – an aberration in epigenotype (stable / heritable change in gene expression) causing a disease phenotype in the absence of nucleotide aberration
- **Both** – through altered gene expression

GENOMIC IMPRINTING IN 15q11-q13



UBE3A ENCODES E6-AP

- E6-AP discovered as a protein that interacts with papilloma E6 to promote degradation of p53
- E6-AP is a ubiquitin-protein ligase; gene symbol *UBE3A*
- Maternal deficiency is the cause of AS
- Imprinted with tissue-specific silencing of the paternal copy in brain

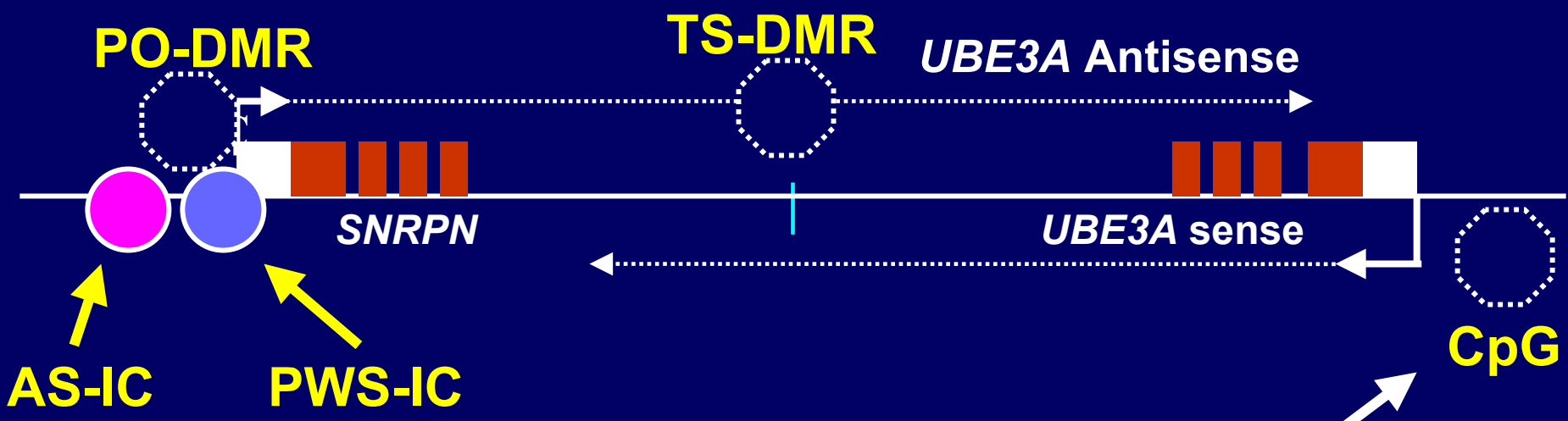
RELATIONSHIP OF *SNRPN*, *UBE3A* AND IMPRINTING CENTER

Invariant

Mat = meth
Pat = unmeth

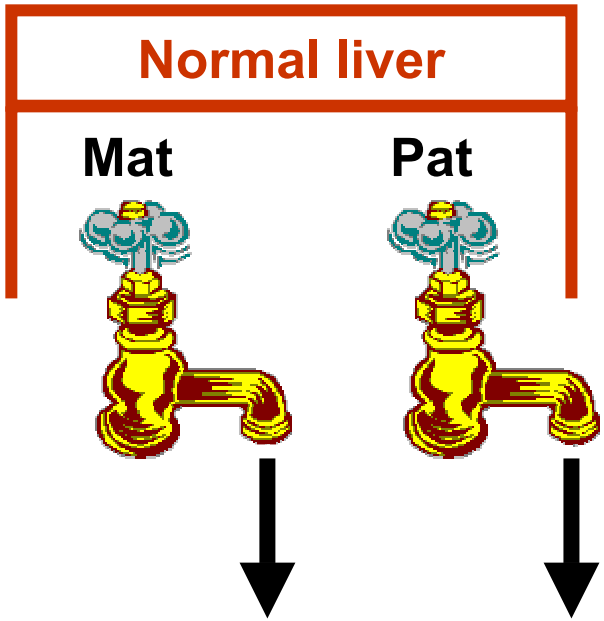
Plasticity

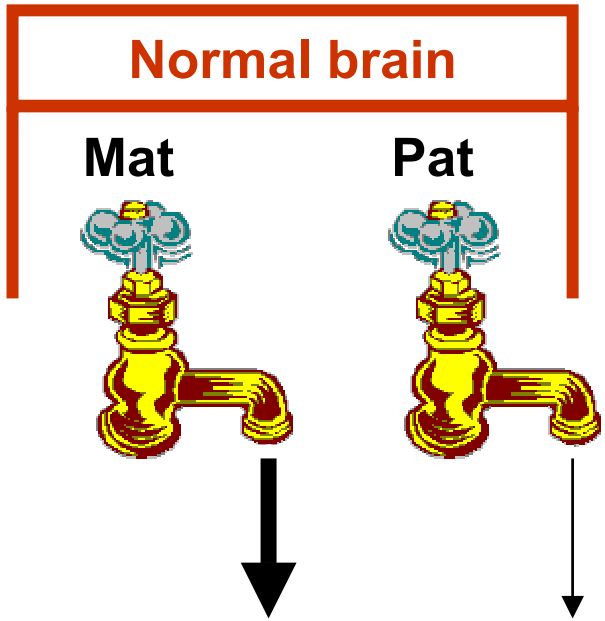
Brain = unmeth/meth
Other = meth

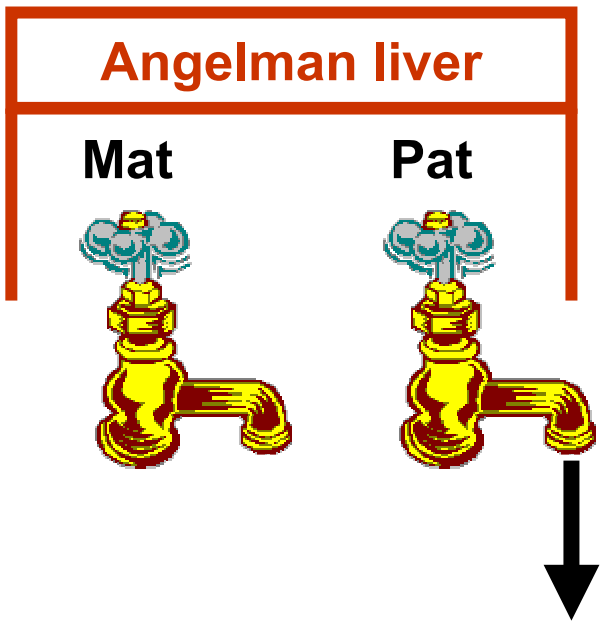


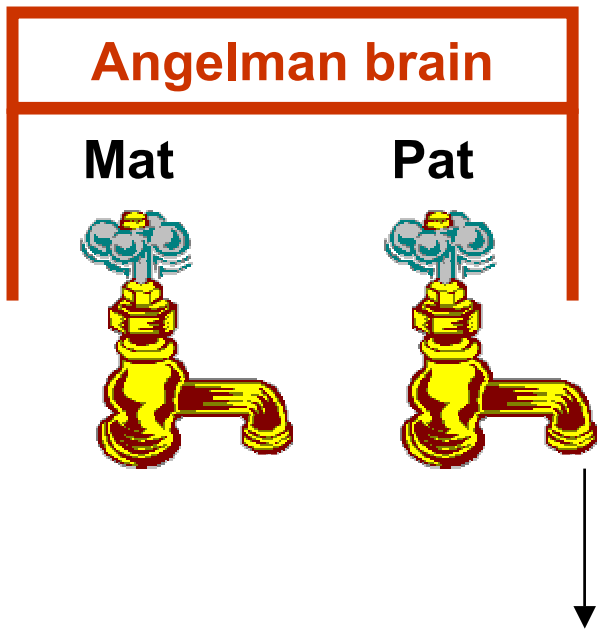
1 or 2 of 11
autism brains
abnormal vs 0/60
control brains

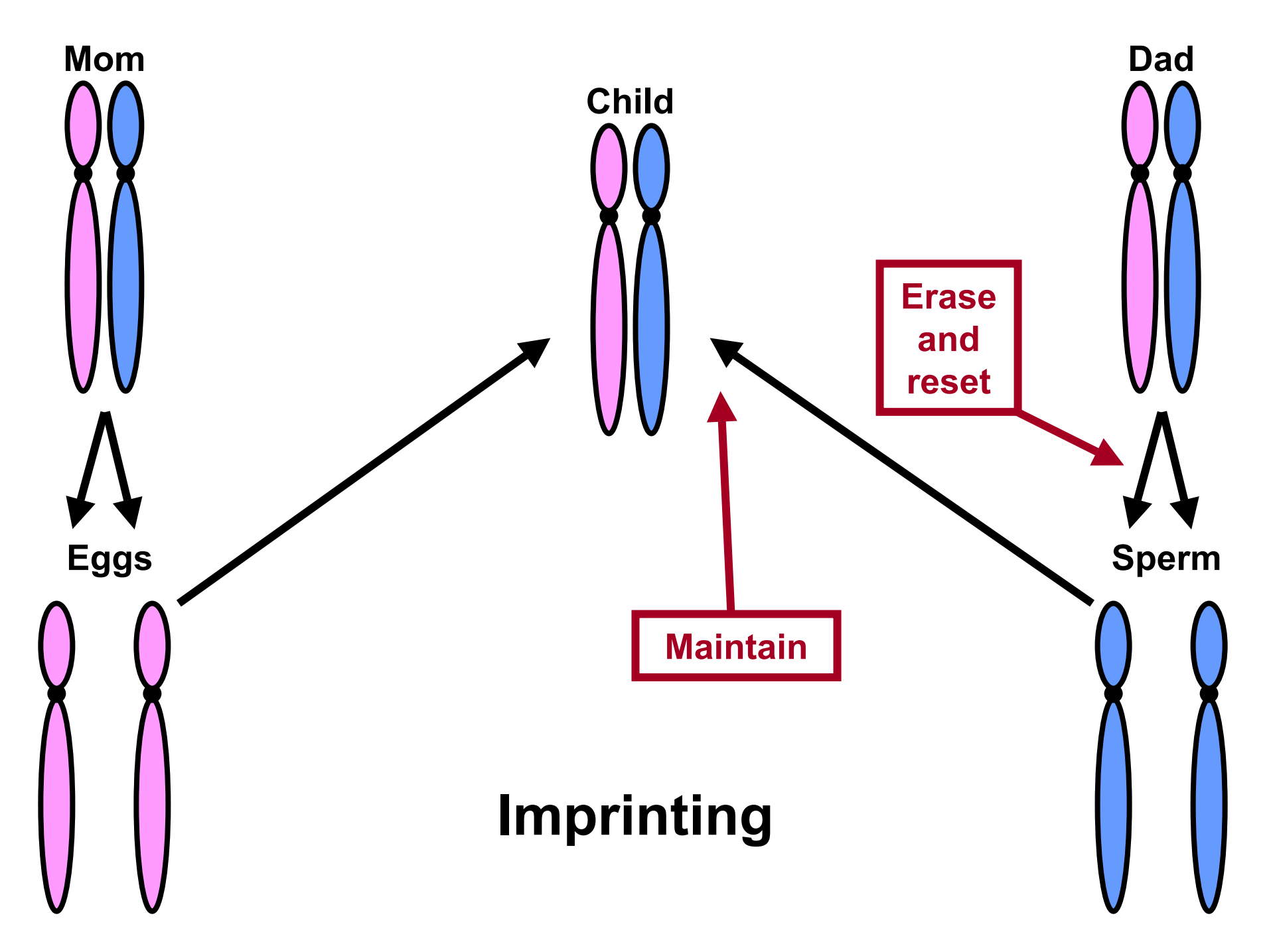
Yong-hui Jiang

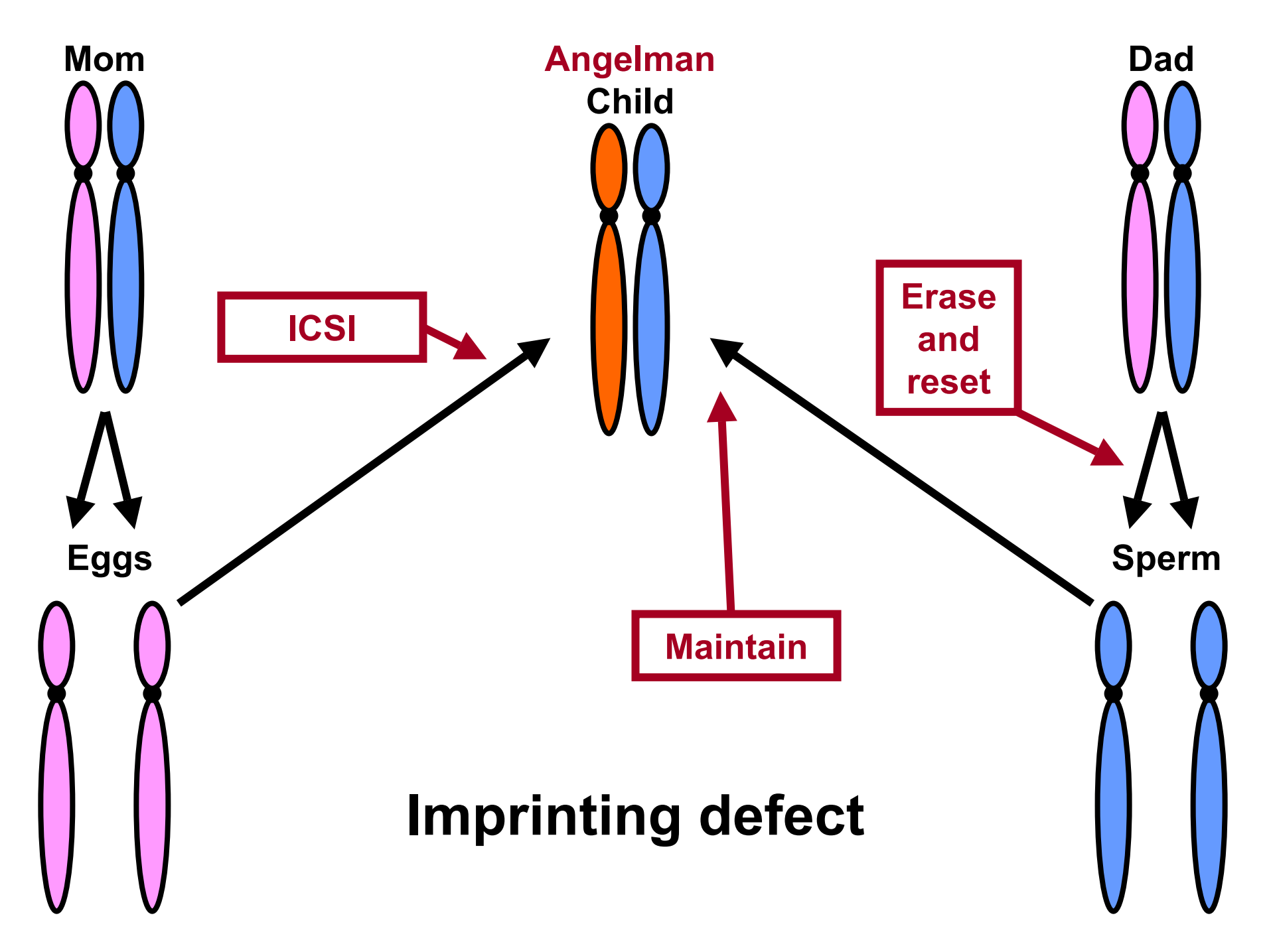

Normal liver
Mat
Pat

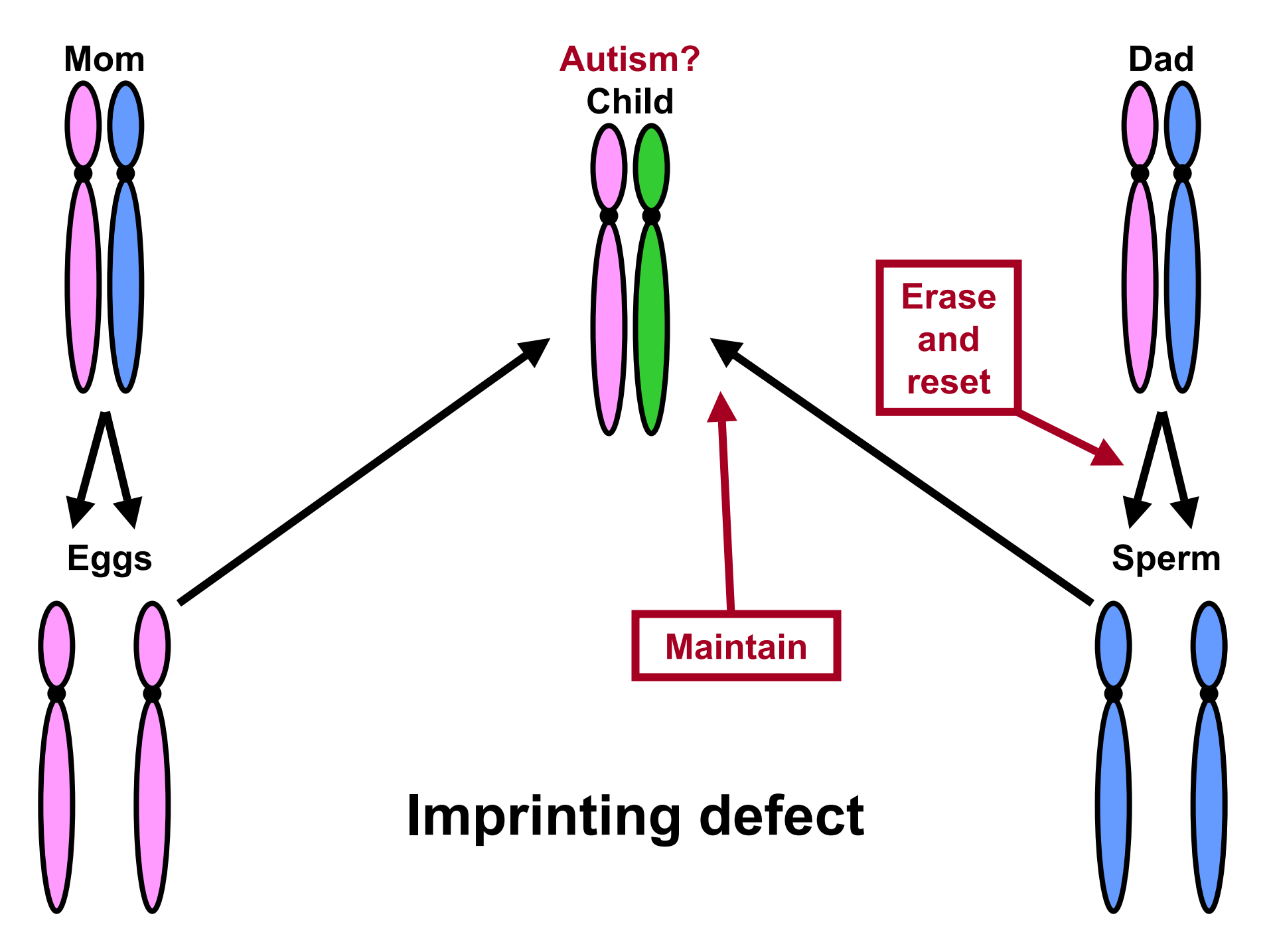

Normal brain
Mat
Pat


Angelman liver
Mat
Pat

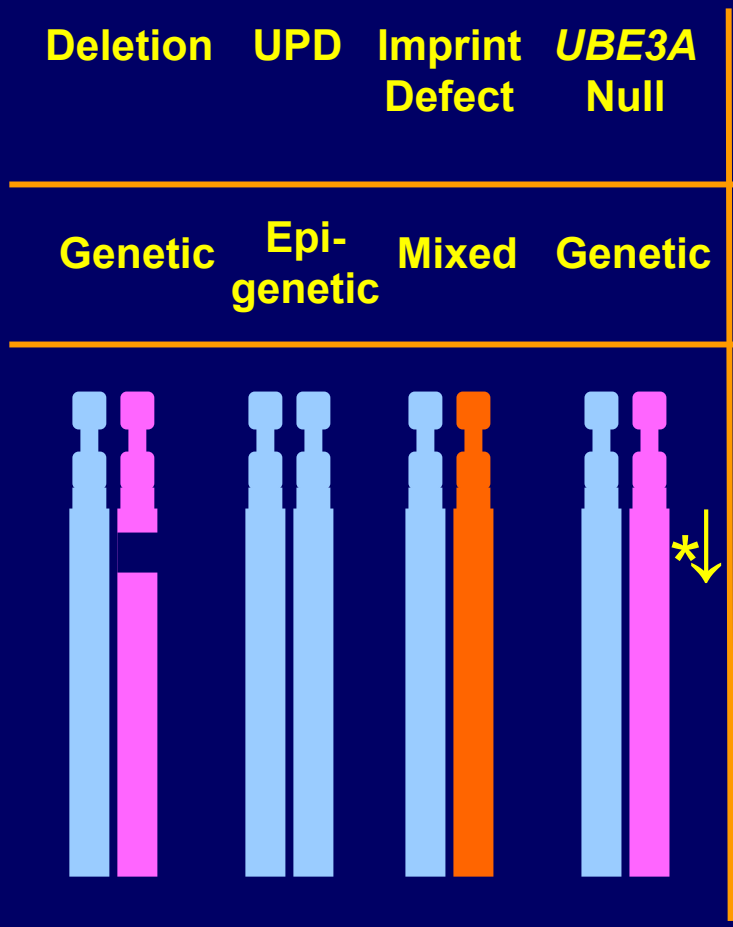

Angelman brain
Mat
Pat


Mom
Child
Dad
Erase and reset
Eggs
Sperm
Maintain
Imprinting


Mom
Angelman
Child
Dad
ICSI
Erase
and
reset
Eggs
Sperm
Maintain
Imprinting defect


Mom
Autism?
Child
Dad
Erase and reset
Eggs
Sperm
Maintain
Imprinting defect

Angelman



An epigenetic defect can give the same phenotype as a genetic defect

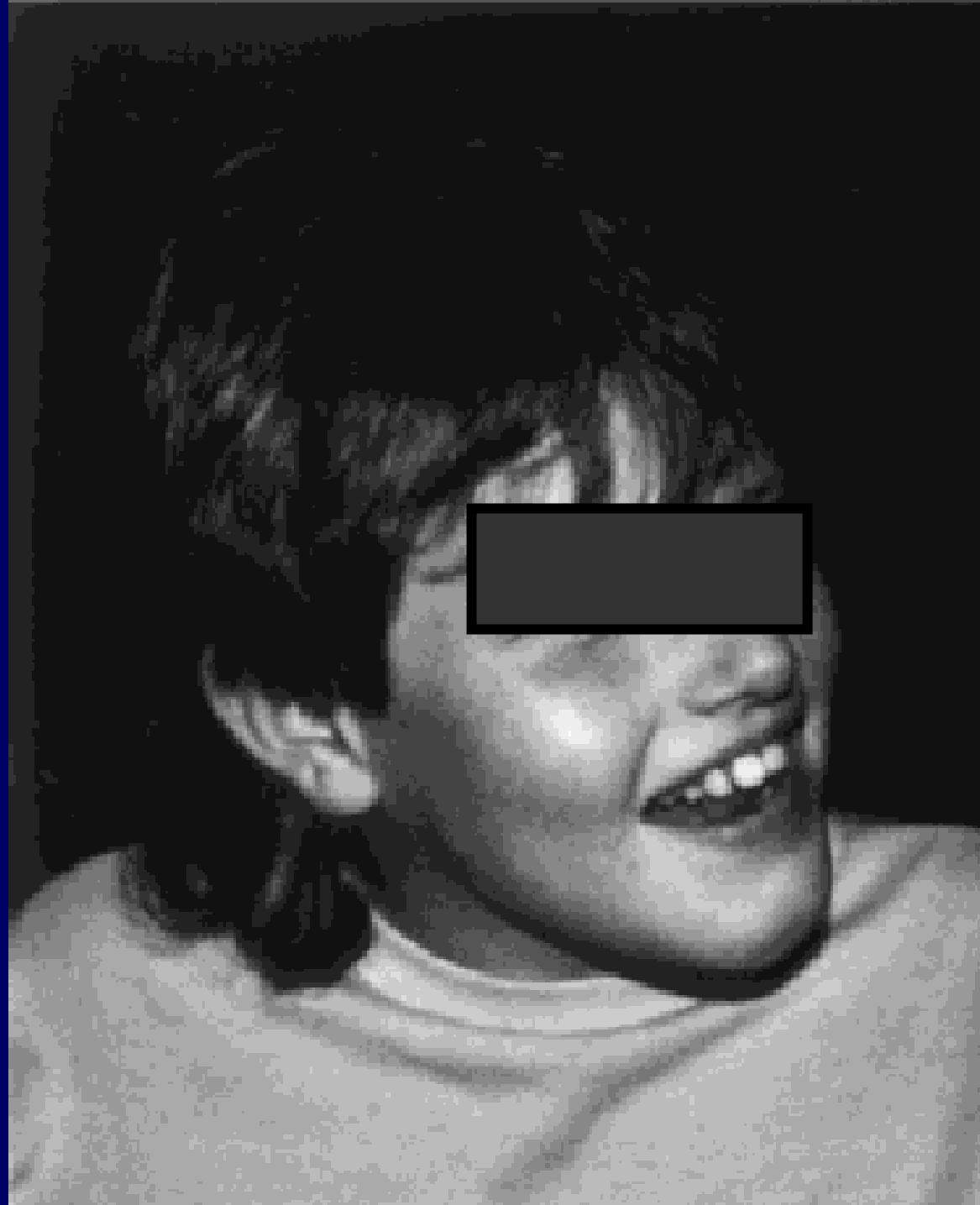
Heterogeneity of types of defects causing one phenotype

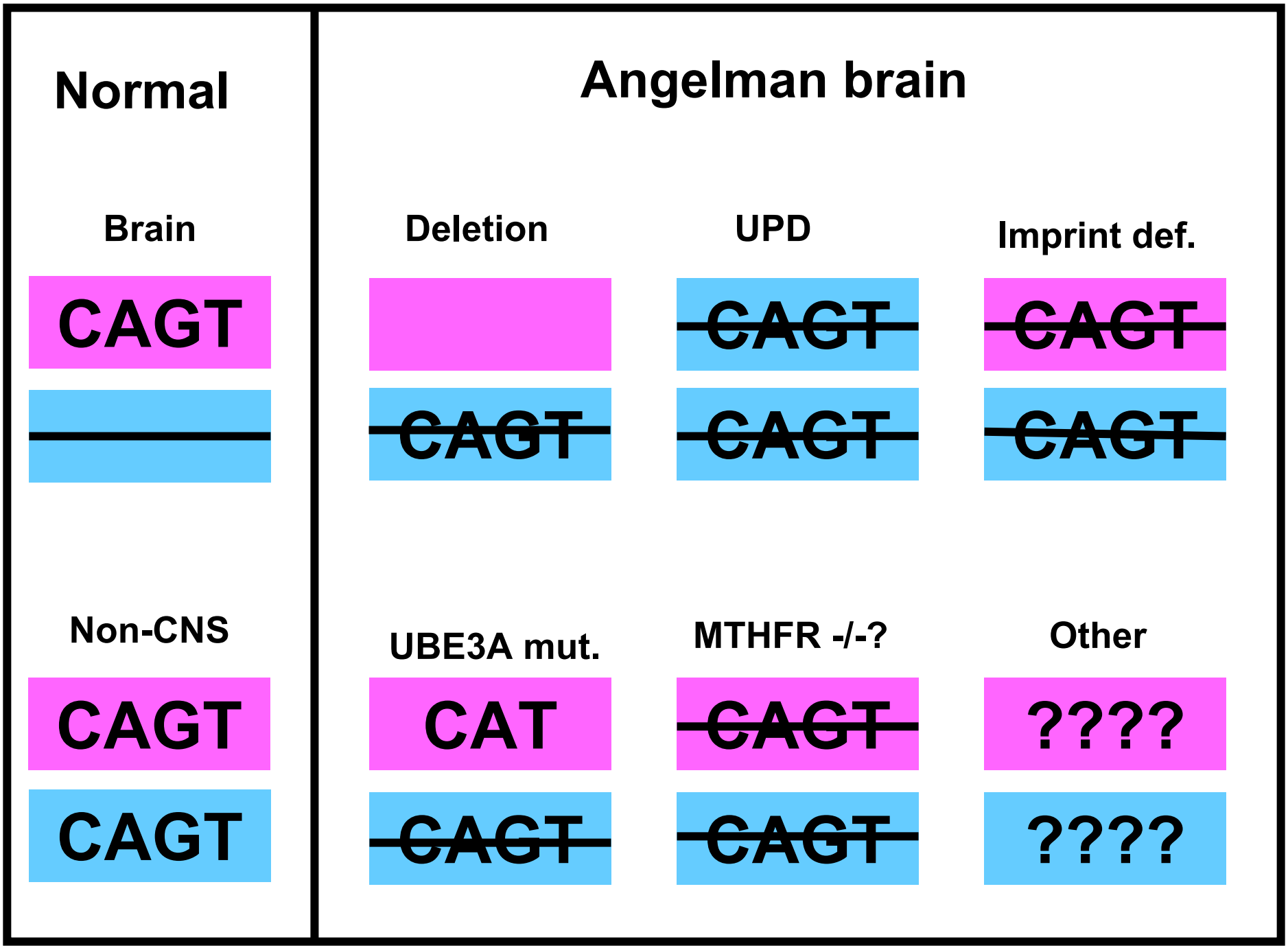
MTHFR deficiency in a patient with typical AS but no identifiable defect.

Arn et al., 1998

PMID 9605586

Does *MTHFR* deficiency silence (maternal) *UBE3A*?




Normal
Angelman brain
Brain
Deletion
UPD
Imprint def.
CAGT
CAGT
CAGT
CAGT
CAGT
CAGT
CAGT
Non-CNS
UBE3A mut.
MTHFR -/-?
Other
CAGT
CAGT
CAT
CAGT
CAGT
CAGT
????
????

AUTISM (NARROW) AND AUTISM SPECTRUM DISORDER (BROAD)

- A neurological or brain disorder that profoundly affects a person's ability to communicate, form relationships with others and respond appropriately to the environment.
- Look perfectly normal.
- Abnormal behaviors such as hand flapping.

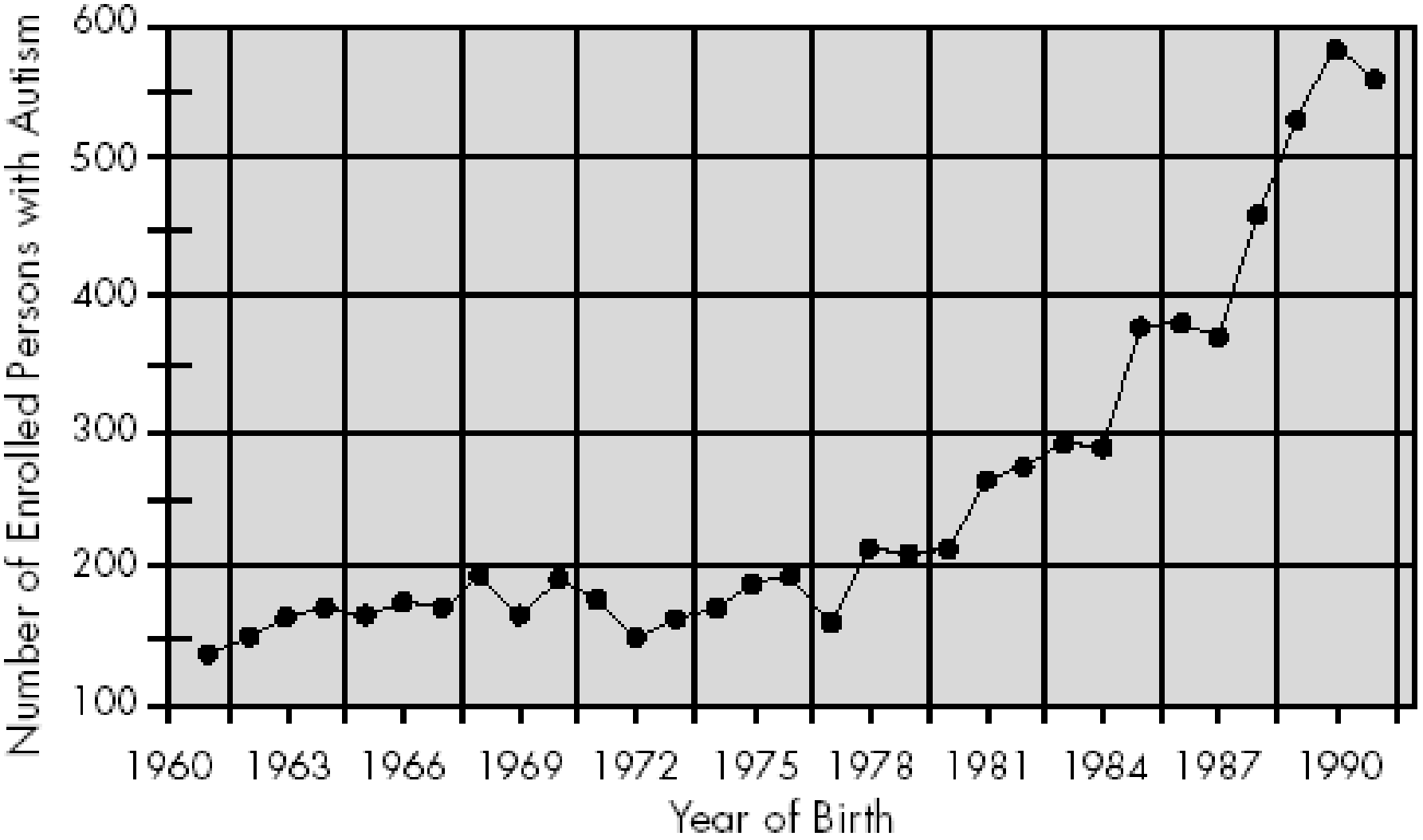
GENETIC AND OTHER FACTORS IN AUTISM?

- Incidence 10-15 in 10,000 (16-24 in 10,000 males; 4 to 6 in 10,000 females)
- Male predominance (4:1 ratio)
  - Unknown
- High concordance MZ twins but low in DZ twins
  - 10-20 loci?
- Association with higher mat. education
  - Unknown

AUTISM, 15q11-q13, AND GENOMIC IMPRINTING

- Maternal but not paternal dup15q11-q13 cause autism; often maternally inherited
- Inv dup 15q causing autism are always of maternal origin
- Evidence for genomic imprinting & parent of origin effect
- Nurmi et al., linkage disequilibrium with D15S122 at 5′-*UBE3A*
- Shao et al., LOD 4.71 at *GABRB3* using ordered-subset analysis (OSA); 5′ of *UBE3A;* maternal sharing

Figure 1. *Distribution of birth dates of regional center eligible persons with autism*



(from "Changes in the Population of Persons with Autism and Pervasive Developmental Disorders in California's Developmental Services System: 1987 through 1998")

Report to CA legislature M.I.N.D. Institute Oct. 17, 2002

- Without evidence for an artificial increase in autism cases, we conclude that some, if not all, of the observed increase represents a true increase in cases of autism in California, and the number of cases presenting to the Regional Center system is not an overestimation of the number of children with autism in California.
- http://www.dds.cahwnet.gov/autism/mindreport.cfm

Fombonne JAMA Jan. 2003 / PMID 12503982

- **“Therefore, from available evidence it can be concluded that recent rates for both ASD (autism spectrum disorder) and autism disorder are 3 to 4 times higher than 30 years ago.”**

Fombonne JAMA Jan. 2003 / PMID 12503982

- Unless comparisons also control rigorously for changing case definitions, interpretation of differences in prevalence rates over time and across surveys will be virtually impossible.
- Moreover, there is strong evidence that differences in methods for case finding can account for a huge proportion of the variability of prevalence estimates between surveys.
- Claims about an epidemic of autism and its putative causes have the most weak empirical support.

High concordance MZ twins but low in DZ suggests de novo factor

Disorder	MZ	DZ
Down syndrome	100%	<5%
Achondroplasia and Rett de novo	100%	nil
Autism narrow	~60%	nil
Autism broad	~90%	~10%
De novo gametic or preMZ imprinting defect	100%	<5%

THE USUAL GENETIC HYPOTHESIS FOR AUTISM

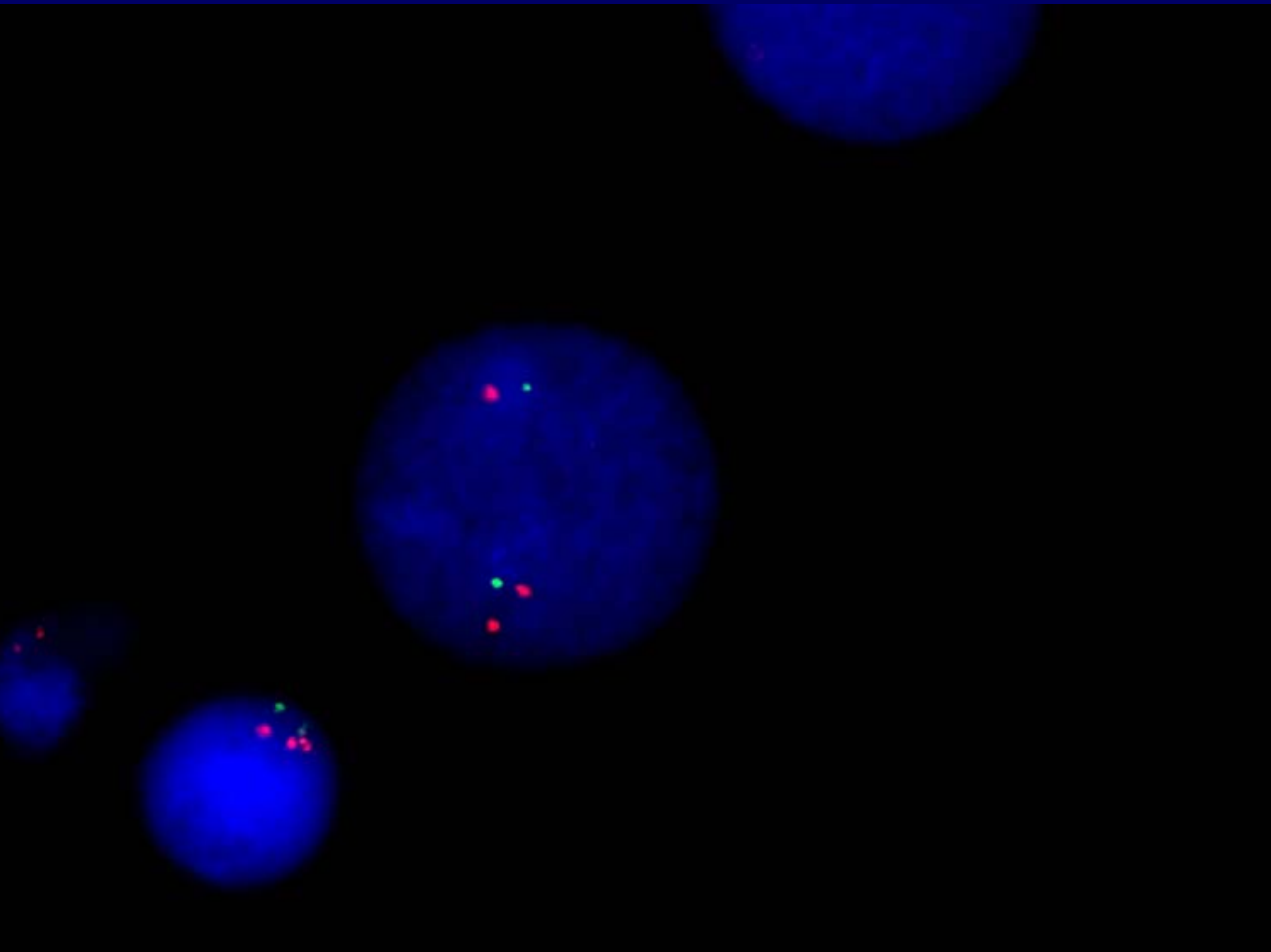
- Risch et al. *Am J Hum Genet* 65:493,1999. A genomic screen of autism: Evidence for a multilocus etiology.
- "These results are most compatible with a model specifying a large number of loci (perhaps ≥15) and are less compatible with models specifying ≤10 loci."

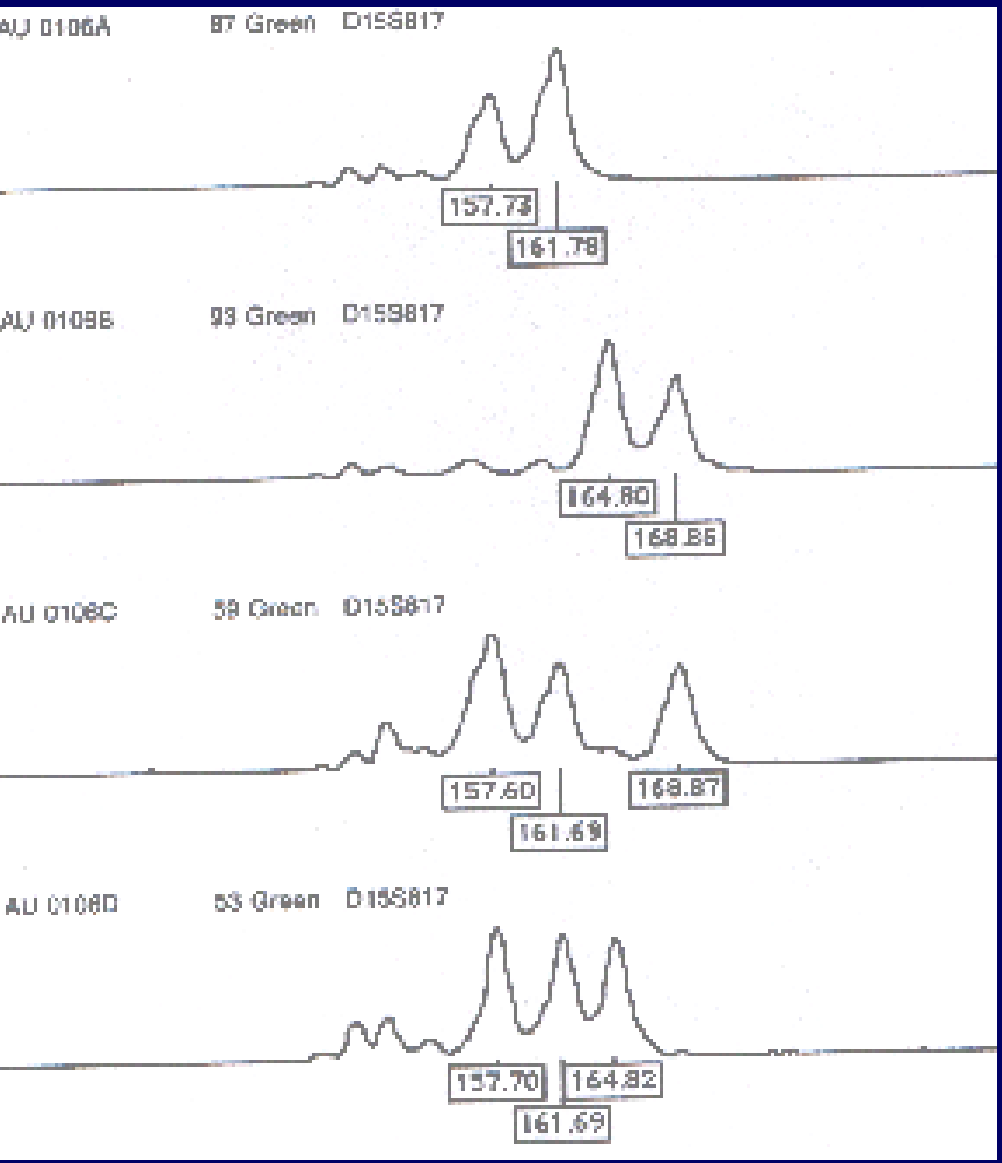
ALTERNATIVE HYPOTHESIS

- Autism is an oligogenic disorder (perhaps even one major locus with modifiers) caused in most cases (e.g., singleton families) by de novo genetic or epigenetic defects arising in germ cells or the early embryo (prior to MZ twinning).
- Over-expression of *UBE3A* may be the unifying pathophysiology; the major gene?

EVIDENCE

- Mat. but not pat dupes 15q cause autism
- Increased sharing of parental alleles in affected sib pairs
- Tissue specific DNA methylation in 15q
- Abnormal DNA methylation in 1-2 of 11 autism brains
- Hypomorphic allele for *MTHFR* may be protective



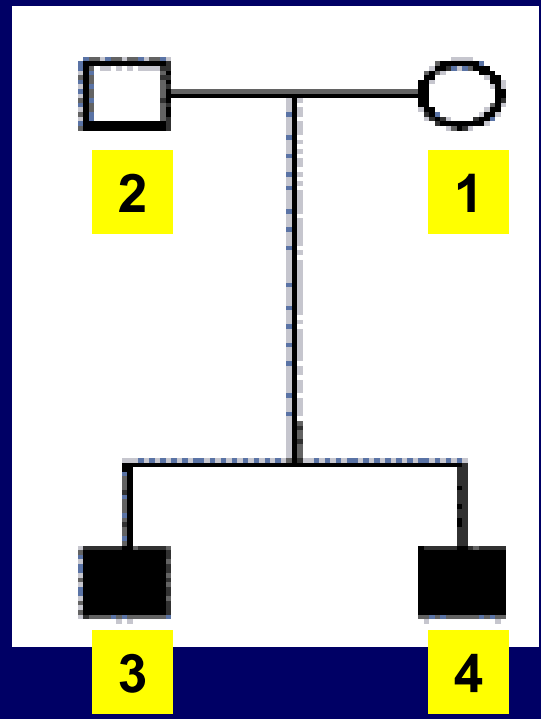


1

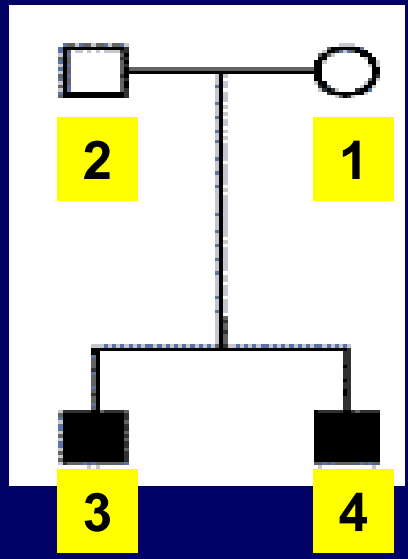
2

3

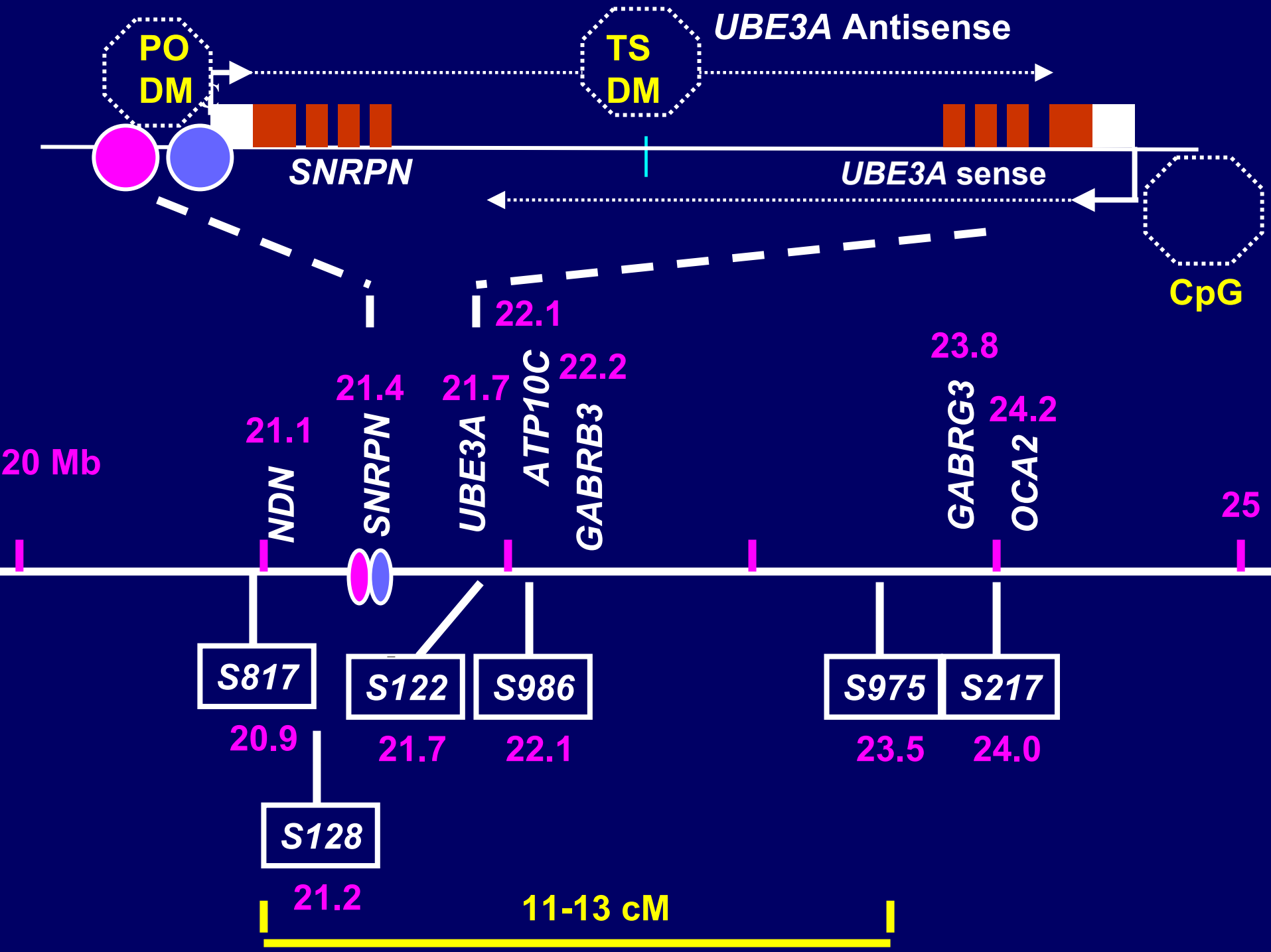
4

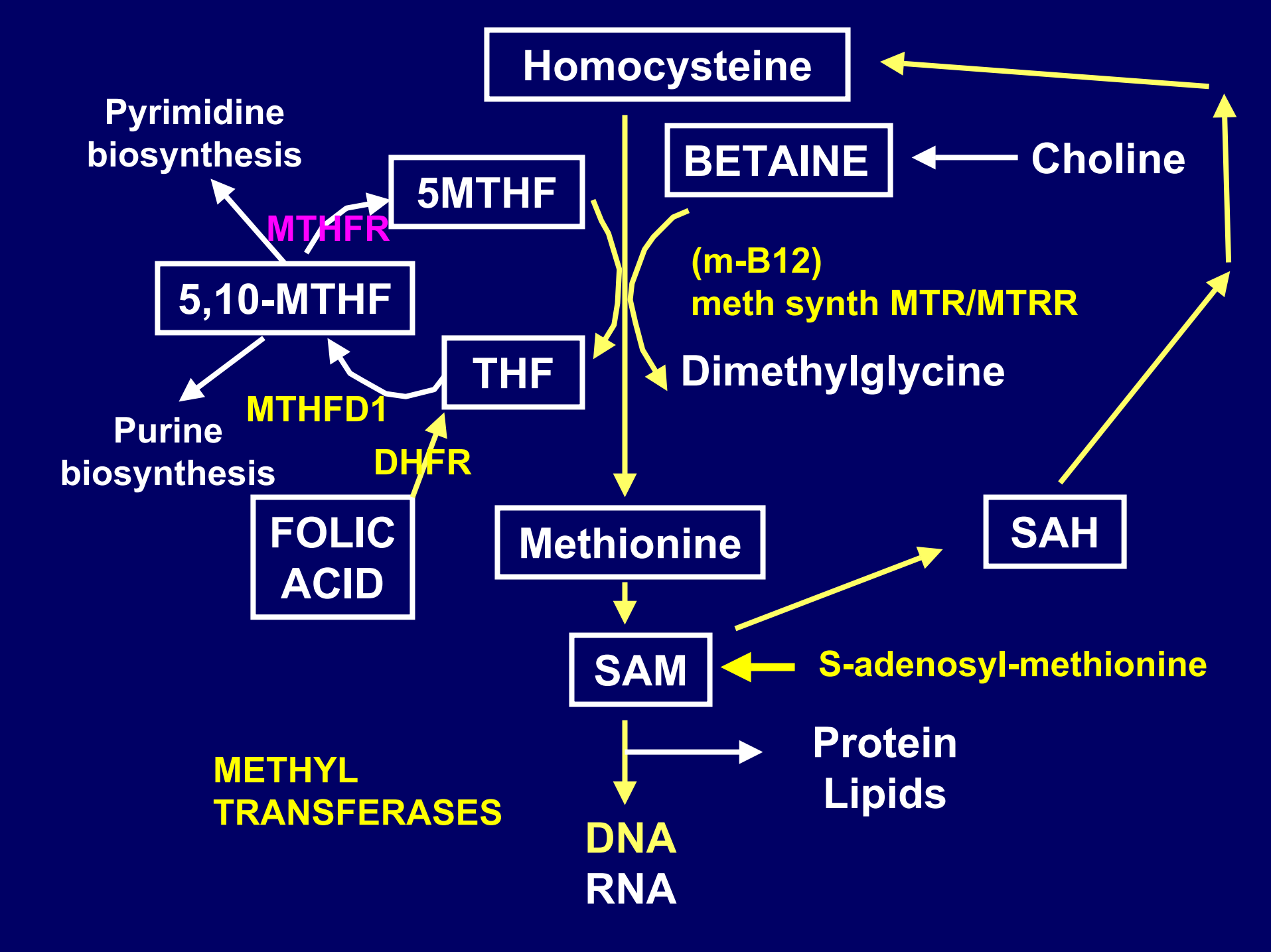


D15S817



C C C 1 2 3 4 C C


PO
DM
TS
DM
UBE3A Antisense
SNRPN
UBE3A sense
CpG
22.1
21.4
21.7
22.2
23.8
21.1
24.2
20 Mb
NDN
SNRPN
UBE3A
ATP10C
GABRB3
GABRG3
OCA2
25
S817
S122
S986
S975
S217
20.9
21.7
22.1
23.5
24.0
S128
21.2
11-13 cM


Homocysteine
Pyrimidine biosynthesis
BETAINE
Choline
5MTHF
MTHFR
(m-B12)
meth synth MTR/MTRR
5,10-MTHF
THF
Dimethylglycine
MTHFD1
Purine biosynthesis
DHFR
FOLIC ACID
Methionine
SAH
SAM
S-adenosyl-methionine
Protein
Lipids
METHYL TRANSFERASES
DNA
RNA

High folic acid

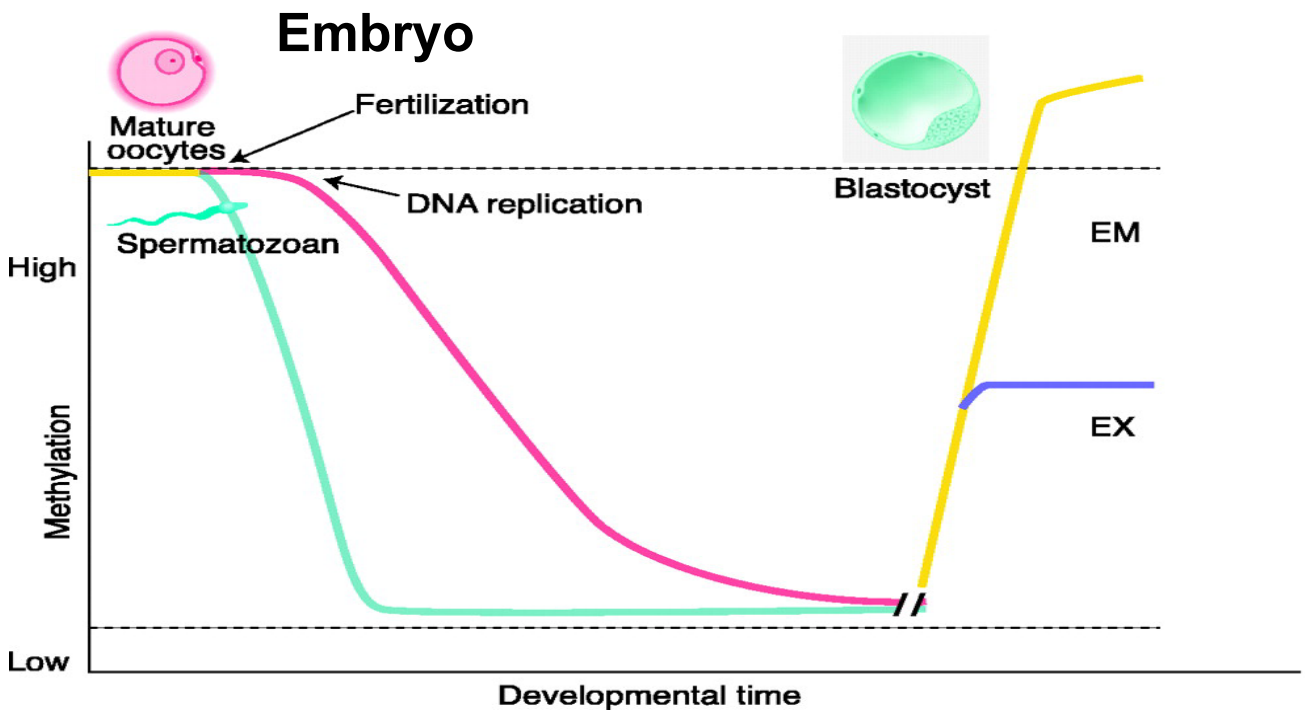
Low folic acid

methylation

Wolff *et al.*, *FASEB J* 1998; 12:949-957

MTHFR AND FOLATE

- Use transmission disequilibrium test (TDT) to avoid matched control problems and allow use of parent child trios
- Is the hyopmorphic V allele of *MTHFR* protective for autism?



From Reik et al. Science 293:1089,2001

Buiting et al. Am J Hum Genet 2003 / PMID 12545427

Table 3

Grandparental Origin of the Chromosome Carrying the Imprinting Defect

	AS		PWS	
Origin	IC Mutation	No IC Mutation	IC Mutation	No IC Mutation
Maternal:				
Grandfather	5[a]	7	0	0
Grandmother	1[a]	11	0	0
Paternal:				
Grandfather	0	0	1[b]	0
Grandmother	0	0	4[a]	19[c]

TESTABLE HYPOTHESIS

- **Autistic children more often inherit paternal 15q11-q13 from their grandmother than from their grandfather because the need to switch imprint increases risk of an imprinting defect.**

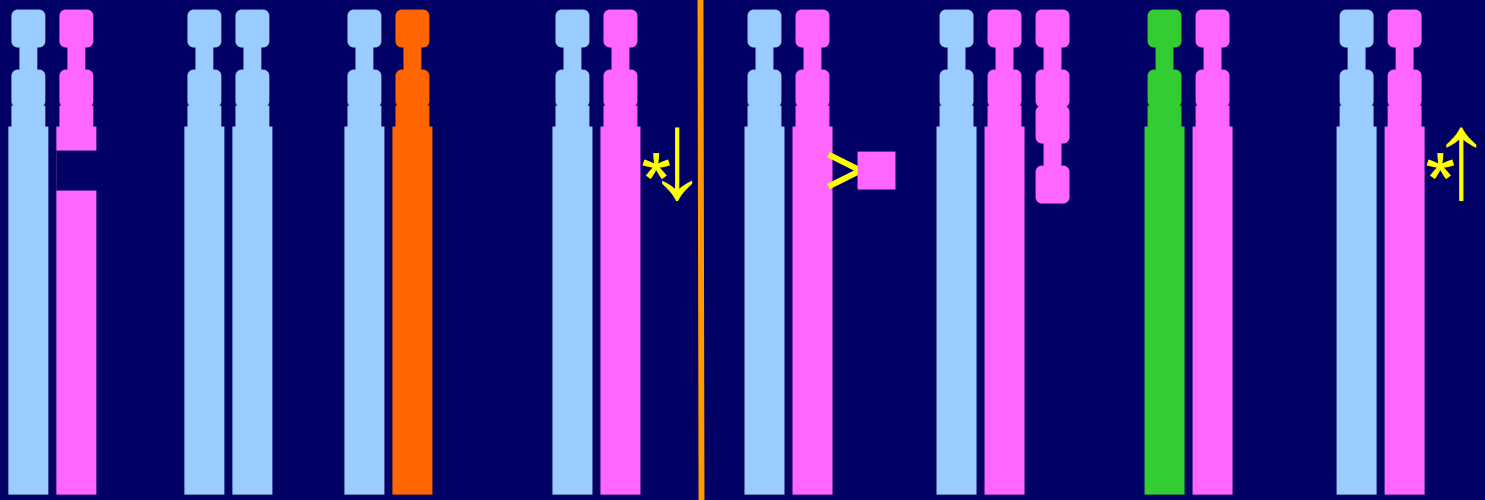
PGEMN HYPOTHESIS / MODEL

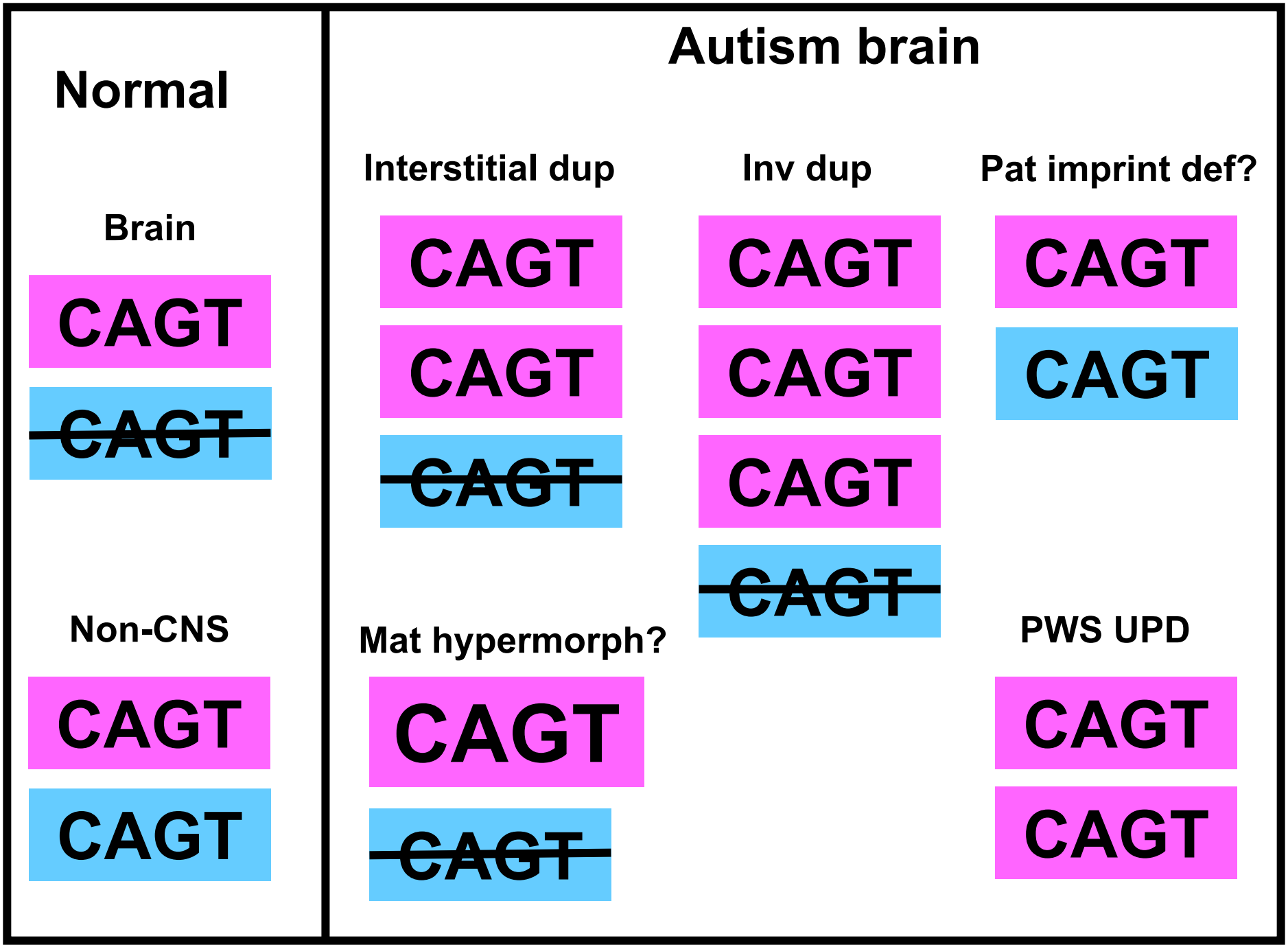
- Unpublished model for autism presented

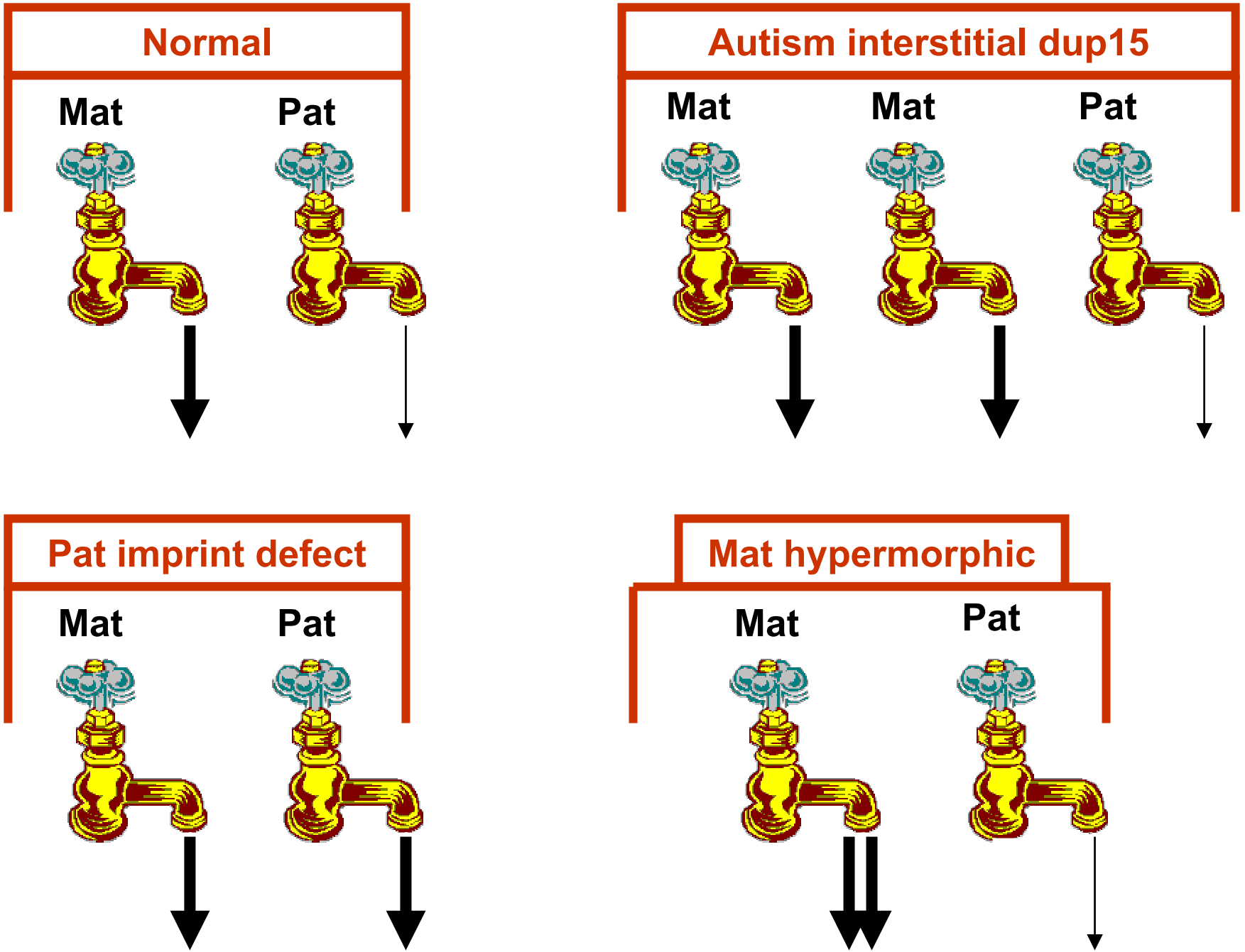
Angelman

Autism?

Deletion	UPD	Imprint Defect	*UBE3A* Null	Duplic-ation	Isodi-centric	Imprint Defect?	*UBE3A*? Hypermorph
Genetic	Epi-genetic	Mixed	Genetic	Genetic	Genetic	Mixed?	Genetic?




Normal
Brain
CAGT
CAGT
Non-CNS
CAGT
CAGT
Autism brain
Interstitial dup
CAGT
CAGT
CAGT
Inv dup
CAGT
CAGT
CAGT
CAGT
Pat imprint def?
CAGT
CAGT
Mat hypermorph?
CAGT
CAGT
PWS UPD
CAGT
CAGT


Normal
Mat
Pat
Autism interstitial dup15
Mat
Mat
Pat
Pat imprint defect
Mat
Pat
Mat hypermorphic
Mat
Pat

ICSI AND IMPRINTING

- 19 Hits in PubMed
- 1995 – Theoetical concerns
- 1998 – Yanagimachi: success in mouse even with round spermatids and secondary spermatocytes
- 1998 – Steirteghem: no imprinting defects found in first 165 cases
- 2000 - Steirteghem: normal DNA methylation at 15q11-q13 normal in 95 children

ICSI AND IMPRINTING

- 2002 – Horsthemke: two cases of Angelman "sporadic" imprinting defect
- 2003 – 6 of 149 Beckwidth-Weidemann (3 ICSI & 3 IVF) vs expected 1.7; *P* ~ 0.01; similarity to large offspring syndrome.
- 2003 – Another case of AS and ICSI
- About 50 % of ART uses ICSI at present.

RECOGNITION

PWS/AS/Autism

- Trilochan Sahoo
- Yong-hui Jiang
- Jan Bressler
- Dani Bercovich

FISH

- Lisa Shaffer
- Cathy Kashork

AGRE

NIMH/Stanford

Genotyping

- Igne Buyse
- David Stockton
- Ben Roa

Greenwood SC (SCAP)

- Roger Stevenson
- Ron Michaelis
- Dick Schroer

Statistical analysis

- Richard Speilman