

# Family-Based Studies

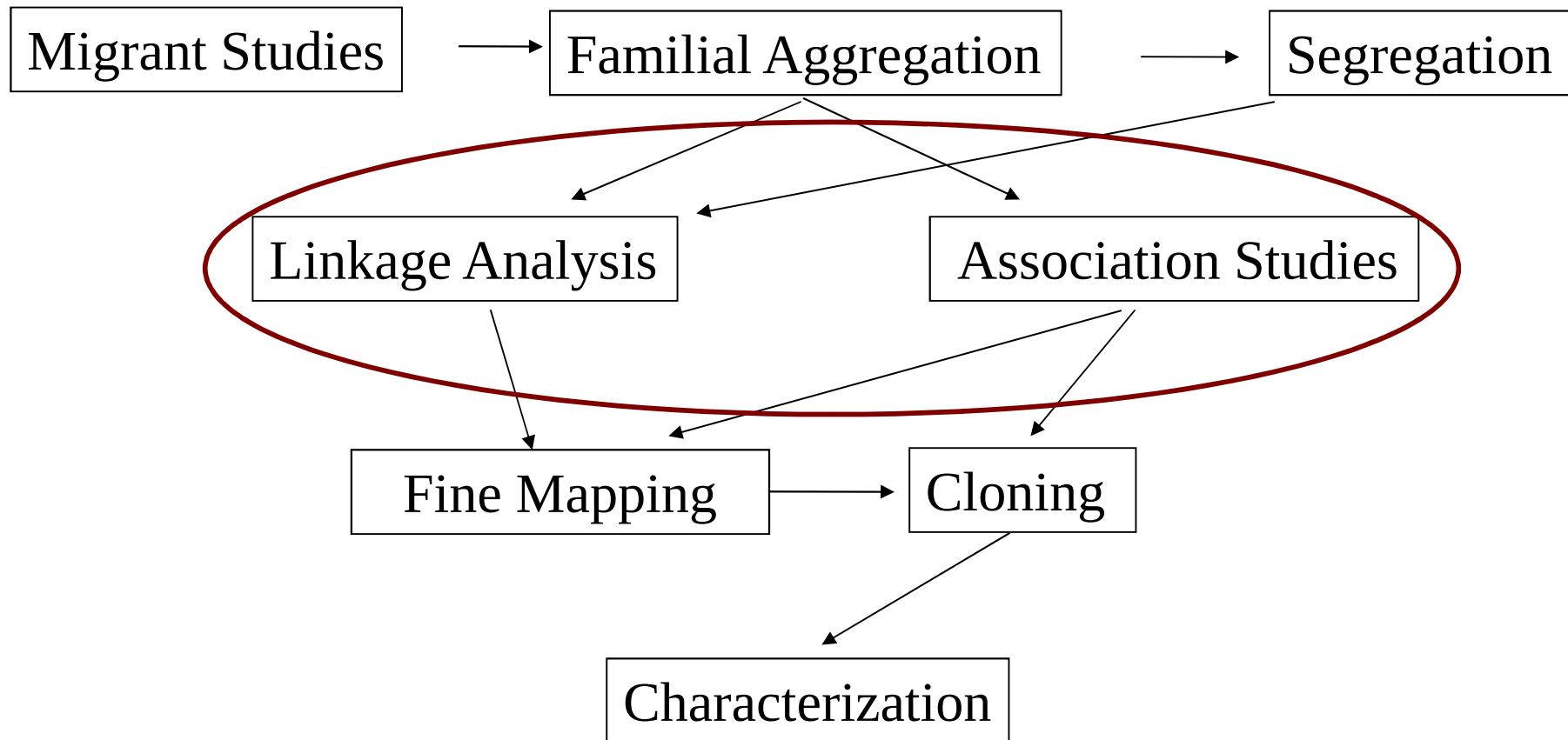
*“If you cannot get rid of the family skeleton, you may as well make it dance”  
(G.B. Shaw)*

# Outline

- Review last weeks homeworks
- **Linkage analysis, with PTC testing example**
  - **Background/Review**
  - Linkage analysis
- Family-based association tests
  - Overview
  - Trios/TDT
  - Discordant sibships

# Review: Process of Genetic Epidemiology

Defining the Phenotype



# Review: Population stratification

- **Population stratification** is a form of confounding in genetic studies where a gene under study shows marked variation in allele frequency across subgroups of a population and these subgroups differ in their baseline risk of disease
- Previous e.g. of Pima & Papago Indians: Significant allele frequency difference *because of ancestry, not disease status*
- **Family-based tests** today tend to be **robust to this** issue (not require any special adjustment)



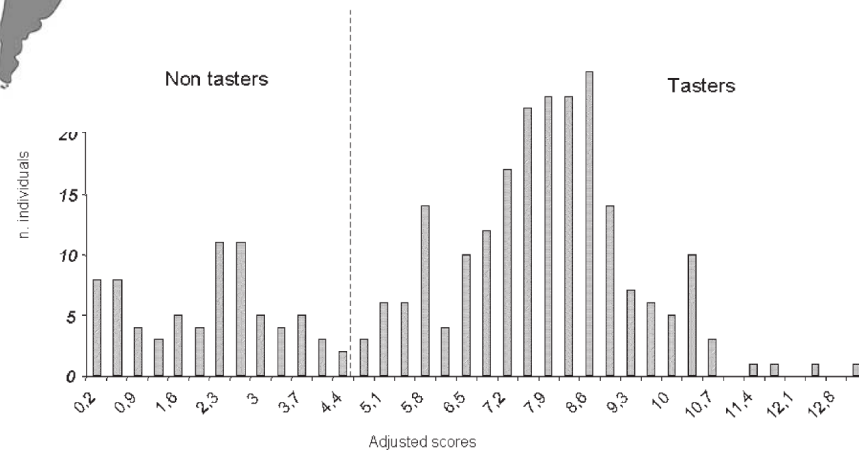
# Outline

- Review last weeks homeworks
- **Linkage analysis, with PTC testing example**
  - Background/Review
  - **Linkage analysis**
- Family-based association tests
  - Overview
  - Trios/TDT
  - Discordant sibships

# Geographic Distribution of PTC Phenotype



Wooding Genetics 2006, adapted from Cavalli-Sforza 1994



**Figure 1** Distribution of PTC sensitivity in Talana. The x-axis indicates the PTC scores, adjusted for age and sex using the following formula:  $n + [(a - a_M) / 20] - 0.73$  female, where  $n$  is the PTC tasted solution,  $a$  is the age of the individual and  $a_M$  is the average age of participants. The value of 0.73 was deducted in females since women are 0.73 dilution steps more sensitive than men at all ages. Distribution of corrected PTC scores showed significant bimodality with a cut-off value of 4.5 that separates the group of tasters from the group of non-tasters, with estimated means of 2.19 (SD = 1.12) and 7.99 (SD = 1.44), respectively.

# First PTC Family Study

|                                         | Number of Families | Children  |               |
|-----------------------------------------|--------------------|-----------|---------------|
|                                         |                    | Can taste | Can not taste |
| Both parents can taste                  | 40                 | 90        | 16            |
| One parent can taste, the other can not | 51                 | 80        | 37            |
| Neither parent can taste                | 9                  | 0         | 17            |

L. H. Snyder Science 1931

# Types of Genetic Studies

## Family Studies

Compare trait values across family members

## Linkage Analysis

Compare trait values with inheritance patterns

## Association

Compare trait values against genetic variants

# Family Studies

## Familial Relationships

- Twins
- Siblings
- Parents/offspring

## Phenotype information

- Affected/Unaffected (Prostate Cancer)
- Quantitative measure (Blood Pressure)

No Genotype information required for some things (e.g., lecture 2)

# Why do Family Studies?

Is the trait genetic?

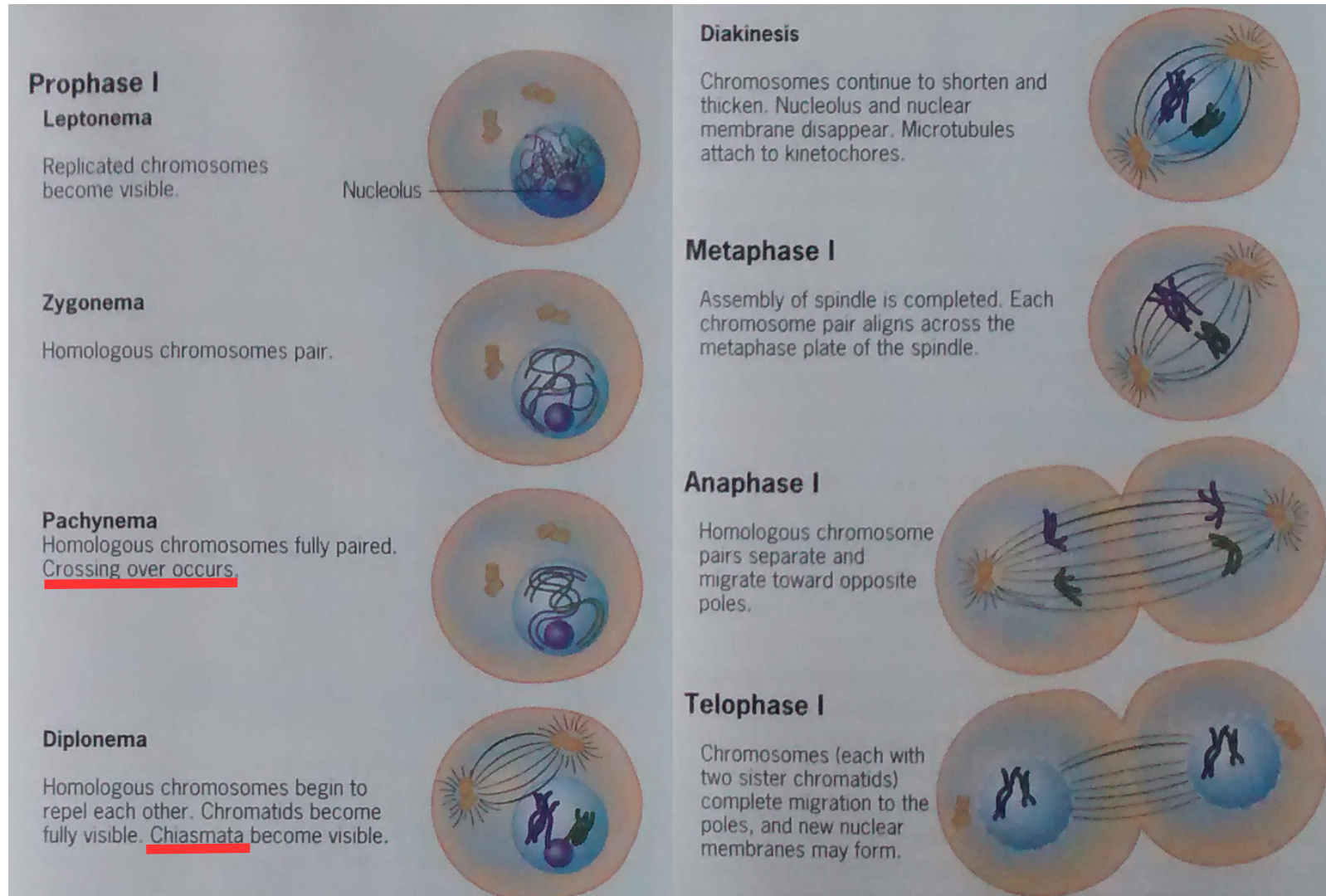
What is the mode of transmission?

- Dominant
- Recessive
- Additive
- Polygenic (Multiple genes involved)

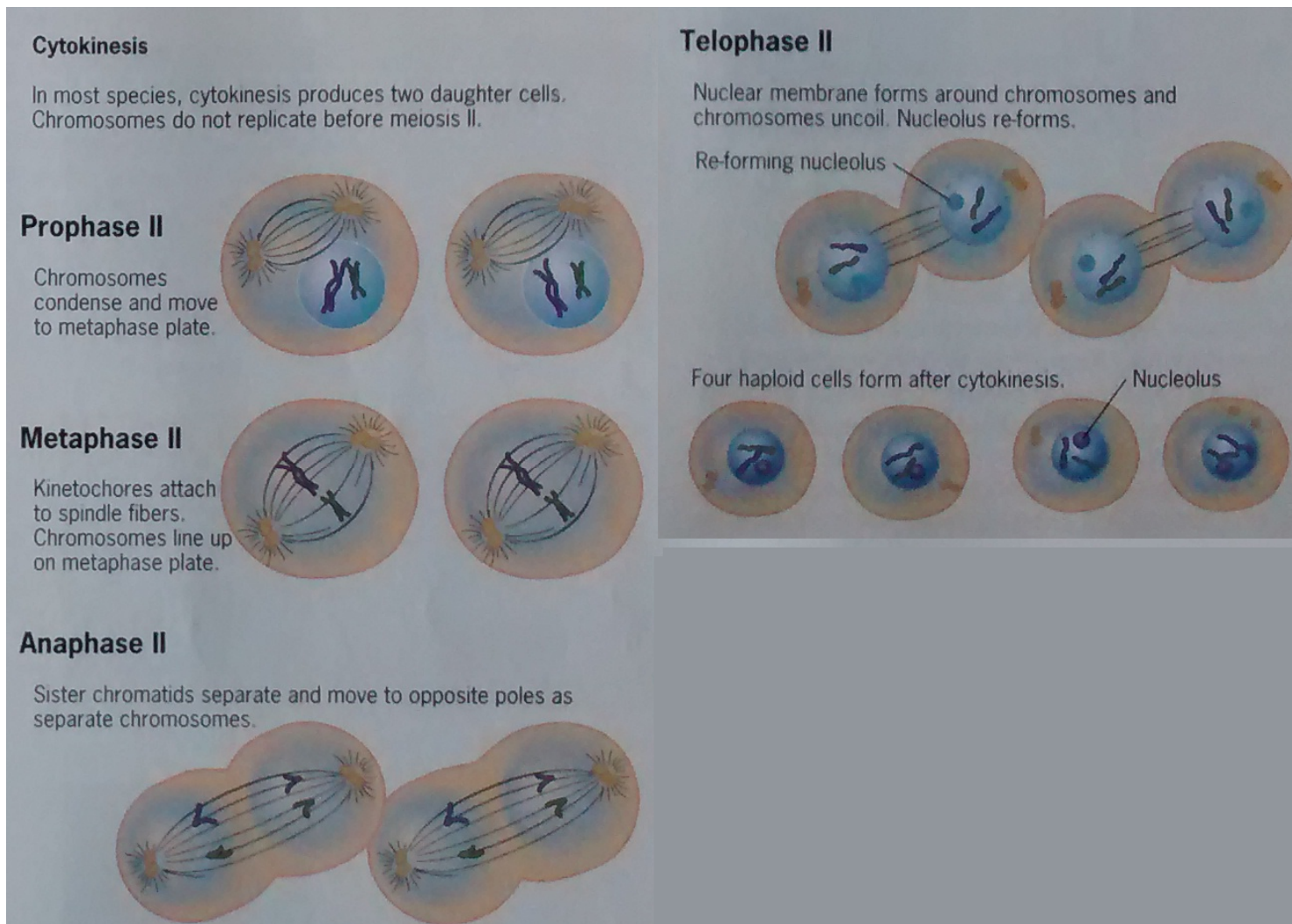


<https://belkazan.files.wordpress.com/2012/04/stripe-shirts-mother-father-child-at-the-beach-boardwalk.png?w=620>

# Meiosis – Crossing over

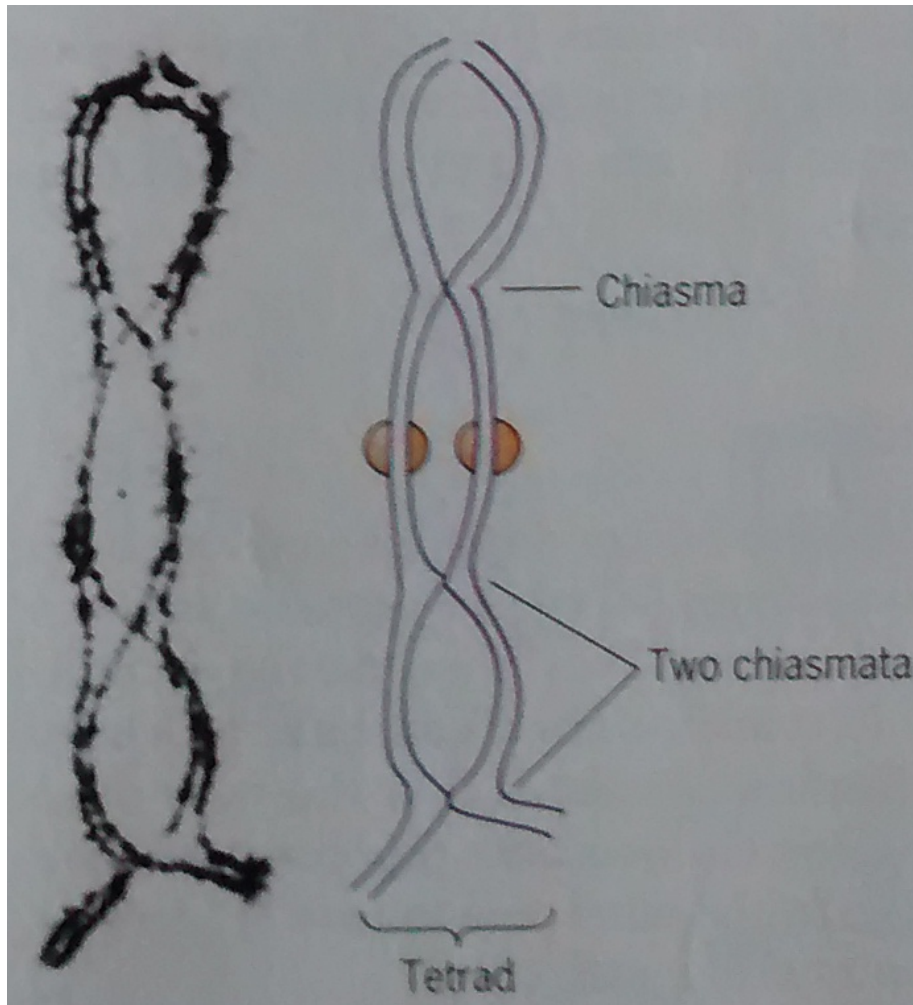


# Meiosis – Crossing over

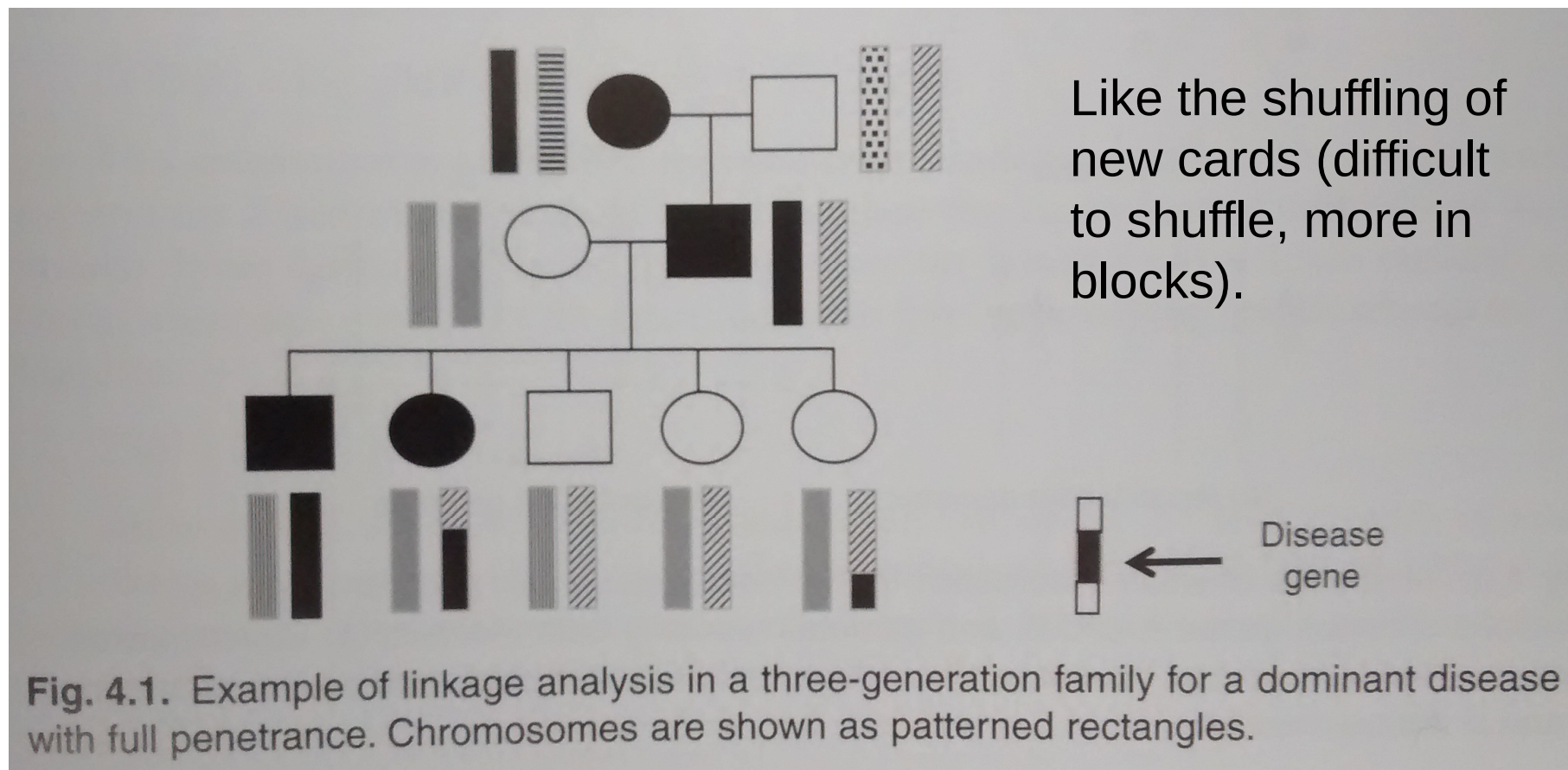




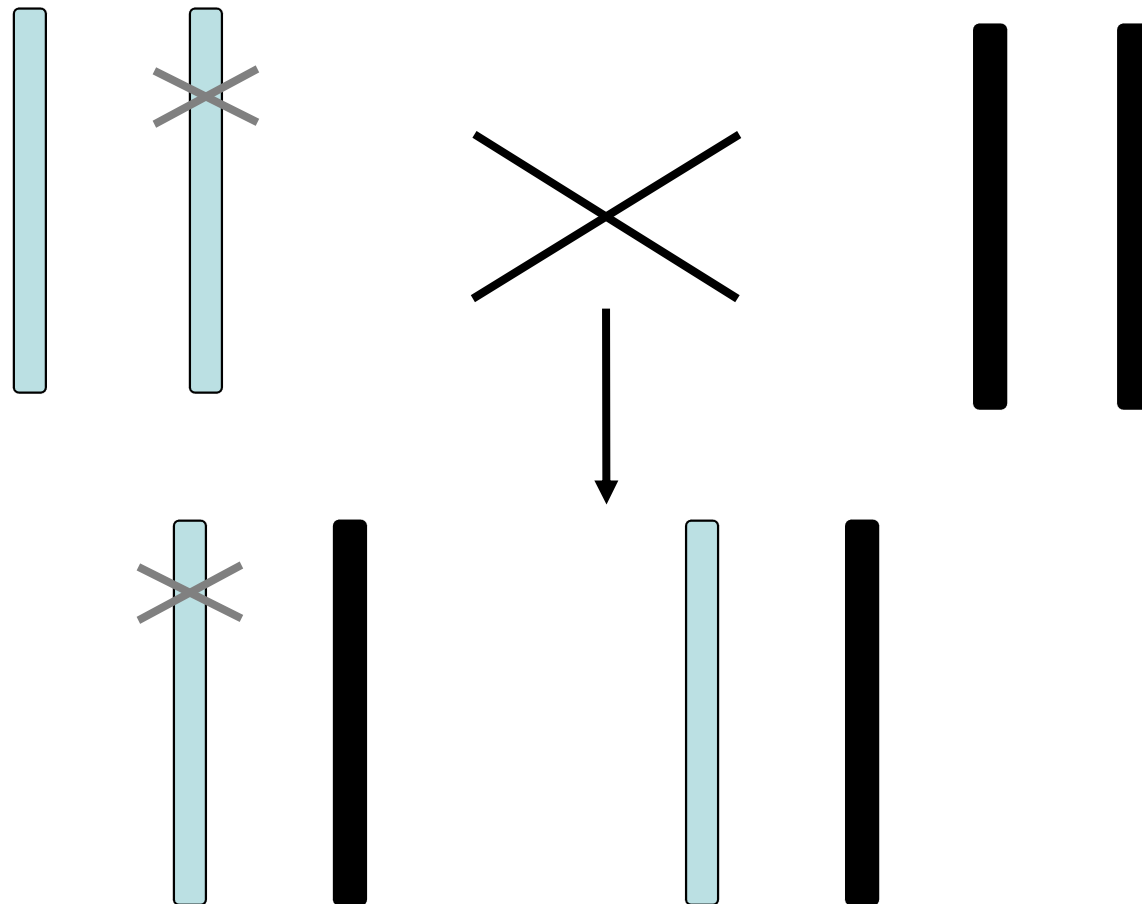
# Crossing over – Chiasmata



# Idea behind linkage

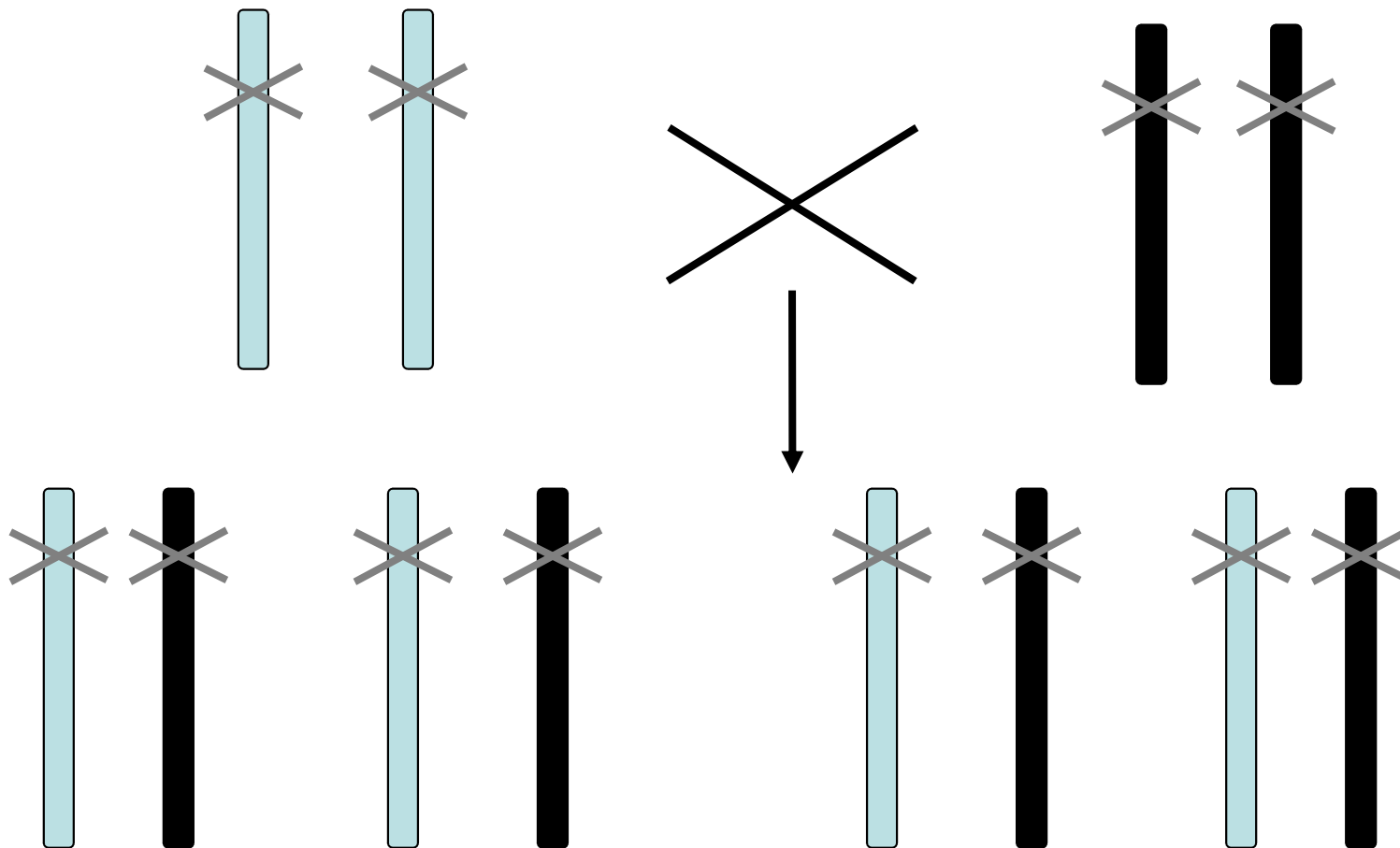


# Recall: Mutation and Meiosis



# Recall: E.g., Recessive trait

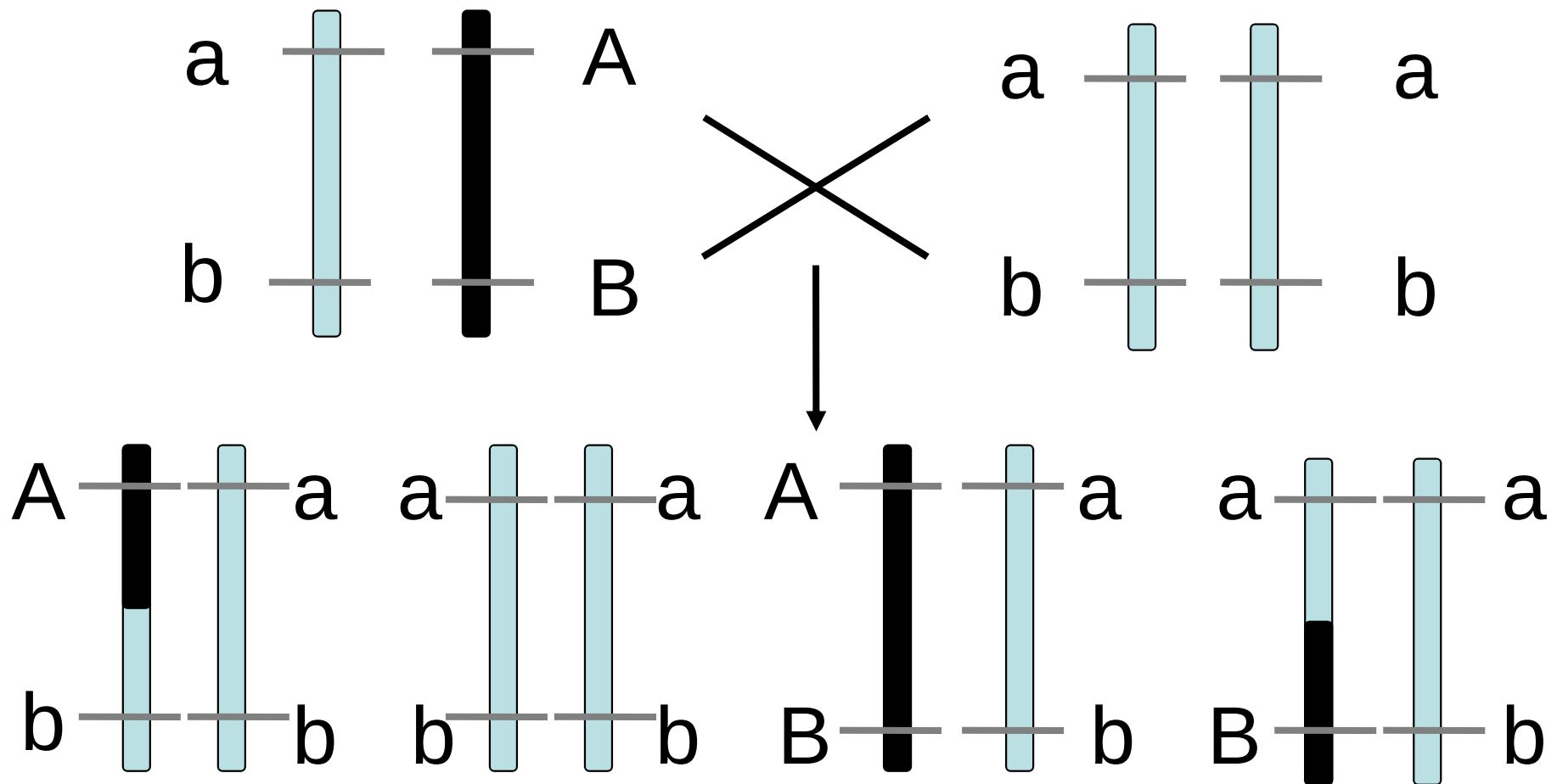
(Know the trait, know the genotype at the disease loci)



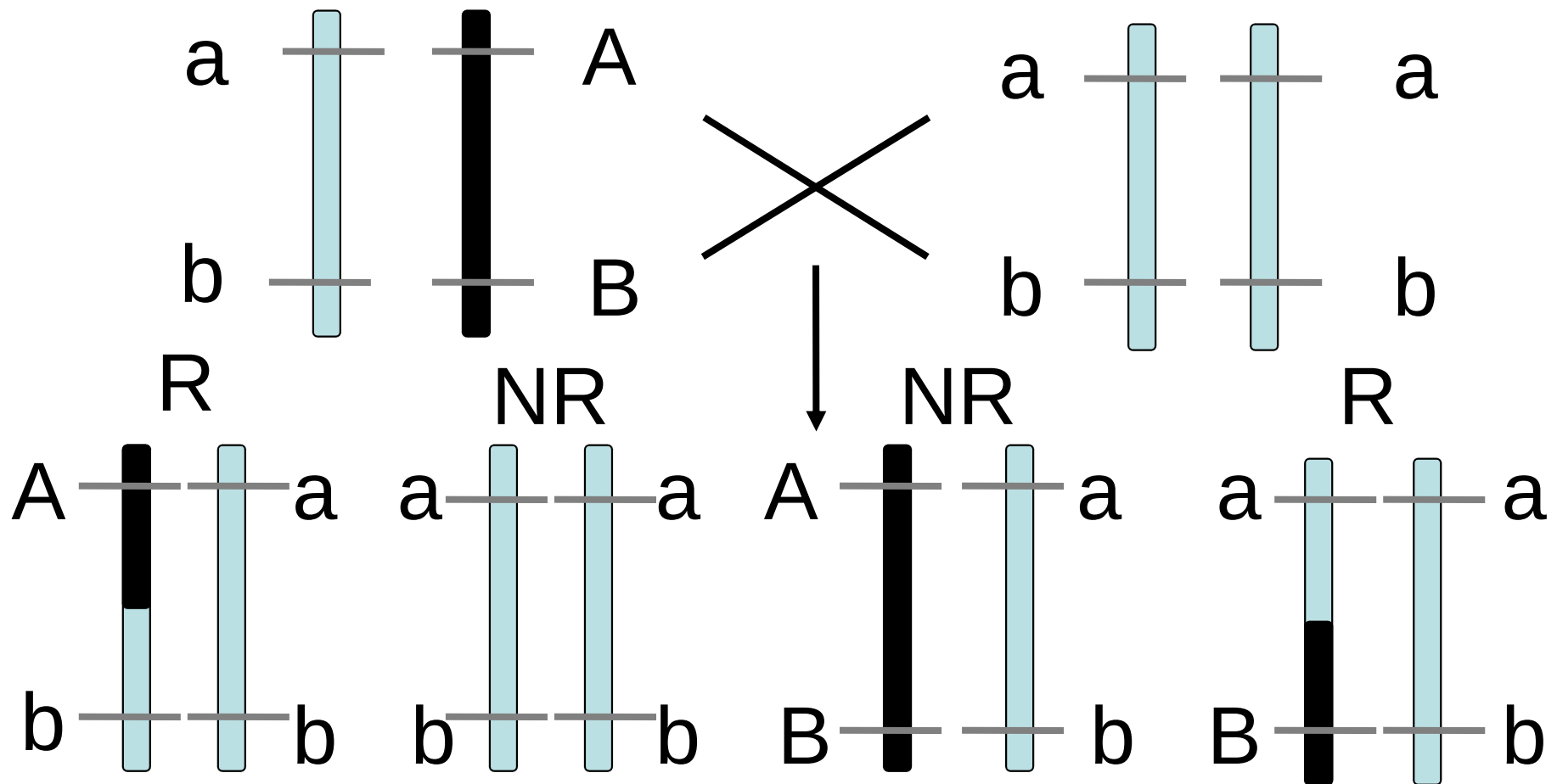
# Linkage Analysis

- Narrow down position of disease gene
- No biological knowledge needed
- Genetic markers (not disease gene)
- Recombination

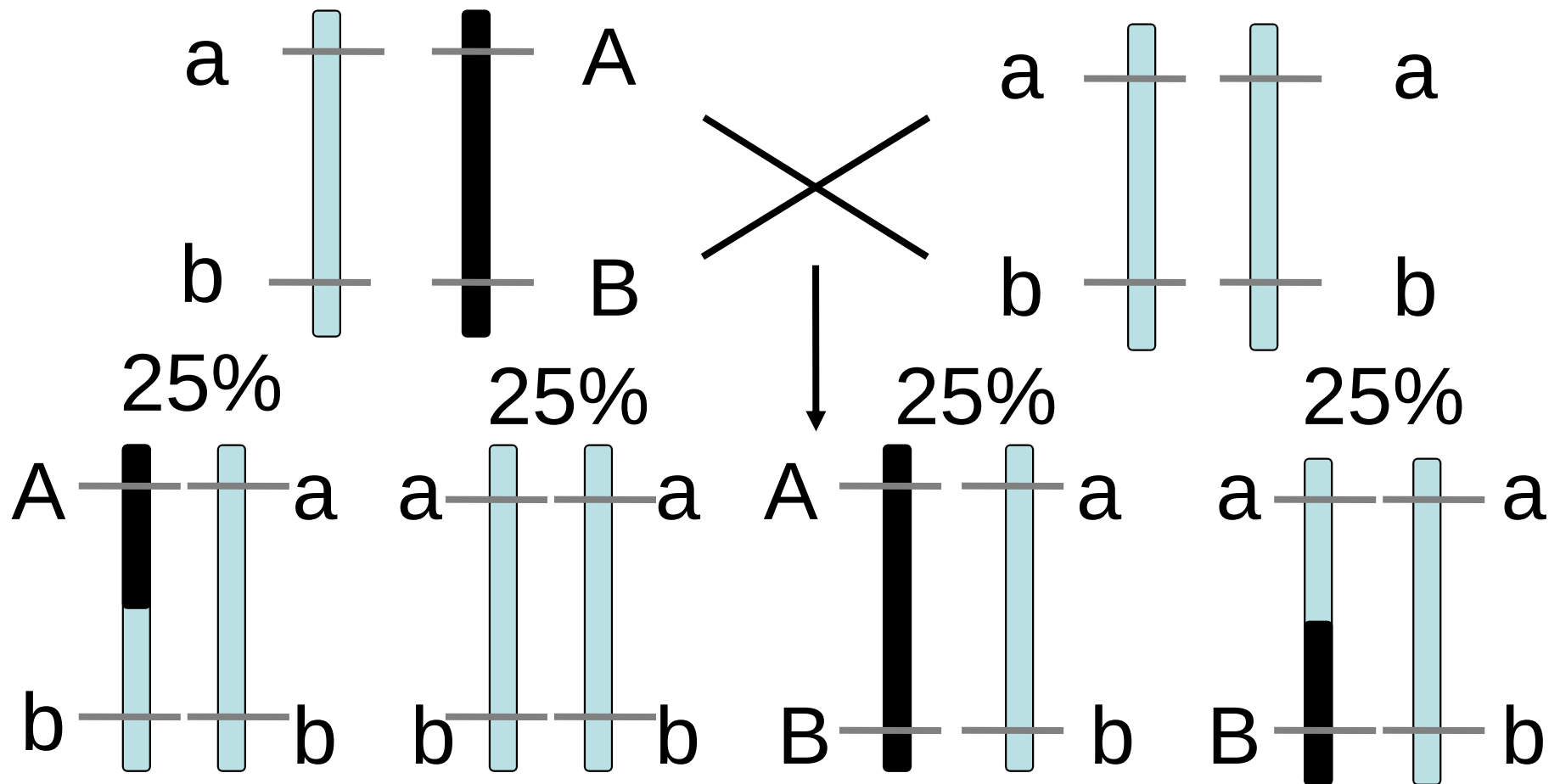
# Recombination – was there recombination?



# Recombination– was there recombination?

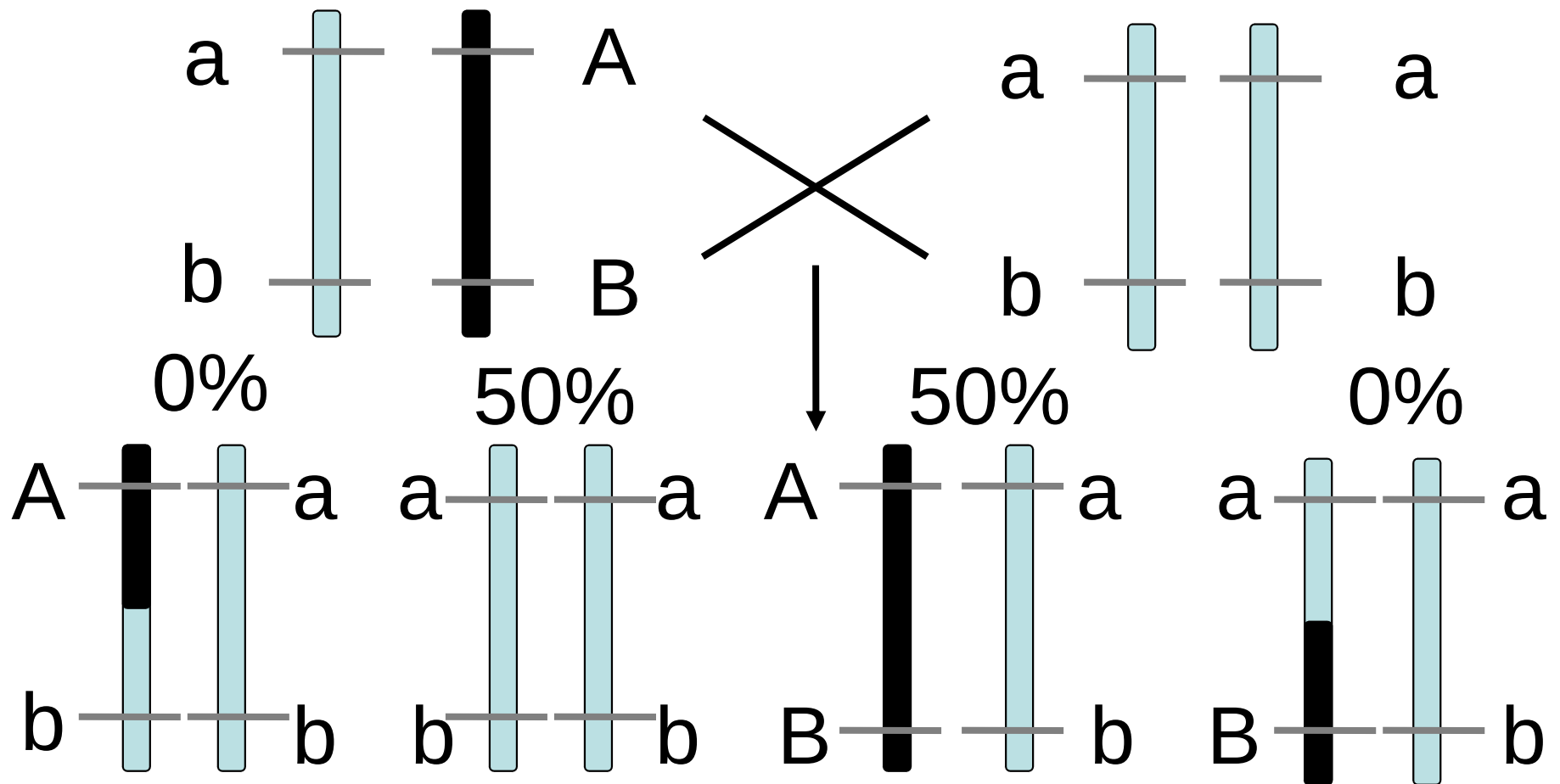


# Independent Assortment

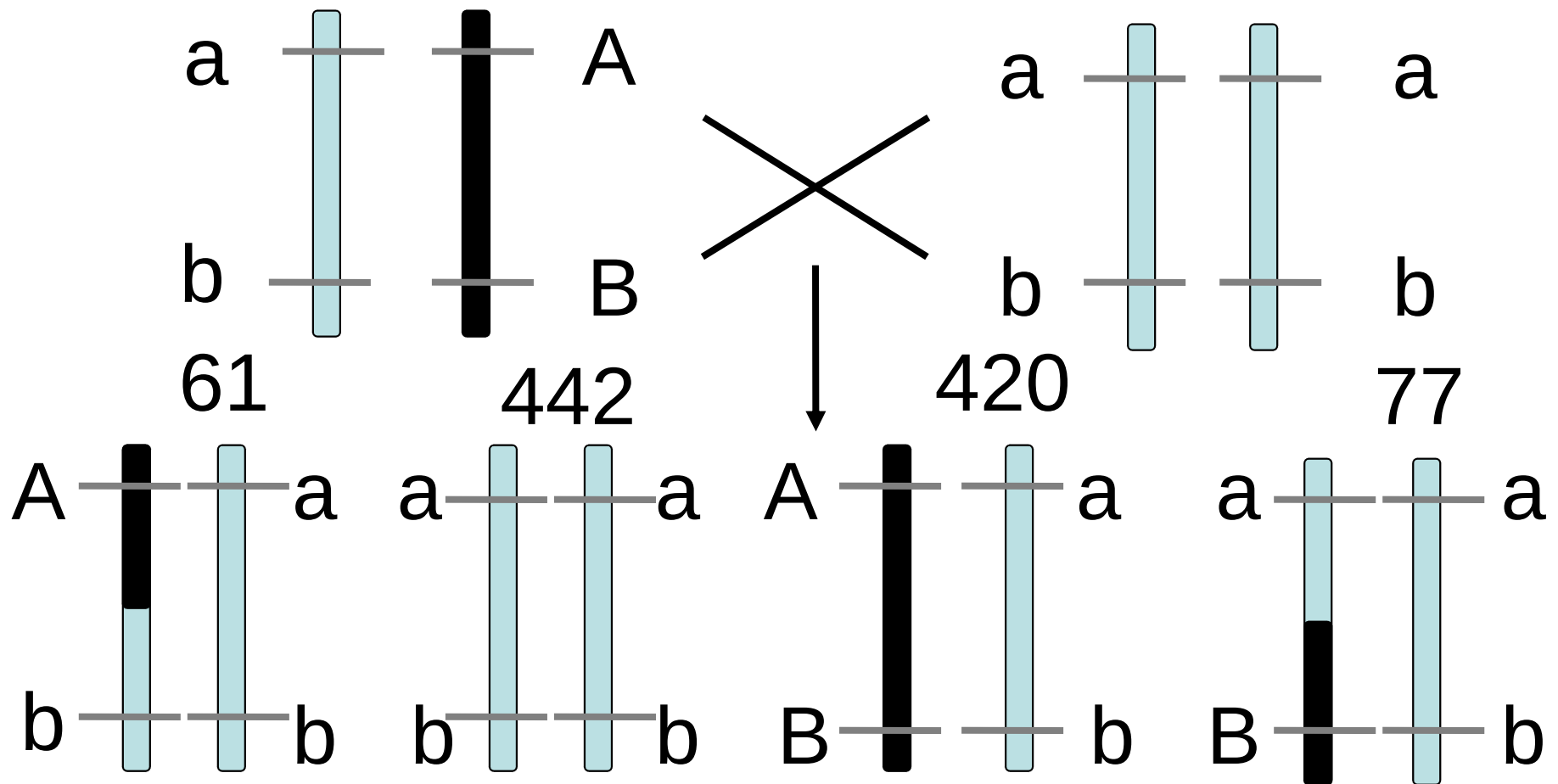




# No recombination

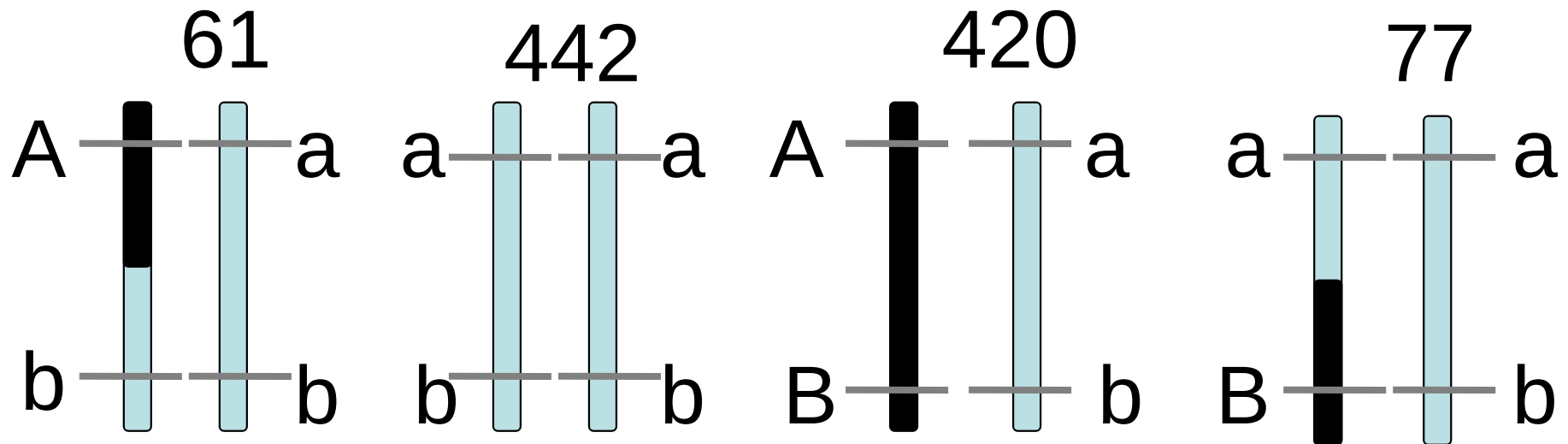


# Recombination Fraction



# Recombination Fraction

$$\theta = \text{Pr}(\text{observing a recombinant gene}) = \frac{\{\# \text{ Recombinants}\}}{\text{Total}} =$$



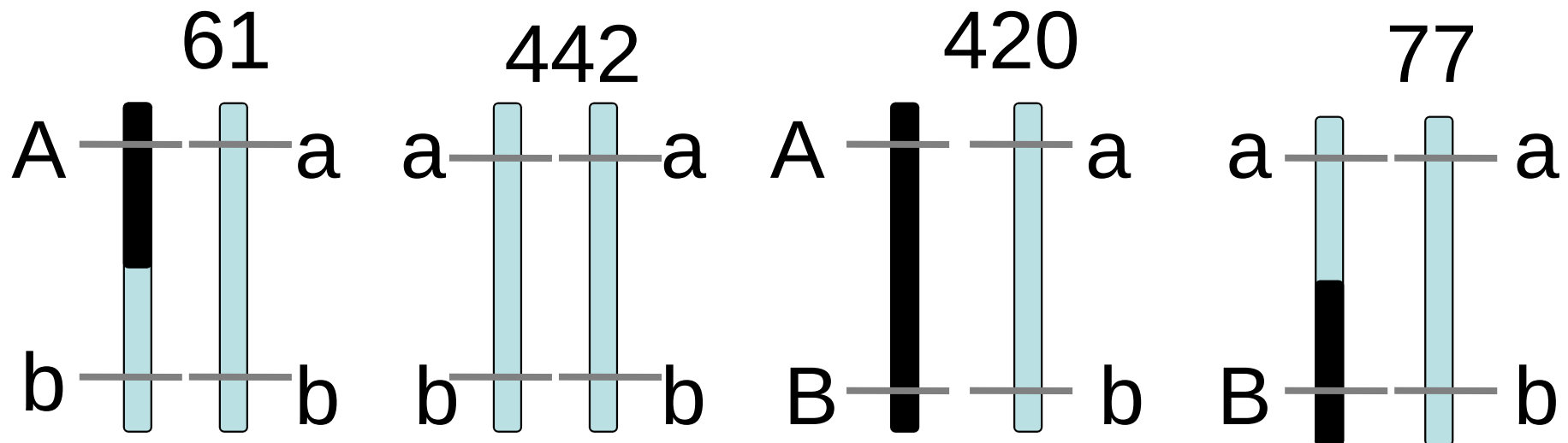
# Recombination Fraction

$\theta = \text{Pr}(\text{observing a recombinant gene}) =$

$\{\# \text{ Recombinants}\} / \text{Total} =$

$(61+77)/(61+77+442+420)=138/1000$

$= 13.8\% < 50\%$ , indicating  
some linkage

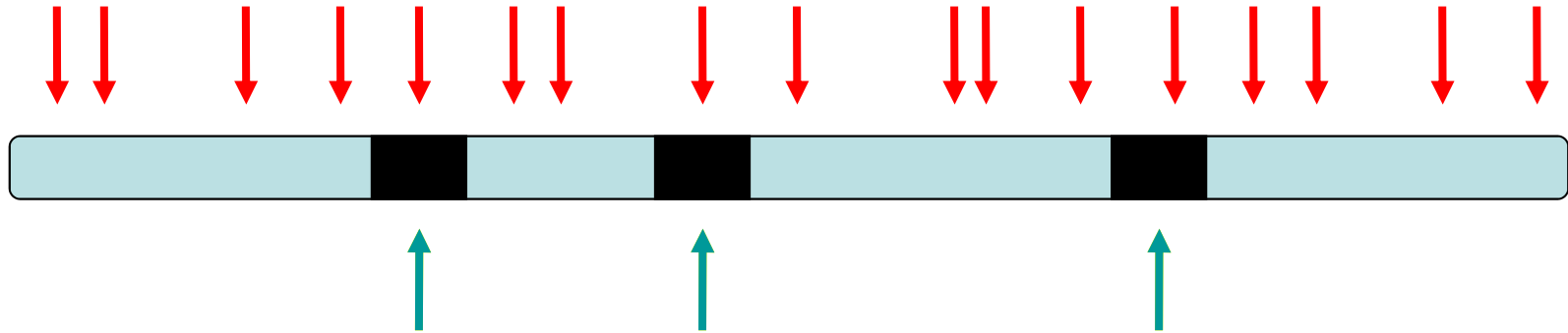


# Human Linkage Analysis

- RFLP Markers for Linkage (1980)
- Huntington's Disease Linkage (1983)
- Cystic Fibrosis Linkage (1985)
- Cystic Fibrosis Gene (1989)
- Huntington's Disease Gene (1993)

# Genome-wide linkage analysis

Genetic Markers



Genes

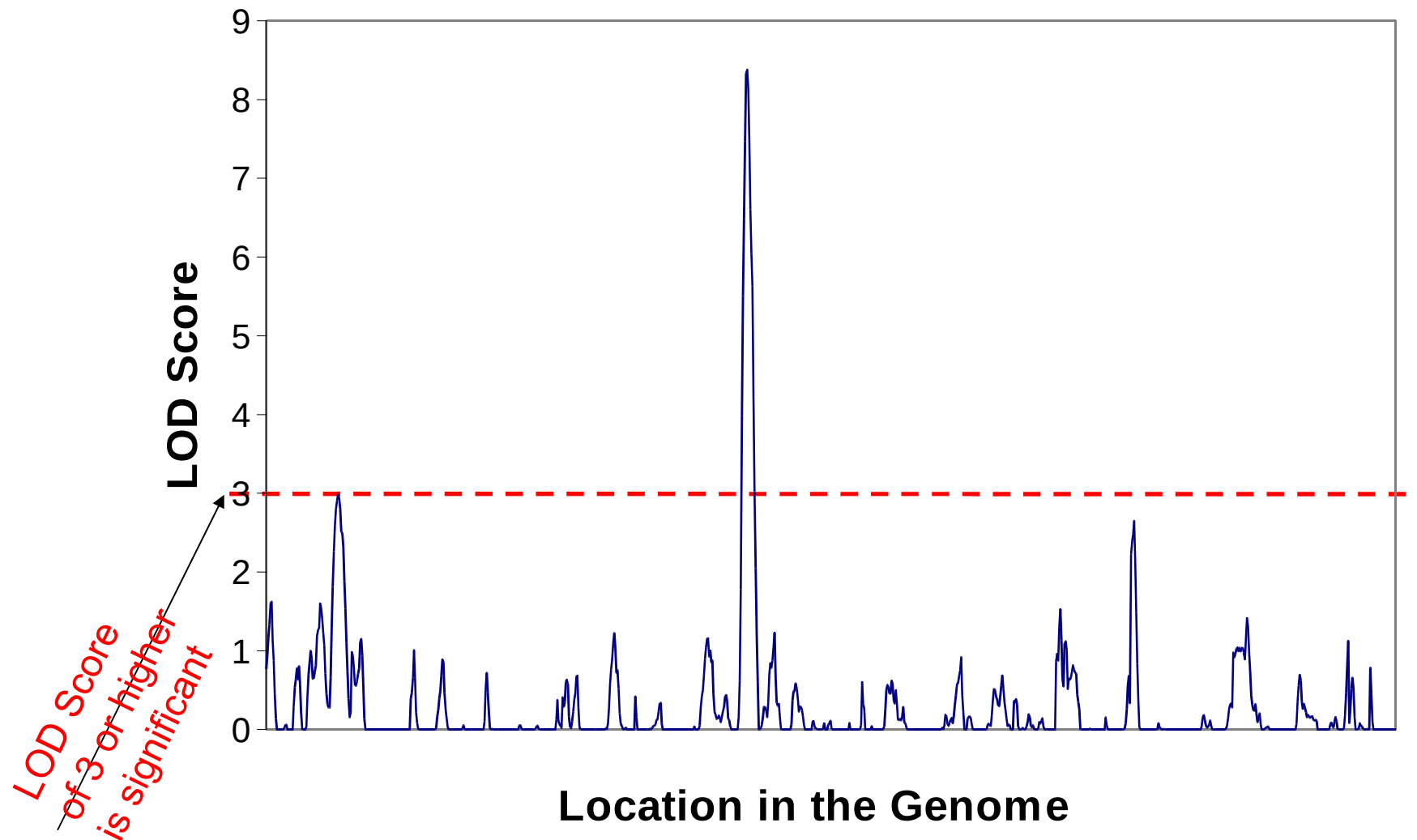
# Linkage Analysis

LOD score based on recombination

$$\text{LOD } (\theta) = \log \left( \frac{(\theta)^R (1 - \theta)^{NR}}{(\theta_{\text{null}} = 1/2)^{R + NR}} \right)$$

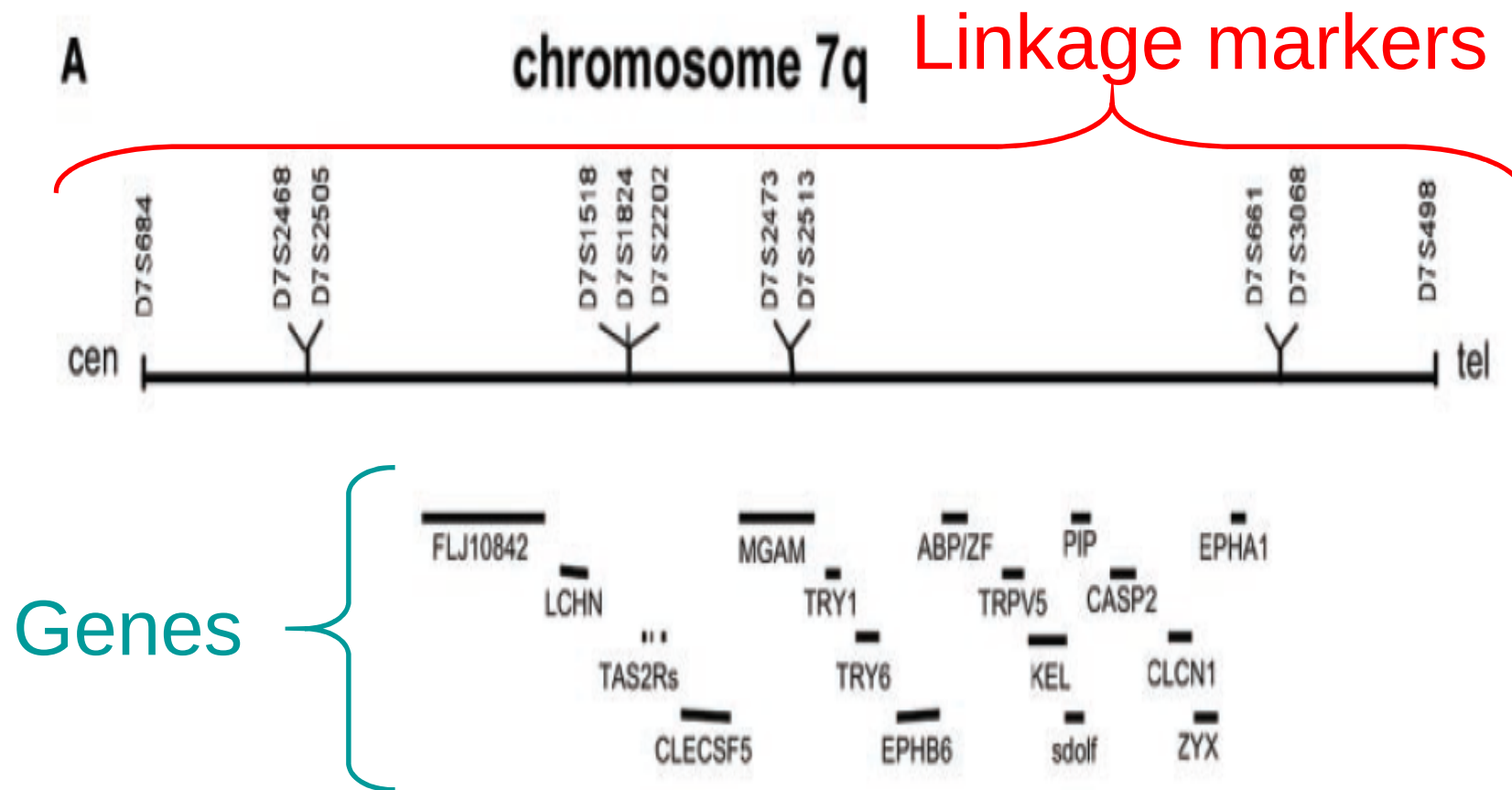
(Likelihood ratio test)

# PTC Linkage Analysis





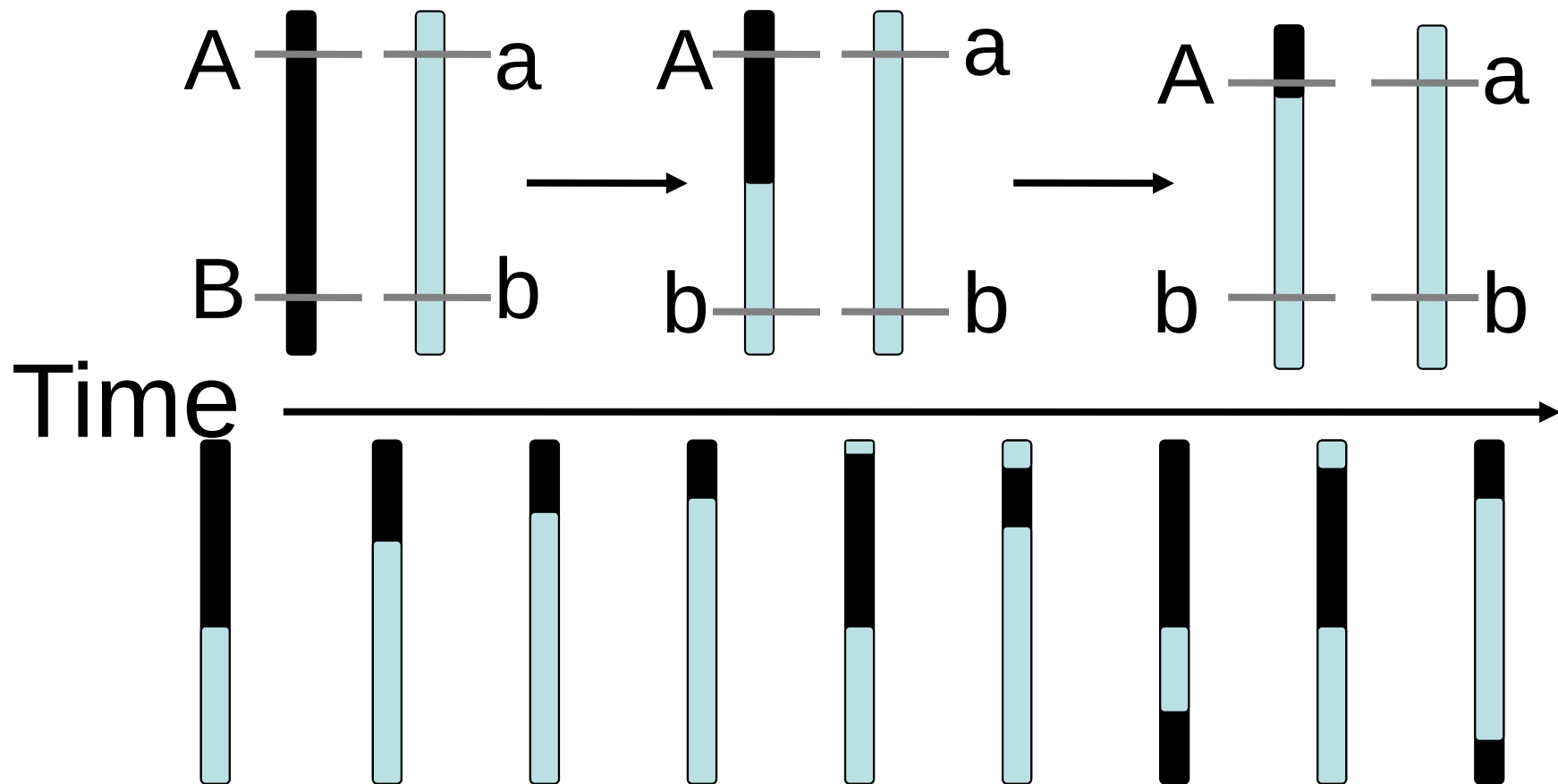
After you have found a linkage peak:  
Fine Mapping (more details later)



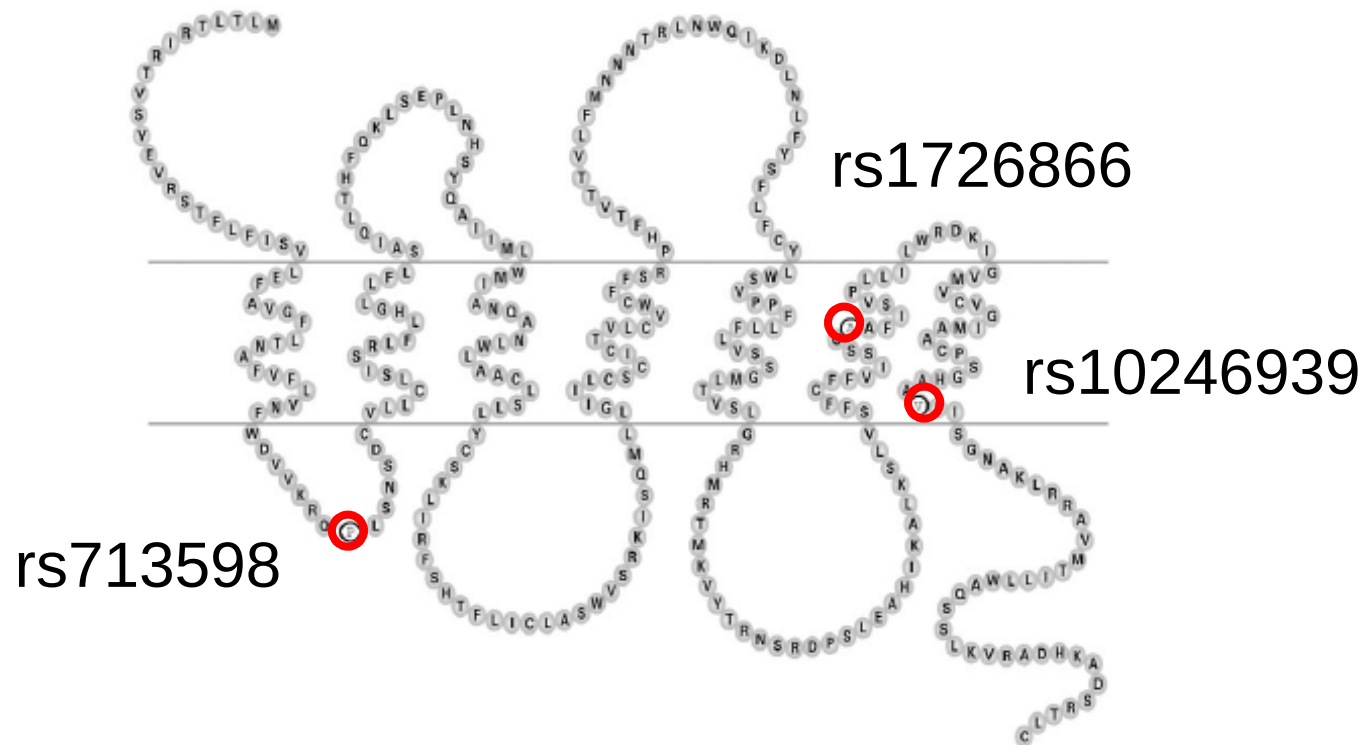
Kim et al. Science 2003

# Key Idea: Linkage and Linkage Disequilibrium

Linkage Disequilibrium blocks (LD) are much finer resolution than just linkage (basically the effect of linkage over time, over many more generations) [Shuffle cards more and more...]



# TAS2R38 Receptor Structure



Kim et al. J Dent Res 2004

# 3 SNPs form 3 Haplotypes

Taster



Non-taster



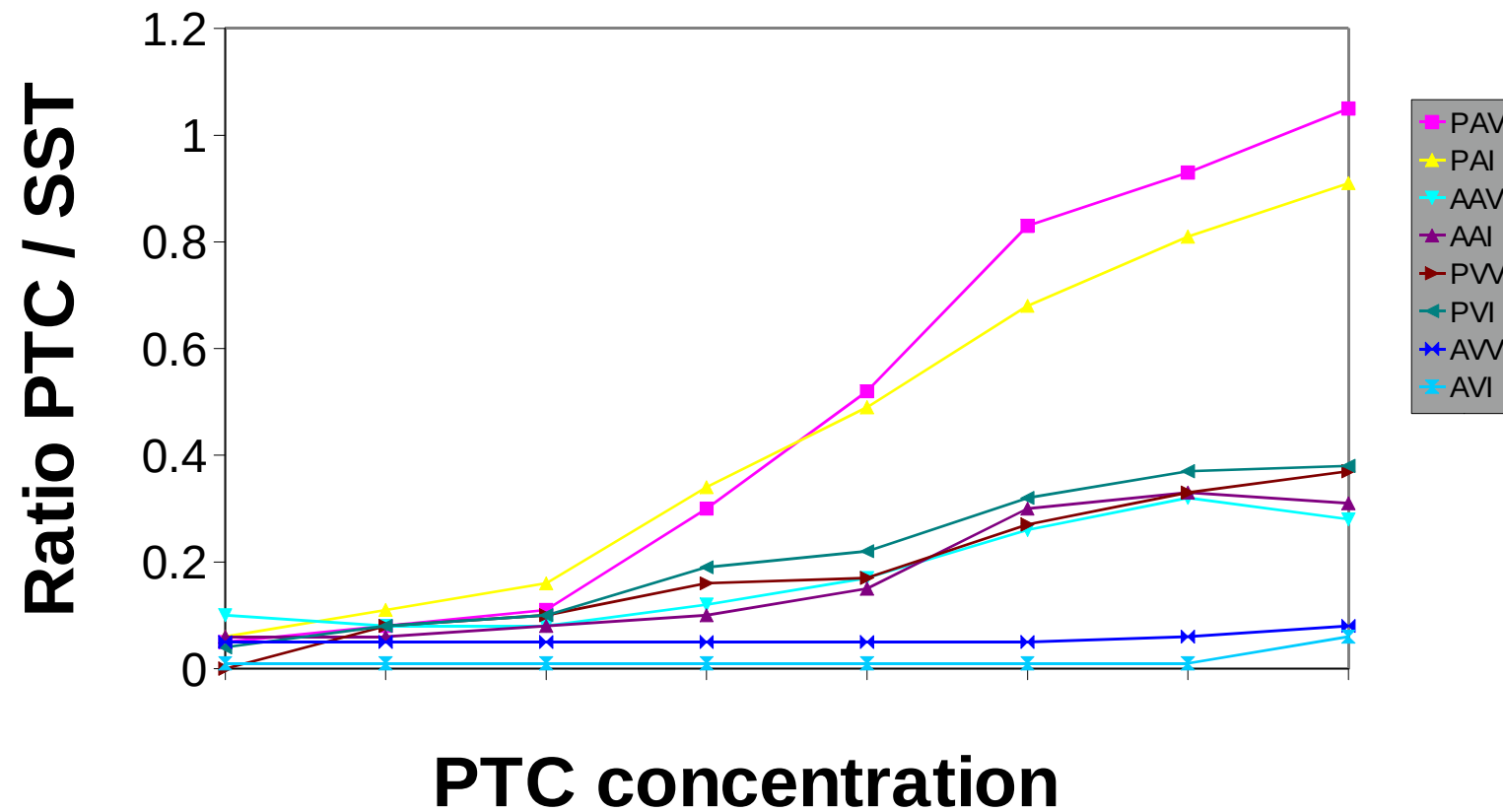
Rare



# Predicted Effect of the 3 SNPs

| Amino Acid Substitution |       | PAM250 |
|-------------------------|-------|--------|
| P                       | --> A | 1      |
| A                       | --> V | 0      |
| V                       | --> I | 4      |

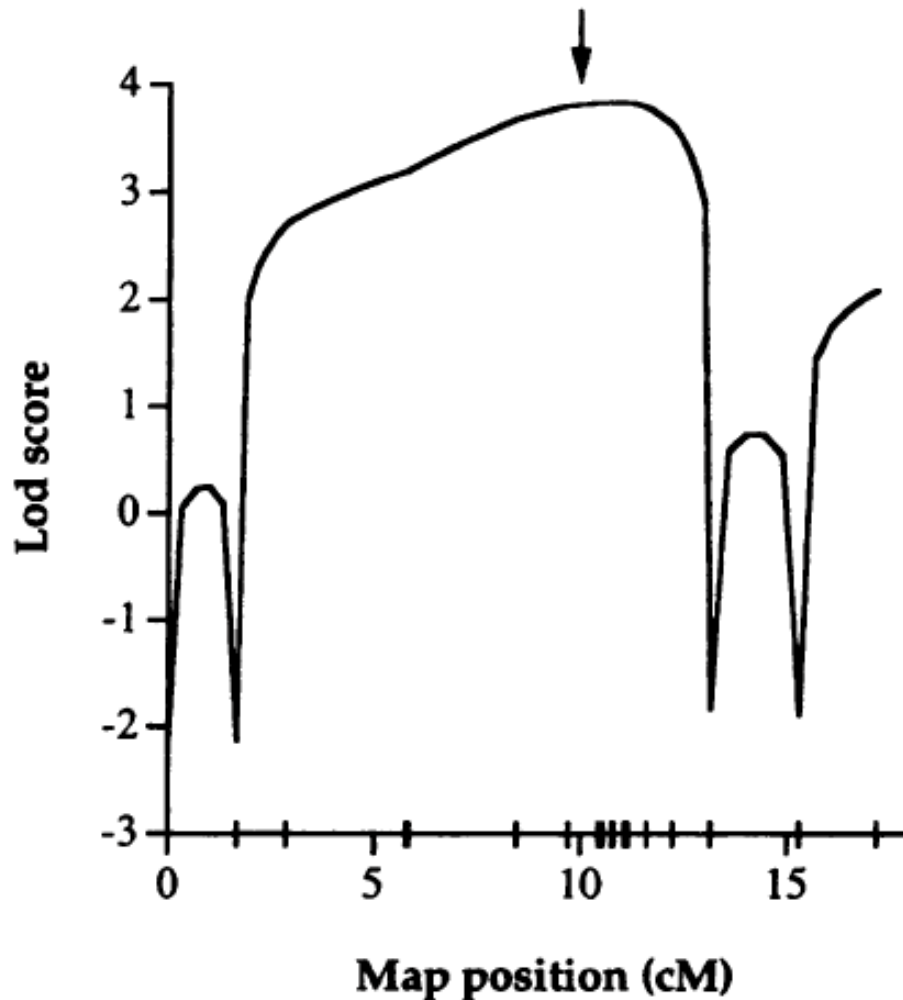
## TAS2R38 Haplotype function in vitro



# Exome sequencing and examples in Austin book following up on linkage peaks:

- BRCA2 Breast cancer
- HOXB13 G84E
  - Took so long to find because they were initially looking in the wrong place!

# Multipoint Linkage Analysis



- Can base LOD score on multiple surrounding markers, same basic idea
- Austin refers to other articles
  - Khoury et al. (1993)
  - Ott (1999)



# Non-parametric linkage analysis

- Large multi-generational families most powerful for parametric linkage analysis
  - Difficult to ascertain!!!
- Non-parametric tests available for  $\geq 2$  affected siblings
- Instead of testing  $\theta$  directly, test excessive sharing identity by descent (IBD)
  - Always share exactly one allele IBD with parent, and so on

# Outline

- Review last weeks homeworks
- Linkage analysis, with PTC testing example
  - Background/Review
  - Linkage analysis
- **Family-based association tests**
  - **Overview**
  - **Trios/TDT**
  - **Discordant sibships**

# Moving from linkage to association...

- Linkage: Estimating & Testing recombination rates  $\theta$
- Association:
  - Difference in genotype frequencies (next lecture)
  - Difference in genotype transmission frequency (discuss next)
- Family based tests next based on composite hypothesis: No linkage, no association
- LD block much smaller than linkage, so much finer resolution (recall slide earlier over time...)
- *Much smaller* family structures than for linkage!

# Mendel's laws

- Recall the playing cards example...
- One allele from each parent for each gene
  - Many family based tests based on this, rather than estimating allele frequencies (case-control)

# Mendelian transmission: Ex

- E.g., parents are Aa, Aa:
  - $P(\text{offspring}=AA \mid \text{Mother}=Aa, \text{Father}=Aa)=?$
  - $P(\text{offspring}=Aa \mid \text{Mother}=Aa, \text{Father}=Aa)=?$
  - $P(\text{offspring}=aa \mid \text{Mother}=Aa, \text{Father}=Aa)=?$

| M\F | A  | a  |
|-----|----|----|
| A   | AA | Aa |
| a   | Aa | aa |

# Mendelian transmission: Ex

- E.g., parents are Aa, Aa:
  - $P(\text{offspring}=AA \mid \text{Mother}=Aa, \text{Father}=Aa)=1/4$
  - $P(\text{offspring}=Aa \mid \text{Mother}=Aa, \text{Father}=Aa)=1/2$
  - $P(\text{offspring}=aa \mid \text{Mother}=Aa, \text{Father}=Aa)=1/4$

| M\F | A  | a  |
|-----|----|----|
| A   | AA | Aa |
| a   | Aa | aa |

Conditioning on  
parents...

# Mendelian transmission: Ex

- E.g., parents are AA, Aa:
  - $P(\text{offspring}=AA \mid \text{Mother}=AA, \text{Father}=Aa)=?$
  - $P(\text{offspring}=Aa \mid \text{Mother}=AA, \text{Father}=Aa)=?$
  - $P(\text{offspring}=aa \mid \text{Mother}=AA, \text{Father}=Aa)=?$

| M\F | A  | a  |
|-----|----|----|
| A   | AA | Aa |
| A   | AA | Aa |

# Mendelian transmission: Ex

- E.g., parents are AA, Aa:
  - $P(\text{offspring}=AA \mid \text{Mother}=AA, \text{Father}=Aa)=1/2$
  - $P(\text{offspring}=Aa \mid \text{Mother}=AA, \text{Father}=Aa)=1/2$
  - $P(\text{offspring}=aa \mid \text{Mother}=AA, \text{Father}=Aa)=0$

| M\F | A  | a  |
|-----|----|----|
| A   | AA | Aa |
| A   | AA | Aa |



# Mendelian transmission: Ex

- E.g., parents are AA, AA:
  - $P(\text{offspring}=AA \mid \text{Mother}=AA, \text{Father}=AA)=?$
  - $P(\text{offspring}=Aa \mid \text{Mother}=AA, \text{Father}=AA)=?$
  - $P(\text{offspring}=aa \mid \text{Mother}=AA, \text{Father}=AA)=?$

| M\F | A  | A  |
|-----|----|----|
| A   | AA | AA |
| A   | AA | AA |

# Mendelian transmission: Ex

- E.g., parents are AA, AA:
  - $P(\text{offspring}=AA \mid \text{Mother}=AA, \text{Father}=AA)=1$
  - $P(\text{offspring}=Aa \mid \text{Mother}=AA, \text{Father}=AA)=0$
  - $P(\text{offspring}=aa \mid \text{Mother}=AA, \text{Father}=AA)=0$

| M\F | A  | A  |
|-----|----|----|
| A   | AA | AA |
| A   | AA | AA |

*Homozygote parents are “non-informative” (no variation in offspring’s conditional genotype distribution)*

# Trios: Transmission Disequilibrium Test (TDT)

- Test based on transmissions from parents to offspring
- Assumptions:
  - Parents' and offspring genotypes are known
  - Dichotomous phenotype (thought Q-TDT), only affected offspring
- Count transmissions from heterozygote parents, and compare to expected transmissions
  - Mendel's laws of segregation (previous slides) not control group
  - Test for over/under-transmission of alleles in cases (intuition)
- Conditional test
  - Offspring affection status
  - Parental genotypes (conditions out allele frequencies, which is what case-control is based on testing)
- Composite null hypothesis: No linkage, no association

# Trios: Transmission Disequilibrium Test (TDT)

|                             |   | Non-transmitted parental allele |   |
|-----------------------------|---|---------------------------------|---|
| Transmitted parental allele | A | w                               | x |
|                             | a | y                               | z |

- w AA parents (transmit one A, do not transmit other A)
- z aa parents (transmit one a, do not transmit other a)
- x Aa parents that transmit A, do not transmit a
- y Aa parents that transmit a, do not transmit A

# Possible Parental Configurations

- **AA-AA, AA-Aa, AA-aa, Aa-AA, Aa-Aa, Aa-aa, aa-AA, aa-Aa, aa-aa**
  - (Ones not bolded are symmetric for what we will do next, e.g., AA-Aa == Aa-AA)
  - Six possible configurations

# Both parents homozygous

|                             |   | Non-transmitted parental allele |   | AA-AA  |
|-----------------------------|---|---------------------------------|---|--------|
| Transmitted parental allele | A | A                               | a | <br>AA |
|                             | a | 2                               | 0 |        |
|                             |   | 0                               | 0 |        |

- Offspring genotype is deterministic, no variation, not informative!

# Both parents homozygous

|                             |   | Non-transmitted parental allele |   |        |
|-----------------------------|---|---------------------------------|---|--------|
|                             |   | A                               | a | aa-aa  |
| Transmitted parental allele | A | 0                               | 0 | <br>aa |
|                             | a | 0                               | 2 |        |

- Offspring genotype is deterministic, no variation, not informative!

# Both parents homozygous

|                             |   | Non-transmitted parental allele |   |        |
|-----------------------------|---|---------------------------------|---|--------|
|                             |   | A                               | a | AA-aa  |
| Transmitted parental allele | A | 1                               | 0 | <br>Aa |
|                             | a | 0                               | 1 |        |

- Offspring genotype is deterministic, no variation, not informative!



# One parent heterozygous

|                             |   |                                 |   |        |    |      |
|-----------------------------|---|---------------------------------|---|--------|----|------|
|                             |   | Non-transmitted parental allele |   | AA-Aa  |    |      |
|                             |   | A                               | a |        |    |      |
| Transmitted parental allele | A | 1                               | 1 | AA, Aa |    |      |
|                             | a | 0                               | 0 | .5     | .5 | ← Pr |
|                             |   | Non-transmitted parental allele |   |        |    |      |
|                             |   | A                               | a |        |    |      |
| Transmitted parental allele | A | 1                               | 0 |        |    |      |
|                             | a | 1                               | 0 |        |    |      |

- Variation from one parent

# One parent heterozygous

|                             |   |                                 |   |         |    |      |
|-----------------------------|---|---------------------------------|---|---------|----|------|
|                             |   | Non-transmitted parental allele |   | Aa - aa |    |      |
|                             |   | A                               | a |         |    |      |
| Transmitted parental allele | A | 0                               | 1 | Aa, aa  |    |      |
|                             | a | 0                               | 1 | .5      | .5 | ← Pr |

|                             |   |                                 |   |
|-----------------------------|---|---------------------------------|---|
|                             |   | Non-transmitted parental allele |   |
|                             |   | A                               | a |
| Transmitted parental allele | A | 0                               | 0 |
|                             | a | 1                               | 1 |

- Variation from one parent

# Both parents heterozygous

|                             |   | Non-transmitted parental allele |   | Aa - Aa<br>                     |
|-----------------------------|---|---------------------------------|---|---------------------------------|
|                             |   | A                               | a |                                 |
| Transmitted parental allele | A | 0                               | 2 | AA, Aa, aa<br>.25, .5, .25 ← Pr |
|                             | a | 0                               | 0 |                                 |

|                             |   | Non-transmitted parental allele |   |                             |   | Non-transmitted parental allele |   |
|-----------------------------|---|---------------------------------|---|-----------------------------|---|---------------------------------|---|
|                             |   | A                               | a |                             |   | A                               | a |
| Transmitted parental allele | A | 0                               | 1 | Transmitted parental allele | A | 0                               | 0 |
|                             | a | 1                               | 0 |                             | a | 2                               | 1 |

- Variation from both parents

# Trios: Transmission Disequilibrium Test (TDT)

|                             |   | Non-transmitted parental allele |   |
|-----------------------------|---|---------------------------------|---|
|                             |   | A                               | a |
| Transmitted parental allele | A | w                               | x |
|                             | a | y                               | z |

- w AA parents (transmit one A, do not transmit other A)
- z aa parents (transmit one a, do not transmit other a)
- x Aa parents that transmit A, do not transmit a
- y Aa parents that transmit a, do not transmit A

# Transmission Disequilibrium Test (TDT)

|                             |   | Non-transmitted parental allele |   |
|-----------------------------|---|---------------------------------|---|
|                             |   | A                               | a |
| Transmitted parental allele | A | w                               | x |
|                             | a | y                               | z |

- No variation in w or z (recall homozygous parents non informative)
- $(x-y)^2/(x+y) \sim \chi_1^2$ ; it's just special case of McNemar's test
- Think of it as testing are there an excess of the A allele in the affected offspring than would happen by Mendel's laws?

# Transmission Disequilibrium Test (TDT)

|                             |   | Non-transmitted parental allele |    | Insulin Dependent Diabetes Mellitus (IDDM) |
|-----------------------------|---|---------------------------------|----|--------------------------------------------|
|                             |   | A                               | a  |                                            |
| Transmitted parental allele | A | ?                               | 78 |                                            |
|                             | a | 46                              | ?  |                                            |

- Example from the text: 94 families, 78 parents transmit allele A, 46 transmit allele a
- $(78-46)^2/(78+46)=8.26$ , p-value=0.004

# Limitations of TDT

- Limitations
  - Only affected offspring
  - Only dichotomous phenotypes
  - Bi-allelic markers
  - Additive genetic model
  - No missing parents
  - Can't do multiple markers, multiple phenotypes
- Extensions exist for most everything you'd want to do, e.g., FBAT, ...*(next slide)*...

# Discordant sibships

- Conditional logistic regression
  - $P(Y_1=1|Y_1+Y_2=1, g_1, g_2, \dots)$
  - Matching each sib together, conditions on the fact that *they have discordant phenotypes*
  - Standard model for disease as in logistic regression, just matching based on family strata
    - Interpret coefficients, etc., in the same way
- You will go through an example in the homework



# Summary

- Family-based studies can be robust to population substructure (based on Mendelian transmissions, not population allele frequencies)
- Robust to HWE failure
- **Powerful for very rare, highly penetrant traits**