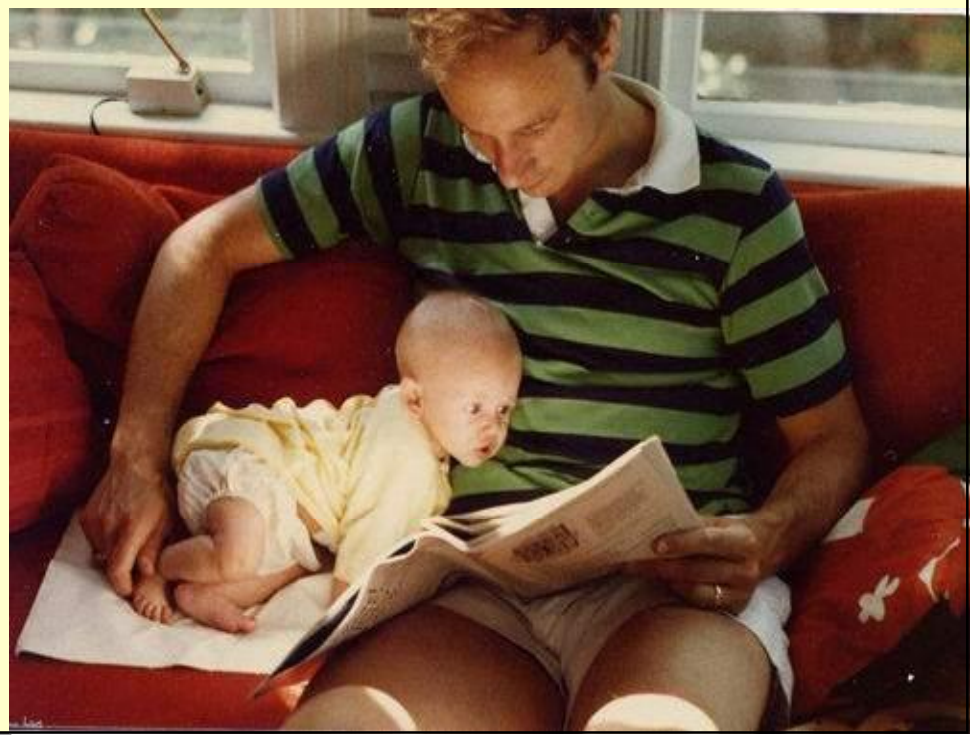
The Search for Genes Contributing to Specific Reading Disabilities and ADHD: What do we know and what do they tell us?

Dr. Cathy Barr

Senior Scientist
The Hospital for Sick Children
and
The Toronto Western Hospital

Associate Professor
Department of Psychiatry, University of Toronto



Family Studies of RD

- Thomas 1905
- Fisher 1905
- Hallgren 1950
- Finucci 1976
- Pennington 1991
- Schulte-Korne 1996

•Reading and Spelling difficulties are clearly familial

•Family studies cannot disassociate genetic and environmental factors in families

Twin Studies

- Compares identical twins (monozygotic) to fraternal twins (dizygotic)
- If MZ twins share a trait more than DZ twins provides evidence for a genetic component
- twin studies can dissect the variance of the phenotype into genetic, common environment, and non-shared environment (which also includes measurement error)

Twin Studies

- Early twin studies compared concordance rates in MZ vs DZ twins using a categorical cut off
- Heritability estimates of approximately 30 to 70% for reading or spelling disabled twins

DeFries et al., 1987
DeFries et al., 1993
Wadsworth et al., 2000
Stevenson et al., 1987
Gayan and Olson 2001
Harlaar 2005

Twin Studies of Reading Ability

More recent studies have investigated reading ability and components of reading

	genetic	shared environment	non-shared environment
Word Recognition	.45	.49	.06
Orthographic coding	.58	.20	.22
Phonological Decoding	.61	.24	.15
Phonological Awareness	.56	.24	.20

Multivariate Analyses

Multivariate analyses indicate there will be common as well as independent genes contributing to these skills

	genetic correlation
Word Recognition -- decoding	.99
Orthographic coding – word recognition	.81
Phonological Decoding -- Orthographic coding	.73
Phonological Awareness -- Decoding	.67
Phonological Awareness -- Orthographic coding	.28

Environmental Factors

- Significant influence of shared (20-45%) and non-shared environmental factors (10%)

- Education
- English as a second language
- Print exposure
- Education of the parents
- Quality of the school?

Gene by Environmental effects

Parental environment

- parents with reading disabilities will be less likely to read to children

Child self selecting environment

- print exposure influences reading ability
- poor readers will avoid reading

Genetics

- genes do not operate in vacuum
- environment determines their expression
- the DNA structure does not change with environmental influences but the expression does
- plasticity is influenced by genes
- the ability to respond to environmental stimuli is often genetically influenced

Steps in a molecular genetic study

1) Design of study and assessment

2) Extensive assessment of subjects and families

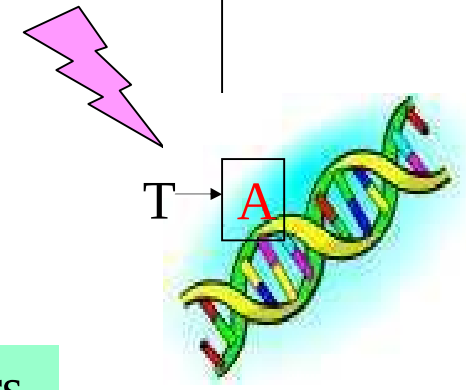
3) Isolate DNA from blood or cheek swab sample

4) Identify and genotype polymorphisms for the gene or area of interest

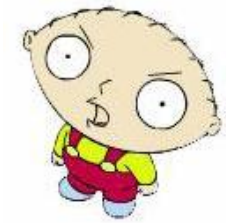
5) Look for sharing of chromosome regions in families comparing individuals with and without the phenotype -- **linkage studies**

6) Determine if there are shared DNA variants in the people with the phenotype compared to without (sharing of genes in the population) -- **association studies**

How it works



DNA change occurs that results in change of gene function and change in phenotype



DNA variation

Mutation --

Minor allele frequency less than 1%

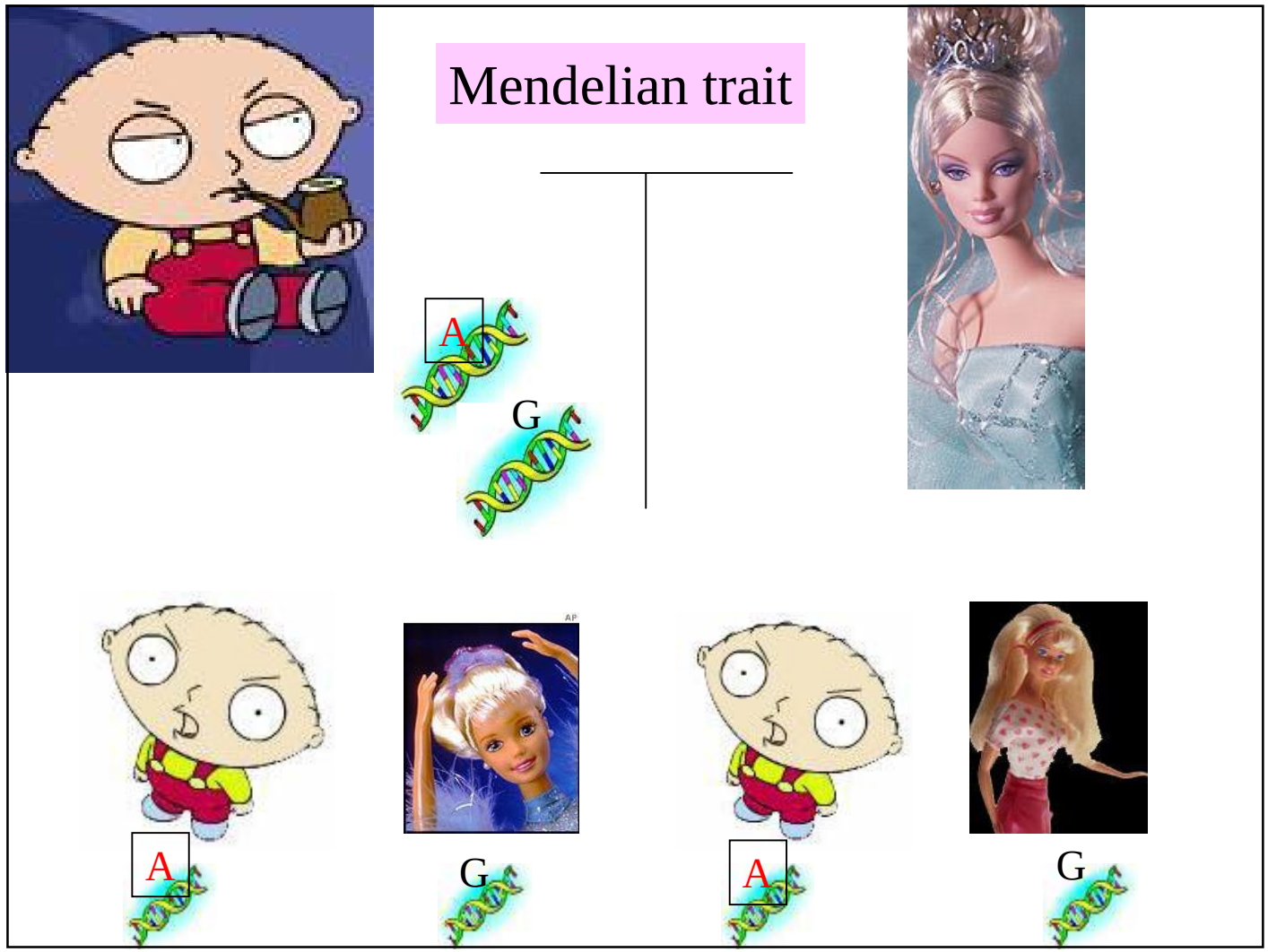
Polymorphism --

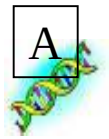
Minor allele frequency greater than 1%

Does not indicate function

Mutation can become a polymorphism

The same DNA change can be a mutation in one population and a polymorphism in another





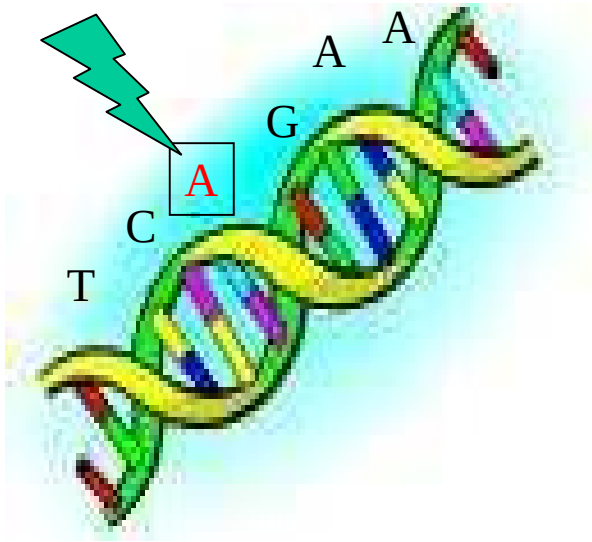
Complex Traits

- Incomplete penetrance
- Mode of inheritance
  (dominant, recessive, additive)
- Interaction of other genes
- Gender effects
- Environmental influences

DNA Markers

Functional DNA change usually unknown.

Inheritance of chromosome tracked with surrounding polymorphic markers



An identifiable physical location on a chromosome (e.g., restriction enzyme cutting site, gene, microsatellite) whose inheritance can be monitored.

Candidate Gene Studies

selection of candidate genes

~~**animal models**~~
~~**pharmacological interventions**~~
biology → **brain expressed**

related disorders → ADHD language impairment

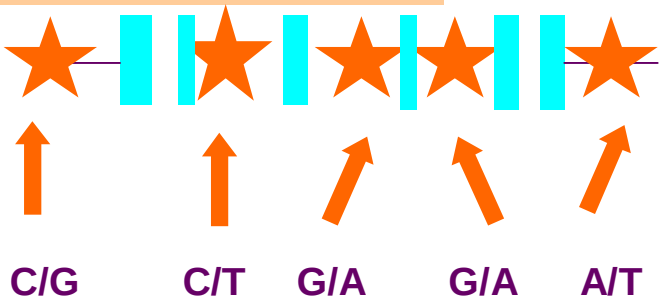
↓

candidate genes in linked regions

Candidate Gene Studies

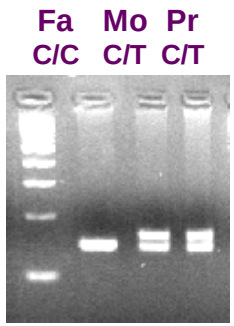
select candidate gene

- ~~animal models~~
- ~~pharmacological interventions~~
- biology
- related disorders



identify polymorphisms (common DNA changes)

genotype multiple polymorphisms across the gene



statistical analysis

Genome Scan

D19S229
D19S247
D19S204
D19S221
D19S179
D19S248
D19S178
D19S246
D19S180
D19S254

Systematically screen all of the chromosomes for linkage using DNA markers spaced at regular intervals

Issues for Genetic Studies -- Heterogeneity

population studied ethnic differences

- **linkage disequilibrium between marker and functional variant**

population studied phenotypic differences

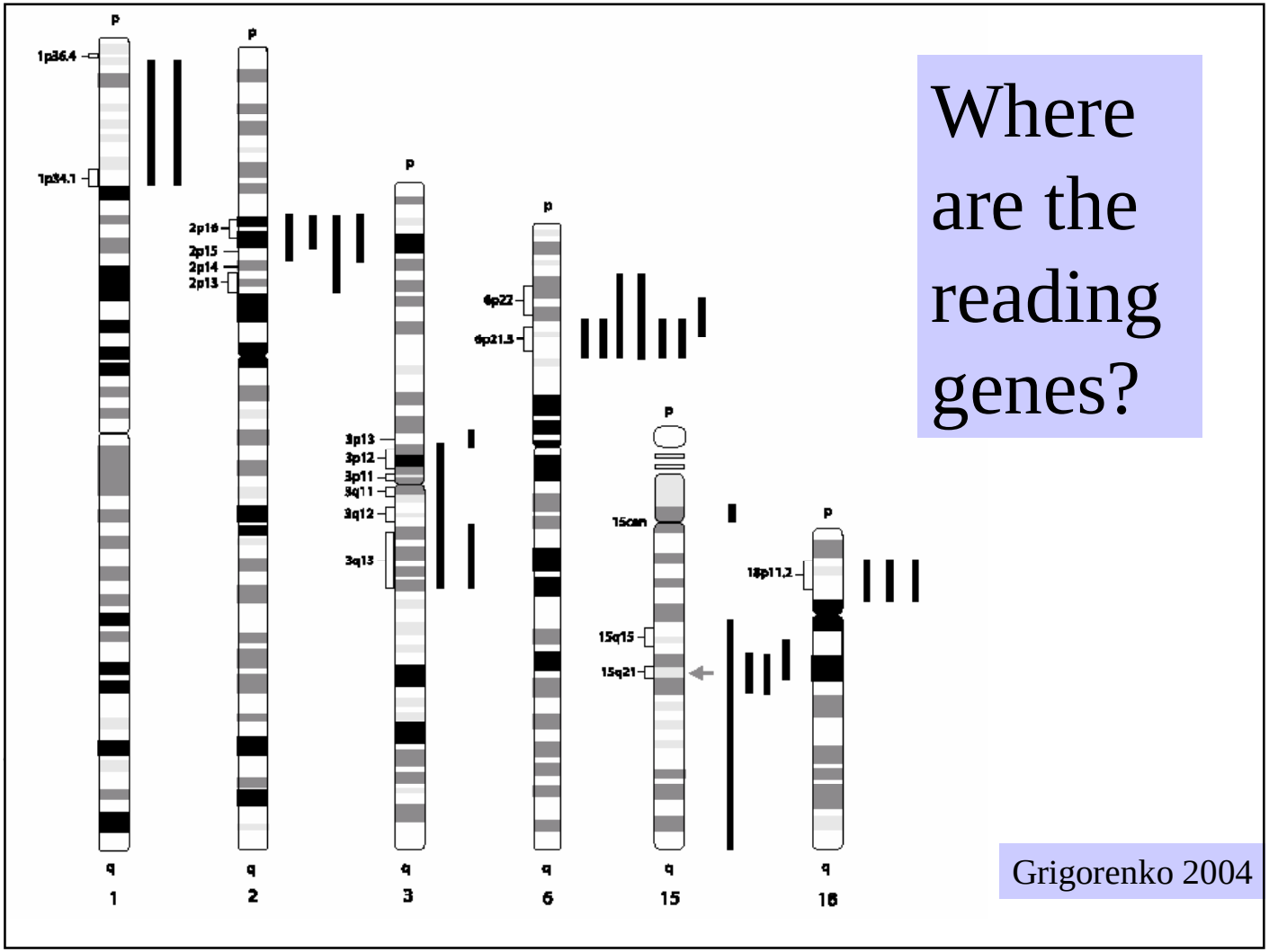
- **ascertainment**
- **inclusion/exclusion criteria**
- **comorbidity**

Issues for Genetic Studies -- Power

All studies thus far have used relatively small sample sizes

- *** false positive
- *** false negatives

Genes of small effect may not be identified in these samples



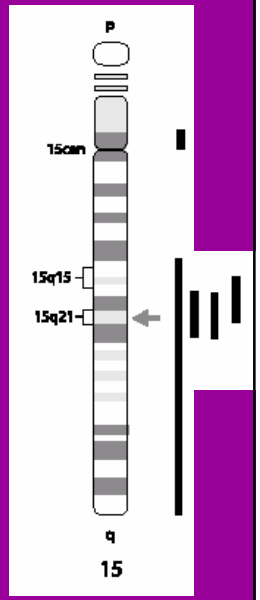
Where are the reading genes?

Grigorenko 2004

Linkage Findings for Reading Disabilities on 15q15-21

Studies reporting linkage/association for RD or spelling to 15q region

Smith et al., 1983
Grigorenko et al., 1997
Schulte-Korne et al., 1998
Morris et al., 2000
Chapman et al., 2004
Smith 2005 linked to Speech Sound Disorder



Studies not finding evidence for linkage

Bisgaard et al., 1987
Rabin et al., 1993
Cardon et al., 1994
Fisher et al., 2002

What are the Reading Genes?

Despite replicated findings for linkage (sharing of chromosomal regions in affected family members), until recently there have been no genes identified as contributing to RD

Is *EKN1* the first gene for RD?

EKN1 (or ENK1 to the Dyslexic)

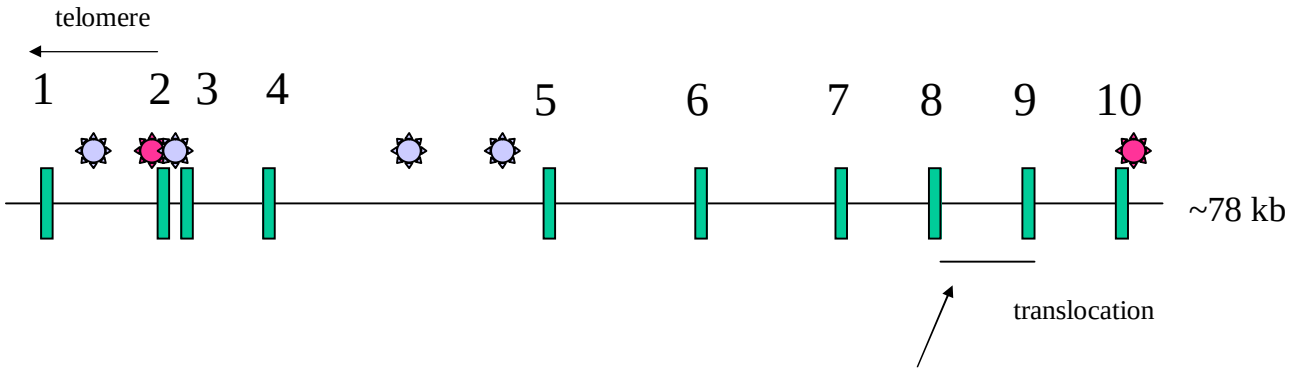
Taipale et al., 2003 PNAS 100(20):11553-8

What is EKN1?

"En Keksi Nimeä 1", translated into English approximately "Can't Name It 1"

Gene of unknown function

What is the evidence that EKN1 is a gene for RD?



- translocation t(2;15)(q11;q21) cosegregates with dyslexia in one Finnish family (father and 3 kids)
- identified breakpoint in gene of unknown function, EKN1, between exons 8 & 9
- Conclude that -3A and/or 1249T are contributing to RD

EKN1 Expression -- everywhere

Taipale et al., 2003
heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, leukocytes

NCB1 UniGene

cDNA sources: pooled ; Pituitary ; Testis ; head_neck ; pooled germ cell tumors ; carcinoma, cell line ; testis ; glioblastoma (pooled) ; glioblastoma ; hypernephroma ; Trabecular meshwork ; lung ; kidney ; pooled colon, kidney, stomach ; Stomach ; Primary Lung Epithelial Cells ; adenocarcinoma ; Liver ; melanotic melanoma ; Purified pancreatic islet ; Human Lung Epithelial cells

Homology

Listed below are the nucleotide sequence comparisons used in determining homology.The pairs below represent reciprocal best hits; each alignment is the best one for both organisms. The percent ID below represents identity over an aligned region.

Organism-Gene	Organism-Gene	PercentID
H.sapiens -EKN1	M.musculus - 1700010I24Rik	82.8
H.sapiens -EKN1	C.intestinalis - Cin.5317 (sea squirts)	74.1
H.sapiens -EKN1	Danio rerio - Dr.15037 (zebrafish)	70.7

	differs from humans by ## AA
chimps	3
pygmy chimps	2
gorilla	5
orangutan	6

Toronto Genetics of Reading Disabilities Project

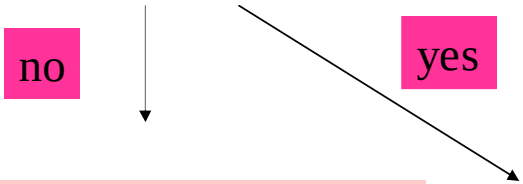
Year 5 of a CIHR funded project to collect nuclear families identified through a proband with reading problems.

Investigators	
Cathy Barr (P.I.)	**TWH, Sickkids**
Rosemary Tannock	**SickKids**
Maureen Lovett	**SickKids**
Tom Humphries	**SickKids**
Coordinator	
Barbara Anderson	**SickKids**

Recruitment of Subjects

Probands identified with reading problems (age 6-16) are recruited from local schools

prescreened for Attention Deficit Hyperactivity Disorder (ADHD)



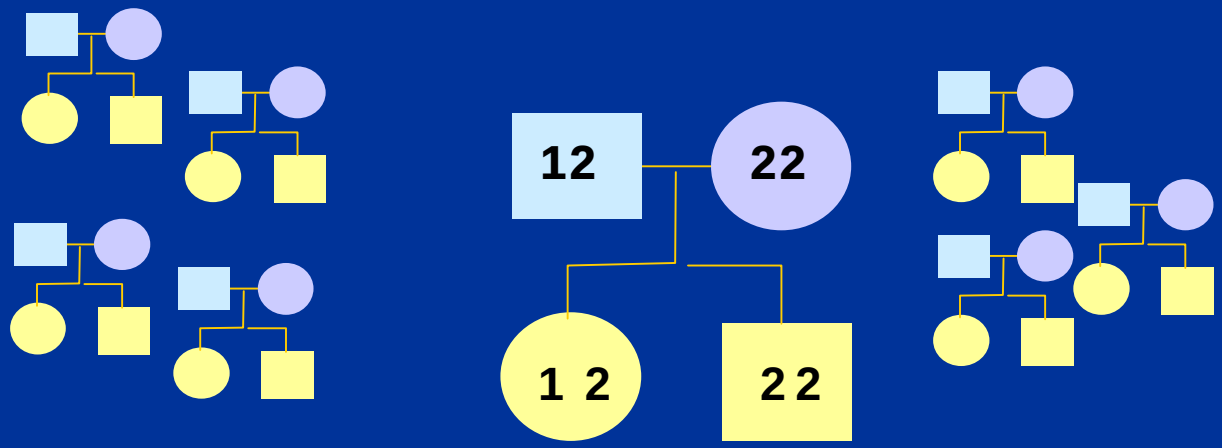
proband and siblings in the age range recruited to study

ADHD genetics study

n=273 families, with 107 siblings

Transmission Disequilibrium Test (TDT)

Biased transmission of alleles from heterozygous parents to affected offspring



$$\text{TDT } \chi 2 = \frac{(\text{1 allele transmitted - 1 allele not transmitted})^2}{\text{1 allele transmitted + 1 allele not transmitted}}$$

TDT Categorical Analysis

	Allele	Allele Frequency	Transmissions	Non-Transmissions	$\chi 2$	p-value
rs2007494	A	0.745	29	23	0.692	0.405
	C	0.255	23	29	0.692	0.405
-3G>A	**G**	**0.922**	**15**	**8**	**2.130**	**0.145**
	A	0.078	8	15	2.130	0.145
rs3743204	C	0.745	29	23	0.692	0.405
	A	0.255	23	29	0.692	0.405
C_1877025	**T**	**0.664**	**20**	**38**	**5.586**	**0.018**
	G	**0.336**	**38**	**20**	**5.586**	**0.018**
rs692691	C	0.594	31	35	0.242	0.623
	T	0.406	35	31	0.242	0.623
1249G>T	G	0.901	15	10	1.000	0.317
	T	0.099	10	15	1.000	0.317

For the categorical analysis, subjects must score below 1.5 Standard Deviations (S.D.) on 2 out of 3 core reading measures: Word Attack, Word ID and WRAT reading, or 1 S.D. below the mean on the average of the three.

83 probands and 18 siblings made this criteria.

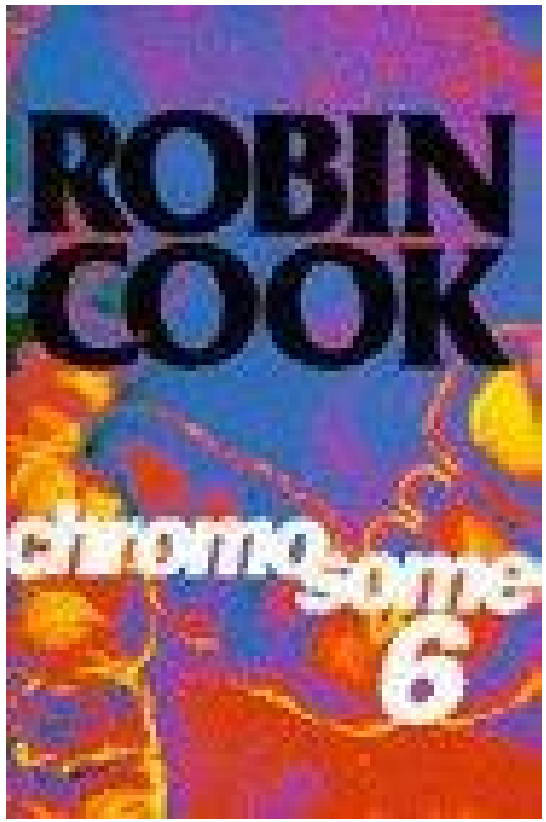
EKN1 in other studies

- Scerri et al., 2004 (Oxford) found association with the common alleles (-3G/1249G)
- Cope et al. 2005 (Wales) no association of these specific alleles
- Marino et al., 2005 (Italy) no association of these specific alleles
- Smith some indication for association of 1249G/T

Is EKN1 the first gene for reading disabilities?

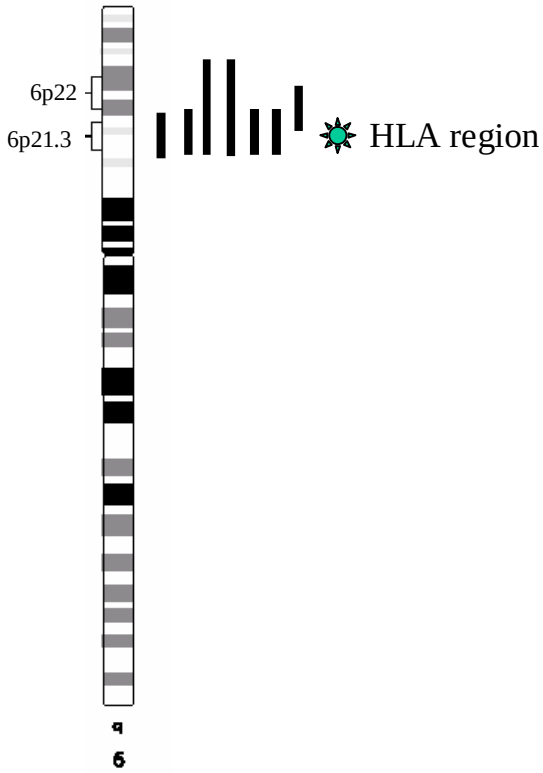
- some support for this gene as a susceptibility locus
- does not confirm the two previously reported variants as contributing to RD – can rule these out as the DNA change contributing to RD
- Is this the gene or could it be a gene nearby? a positional effect?

Chromosome 6p



"A typical Robin Cook medical thriller - ***another real page turner.***"

6p Linkage and Association Findings



Evidence for linkage or association

Smith and Kimberling, 1991
Cardon et al., 1994
Grigorenko et al., 1997
Gayan et al., 1999
Kaplan et al., 2002
Grigorenko et al., 2003
Turic et al., 2003
Deffenbacher et al., 2004
Francks et al., 2004
Cope et al., 2005

No evidence for linkage

Field and Kaplan, 1998
Kaminen et al., 2003
Nopola-Hemmi et al., 2001
Chapman et al., 2004

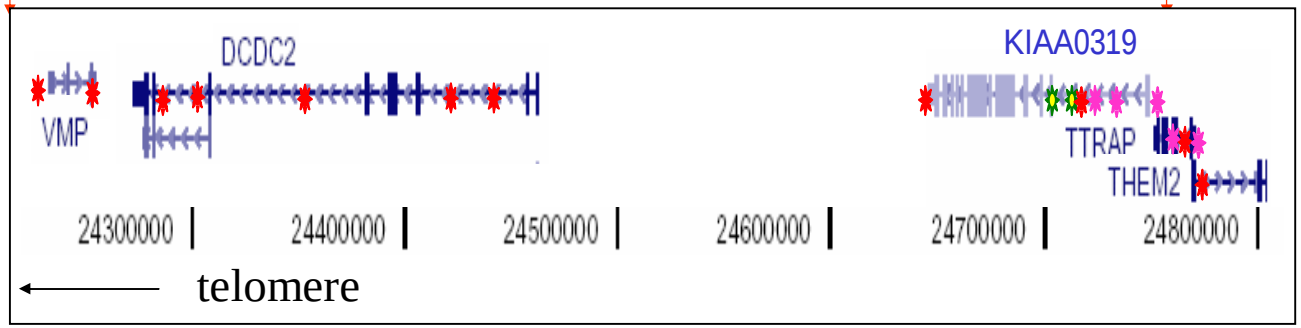
The 589 Kb Region on Chromosome 6p

Three independent studies have found evidence for association of DD to a region containing brain-expressed genes

USA

Oxford (UK)

Cardiff (UK)



Chromosome 6p candidates

- VMP
- DCDC2 ⟺
- GPLD1
- ALDH5A1
- KIAA0319
- TTRAP
- THEM2

DCDC2 contains a doublecortin-homology domain that is noteworthy because the gene doublecortin (*DCX*) has been implicated in X-linked lissencephaly and is required for neuronal migration

Highly expressed in the brain
Specifically in regions implicated in reading

Chromosome 6p candidates

- VMP
- DCDC2
- GPLD1
- ALDH5A1
- KIAA0319 ⟺ Gene of unknown function highly expressed in the brain
- TTRAP
- THEM2

Chromosome 6p summary

- Studies are converging on a few genes
- Both DCDC2 and KIAA0319 have some evidence for being under expressed in lymphocytes from people with RD
- Both genes seem to be involved in the migration of neurons

The real reason we invaded Iraq

To defeat terrorism we have no choice but to invade ~~Afriganist~~ Afgan ~~Aphghanis~~ ~~Afgah~~ Iraq.

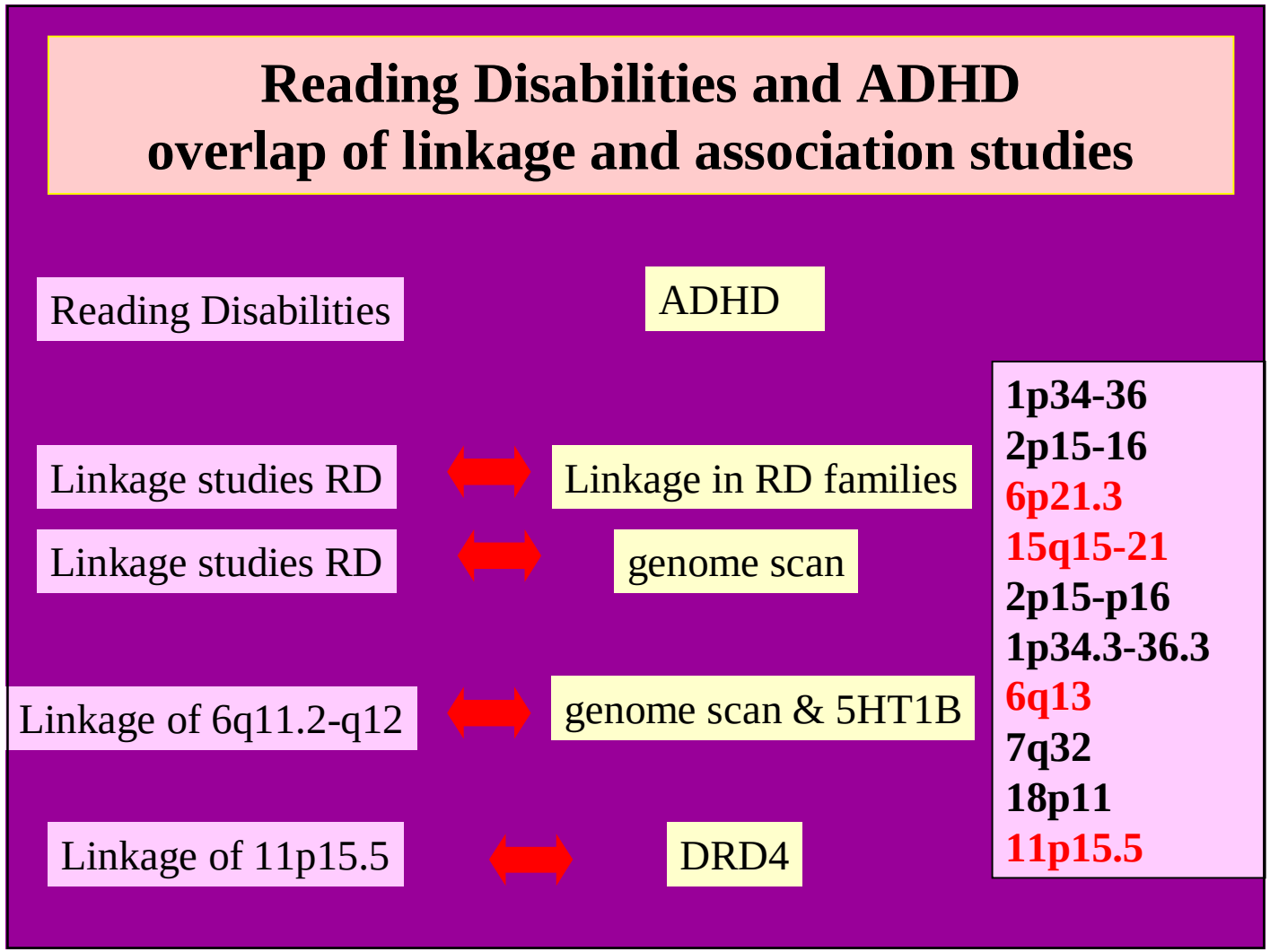
ADHD is characterized by extremes in the domains of inattention, hyperactivity and impulsivity

Evidence for a Genetic Relationship of ADHD & Reading Disabilities

Comorbidity
25 to 40% of individuals with RD have symptoms of ADHD
15 to 26% of individuals with ADHD have RD

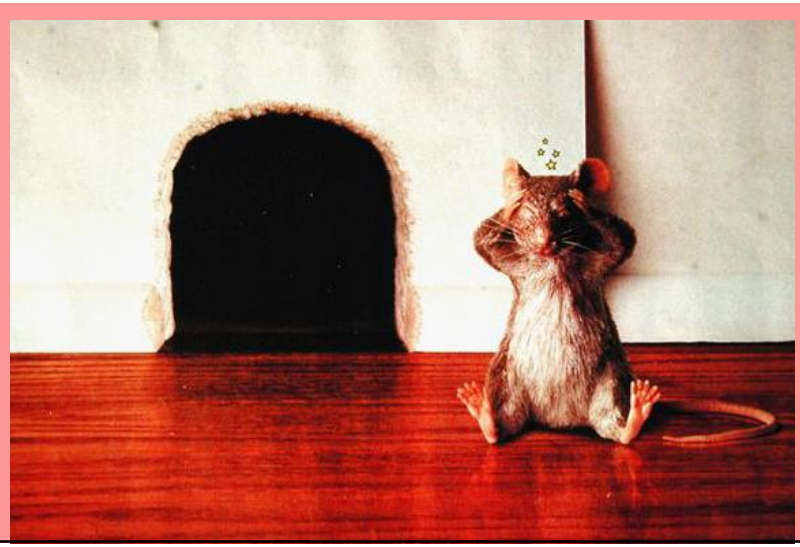
Twin Studies
Gilger et al., 1992 Twin study using RD twins suggested ADHD with RD is a genetic subtype

Wilcutt et al., 2001. twin studies suggests genetic overlap differs for dimensions of ADHD.
95% of the overlap between inattention and RD &
21% of the overlap for hyperactive/impulsive symptoms and RD due to common genetic influences.

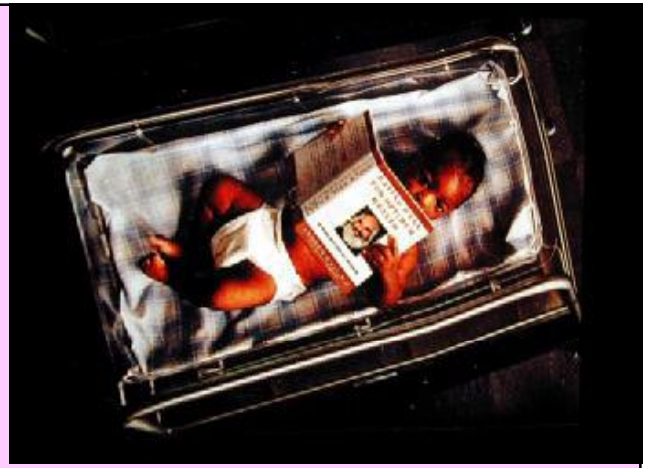


Question

What do you think is more heritable?
ADHD or reading disabilities

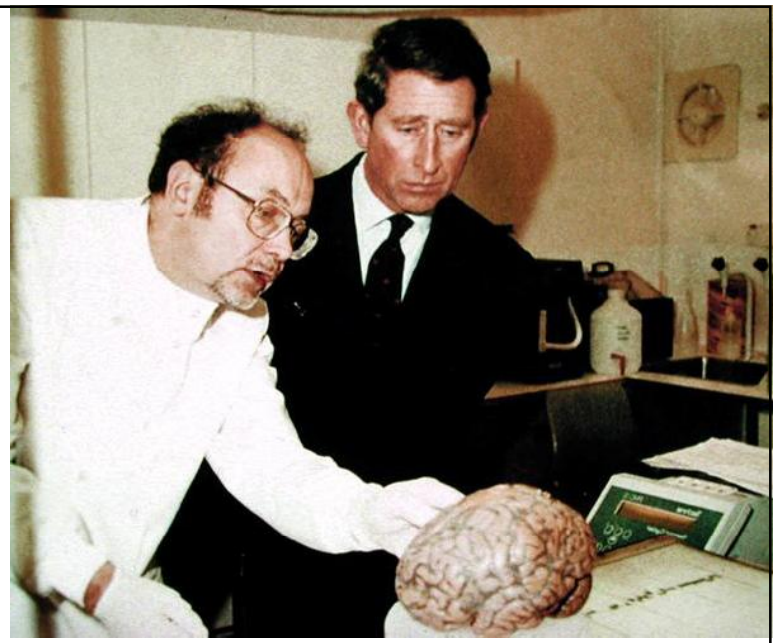


Complications in the Genetic Study of ADHD



- **Diagnosis is based on description of the behaviour**
  - **Informant Bias**
- **Clinically heterogeneous Disorder**
- **DSM-IV subtypes -- H/I, inattentive, combined**
- **Genetic relationship of co-morbid disorders unclear**
- **Developmental changes and gender effects**

Hypothesis



The genetic susceptibility to ADHD is related to an inherent dysregulation of the neurotransmitter systems

Primary Systems involved:

Dopamine
Adrenergic
Serotonin

Molecular Genetic Studies of ADHD

Published Studies by year

1995	**n=3**
1996	**n=2**
1997	**n=2**
1998	**n=7**
1999	**n=8**
2000	**n=19**
2001	**n=19**
2002	**n=19**
2003	**n=12**

Genetic Studies of ADHD

Published Studies since 1995	**n=91**
Number of Genes Studied	**n=28**
Number of Genes Reported to be Linked or Associated	**n=13**

Genes Reported to be Linked or Associated with ADHD

Gene	Positive reports/Number of studies
Dopamine Receptor D4 (DRD4)	11/19
Dopamine Transporter (DAT1)	7/13
Dopamine Receptor D5 (DRD5)	3/5
Dopamine Receptor D1 (DRD1)	1/2
Synaptosomal associated protein of 25 kd	3/3
serotonin receptor 2A	1/2
serotonin receptor B	2/2
Dopamine Beta Hydroxylase (DBH)	3/5
Dopamine Receptor D2 (DRD2)	1/5
Cathecol-O-Methyl Transferase (COMT)	1/6
serotonin transporter	3/4

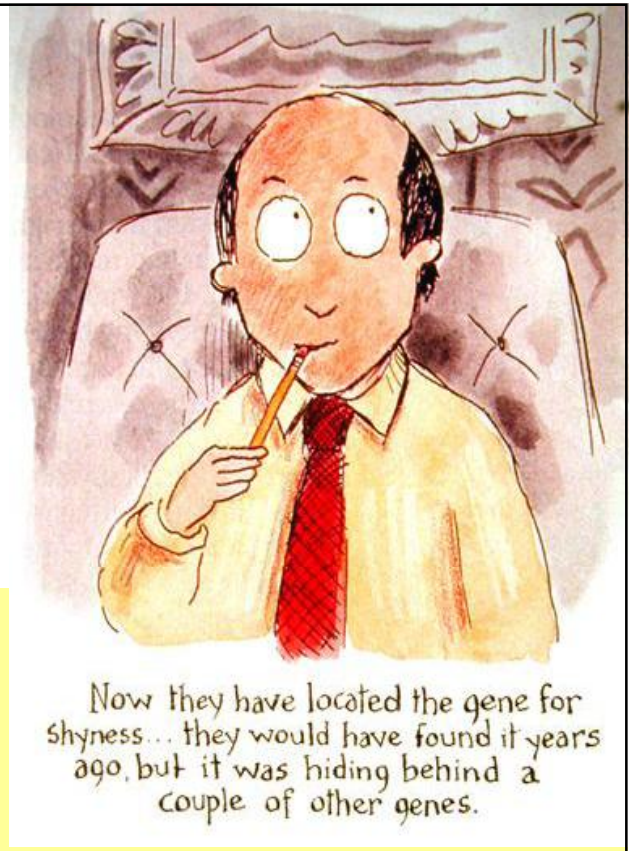
Toronto ADHD Project

Canadian Institutes of Health Research funded project to collect nuclear families with an ADHD proband

Progress Year 7:
Assessed over 590 children
383 probands ADHD
80 affected siblings

Cathy Barr (P.I.)	**SickKids, TWH**
Russell Schachar	**SickKids**
Wendy Roberts	**SickKids**
Molly Malone	**SickKids**
Rosemary Tannock	**SickKids**
Abel Ickowicz	**SickKids**
James Kennedy	**CAMH (Clarke)**

Genes with evidence for linkage in our sample



Dopamine Receptor D4 (DRD4)
Dopamine Transporter (DAT1)
Dopamine Receptor D5 (DRD5)
Dopamine Receptor D1 (DRD1)
Serotonin receptor 2A (HTR2A)
Serotonin receptor 1B (HTR1B)
Calcyon
Synaptosomal associated protein of 25 kd (SNAP-25)

How do we know if a gene is really involved?

Replication

Weight of the evidence in favor of the association

Functional Studies

evidence that change in DNA results in change in function that results in change in phenotype

Genes Associated with ADHD

Gene	Odds Ratio
Dopamine Receptor D4 (DRD4)	1.16-1.45
Dopamine Transporter (DAT1)	1.13
Dopamine Receptor D5 (DRD5)	1.2
SNAP25	1.19
serotonin receptor 2A	1.1
serotonin receptor 1B	1.44
Dopamine Beta Hydroxylase (DBH)	1.33
serotonin transporter	1.31

Odds Ratio 1.0 indicates no association >1.0 in increase in risk

Faraone Biol Psych 57:1313-1323 (2005)

Summary: What we know so far

Despite ADHD being a complex phenotype with multiple genes and environmental risk factors, progress has been made in gene identification

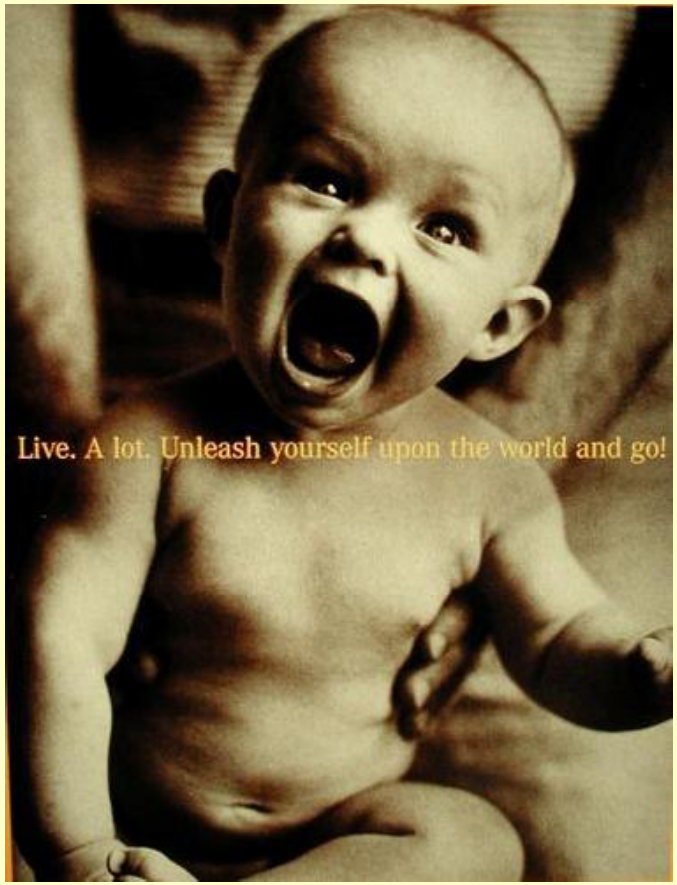
Each gene identified so far only contributes a small risk for the development of the disorder

Steps after a Linkage or Association Finding

- **Confirm linkage in a larger/independent samples**
- **Determine the molecular basis for the linkage findings** *Is there a functional change in the protein? Is there a change in the amount of the mRNA or protein?*
- **Functional studies**
- **Correlate the genotype with the phenotype**

Implications for Families

- Runs in families (familial)
- Highly heritable (twin studies)
- but inheritance not in a clear or predictably Mendelian manner
- Severity in parents does not predict severity in child

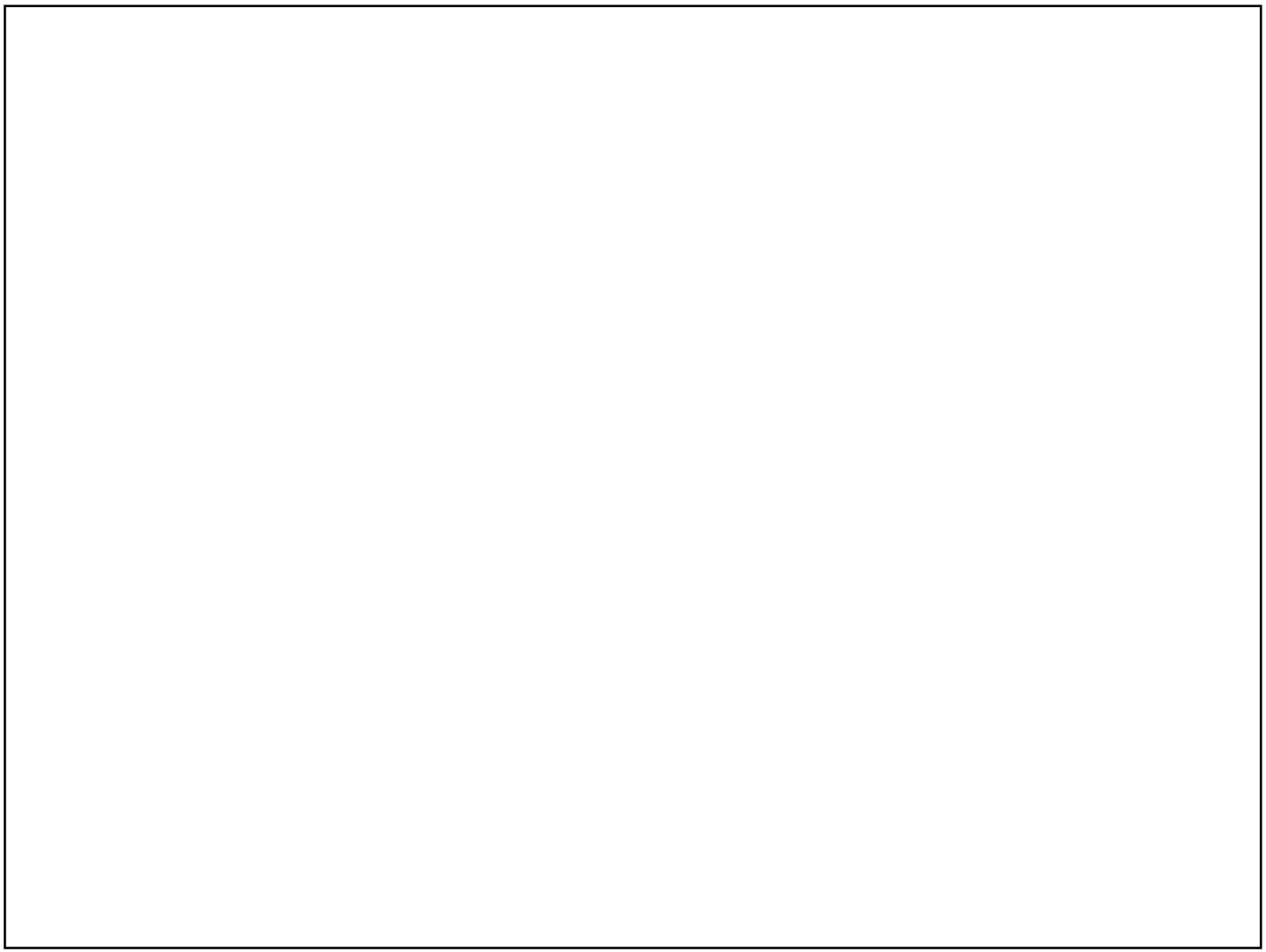


Implications for Families

- Genetic risk for the genes identified thus far very low
- cannot be used for diagnosis or prediction

Why are molecular genetic studies important?

- we need to know how it works
- knowledge of biological underpinnings reduces stigma and can improve self esteem
- understanding of the biology may allow for intervention by reduction of environmental risks
- may influence our thinking on interventions or help with the development of new treatments



Helpful References

O'Donovan, M.C. and Owen M. J. Basic Molecular Genetics. In: Psychiatric Genetics and Genomics. pp 3-29. Oxford University Press, 2002.

Cardon L., and Bell J.I. Association Study Designs for Complex Diseases. *Nature Reviews Genetics* 2:91-99, 2001.

Fisher SE, DeFries JC (2002): Developmental dyslexia: genetic dissection of a complex cognitive trait. *Nat Rev Neurosci* 3:767-80.

Helpful References

Grigorenko EL (2001): Developmental dyslexia: an update on genes, brains, and environments. *J Child Psychol Psychiatry* 42:91-125.

Olson RK (2004): SSSR, Environment, and Genes. *Scientific Studies of Reading* 8:111-124.

Smith SD, Kimberling WJ, Pennington BF (1991): Screening for multiple genes influencing dyslexia. *Reading and Writing: An Interdisciplinary Journal* 3:285-298.

Helpful References

Gayan J, Olson RK (1999): Reading disability: evidence for a genetic etiology. *Eur Child Adolesc Psychiatry* 8 Suppl 3:52-5.

Gayan J, Olson RK (2001): Genetic and environmental influences on orthographic and phonological skills in children with reading disabilities. *Dev Neuropsychol* 20:483-507.

Francks CI, MacPhie IL, Monaco AP. *The Genetic Basis of Dyslexia*. Lancet Neurology 1:483-490, 2002.