Molecular Genetic Studies of ADHD: What We Know So Far

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Conflict of Interest/Disclosures

None

Learning Objectives

At the end of this presentation, the attendee will be able to:

1. Understand what we currently know about the genetics of ADHD
2. Understand the methods used for genetic studies
3. Interpret new genetic findings as they are reported
4. Understand the caveats associated with genetic research in complex behavioural traits
5. Interpret this knowledge to provide information for families

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

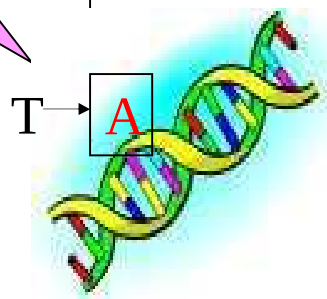
4) Identify polymorphisms for the gene or area of interest

Steps in a molecular genetic study II

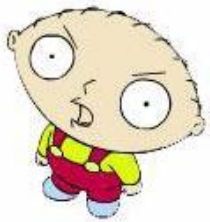
5) Look for sharing of chromosome regions in families comparing individuals with and without the phenotype -- **linkage studies**

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**

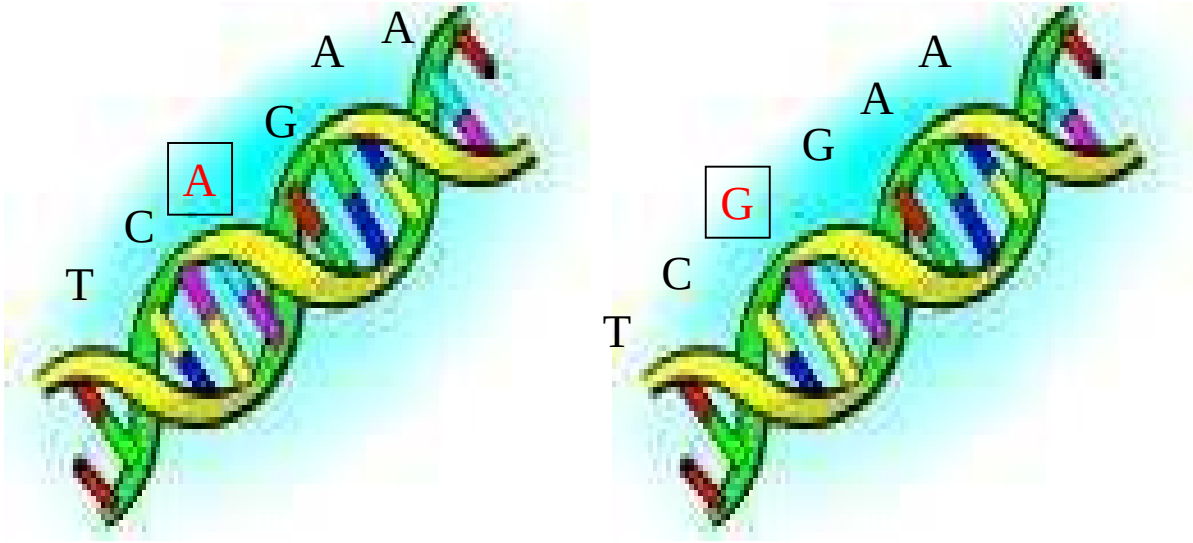
How it works



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



Allele



One of two or more alternatives DNA sequences at a chromosomal position

Here the alleles are A and G

DNA variation

Mutation --

Minor allele frequency less than 1%

Polymorphism --

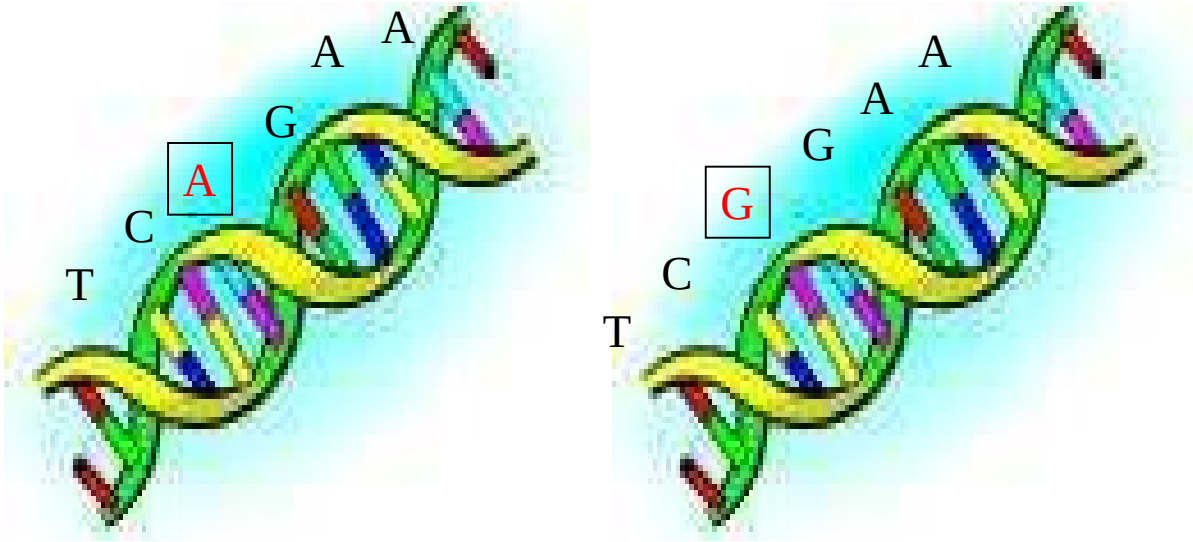
Minor allele frequency greater than 1%

Does not indicate function

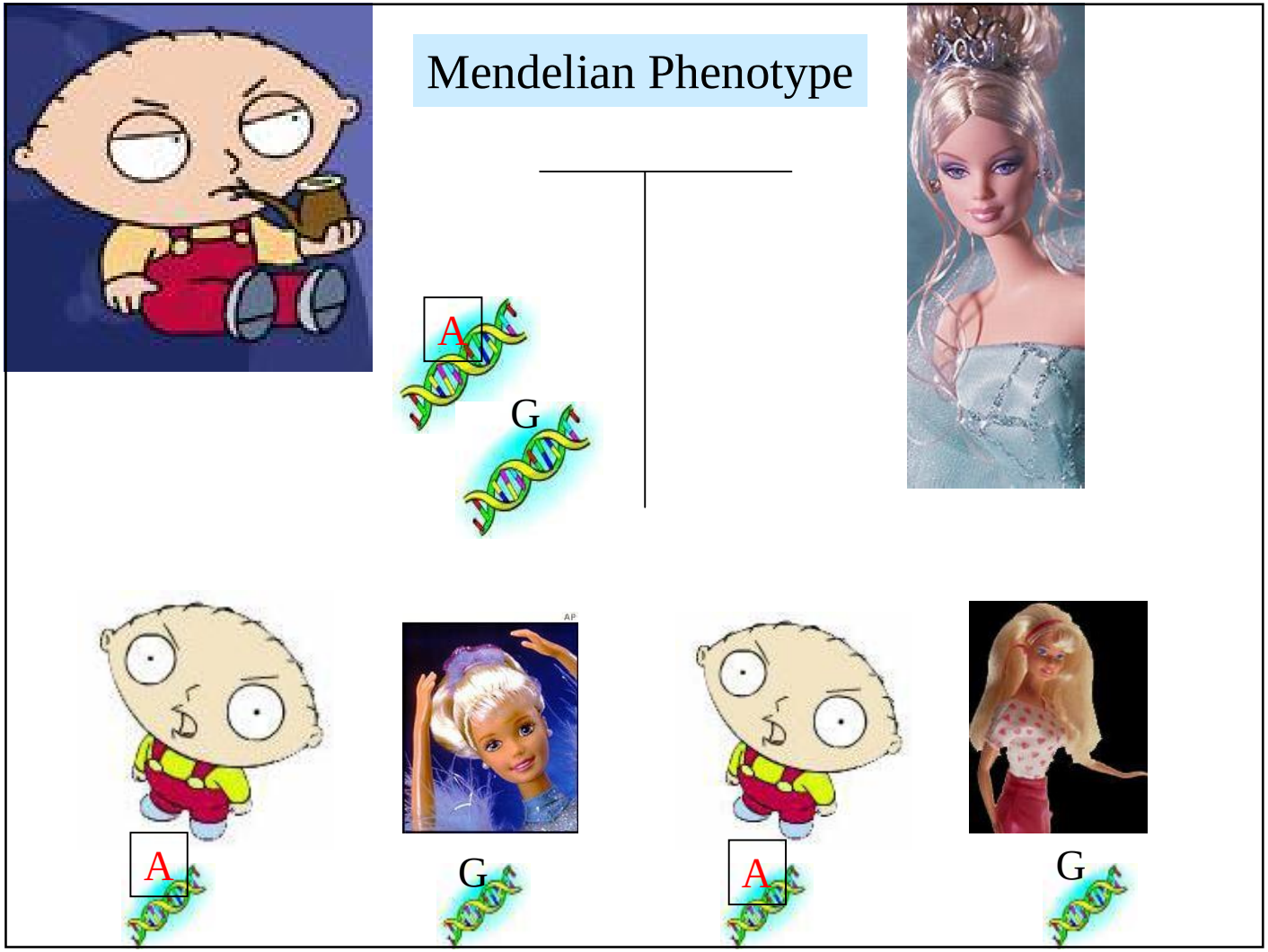
Mutation can become a polymorphism

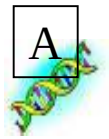
The same DNA change can be a mutation in one population and a polymorphism in another

Genotypes



The set of alleles of the individual
For a two allele system an individual could have one of the following genotypes
AA, AG or GG





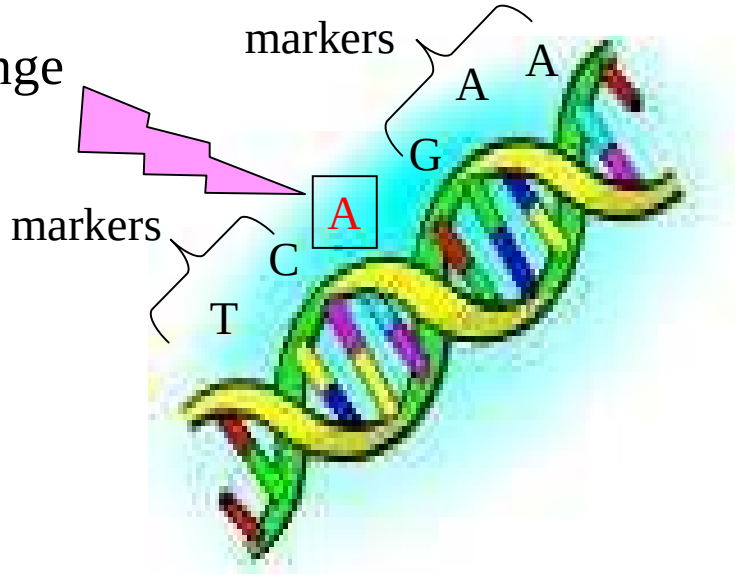
Complex Traits

- 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.

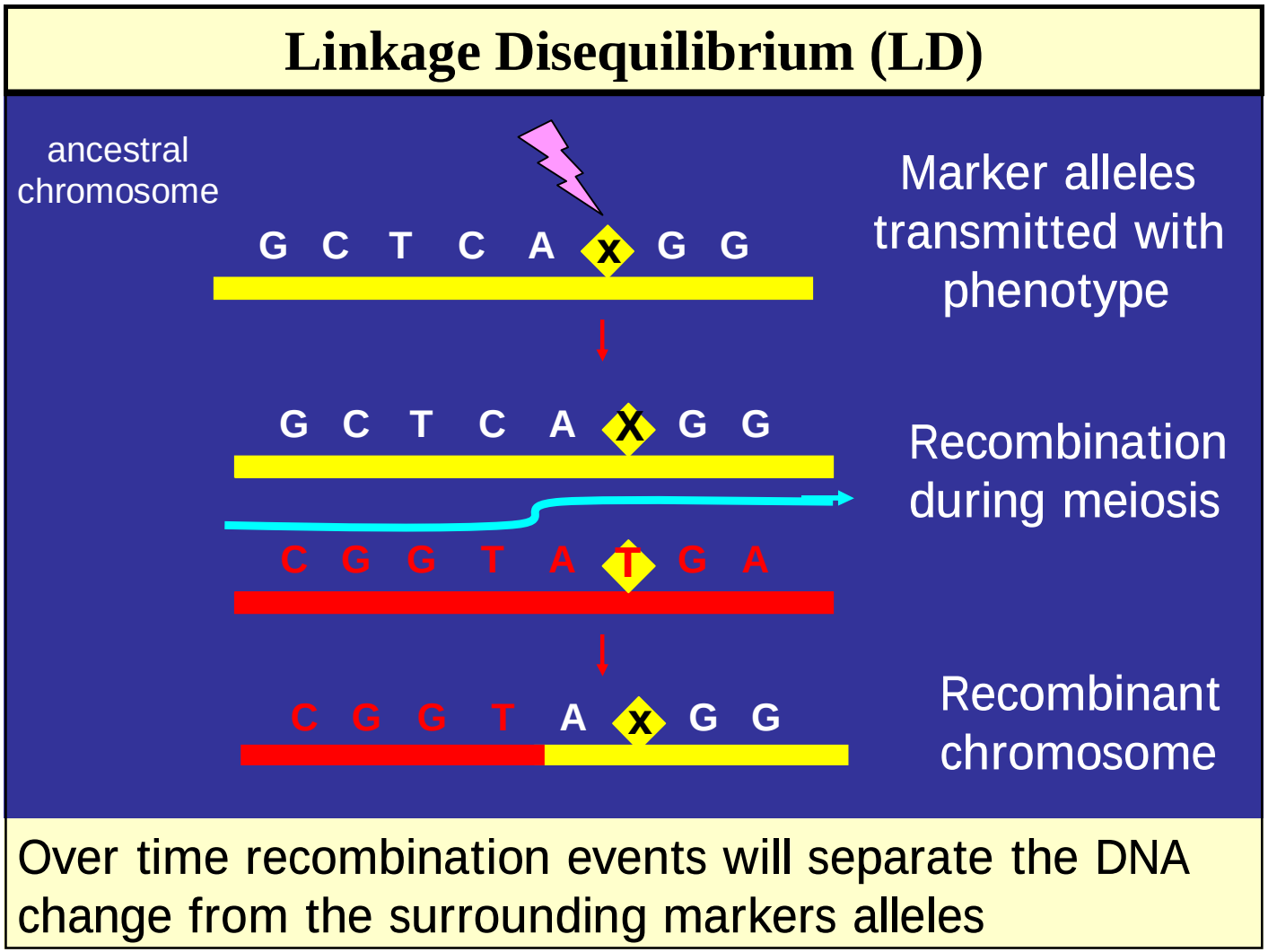
Linkage Disequilibrium (LD)

ancestral chromosome

Marker alleles transmitted with phenotype

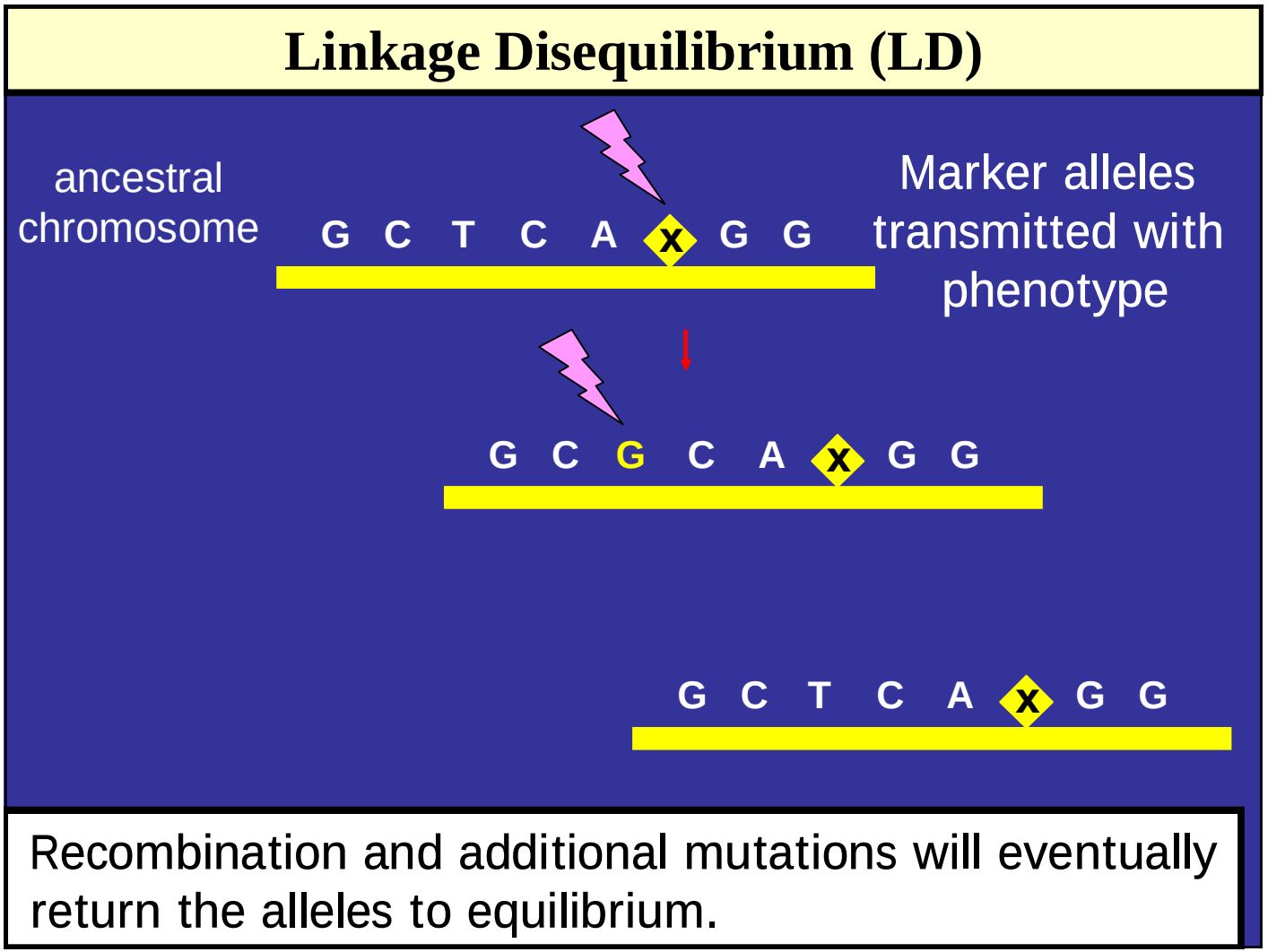
The DNA change studied is close to the functional DNA change such that it is inherited together through many generations.

Linkage Disequilibrium (LD)



Over time recombination events will separate the DNA change from the surrounding markers alleles

Linkage Disequilibrium (LD)



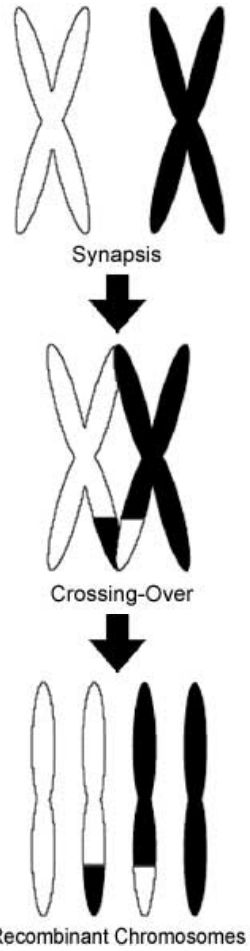
Recombination and additional mutations will eventually return the alleles to equilibrium.

Linkage Disequilibrium (LD)

Why is linkage disequilibrium important?

It allows us to find the location of risk genes through shared ancestral chromosomal regions in populations

Linkage vs Association

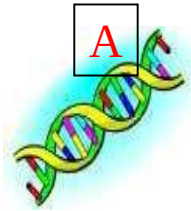


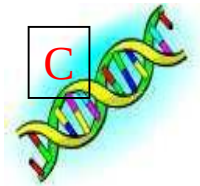
Linkage

Linkage examines the inheritance pattern of DNA markers with known chromosomal location with the pattern of inheritance of the disorder

As the chromosome is passed through the generations the shared segment among affected relatives becomes smaller

Linkage -- examines sharing of alleles **within** families





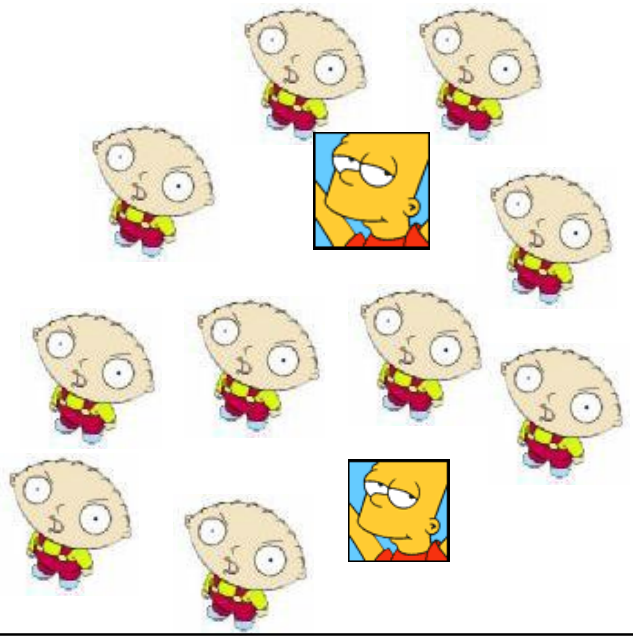
Association Studies

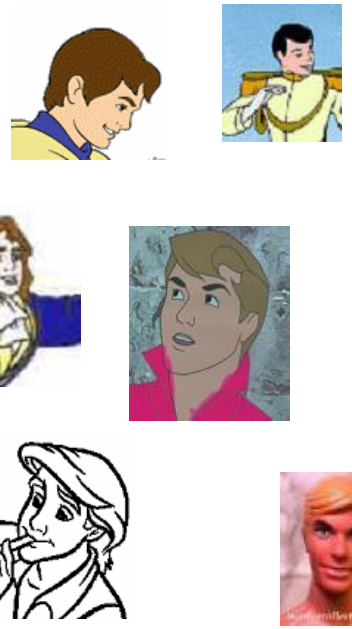
examines sharing of alleles in populations

Cases

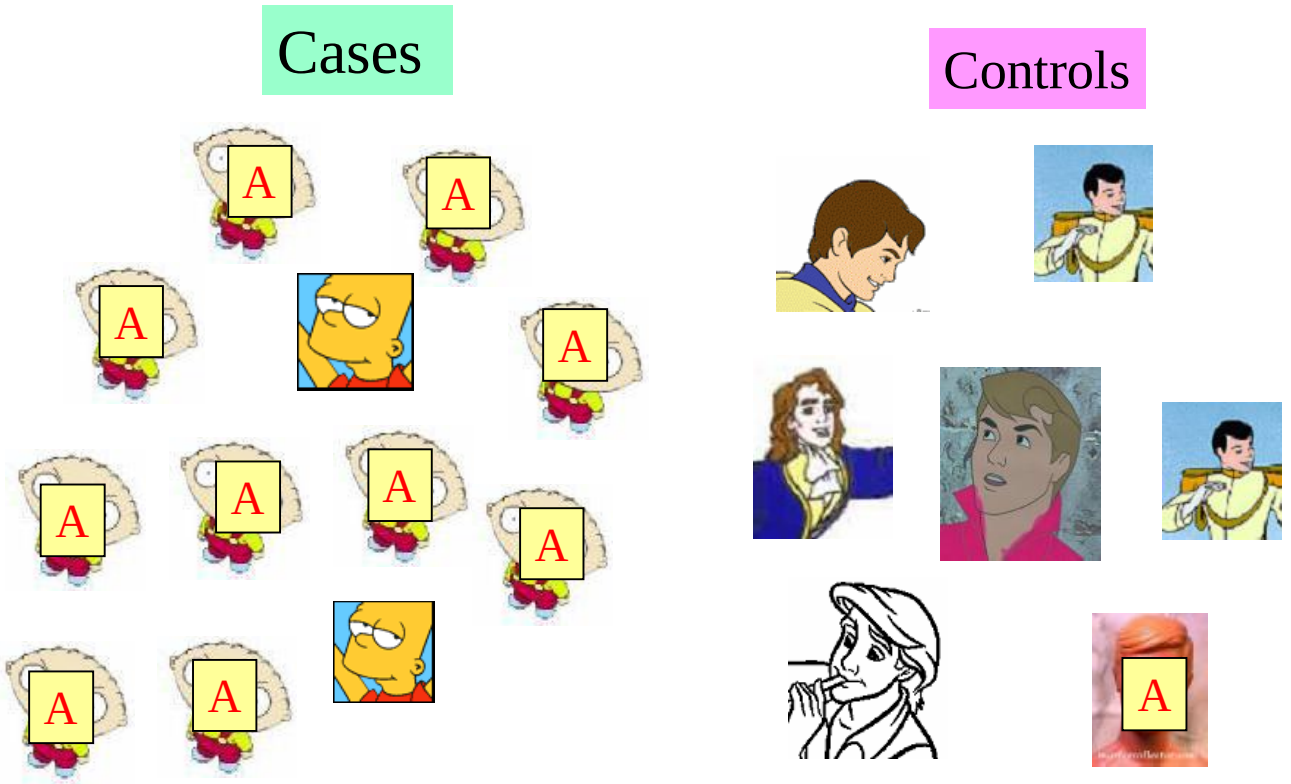
Controls

(selected for bug eyes and evil genius)





Sharing of ancestral haplotype in apparently unrelated individuals



Case-Control Association Studies

Count alleles or genotype frequencies in Cases and compare to Controls

Strengths of Association Studies

more power than linkage study
-- requires less subjects
-- can detect smaller gene effects

Case-Control Association Studies

Limitations

requires that the marker is very close to the disease gene

-- candidate gene or region

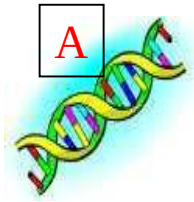
-- or a very dense marker map (>100,000 markers)

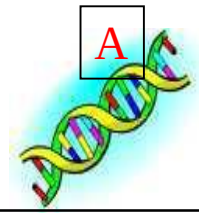
-- the DNA change tested must be the functional change or in LD with the DNA

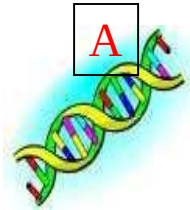
Control must be matched for ethnic group with case

Family Based Control Association Studies

Uses the chromosomes not passed to the proband as the control alleles. Examines the sharing of alleles **between** families. Affected individuals share the same ancestral chromosome in what appears like unrelated families.

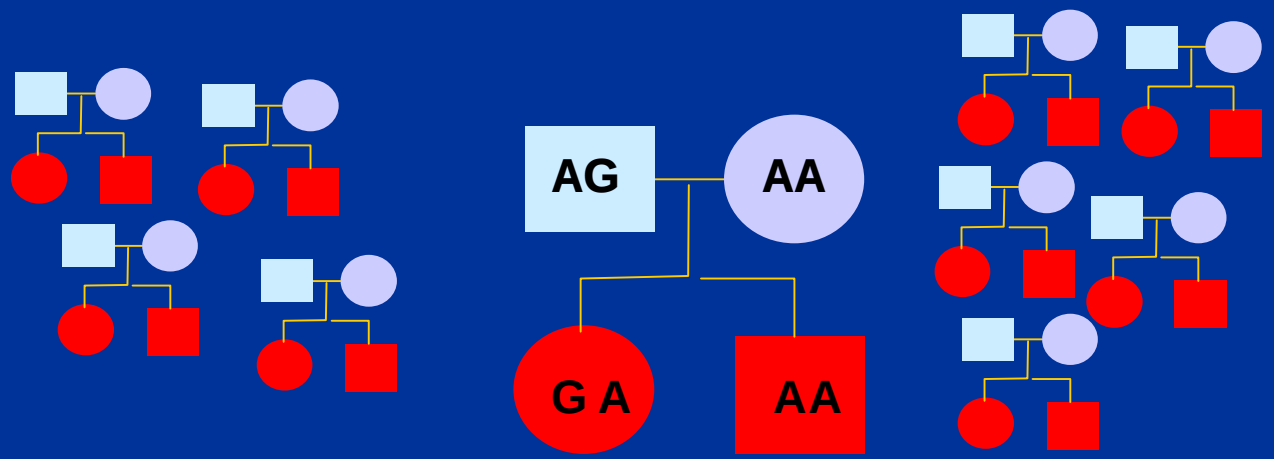






Transmission Disequilibrium Test (TDT)

Biased transmission of alleles from heterozygous parents to affected offspring



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

Genome Scan

D19S229
D19S247
D19S204
D19S221
D19S179
D19S248
D19S178
D19S246
D19S180
D19S254

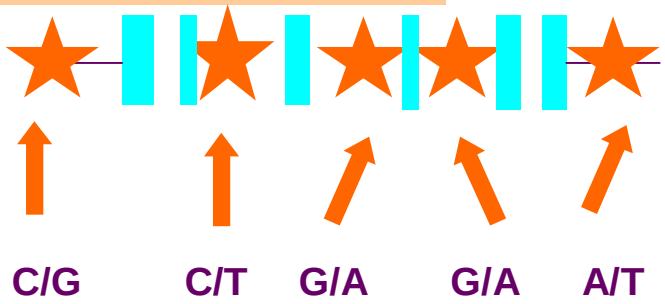
Systematically screen all of the chromosomes for linkage using DNA markers spaced at regular intervals

Advantage?

Candidate Gene Studies

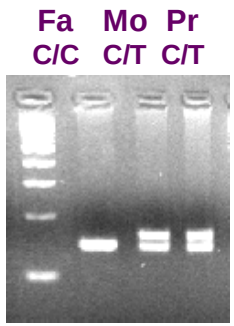
select candidate gene

- animal models
- pharmacological
- biology
- related disorders



identify polymorphisms

genotype multiple polymorphisms across the gene



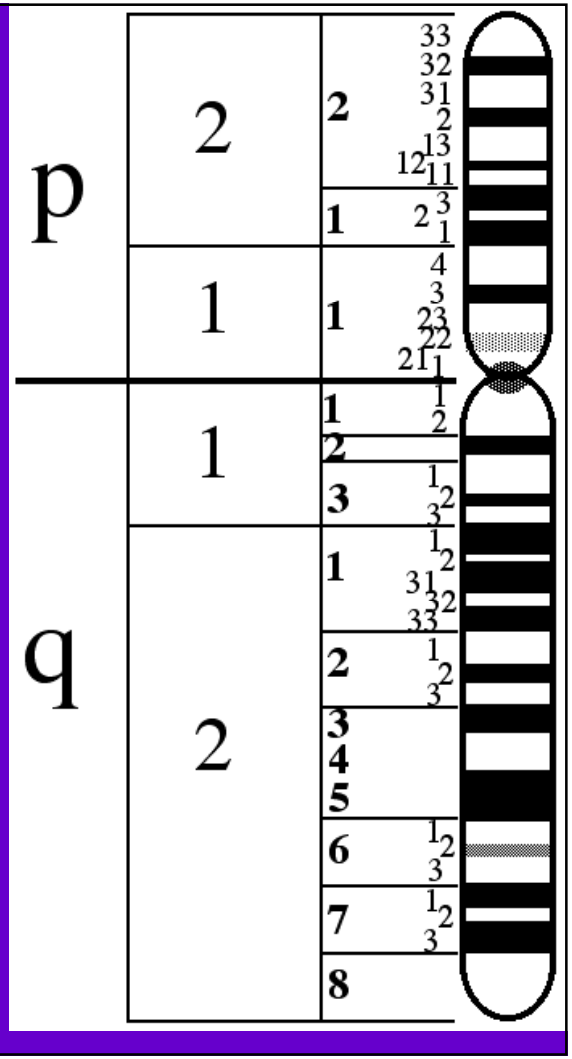
162 bp (T)
144 bp (C)

statistical analysis

Chromosomal Nomenclature

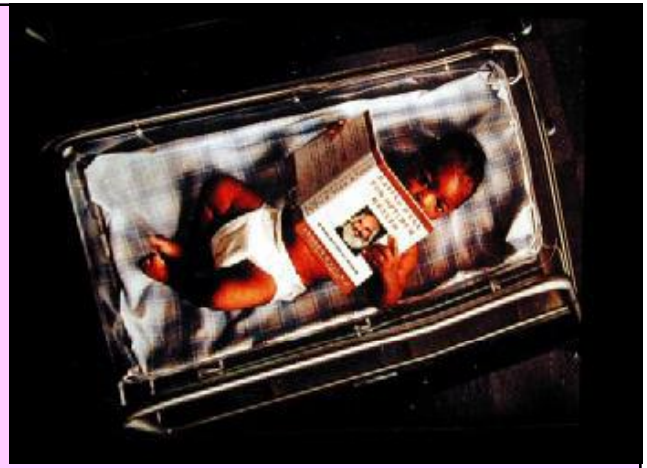
The two chromosome arms are referred to as p for petit (short arm) and q for the next letter in the alphabet (long arm).

Bands are numbered from the centromere. As microscopes improved the coarse banding patterns were refined by the addition of further levels of numbering.



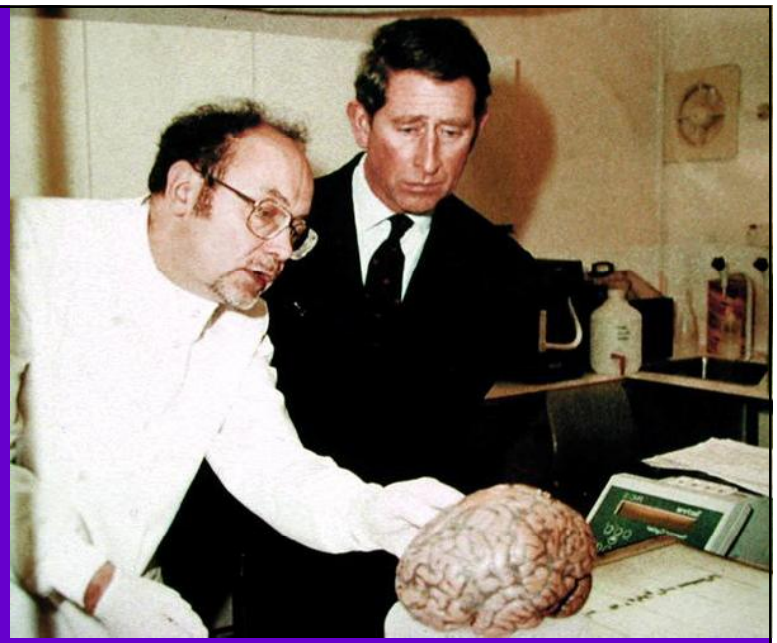
ADHD is characterized by extremes in the domains of inattention, hyperactivity and impulsivity

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

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

Issues for Genetic Studies -- Heterogeneity

population studied ethnic differences

- linkage disequilibrium between marker and functional variant

population studied clinical differences

- ascertainment
- inclusion/exclusion criteria
- comorbidity

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)

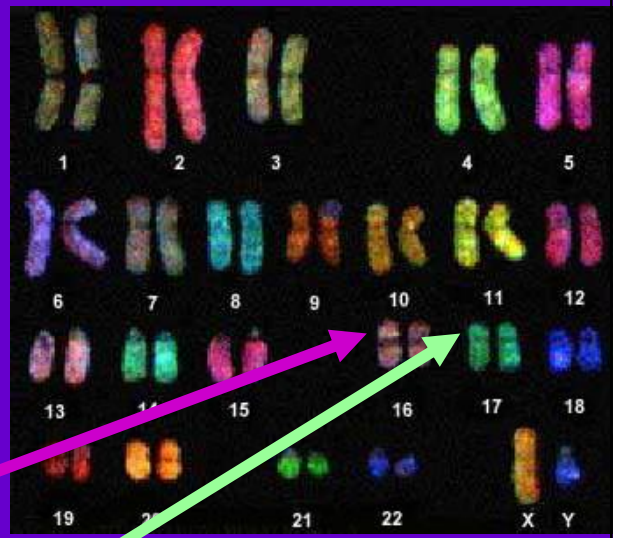
Results from the Genome Scans

Fisher et al., 2002

- 126 families
- some evidence for linkage LOD > 1.5 16p13, 5p12, 10q26, 12q23

Ogdie et al., 2003

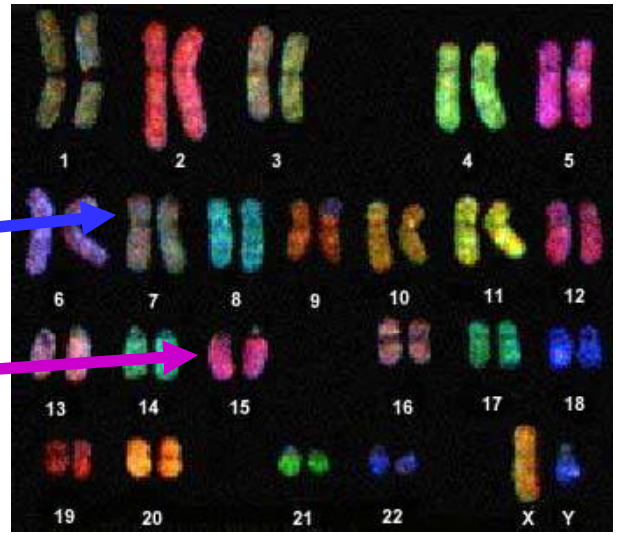
- increased the sample size to 277 families
- increased evidence for linkage to 16p13 and identified linkage to 17p11



Genome Scan
Bakker et al., 2003

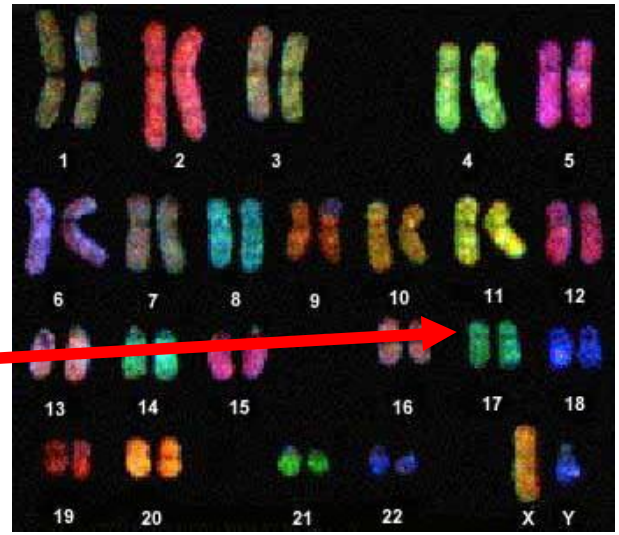
164 Dutch sibling pairs

- 7p13 (LOD 3.04)
- 15q (LOD 3.54) in the region previously linked to reading disabilities



Genome Scan
Arcos-Burgos et al., 2004

- 16 multigenerational families from an isolated region of Colombia
- individual family with evidence for linkage to 17p11
- trends 5q33.3, 8q11.23, 11q22, 4q13.2, 12q23, 8p23



Genome Scan
Regions of overlap – not a lot

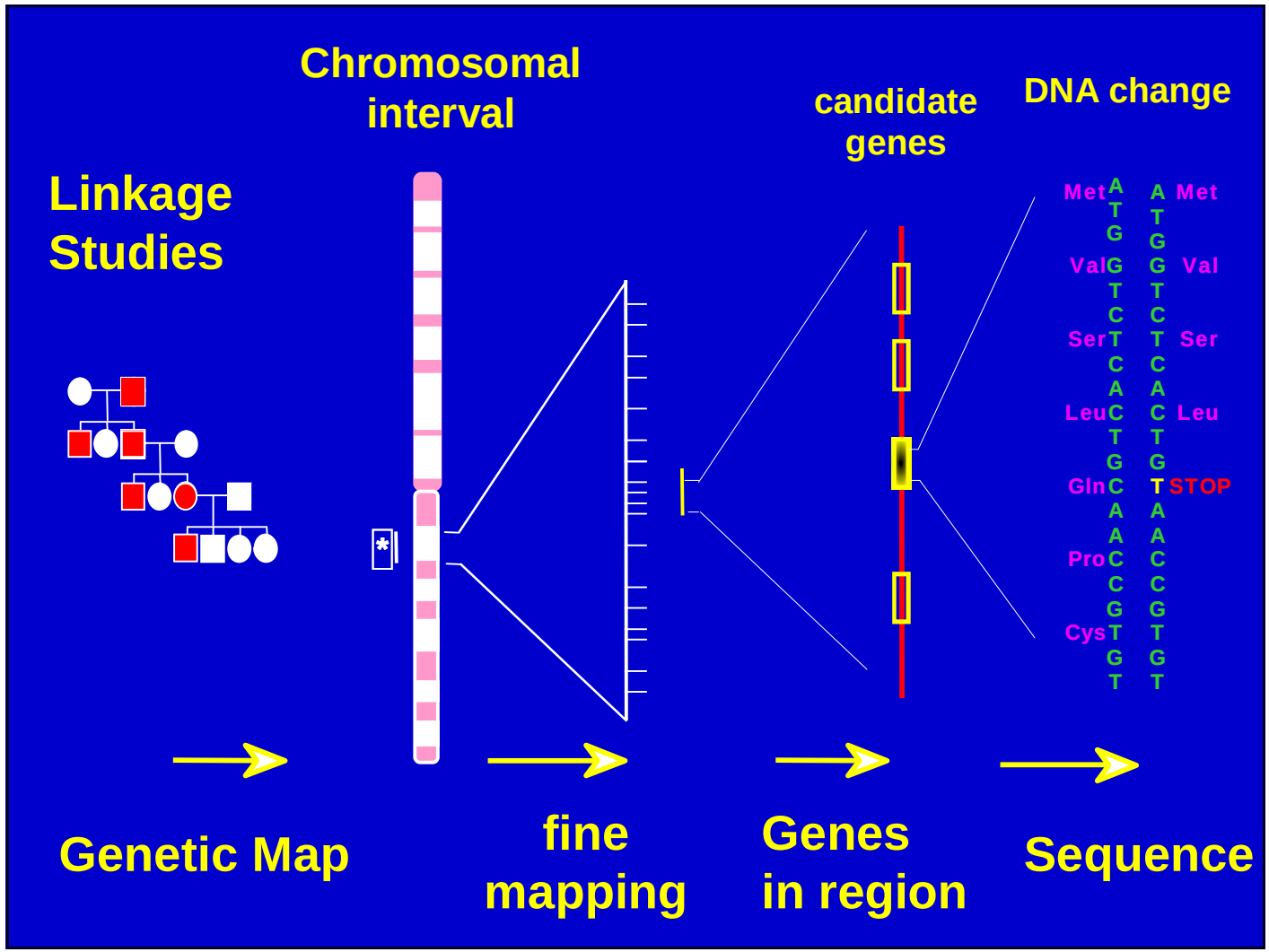
- Bakker and Ogdie 5p13
- Arcos-Burgos and Ogdie 17p11

Issues affecting genome scan results

different ethnicity

clinical Heterogeneity

power



Candidate Genes in Linked Regions from Genome Scan

Serotonin Transporter (Fisher)	17q11.1-q12
Serotonin Receptor 1B (Ogdie)	6q13
Glutamate receptor, ionotropic, N-methyl D-aspartate 2A (Ogdie)	16p13.2
Calcyon (Fisher)	10q25
Glial cell line derived neurotrophic factor (Ogdie, Bakker)	5p13.1-p12
DOPA decarboxylase (Ogdie, Arcos-Burgos)	7p11

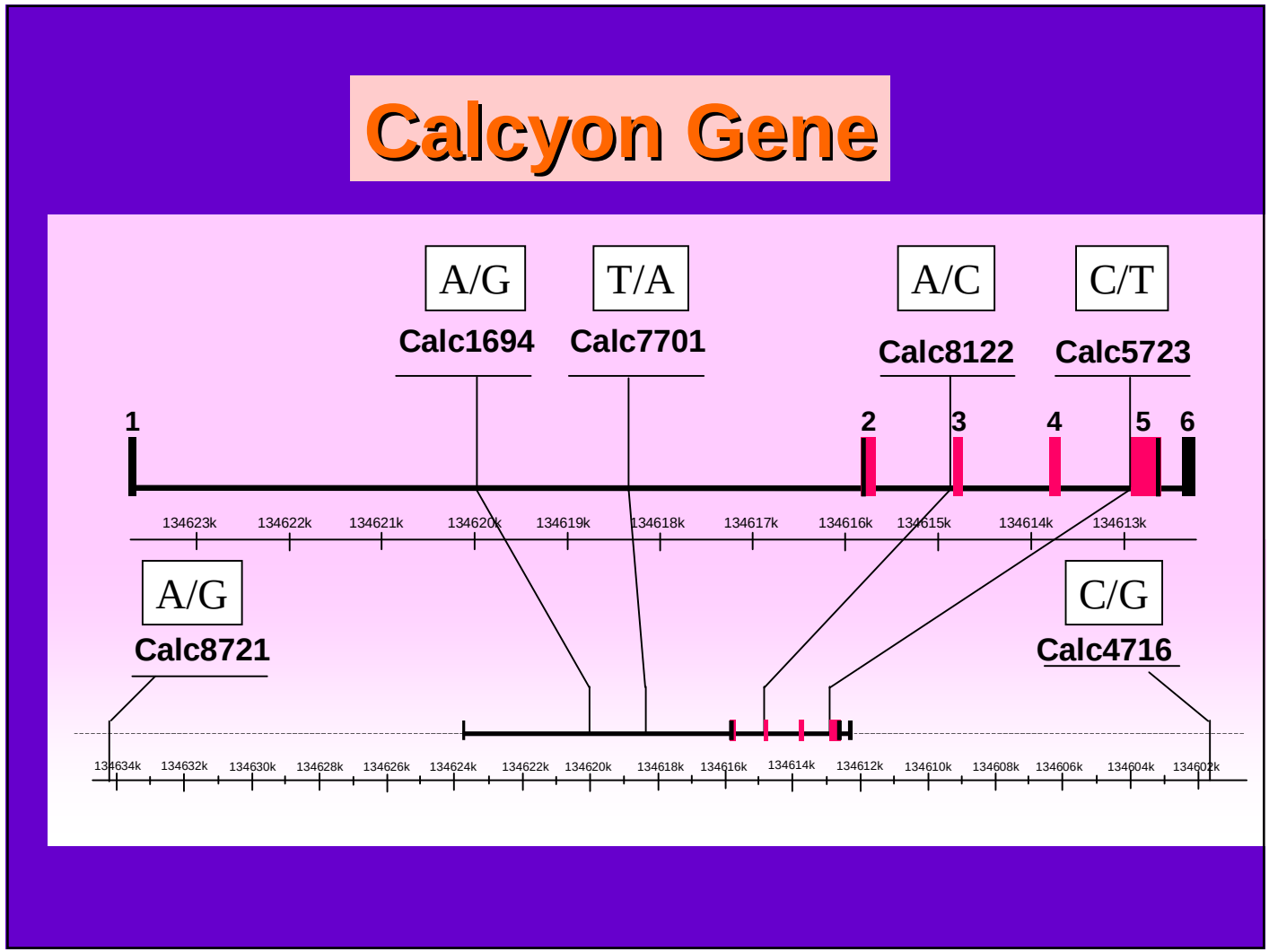
Genetic Evidence supporting Calcyon in ADHD

1. DRD1IP forDRD1 interacting protein
2. Resides at 10q26 – region with weak support for linkage (LOD 1.66) from the first genome scan of ADHD (Fisher, 2002).
3. deletion syndrome of 10q25.2-26

   learning difficulties

   behavioural problems including hyperactivity

3. Involved in the DRD1 and DRD5 receptor calcium signaling



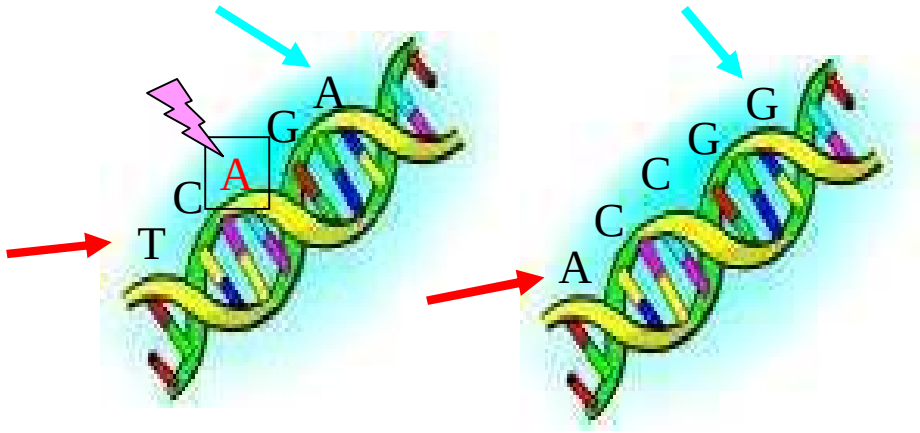
TDT Results for Calcyon Markers

Marker	Allele	Freq	Transmitted	Not Trans	χ^2	*p*-value
Calc8721	A	0.802	59	37	5.042	0.025
	G	0.198	37	59		
Calc1694	A	0.860	42	34	0.842	0.359
	G	0.140	34	42		
Calc7701	T	0.858	46	35	1.494	0.222
	A	0.142	35	46		
Calc8122	A	0.800	57	41	2.612	0.106
	C	0.200	41	57		
Calc5723	C	0.906	39	22	4.738	0.029
	T	0.094	22	39		
Calc4716	C	0.862	48	37	1.424	0.233
	G	0.138	37	48		

Haplotypes

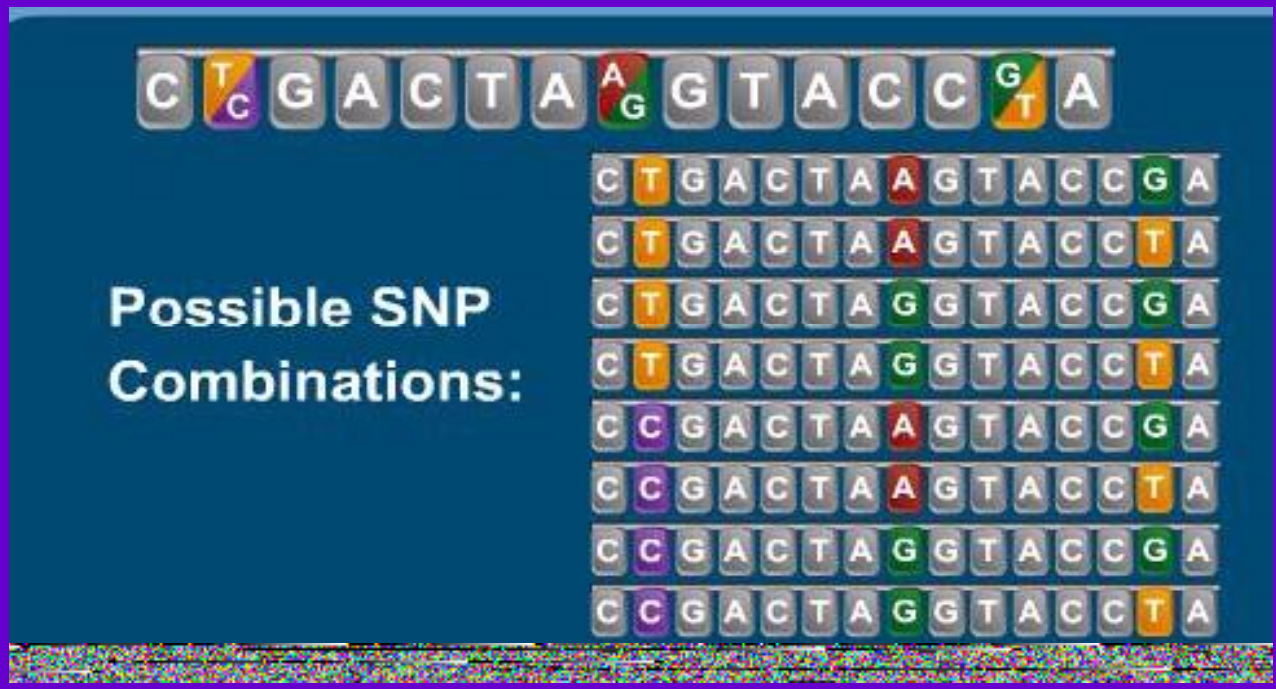
The analysis of haplotypes can in many cases provide more information than the analysis of single markers alone

Haplotypes – What are they?



A set of closely linked genetic markers present on one chromosome which tend to be inherited together (not easily separable by recombination). Some haplotypes may be in linkage disequilibrium.

For three polymorphic markers of two alleles there are 8 possible combinations or haplotypes



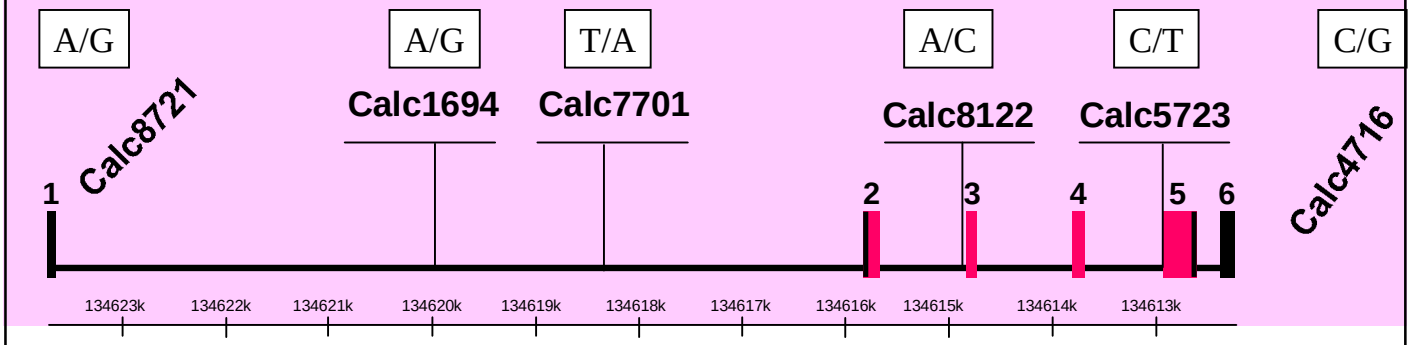
http://gslc.genetics.utah.edu/units/pharma/phsnipping/MakingSNPsMakeSense.swf

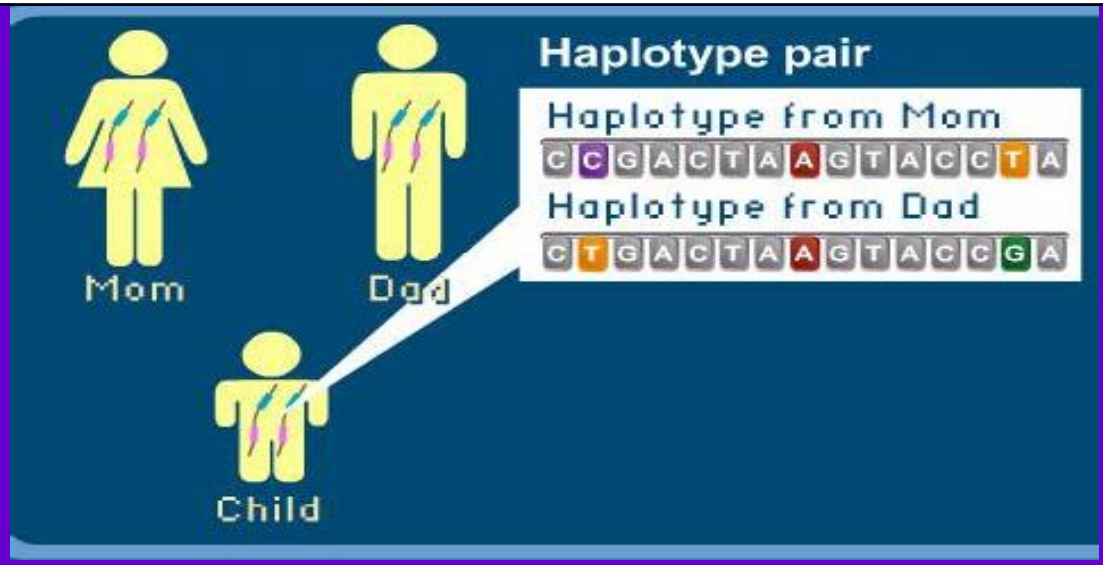
There are 8 possible combinations however only 4 are seen in the population tested in this example

http://gslc.genetics.utah.edu/units/pharma/phsnipping/MakingSNPsMakeSense.swf

Calcyon Haplotypes

Haplotype	8721	-189	1694	7701	5220	8122	5723	4760	4716	frequency
C1	A	C	A	T	C	A	C	C	C	0.754
C2	G	C	G	A	C	C	T	C	G	0.08
C3	G	A	A	T	C	C	C	C	C	0.051
C4	A	C	A	T	C	A	C	T	C	0.037
C5	G	C	G	A	C	C	C	C	G	0.029
C6	A	C	A	T	T	A	C	C	C	0.022





The inheritance of haplotypes in families can provide more information than single marker analysis. Each person inherits one haplotype from the mother and one from the father. We can analyze the inheritance of the haplotypes in the same way as we analyze individual marker alleles using modifications of the TDT.

Calcyon Haplotype Results

Haplotype	Haplotype frequency	Transmission Obs.[d]	Transmission Exp.[e]	Var (O-E)	χ^2 (1 d.f.)	*P*
C1	0.754	303	286.3	32.7	8.434	***0.004***
C2	0.08	20	27.5	15.5	3.634	*0.056*
C3	0.051	14.5	17.5	8	1.126	0.289
C4	0.037	15.1	15.1	7.1	0.000	1.000
C5	0.029	13	10.5	5.2	1.192	0.275
C6	0.022	4.5	8.6	4.4	3.659	*0.056*

[a] Global $\chi 2$ (for haplotypes with frequency greater than 5%) = 8.984, 3 d.f., $P = 0.030$

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

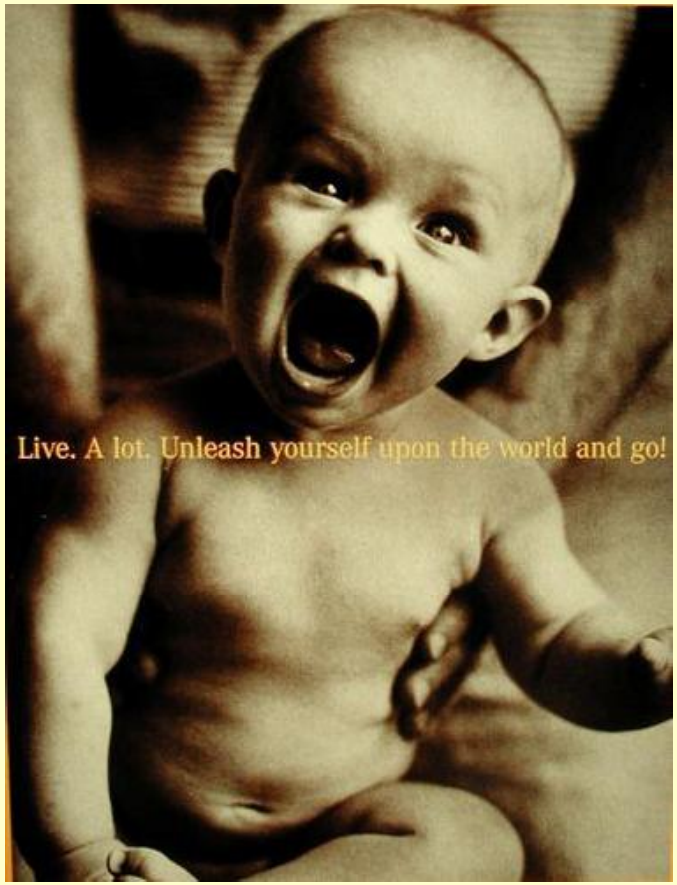
Each gene identified so far only contributes a small risk for the development of the disorder

Steps after a Linkage or Association Finding

- **Replicate 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 pharmacological treatments