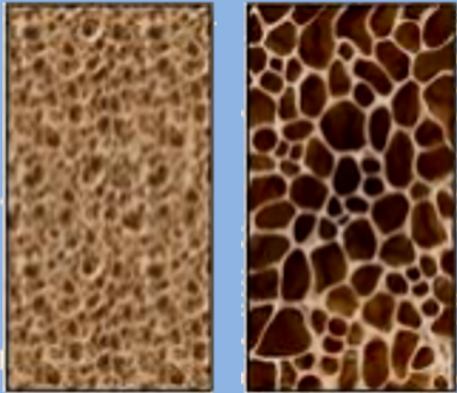


**Abordagens integrativas para se estudar doenças complexas**

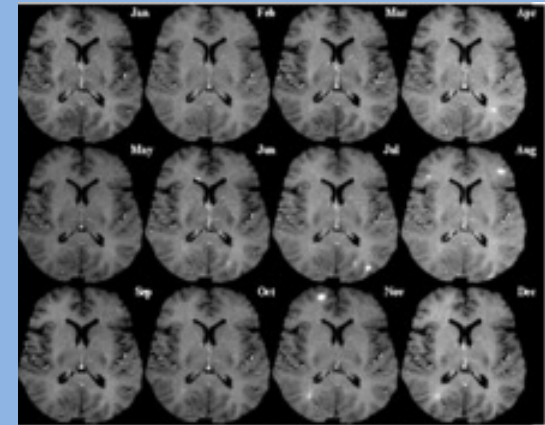
# DOENÇAS COMPLEXAS



osteoporose



lupus



esclerose múltipla



asma



Alzheimer'  
s

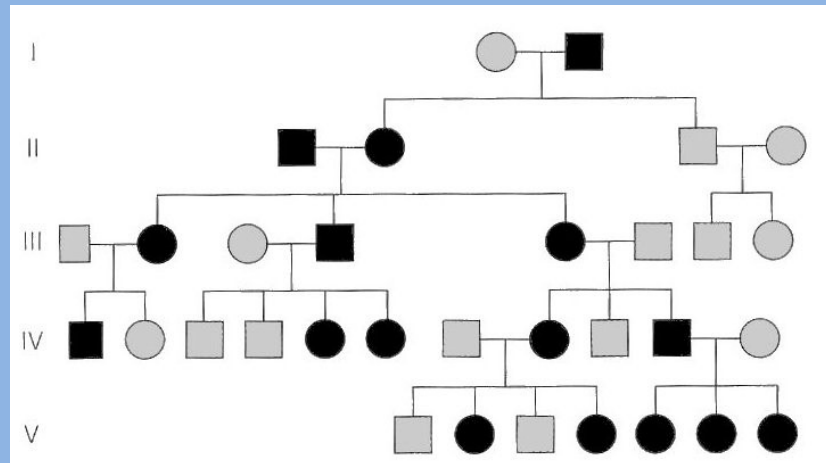


escleroderma

# DOENÇAS COMPLEXAS

## Critério de diagnóstico

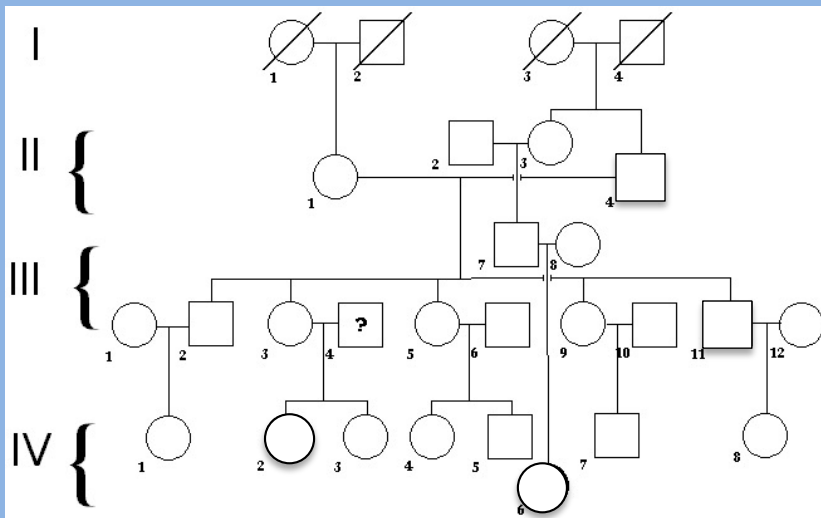
- Indivíduo Afetado ou Não afetado
- Doenças psiquiátricas e do comportamento



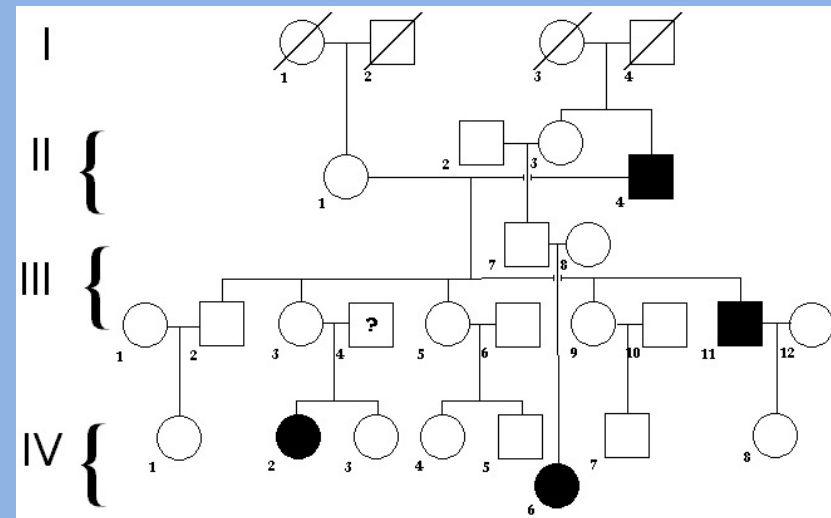
# DOENÇAS COMPLEXAS

## Base Genética

Pessoas que compartilham DNA, tem mais chance de apresentar o mesmo fenótipo



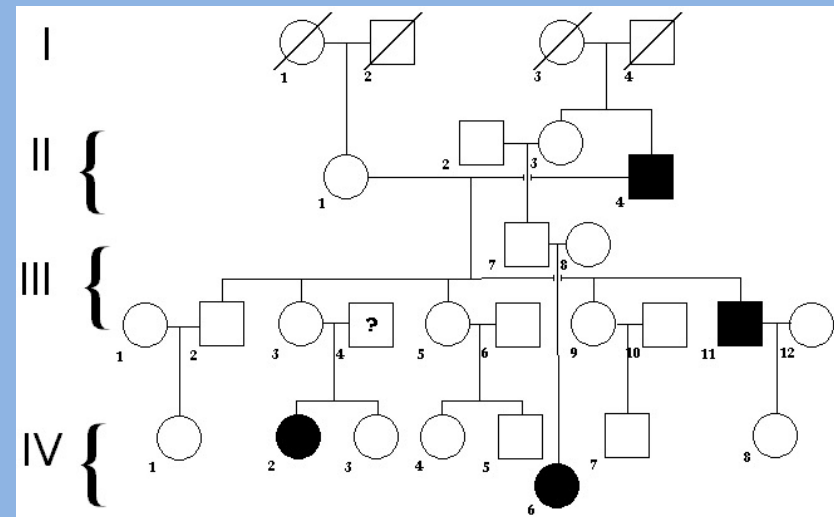
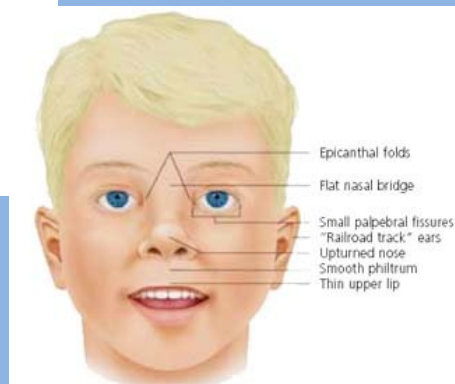
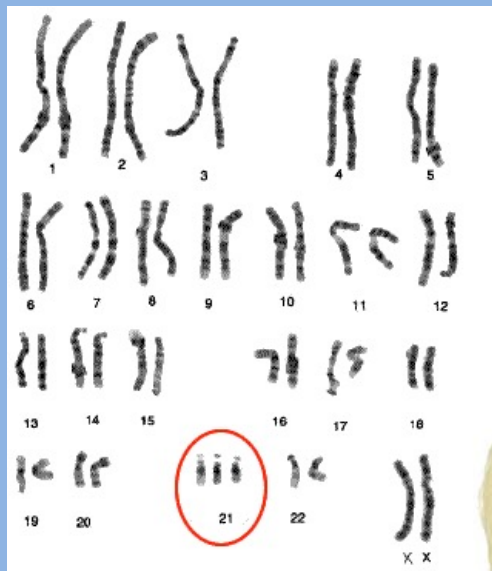
X



# DOENÇAS COMPLEXAS

## Base Genética

Doenças com heranças mendelianas ou associadas com aberrações cromossômicas



# DOENÇAS COMPLEXAS

## Razão de Risco

$\lambda$  = risco do parente do afetado / risco na população geral

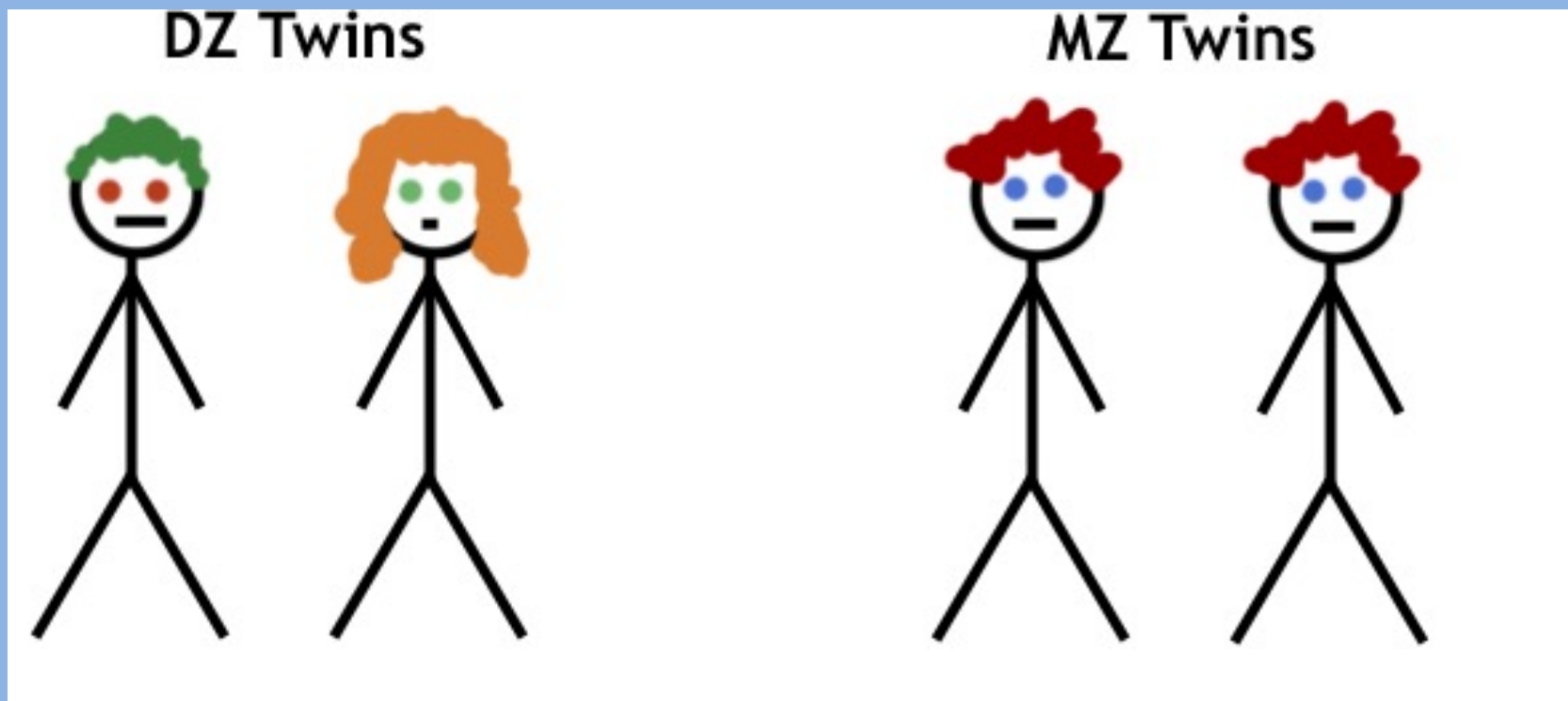
**TABLE 15.1 RISK OF SCHIZOPHRENIA AMONG RELATIVES OF SCHIZOPHRENICS:  
POOLED RESULTS OF SEVERAL STUDIES**

Relative	No. at risk	Risk (%)	$\lambda$
Parents	8020	5.6	7
Sibs	9920.7	10.1	12.6
Sibs, one parent affected	623.5	16.7	20.8
Offspring	1577.3	12.8	16
Offspring, both parents affected	134	46.3	58
Half-sib	499.5	4.2	5.2
Uncles, aunts, nephews, nieces	6386.5	2.8	3.5
Grandchildren	739.5	3.7	4.6
Cousins	1600.5	2.4	3

# DOENÇAS COMPLEXAS

## Estudo de Gêmeos

Estudo de gêmeos dizigóticos e monozigóticos



50%

100%

# DOENÇAS COMPLEXAS

## Estudo de Gêmeos

Estudo de gêmeos dizigóticos e monozigóticos

Study	Country	Concordant pairs	
		MZ	DZ
Kringlen et al. (1968)	Norway	14/50 (0.28)	6/94 (0.06)
Fischer et al. (1969)	Denmark	5/21 (0.23)	4/41 (0.10)
Tienari et al. (1975)	Finland	3/20 (0.15)	3/42 (0.07)
Farmer et al. (1987)	UK	6/17 (0.35)	1/20 (0.05)
Onstad et al. (1991)	Norway	8/24 (0.33)	1/28 (0.04)

Problema

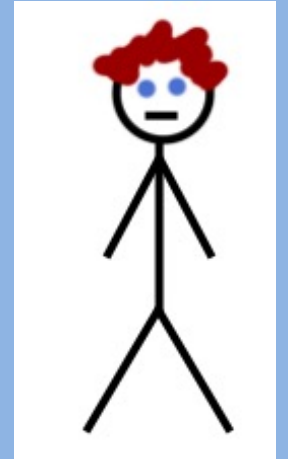
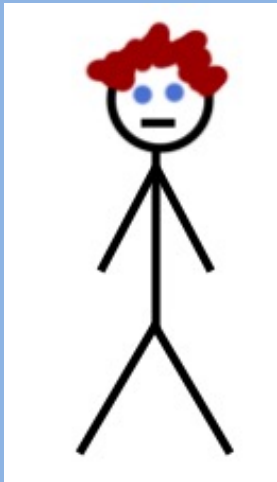
- 1) Monozigóticos tem mais chance de ter mesmo sexo
- 2) Monozigóticos são tratados mais igualmente



# DOENÇAS COMPLEXAS

## Estudo de Gêmeos

Estudo de gêmeos monozigóticos separados ao nascimento



Problema

- 1) Tamanho amostral (N) pequeno
- 2) Separação não total
- 3) Ambiente Intra-uterino

# DOENÇAS COMPLEXAS

## Estudo de Adoção

Estudo com pessoas adotadas



- 1) Procurar pessoas adotadas com DOENÇAS COMPLEXASs e investigar se os pais biológicos ou os adotivos apresentam a mesma doença
- 2) Procurar pais adotivos com DOENÇAS COMPLEXASs e investigar se os filhos adotivos apresentam a mesma doença.

# DOENÇAS COMPLEXAS

## Estudo de Adoção

TABLE 15.3 AN ADOPTION STUDY IN SCHIZOPHRENIA

Case types	Schizophrenia cases among biological relatives	Schizophrenia cases among adoptive relatives
Index cases (47 chronic schizophrenic adoptees)	44/279 (15.8%)	2/111 (1.8%)
Control adoptees (matched for age, sex, social status of adoptive family, and number of years in institutional care before adoption)	5/234 (2.1%)	2/117 (1.7%)

### Problema

- 1) Falta de histórico familiar dos pais biológicos
- 2) Semelhança de características na adoção

# **DOENÇAS COMPLEXAS**

## Identificação da região do genoma

Problema

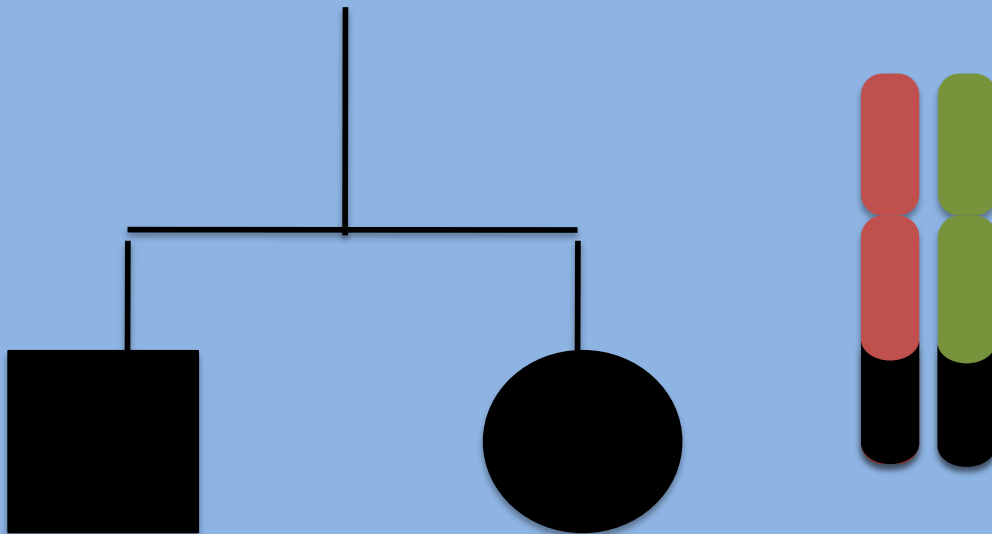
Como identificar a região do genoma responsável pelo fenótipo?

# DOENÇAS COMPLEXAS

## Identificação da região do genoma

Análise de Ligação

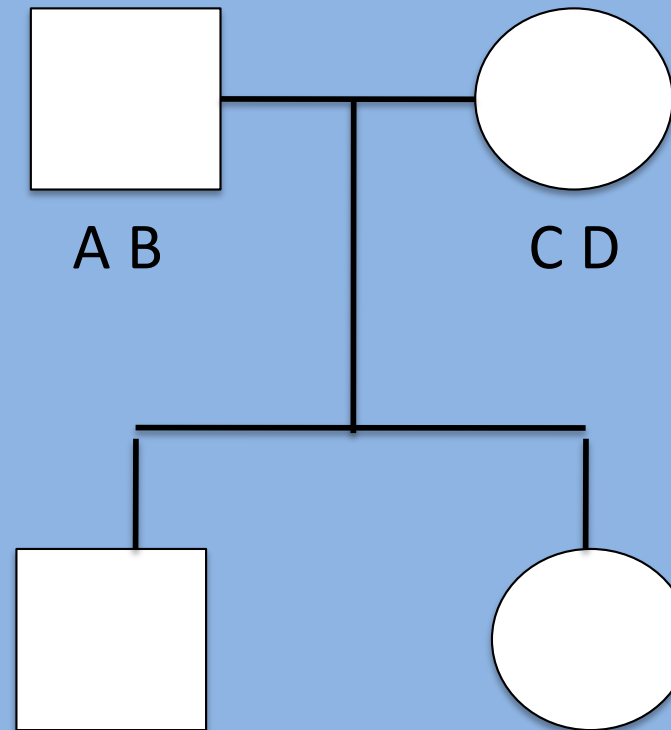
Procura encontrar segmentos dos cromossomos que são compartilhados por membros afetados nas famílias



# DOENÇAS COMPLEXAS

## Identificação da região do genoma

### Análise de Ligação



A C

A C

1/4 (Ambos)

A D

B C

B D

1/2 (Pelo menos um)

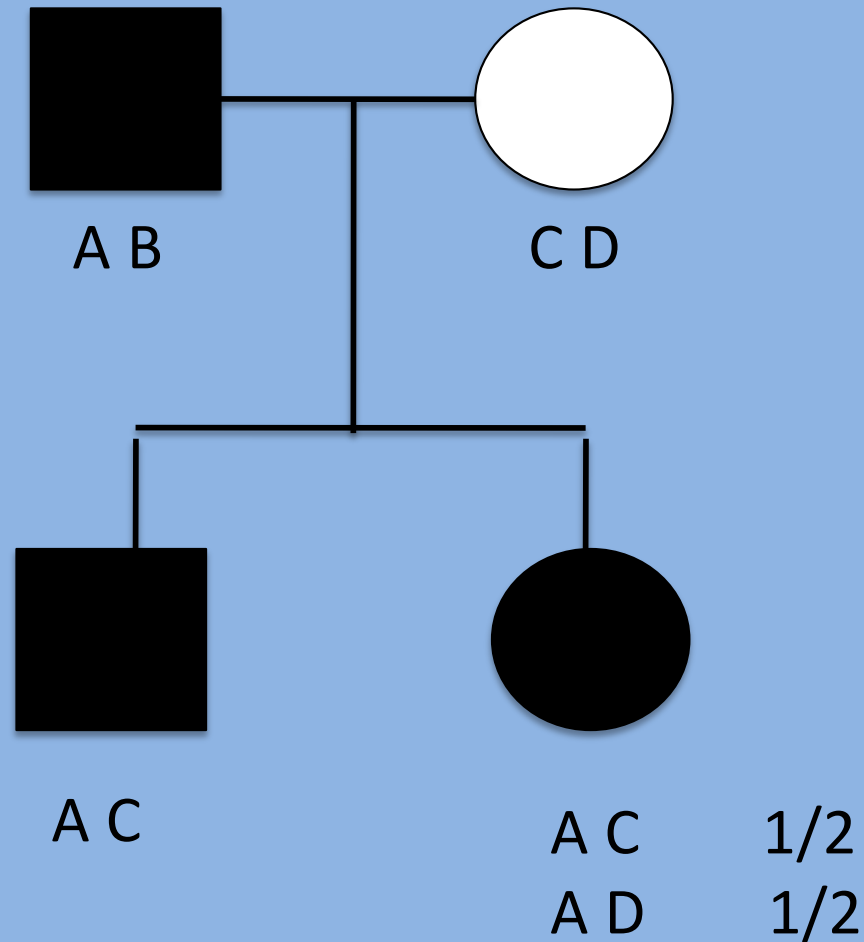
1/4 (Nenhum)

# DOENÇAS COMPLEXAS

## Identificação da região do genoma

### Análise de Ligação

Dominância

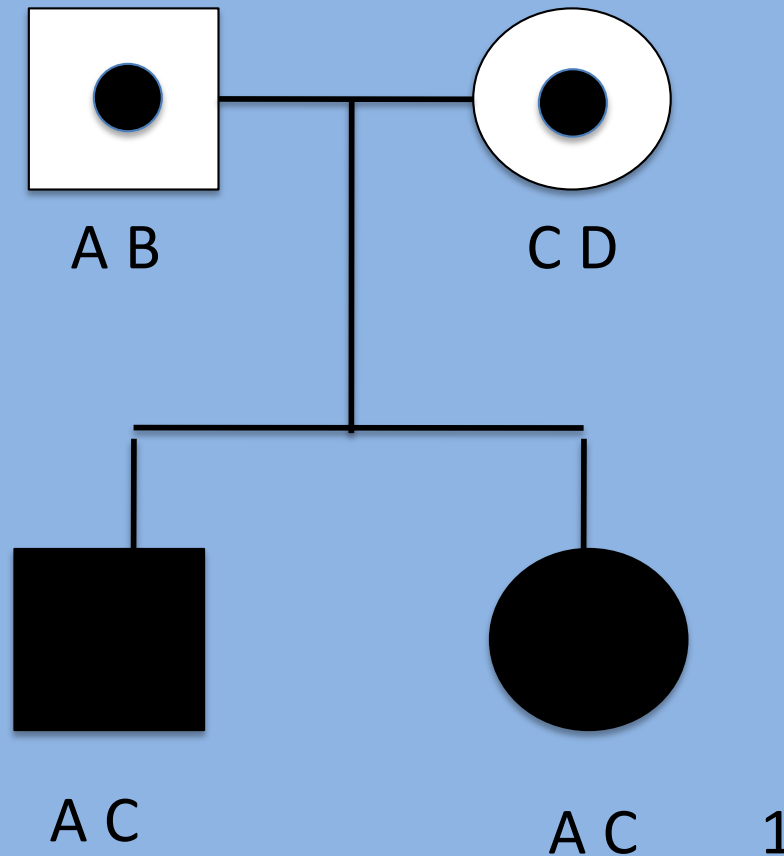


# DOENÇAS COMPLEXAS

## Identificação da região do genoma

### Análise de Ligação

Recessividade



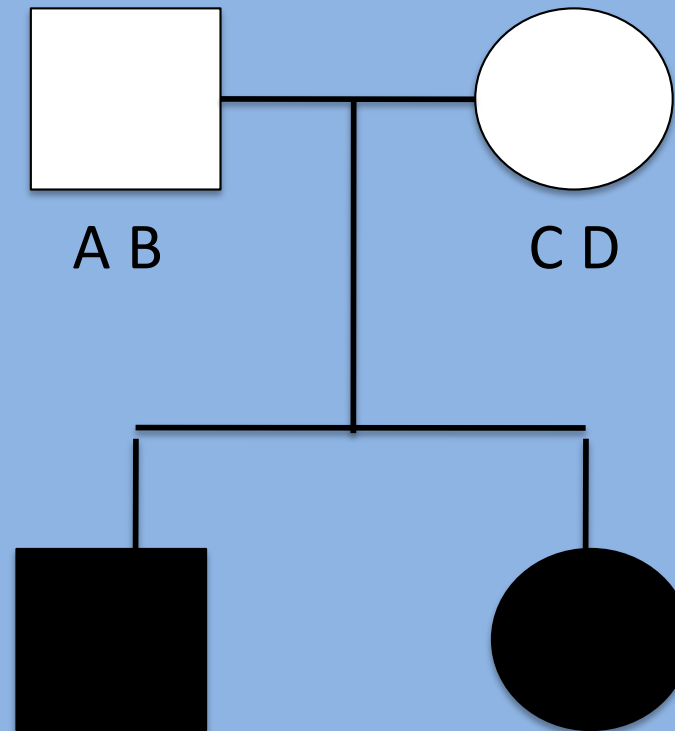


# DOENÇAS COMPLEXAS

## Identificação da região do genoma

### Análise de Ligação

Condição complexa



A C

A C >1/4

A D | >1/2

B C |

B D <1/4

# 4 classes de polimorfismo de DNA

**TABLE 11.1** Classes of DNA Polymorphisms

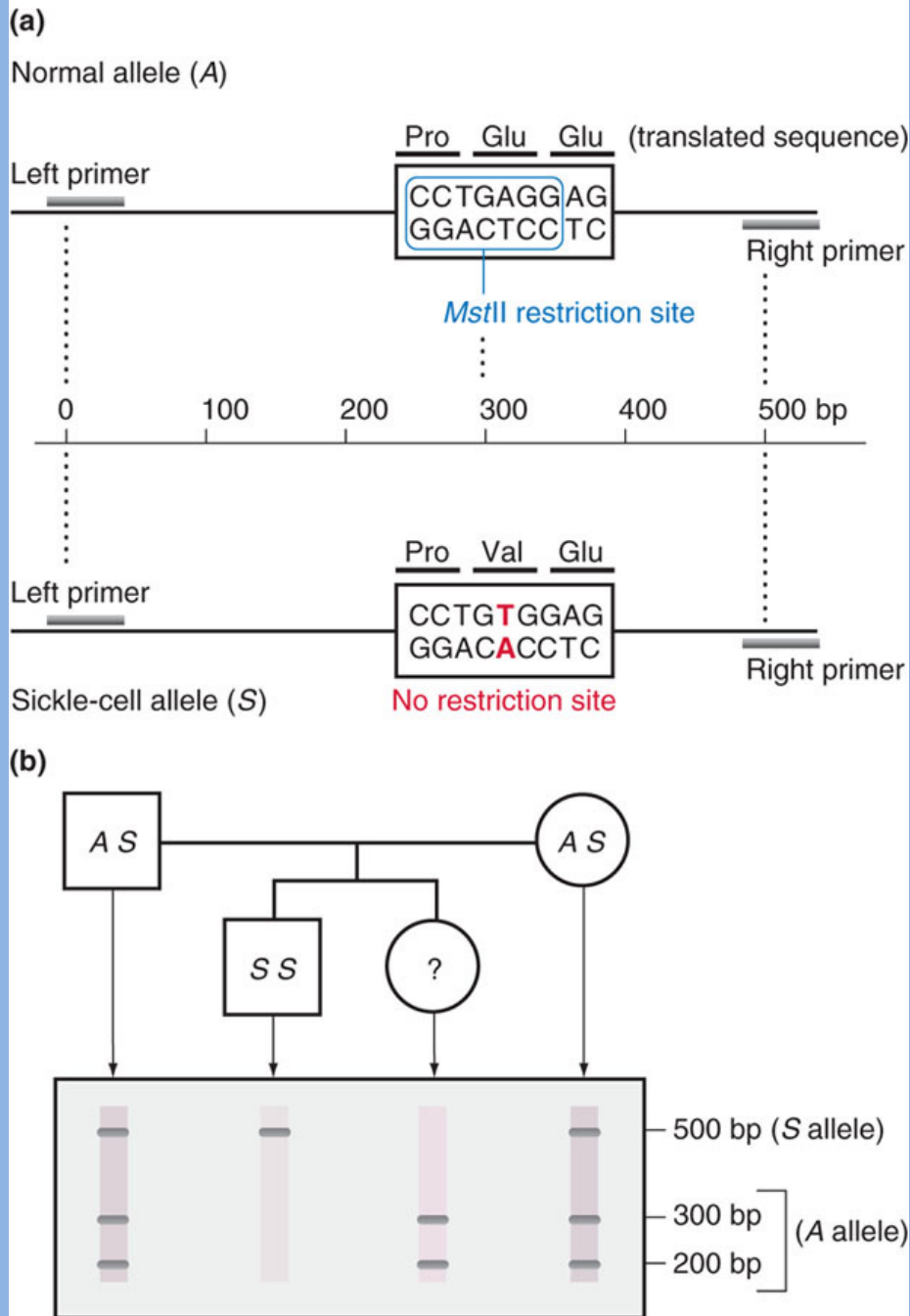
Class	Size of Locus	Number of Alleles	Number of Loci in Population	Rate of Mutation	Use	Method of Detection
<i>SNP</i>	Single base pair	2	100 million	$10^{-9}$	Linkage mapping	PCR followed by ASO hybridization or primer extension
<i>Microsatellite</i>	30–300 bp	2–10	200,000	$10^{-3}$	Linkage mapping	PCR and gel electrophoresis
<i>Multilocus Minisatellite</i>	1–20 kb	2–10	30,000	$10^{-3}$	DNA fingerprinting	Southern blot and hybridization
<i>Small Changes in DNA Content (deletions and duplications)</i>	1–100 bp	2	N/A	$<10^{-9}$	Linkage mapping	PCR and gel electrophoresis

# Single nucleotide polymorphism (SNP)

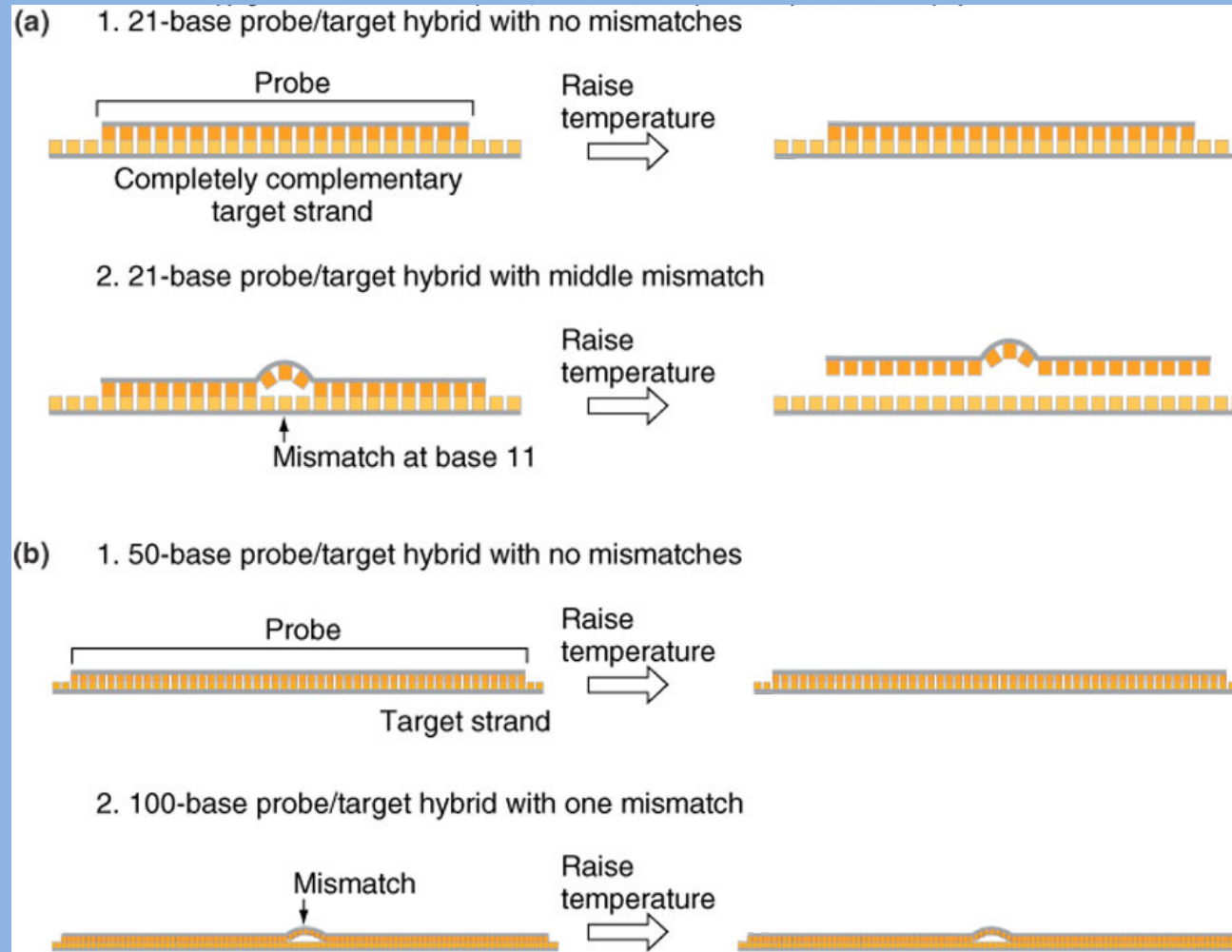
- Single base-pair substitutions
- Arise by mutagenic chemicals or mistakes in replication
- Biallelic – only two alleles
- Ratio of alleles ranges from 1:100 to 50:50.
- 2001 – over 5 million human SNPs identified
- Most occur at anonymous loci.
- Mutation rate of  $1 \times 10^{-9}$  per locus per generation
- Very few are thus new mutation in the species.
- Useful as DNA markers

# SNP detection by PCR

- Must have sequence on either side of polymorphism
  - Amplify fragment
  - Expose to restriction enzyme
  - Gel electrophoresis
- e.g., sickle-cell genotyping with a PCR based protocol



# SNP detection by ASO



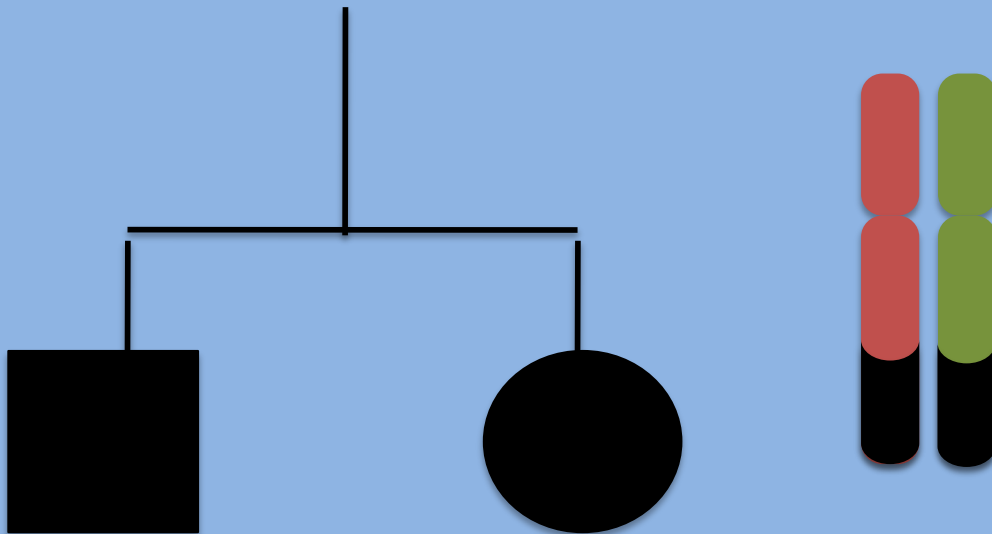
- Very short probes (<21 bp) specific which hybridize to one allele or other
- Such probes are allele-specific oligonucleotides (ASOs).

# DOENÇAS COMPLEXAS

## Identificação da região do genoma

Análise de Ligação

Problemas: regiões muito grandes, pouco recombinantes.



# Estudos de associação e desequilíbrio de ligação

Gene

---

AGTCCTCGTCTCAGCTCGTGAAATTTGTGCCCC  
AGTCGTCGTCTCAGCTCGTGAAATTTGGGCCCC



Gene Mutado

---

AGTCCTCGTCTCAGCCCGTGAAATTTGTGCCCC  
AGTCGTCGTCTCAGCTCGTGAAATTTGGGCCCC

# Estudos de associação e desequilíbrio de ligação

Gene Mutado

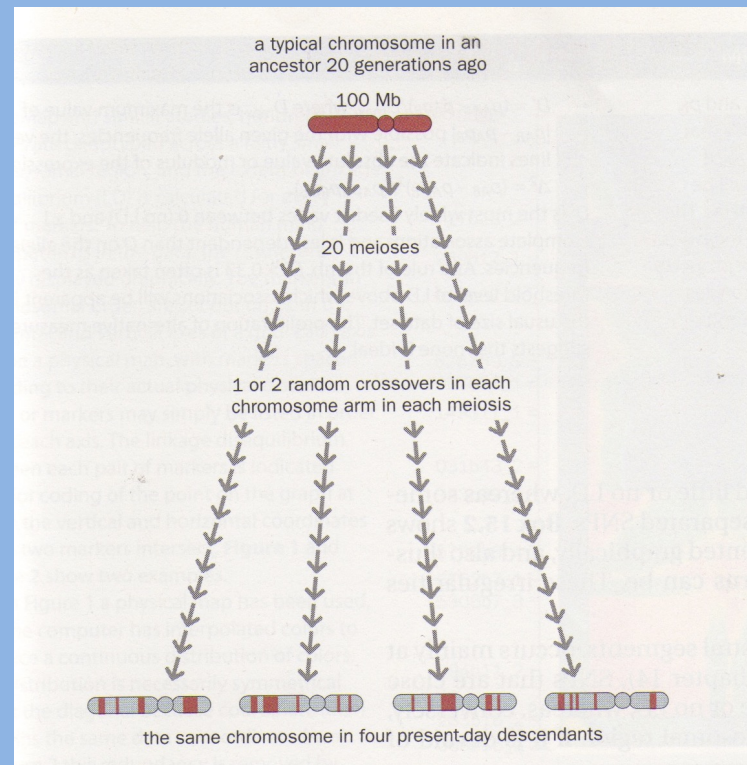
---

AGTCCTCGTCTCAGCCCGTGAAATTTGTGCCCC  
AGTCGTCGTCTCAGCTCGTGAAATTTGGGCCCC

F1 ... F2... F3....Fn

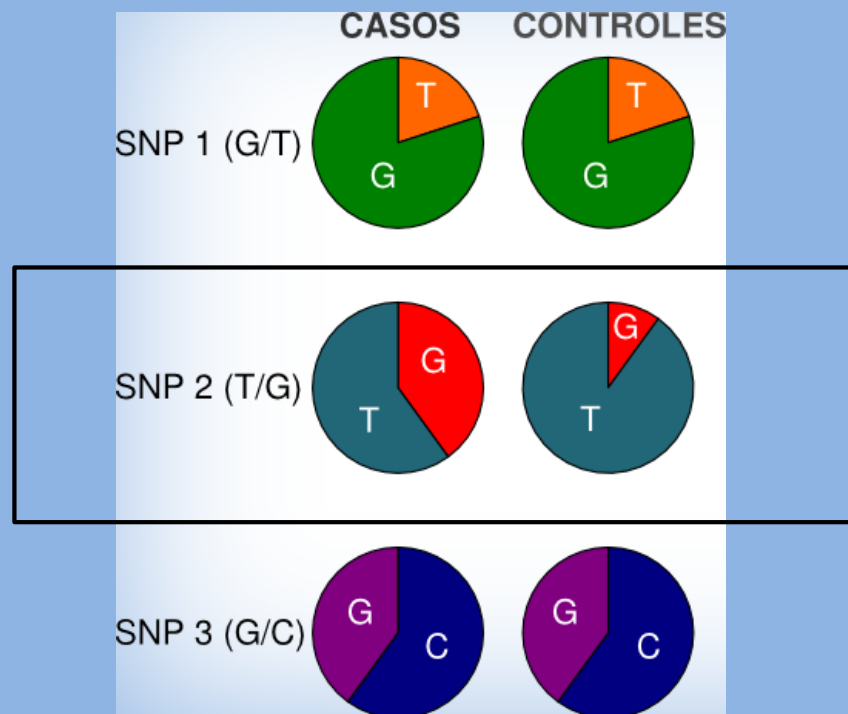
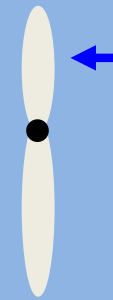


# Estudos de associação e desequilíbrio de ligação



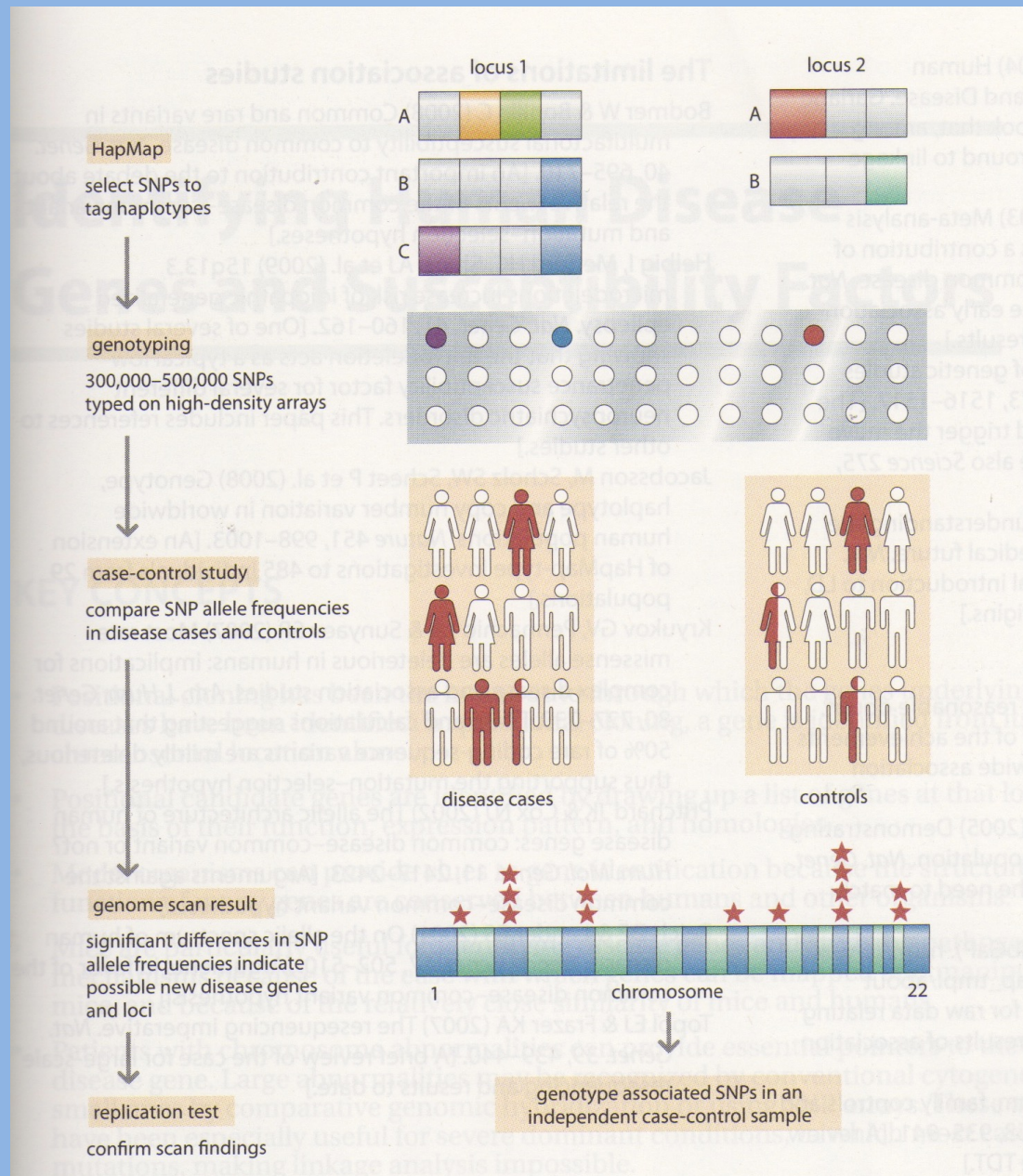
Todos humanos são relacionados em um ancestral em comum. No reino unido, por exemplo, duas pessoas não relacionadas tem um ancestral em comum não mais que 22 gerações atrás.

# Estudos de associação genômica em larga escala

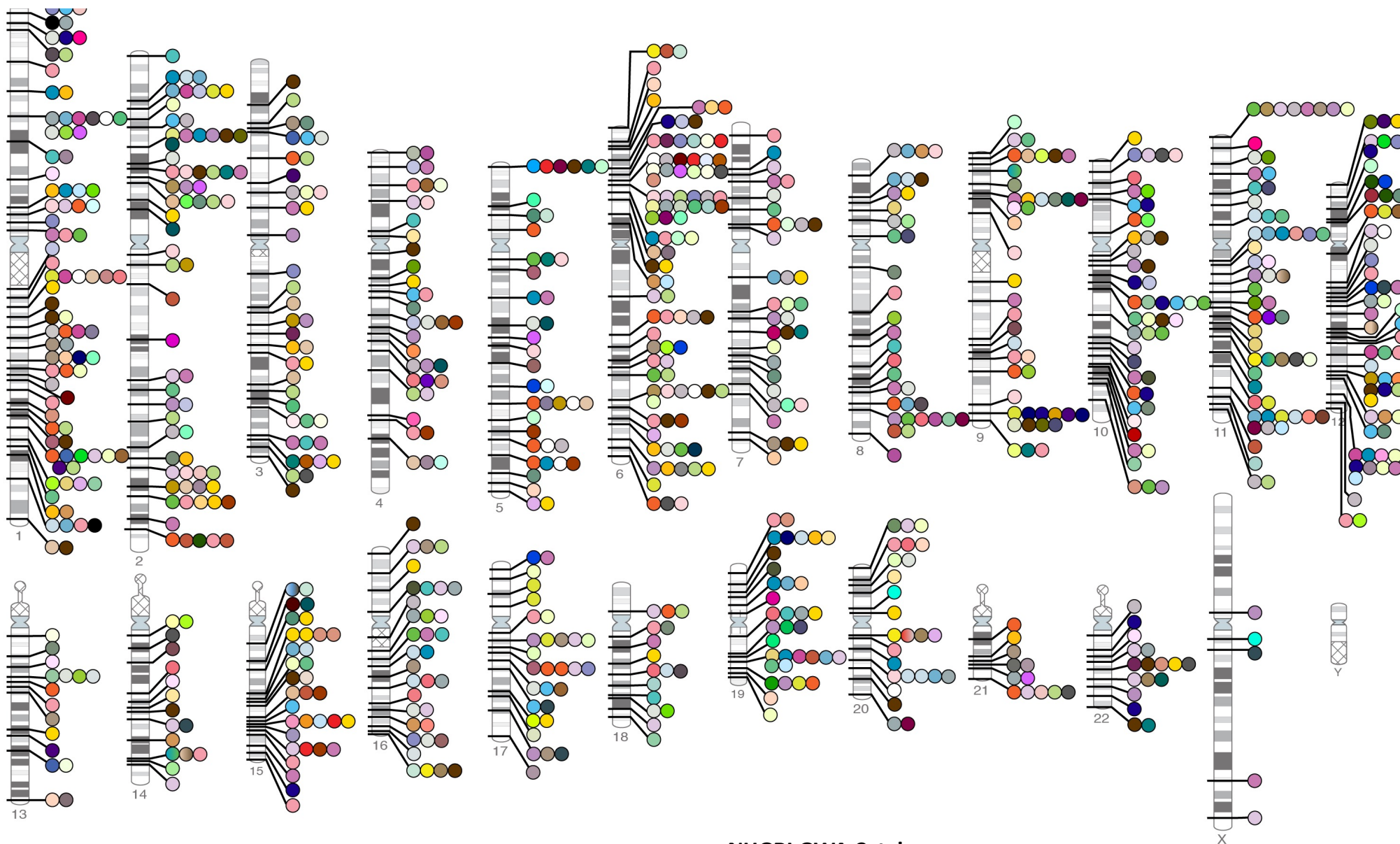


Associação

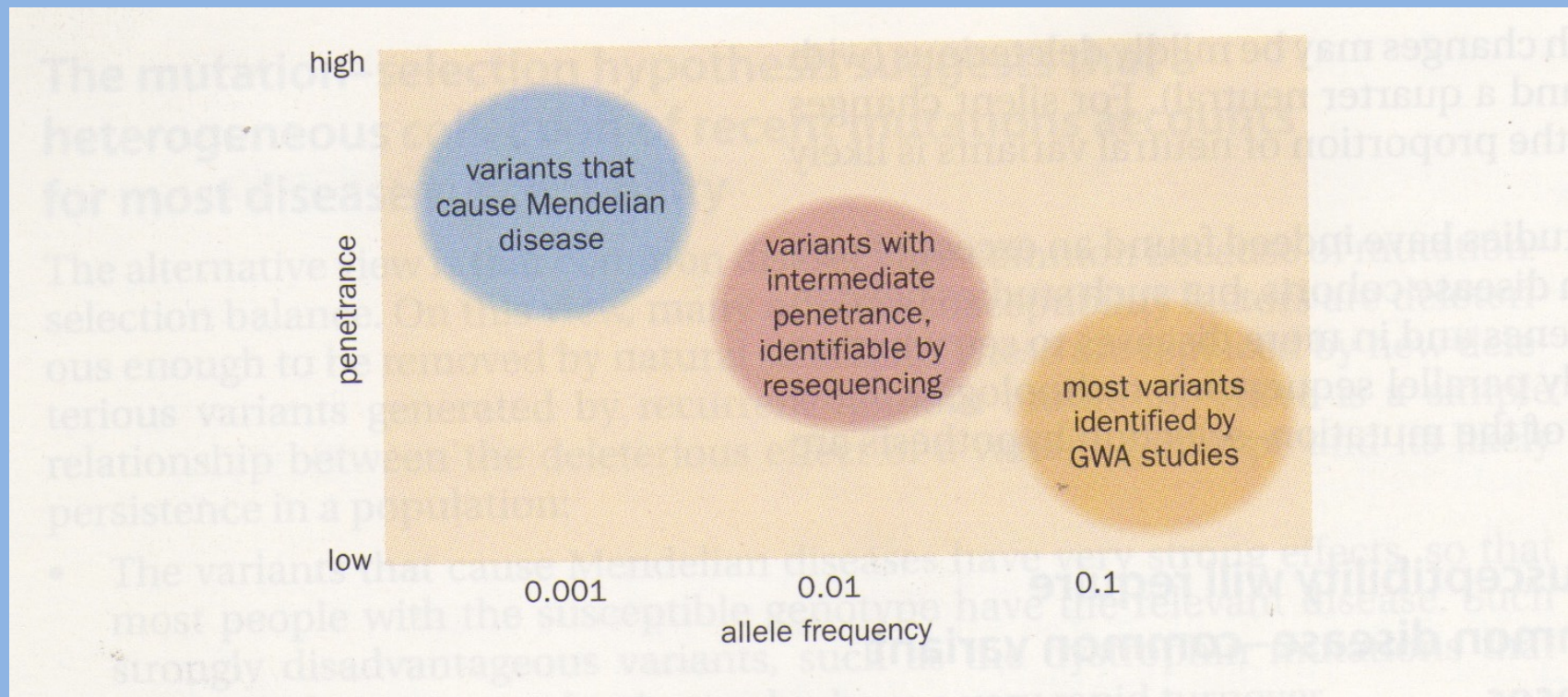
# Estudos de associação genômica em larga escala (GWA)



# Associações genômicas em larga escala publicadas 165 traços



# Estudos de associação genômica em larga escala



# Abordagens integrativas

- Após uma região cromossômica ser identificada, os genes dentro da região devem ser investigados
- Genes candidatos
  - Usualmente existem 10 genes por fragmentos de 1kb
  - Identificar regiões codificantes
    - Análises computacionais para identificar regiões conservadas entre espécies
    - Análises computacionais para identificar seqüência que se parecem com exons, (ORFs, sítios de splice, uso de determinado de codons)
    - Aparecimento de um ou mais clones de ESTs

# Analises computacionais de seqüência genômicas para identificar genes candidatos

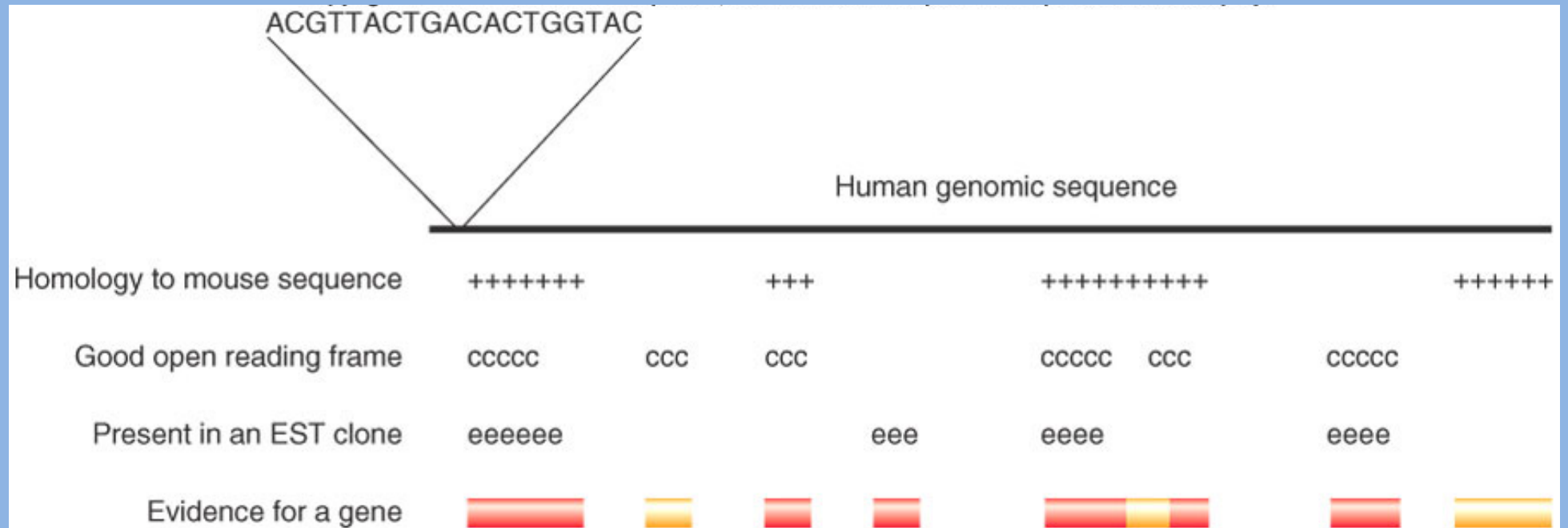


Fig. 11.19

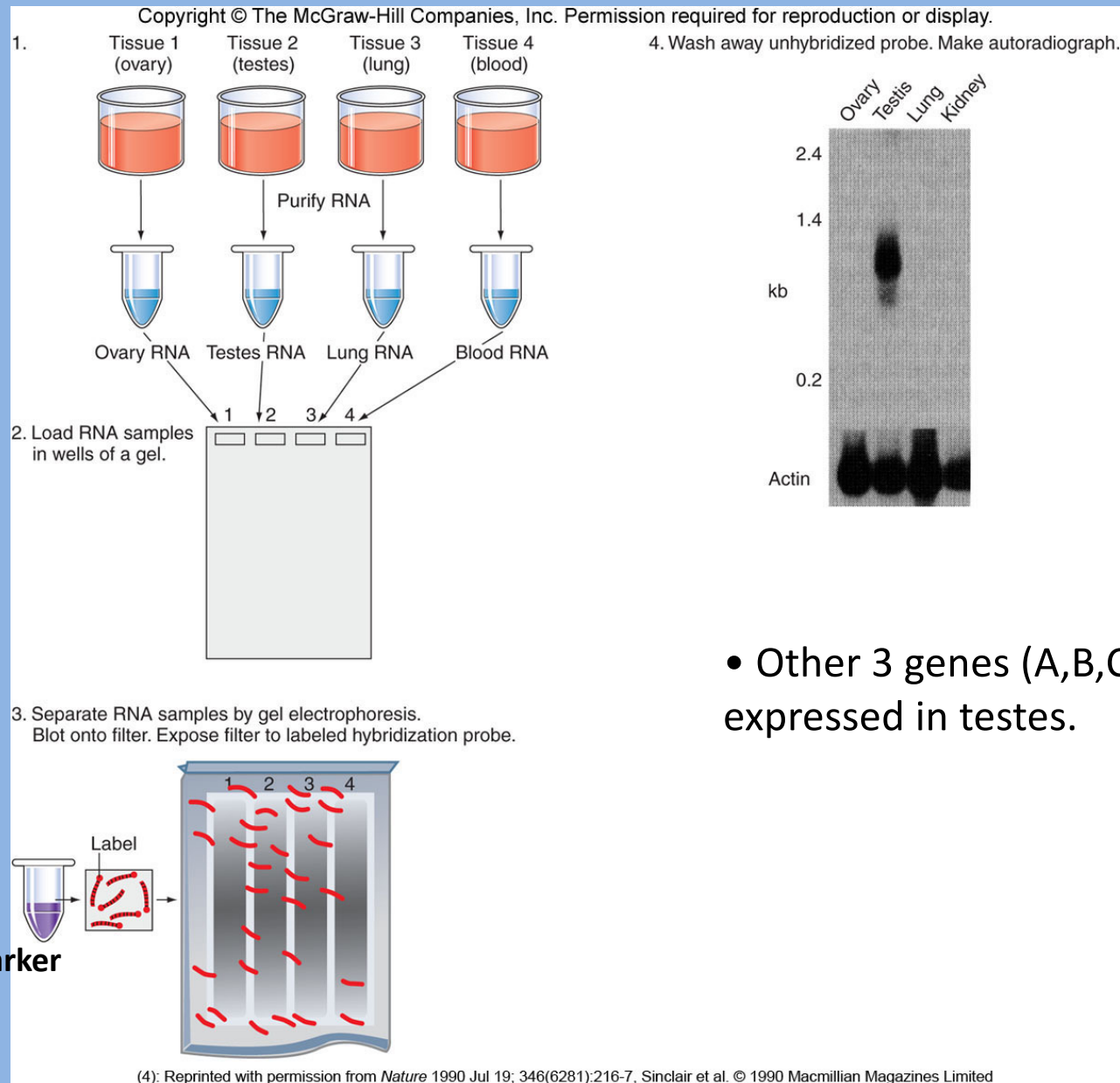
## Padrões de expressão gênica podem apontar para genes candidatos

- Sequências públicas de banco de dados de EST específicos de certos tecidos
- Northern blot
  - Transcritos de RNA de células de um tecido em particular (e.g. com doença) separados por eletroforese e a sonda do gene candidato





# Northern blot example showing *SRY* candidate for testes determining factor is expressed in testes, but not lung, ovary, or kidney.



- Other 3 genes (A,B,C) are not expressed in testes.

- Procurar os genes responsáveis pelo fenótipo.
  - Padrões de expressão
    - Ensaio de expressão de RNA por Northern blot ou amplificação de PCR de cDNA com primers específicos do gene candidato
    - Procurar por “misexpression” (no expression, underexpression, overexpression).
  - Diferenças nas seqüências
    - Identificar mutações sem sentido ao seqüenciar gene candidato em indivíduos afetados e não afetados
  - Modificações transgênicas do fenótipo
    - Inserir gene mutante em organismos modelos.

# Transgenic analysis can prove candidate gene is disease locus.

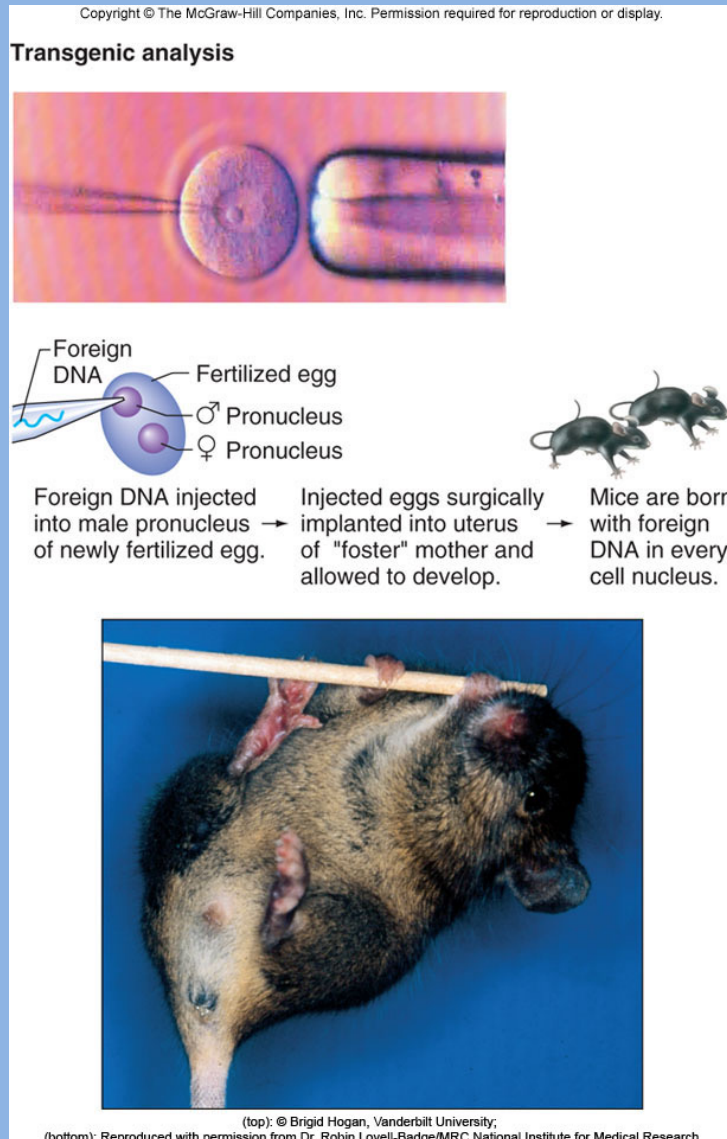


Fig. 11.21

# Variantes em 8q24 são associadas com câncer de próstata

Multiple regions within 8q24 independently affect risk

## Identification of a new prostate cancer susceptibility locus on chromosome 8q24

Meredith Yeager<sup>1,2,\*</sup>, Nilanjan Chatterjee<sup>2</sup>, Julia Ciampa<sup>2</sup>, Kevin B Jacobs<sup>3</sup>, Jesus Gonzalez-Bosquet<sup>2</sup>, Richard B Hayes<sup>2</sup>, Peter Kraft<sup>4</sup>, Sholom Wacholder<sup>2</sup>, Nick Orr<sup>2</sup>, Sonia Berndt<sup>2</sup>, Kai Yu<sup>2</sup>

Matthew L Freedman<sup>2,3</sup>, Simon R Myers<sup>2</sup>, Malcolm C Pike<sup>1,2,4</sup>, Arti Tandon<sup>2,4</sup>, Christine Schirmer<sup>2,4</sup>, Gavin J McDonald<sup>2,4</sup>, Loic Le Marchand<sup>6</sup>, Laurence N Kolonel<sup>6</sup>, Melissa Frasco<sup>1</sup>, Ingrid Ardlie<sup>2,7</sup>, Ingrid Oakley-Girvan<sup>8,9</sup>, Alice S Whittemore<sup>9</sup>, Sue A Ingles<sup>1</sup>, David Altshuler<sup>2,4,12,13</sup>

- Cinco independentes 8q24 loci associados ao câncer de próstata

- Nenhum gene conhecido nessa região

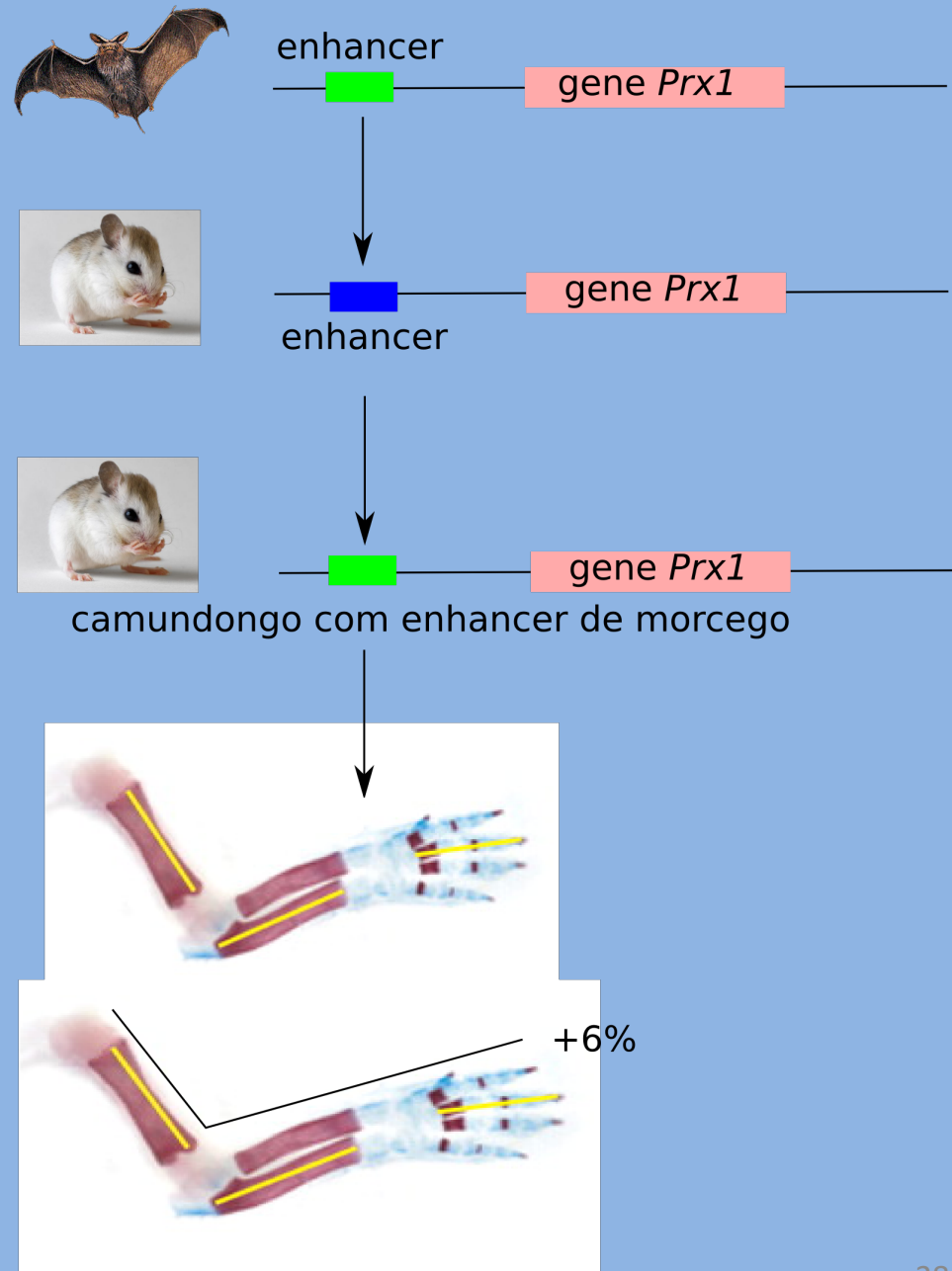
Laurence Kolonel<sup>6</sup>, Loic Le Marchand<sup>6</sup>, Aishan Siddiqui<sup>14</sup>, Michael Robles<sup>15</sup>, Timothy Key<sup>16</sup>, Bradford Knutsen<sup>15</sup>, William Isaacs<sup>16</sup>, Sarah Isaacs<sup>16</sup>, Kathleen E Wiley<sup>10</sup>, Henrik Gronberg<sup>17</sup>, Fredrik Wiklund<sup>17</sup>, Pär Stattin<sup>18</sup>, Jianfeng Xu<sup>19</sup>, S Lilly Zheng<sup>19</sup>, Jieli Sun<sup>19</sup>, Lars J Vatten<sup>20</sup>, Kristian Hveem<sup>20</sup>, Merethe Kumle<sup>21</sup>, Margaret Tucker<sup>2</sup>, Daniela S Gerhard<sup>22</sup>, Robert N Hoover<sup>2</sup>, Joseph F Fraumeni Jr<sup>2</sup>, David J Hunter<sup>4,23</sup>, Gilles Thomas<sup>2</sup> & Stephen J Chanock<sup>2</sup>

We report a genome-wide association study in 10,286 cases and 9,135 controls of European ancestry in the Cancer Genetic Markers of Susceptibility (CGEMS) initiative. We identify a new association with prostate cancer risk on chromosome 8q24 (rs620861,  $P = 1.3 \times 10^{-10}$ , heterozygote OR = 1.17, 95% CI 1.10–1.24; homozygote OR = 1.33, 95% CI 1.21–1.45). This defines a new locus associated with prostate cancer susceptibility on 8q24.

... for prostate cancer risk variants independent of the previously reported association, and ... controlling for ... non genotypes ... ptibility to cancer ... ncer risk variants ... equence of an ... American affected individuals with age at diagnosis <72 years and 837 African American controls (Table 1). We genotyped the same variants in 465 European American cases and 446 European American controls. Analysis of these data identified a cluster of genetic variants that we denote 'region 2' in a span of linkage disequilibrium from 128.14–128.28 Mb. These variants are hundreds of kilobases away from the region 1 described in ref. 2, and the strongest single-SNP association is significant at  $P = 6.5 \times 10^{-7}$  (Fig. 1b and Supplementary Table 2). A third larger study, nested in seven USA and European cohorts and including more than 7,000 prostate cancer cases and 8,000 matched controls, reported an association between rs1447295 and increased risk for prostate cancer in Caucasian men, regardless of age at diagnosis ( $P = 4.00 \times 10^{-19}$ )<sup>6</sup>. We conducted a genome-wide association study (GWAS) of 550,000 SNPs in 1,172 affected individuals (484 with nonaggressive prostate cancer, Gleason <7 and stage A/B; 688 aggressive prostate cancer, Gleason >7 and/or stage C/D) and 1,157 controls using an incidence



Fenótipos nem sempre são produto da alteração de genes codificadores de proteínas.

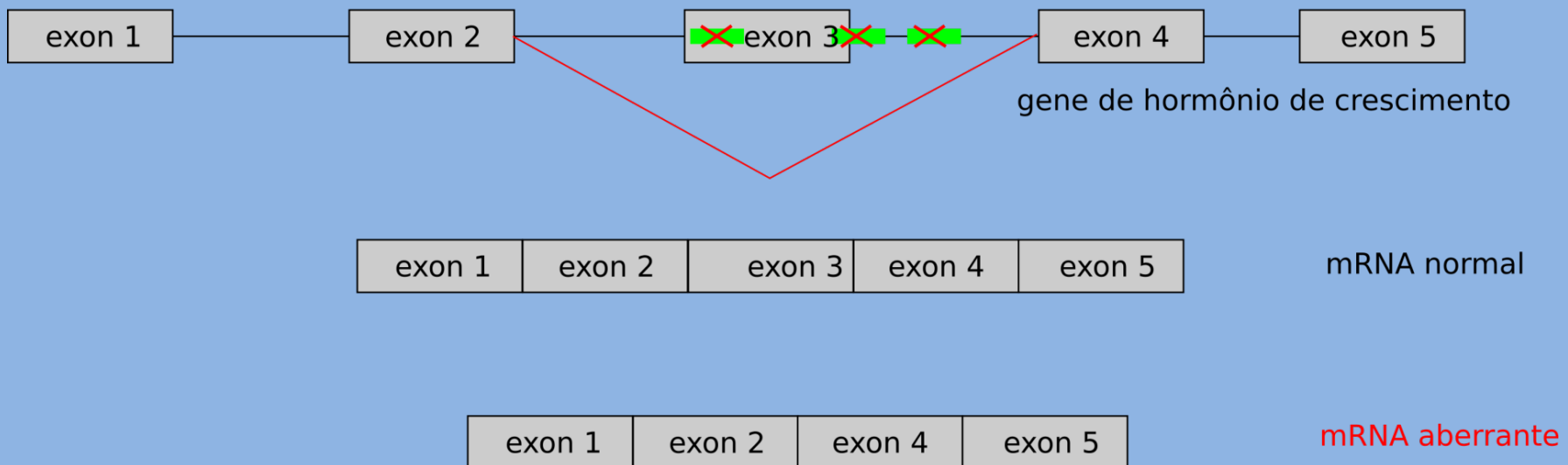
# Elementos reguladores



# Splicing Alternativo

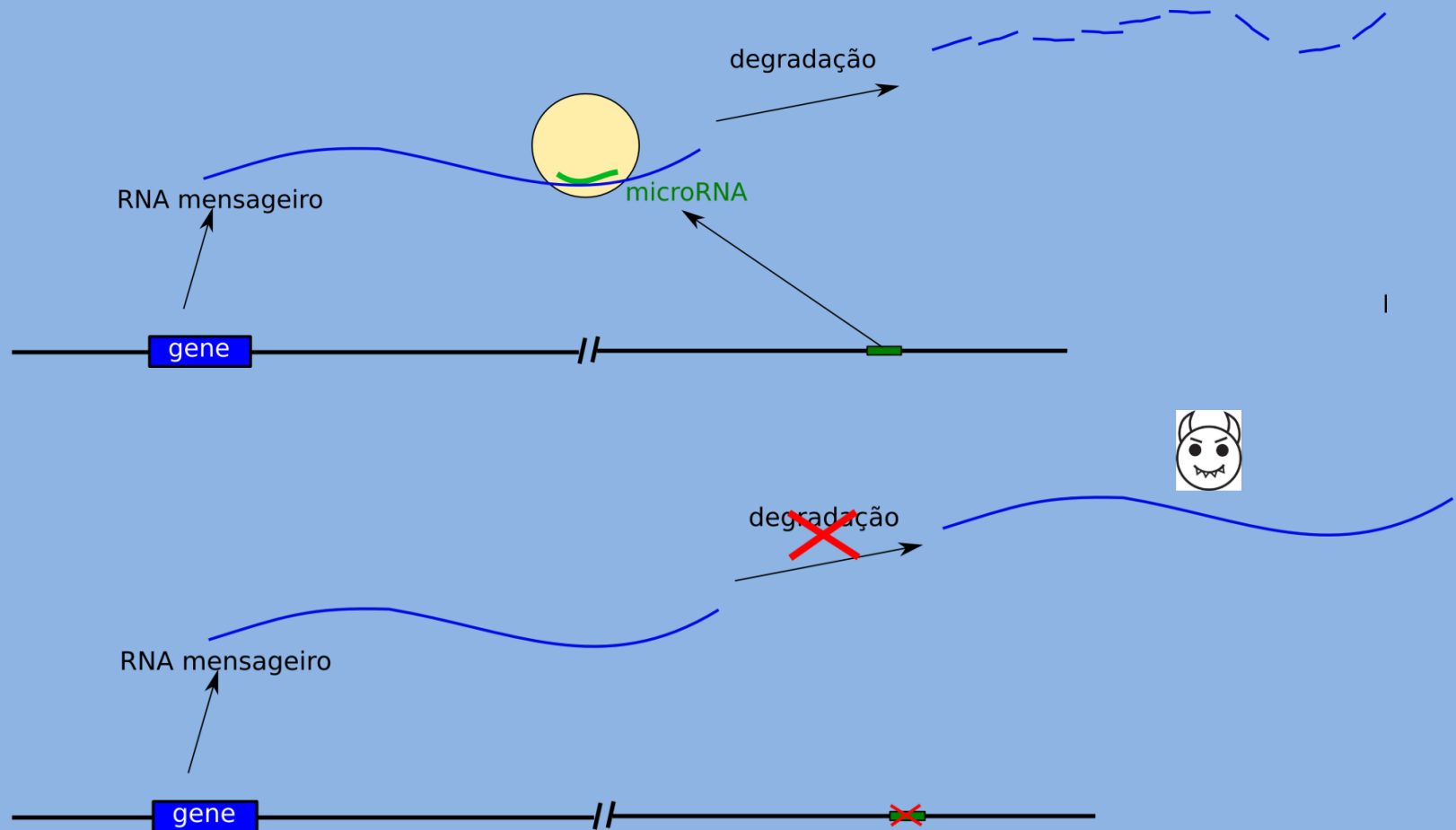
Polimorfismos em sinais de splicing resultam em deficiência de hormônio de crescimento familiar tipo II

-  sinal de splicing
-  polimorfismo em sinal de splicing



## RNA não codificante

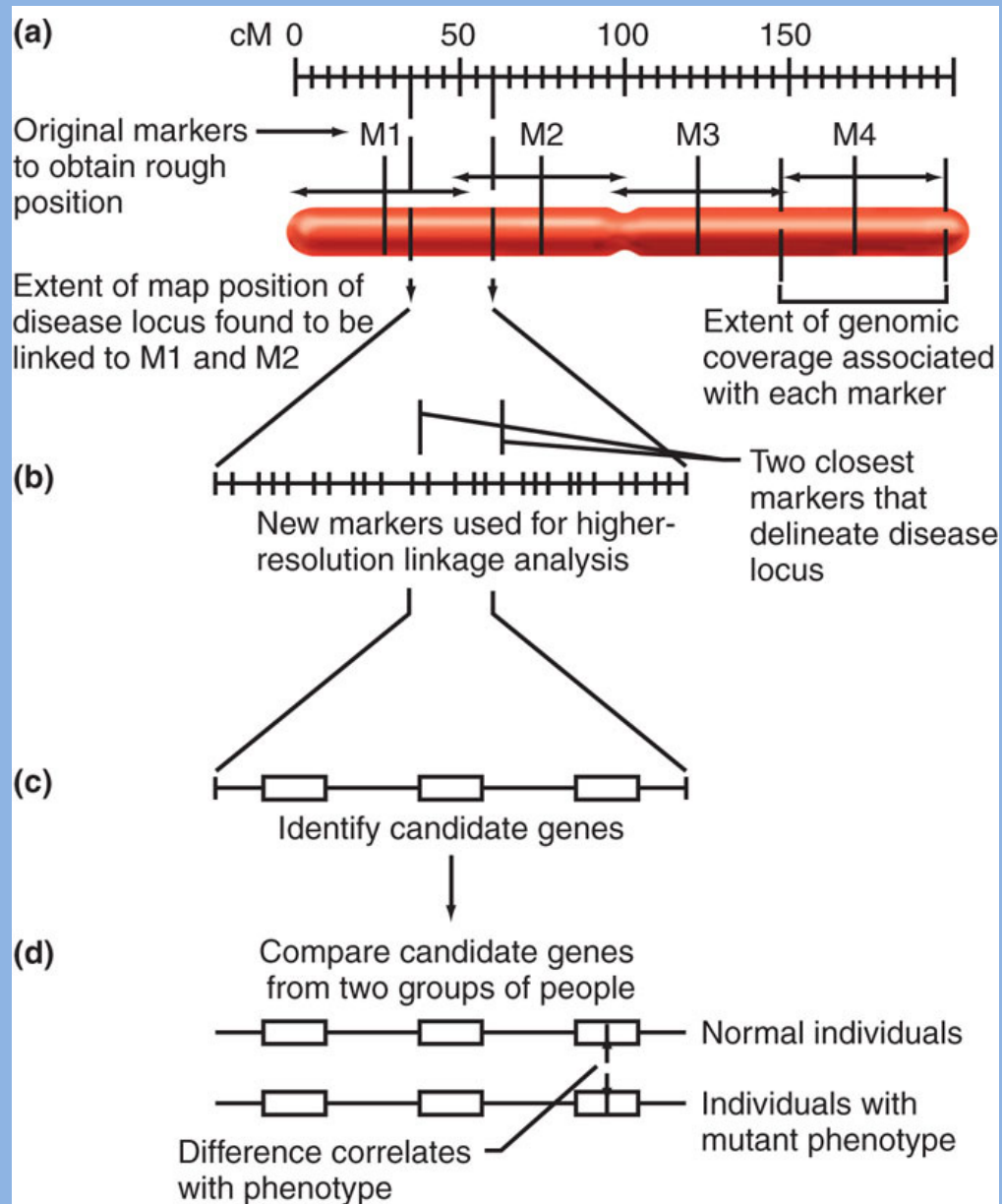
microRNAs, siRNAs (small interfering), lncRNAs (long non-coding), soRNAs (small nucleolar).



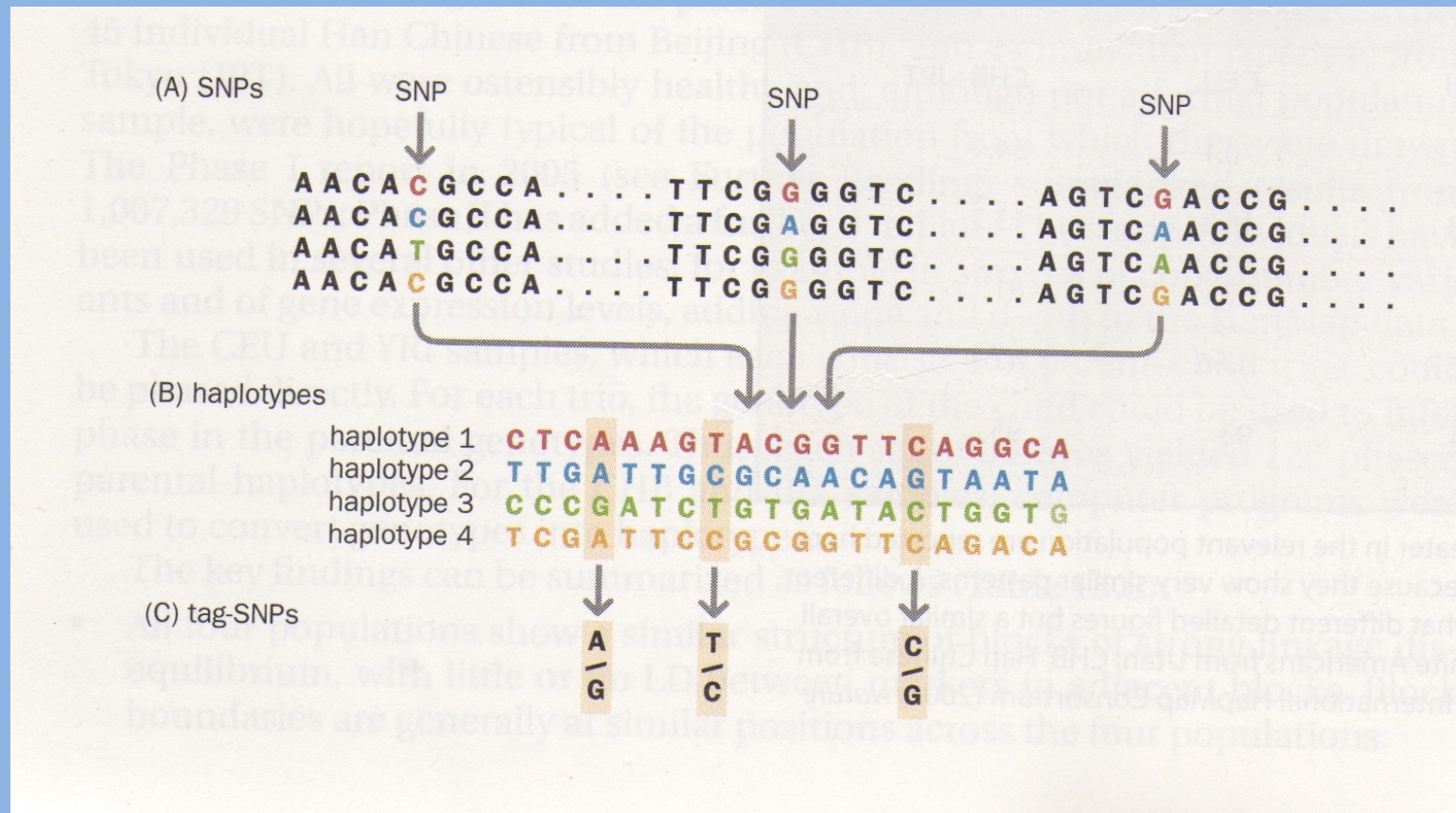




# Positional Cloning

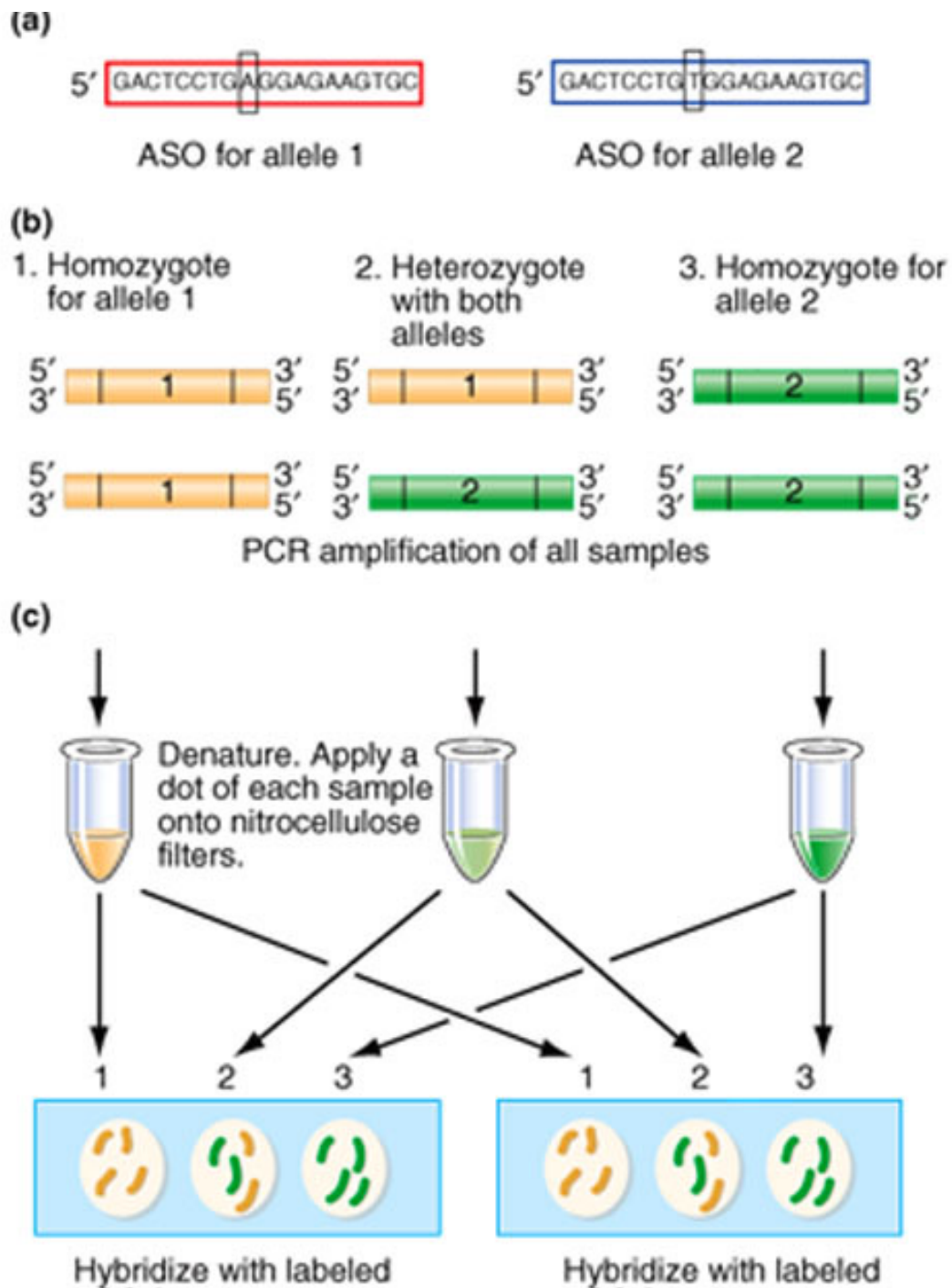


# Estudos de associação genômica em larga escala



# Positional Cloning – Step 1

- Find extended families in which disease is segregating.
- Use panel of polymorphic markers spaced at 10 cM intervals across all chromosomes.
  - 300 markers total
- Determine genotype for all individuals in families for each DNA marker.
- Look for linkage between a marker and disease phenotype.



ASOs can determine genotype at any SNP locus.

Fig. 11.9 a-c

Hybridized and labeled with ASO for allele 1

Hybridized and labeled with ASO for allele 2

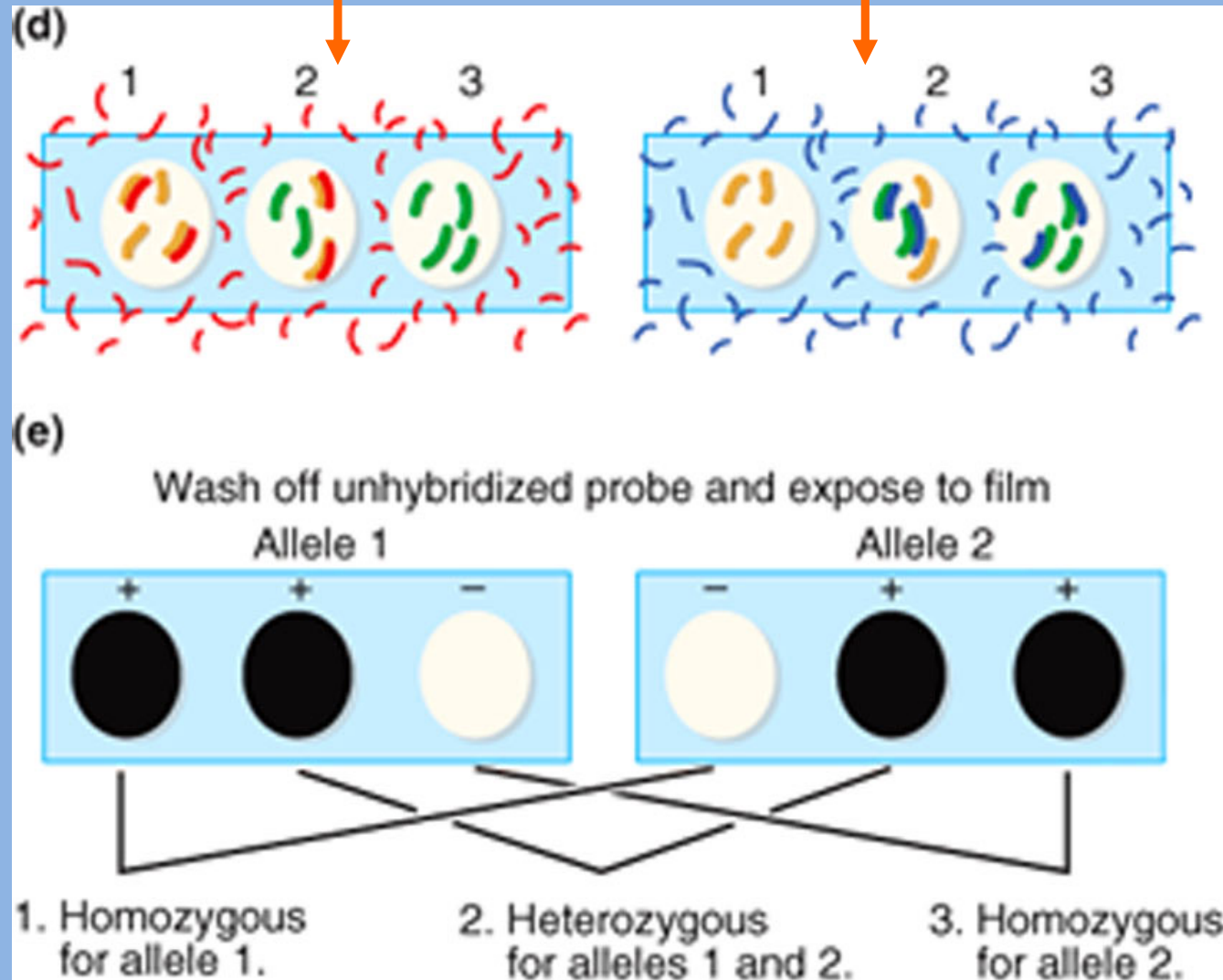


Fig. 11.9 d, e