Nosce te ipsum: the human genome

Part II: genetics, diagnostics, and gene therapy of inherited disease

Important distinction

1. "Monogenic disorders" – human diseases whose etiology can in some more or less linear fashion be traced to a single-locus genetic lesion.
2. Diseases with a "genetic component" or a "genetic predisposition" – disorders that mankind is known to be genetically polymorphic for (in terms of susceptibility) at multiple loci.
3. All other disease (that may or may not be transcription based).

1. Phenomena affecting ploidy (e.g., aneuploidies such as Down, Edwards, Turner, Klinefelter).
2. Phenomena affecting chromosome structure (e.g., translocations in leukemia).
3. Phenomena affecting single loci (genes or relatively small chromosomal segments).

Archibald Garrod (1902)

Higher frequency of children with alkaptonuria (urine turns dark on standing and alkalinization) from consanguineous marriages.

Why?

"There is no reason to suppose that mere consanguinity of parents can originate such a condition as alkaptonuria in their offspring, and we must rather seek an explanation in some peculiarity of the parents, which may remain latent for generations… It has recently been pointed out by Bateson that the law of heredity discovered by Mendel offers a reasonable account of such phenomena. …""

Garrod (1902) *Lancet* **2:** 116.

http://www.esp.org/foundations/genetics/classical/ag-02.pdf

the opposite character. When a recessive gamete meets one of the dominant type the resulting organism (the zygote) will usually exhibit the dominant character, whereas when two recessive gametes meet the recessive character will necessarily be manifested in the zygote. In the case of a rare recessive characteristic we may easily imagine that many generations may pass before the union of two recessive gametes takes place. The application of this to the case in question is further pointed out by Bateson, who, commenting upon the above observations on the incidence of alkaptonuria, writes as follows:[17] "Now there may be other accounts possible, but we note that the mating of first cousins gives exactly the conditions most likely to enable a rare, and usually recessive, character to show itself. If the bearers of such a gamete mate with individuals not bearing it the character will hardly ever be seen; but first cousins will frequently be the bearers of similar gametes, which may in such unions meet each other and thus lead to the manifestation of the peculiar recessive characters in the zygote." Such an explanation removes the question altogether out of the range of prejudice, for, if it be the true account of the matter, it is not the mating of first cousins in general but of those

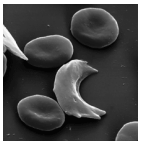
Garrod (1902) *Lancet* **2:** 116.

Sickle-cell anemia – a brief history

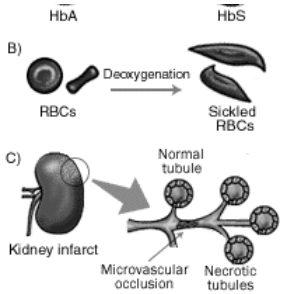
"In the western literature, the first description of sickle cell disease was by a Chicago physician, James B. Herrick, who noted in **1910** that a patient of his from the West Indies had an anemia characterized by unusual red cells that were sickle-shaped."

By 1923, it was realized the condition is hereditary.

In 1949, Neel realized that patients with SCA are homozygous, and heterozygous carriers have a much milder condition (sickle cell trait).



Sickle cell anemia



NIH:

"Sickle cell anemia is the most common inherited blood disorder in the United States, affecting about 72,000 Americans or 1 in 500 African Americans. SCA is characterized by episodes of pain, chronic hemolytic anemia and severe infections, usually beginning in early childhood."

Linus Pauling, 1949: HbS has different charge!!

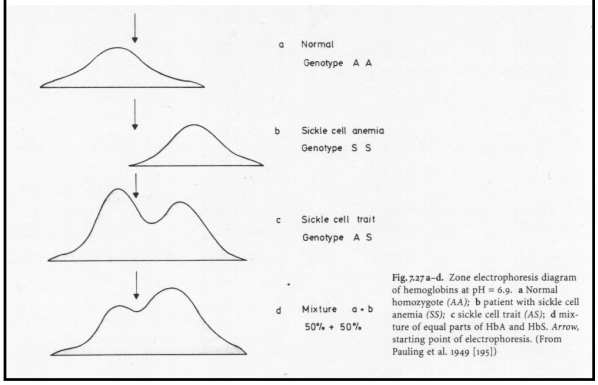


Fig. 7.27a–d. Zone electrophoresis diagram of hemoglobins at pH = 6.9. **a** Normal homozygote (*AA*); **b** patient with sickle cell anemia (*SS*); **c** sickle cell trait (*AS*); **d** mixture of equal parts of HbA and HbS. *Arrow*, starting point of electrophoresis. (From Pauling et al. 1949 [195])

V. Ingram, *Nature* 1956

"On [the existing] evidence alone, it is not possible to decide whether the difference between the proteins, which is in any event small, lies in the amino-acid sequences of the polypeptide chains, or whether it lies in the folding of these chains leading to the masking of some amino-acid side chains."

Experiment:

1. Digest Hb A and Hb S with trypsin (protease – cuts hemoglobin into ~30 peptides).
2. Separate resulting fragments by electrophoresis, and then by chromatography.
3. Trace the peptide map.

V. Ingram (1956) *Nature* **178:** 792.

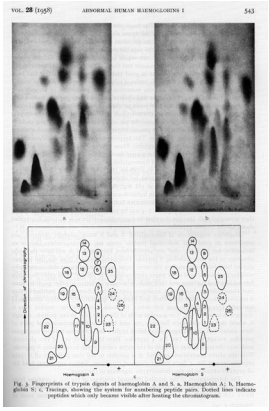
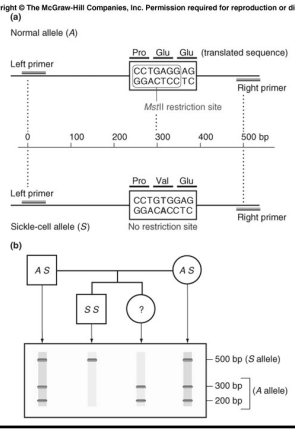


Fig. 3. Fingerprints of trypsin digests of haemoglobin A and S. a, Haemoglobin A; b, Haemoglobin S; c, Tracings, showing the system for numbering peptide pairs. Dotted lines indicate peptides which only became visible after heating the chromatogram.

V. Ingram (1956) *Nature* **178:** 792.

"One can now answer at least partly the question put earlier, and say there there is a difference in the amino-acid sequence in one small part of one of the polypeptide chains. This is particularly interesting in view of the **genetic evidence** that the formation of hemoglobin S is due to a mutation in a single gene."

V. Ingram (1956) *Nature* **178:** 792.



11.7

SCA

1. Fairly homogeneous genetic basis – an A-to-T transversion in the sixth codon of the HBB gene that leads to a glu → val substitution (→ RFLP!)
2. In North America, heterozygosity for mutant allele is largely asymptomatic (sickle cell trait), because concentration of hemoglobin S is not high enough for the erythrocytes to sickle.
3. In areas with high incidence of malaria, the fitness of heterozygotes is greater than of noncarriers or affected individuals (overdominance) because carriers are relatively malaria-resistant, explaining the high frequency of this allele.
4. Therapy – hydroxyurea (wakes up embryonic and fetal globin genes), morphine for the pain, and prophylactic penicillin. "Sickle cell anaemia. A simple disease with no cure" (*Nature* 1989).

"Functional cloning"

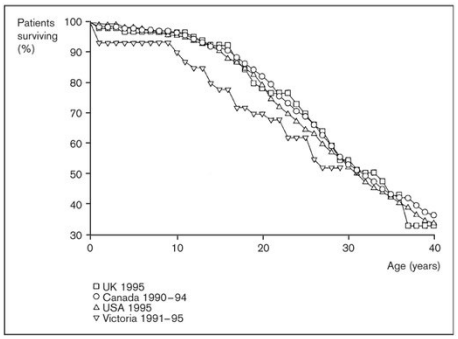
In the case of alkaptonuria, sickle cell anemia, and blood clotting disorders such as hemophilia , the disease genes is identified based on some biochemical or other defect exhibited by the patient.

What if the defect cannot be traced in a simple way to a biochemical phenomenon?

Cystic fibrosis

- Most common monogenic autosomal human genetic disorder – 1 in every 2000 live births.
- q^2=0.05%; q=2.2%; p=97.8%; 2pq=4% carriers.
- Complex dysfunction of the lungs and the pancreas.

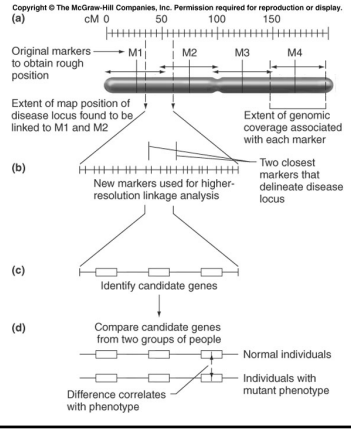
Life expectancy of CF patients



Mapping by linkage ("positional cloning")

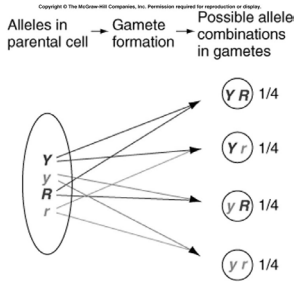
If a given marker is linked (=is on the same chromosome as) to the gene mutations in which cause a certain disease, then one should be able to observe coinheritance of some allelic form of that marker to the occurrence of the disease.

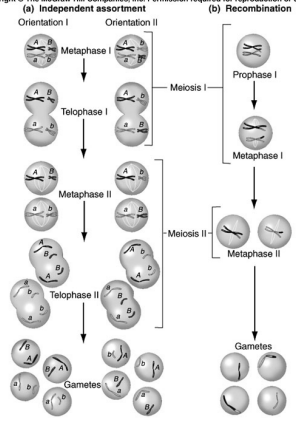
"Coinheritance" = occurrence in genotype of two loci with a frequency higher than Mendel's second law allows.



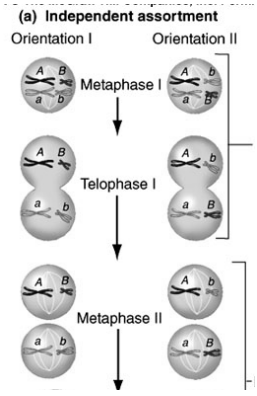
11.17

Mendel's second law





4.17

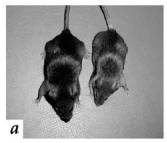


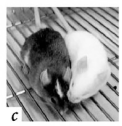
Very simple and astonishingly influential consequence of all this stuff

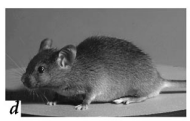
Two markers located on different chromosomes will segregate away from each other in one out of two meioses.

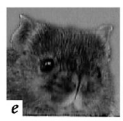
Two markers that are on the same chromosome will tend to stay together.

a, Nanomouse (*Nano*), GENA50. ***b***, Dominant spotting (*KitW-39H*), GENA133. ***c***, A microphthalmia mutant, GENA163. ***d***,***e***, Batface, a craniofacial mutant, GENA123.







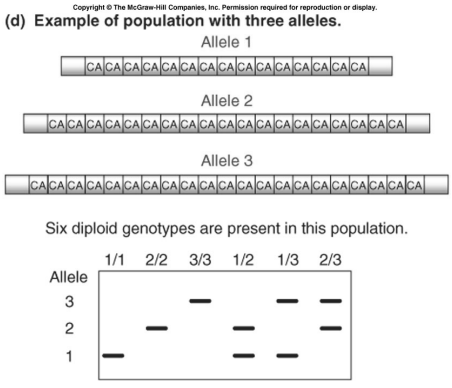


Nolan et al. *Nat. Genet.* (2000). **25:** 440.

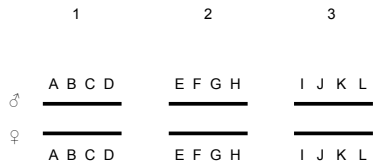
William Ernest Castle – founder of mouse genetics

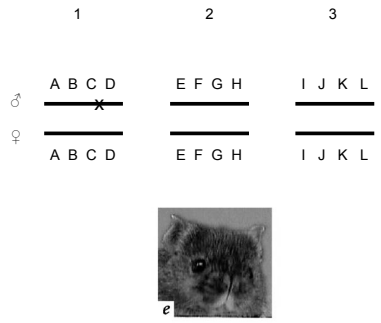
1. Inbreeding as a tool for making genetically uniform strains of mice that are homozygous for every allele in the genome.
2. Brother sister matings – makes 12.5% of all loci in the genome homozygous (Clarence Little).

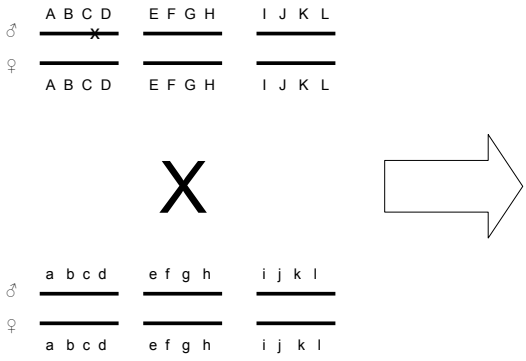
After 40 generations of brother sister mating, >99.98% of genome is homozygous. By F_{60}, mice are considered genetically identical to one another.

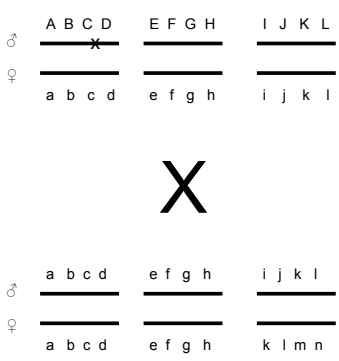


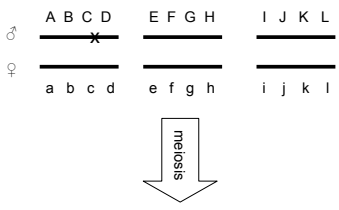
11.12





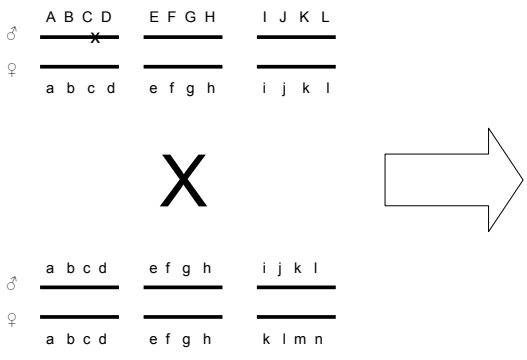






During meiosis, there is a 50% chance that the mutant gene will separate from all markers on unlinked chromosomes. This means that there is an equal likelihood of forming a gamete “X E” and “X e” – similarly, “X J” and “X j.”

On the other hand, the mutant gene will not separate as frequently from markers that lie on the same chromosome as it does. In other words, the “X B” gamete will be more frequent than the “X b” gamete. To identify, on which chromosome, and where exactly, the mutant gene lies, we need to find the specific marker that the disease always inherits with.

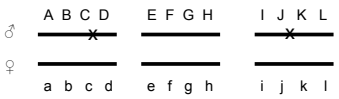


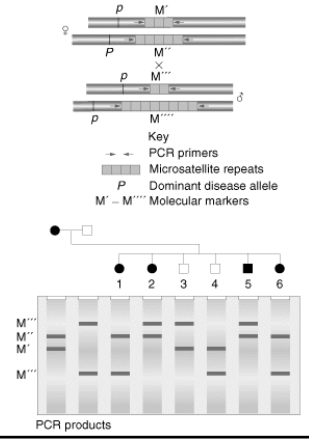
Finally

Take all the mutant children.

Genotype them for markers over the entire genome.

Find the “uppercase marker” that occurs with the highest frequency (>>>50%) in the mutant children.





(a) Three-point cross results

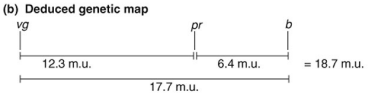
P ♀ *vg b pr / vg b pr* × ♂ *vg⁺ b⁺ pr⁺ / vg⁺ b⁺ pr⁺*

F_1 (all identical) *vg b pr / vg⁺ b⁺ pr⁺*

Testcross ♀ *vg b pr / vg⁺ b⁺ pr⁺* × ♂ *vg b pr / vg b pr*

Testcross progeny			
1779	*vg b pr*	Parental combinations for all three genes
1654	*vg⁺ b⁺ pr⁺*	
252	*vg⁺ b pr*	Recombinants for *vg* relative to parental combinations for *b* and *pr*
241	*vg b⁺ pr⁺*	
131	*vg⁺ b pr⁺*	Recombinants for *b* relative to parental combinations for *vg* and *pr*
118	*vg b⁺ pr*	
13	*vg b pr⁺*	Recombinants for *pr* relative to parental combinations for *vg* and *b*
9	*vg⁺ b⁺ pr*	
4197		

(b) Deduced genetic map



5.12

Problem

Humans are not *Drosophila* or mouse.

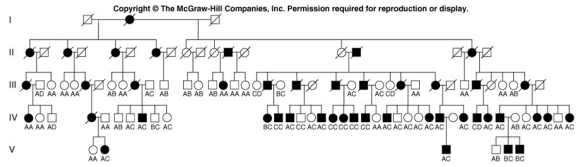
To map genetic distance, one needs to “set up crosses” with known arrangements of genotypes.

The Problem of Phase

In human pedigrees, you cannot “set up a cross” the way you want to! Even in a simple cross:

♂ AaBb × ♀ aabb

… you really need to know whether Dad was AB/ab or Ab/aB in order to calculate map distances (you need to tell which child is recombinant and which one isn’t!!!)



11.18

Lod scores and mapping by linkage

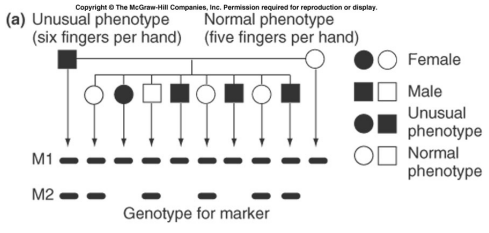
Solution: calculate logarithm of the odds (lod) that the pedigree observed is due to linkage.

For any given trait (disease) and any given marker being tracked in the pedigree, the Lod score is the log_{10} of the following ratio:

probability that this pedigree would be observed if the two loci are linked, and are separated by a certain genetic distance DIVIDED by probability this pedigree would be observed if the two loci were unlinked.

Two loci are linked if the lod score > 3

By the way – a logarithm is taken so that we can add lod scores obtained from different families!!



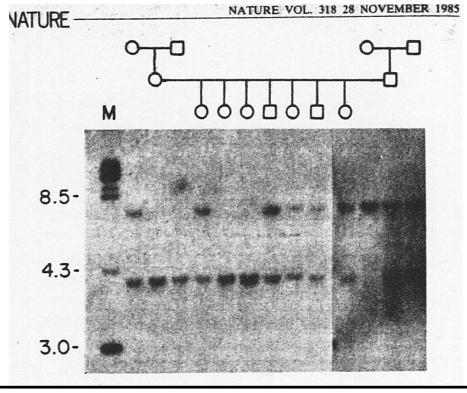
(b)

Odds value (ratios)	LOD score (*Z*)
0.01 (1:100)	-2
0.1 (1:10)	-1
1 (1:1)	0
10 (10:1)	1
100 (100:1)	2
1000 (1000:1)	3

p. 392-393

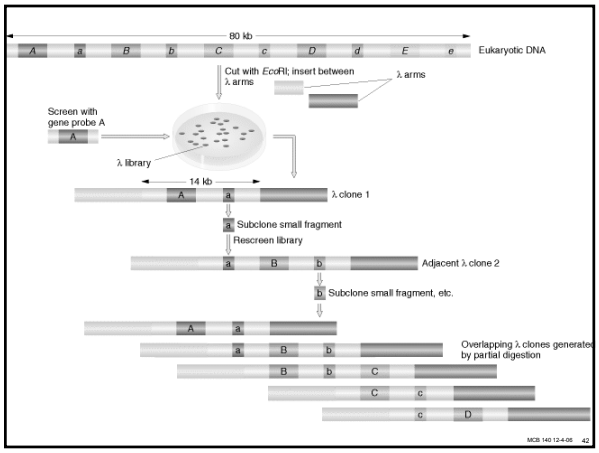
White et al (1985) *Nature* **318:** 382.

A RFLP linked to CF!!! – "middle third of 7q, near *met* "



Finding the CF gene

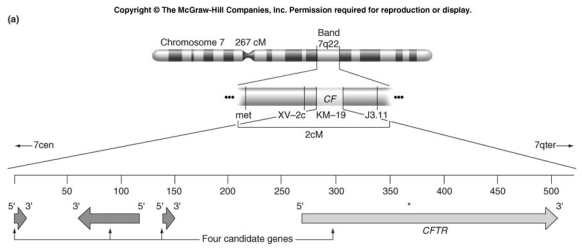
- Linkage analysis placed the CF gene in a rather large (1.5 Mb) fragment of chromosome 7 (next to *met*)
- "Jumping and walking" – Rommens et al (Collins) *Science* **245:** 1059 (1989).



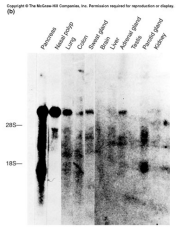
CFTR

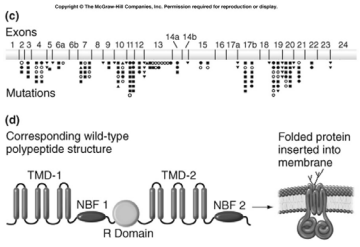
- Large gene (250 kb)
- Large protein (1,480 aa)
- cAMP-regulated chloride channel
- 70% of mutations – Δphe508 (protein stuck in the ER)

Brennan and Geddes (2002) *Curr. Opin. Infect. Dis.* **15:** 175-182.



11.22





11.22

Gene therapy for CF?

2002:

"Since the cloning of the cystic fibrosis gene (CFTR) in 1989, 18 clinical trials have been carried …. Most trials demonstrated proof of principle for gene transfer to the airway. However, gene transfer efficiency … was low, and most likely insufficient to achieve clinical benefit.

A major function of the airway epithelium is to prevent uptake of foreign materials, including gene transfer agents."

Quasi-success – relative of HIV in an Ebola coat!

Griesenbach et al. (2002) *Gene Ther.* **9:** 1344.

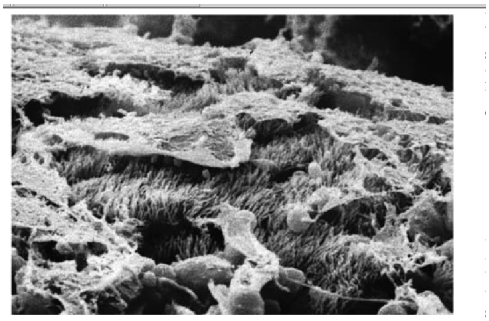
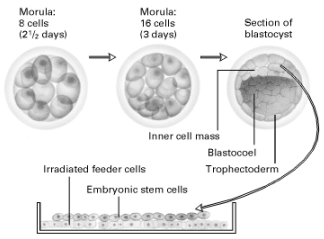


Figure 1 Scanning electron micrograph of human airway epithelium showing fields of cilia covered at their tips by flakes of mucus. In hypersecretory disease the mucus usually forms a continuous sheet or 'blanket'

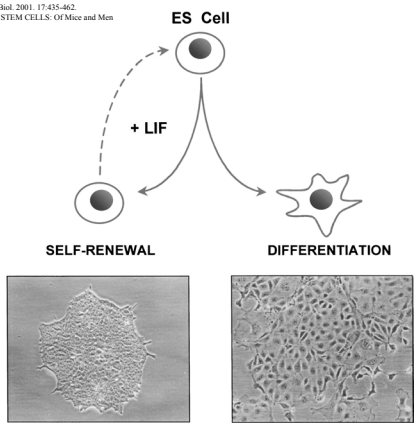
The "knockout" mouse

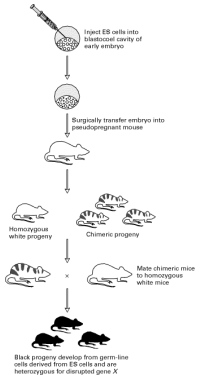
A mouse genotypically uniform and homozygous for an amorphic (full null) allele of a gene of choice.

Embryonic stem cells



Annu. Rev. Cell Dev. Biol. 2001. 17:435-462.
EMBRYO-DERIVED STEM CELLS: Of Mice and Men
Austin G. Smith





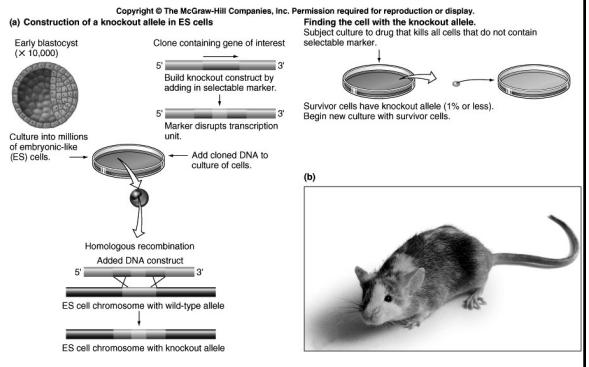
Positive-negative selection (Mario Capecchi, 1989)

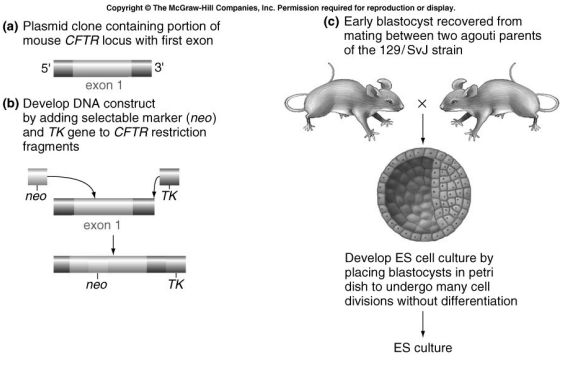
Non-HR events are MASSIVELY more frequent than HR events.

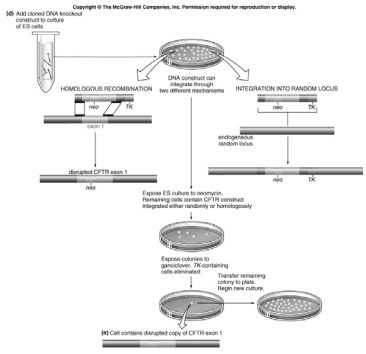
↓

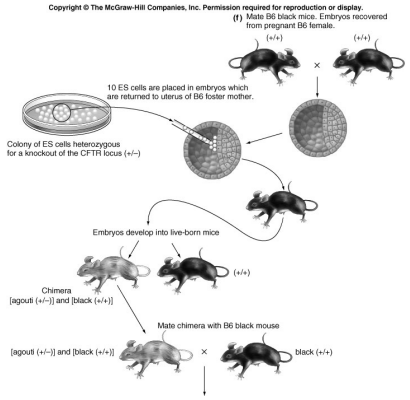
Two sequential selection events to eliminate all cells that underwent non-HR or no recombination at all, and PRESERVE those cells that underwent HR.

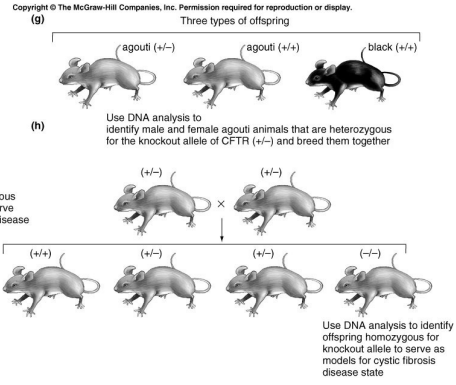
"Targeting construct"











Mouse model?

p. 857: “Animals homozygous for the *CFTR* knockout allele display a mutant phenotype that is very similar to that expressed by humans suffering from cystic fibrosis.”

I beg your pardon?

And now, the truth

“The airways of CF mice are of obvious interest to investigators because ~95% of the morbidity and mortality in CF humans is due to pulmonary manifestations of the disease. … In the CF patient, a consistent finding in the airways is mucus plugging with bacterial infection. …

In all CF mouse models examined, virtually normal lung histology and absence of mucus plugging are consistent findings (36, 39, 70, 78, 92, 103, 114, 119).”

Grubb and Boucher (1999) *Phys. Rev.* **79:** 193-214.

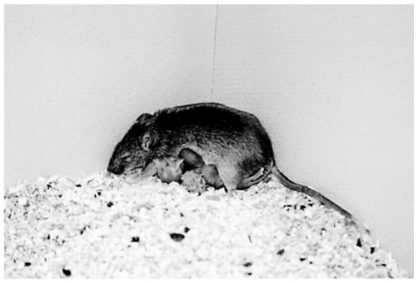
A remarkable example of a phenotype in a knockout mouse

A Defect in Nurturing in Mice Lacking the Immediate Early Gene *fosB*

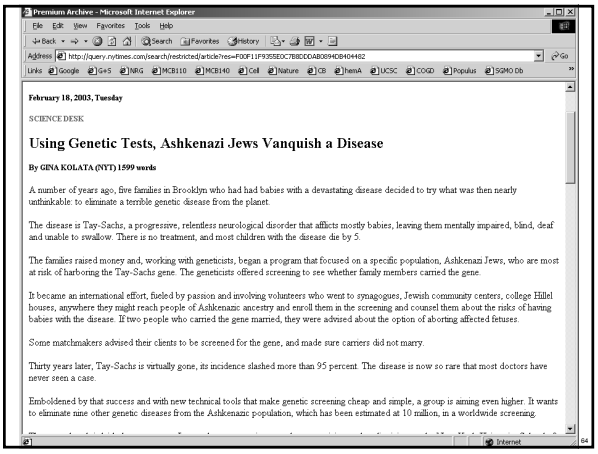
Brown et al.

Cell, Vol. 86, 297–309, July, 1996

C



D



Premium Archive - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Address http://query.nytimes.com/search/restricted/article?res=F00F11F9355EOC7B8DDDAB0894DB404482

February 18, 2003, Tuesday

SCIENCE DESK

Using Genetic Tests, Ashkenazi Jews Vanquish a Disease

By GINA KOLATA (NYT) 1599 words

A number of years ago, five families in Brooklyn who had had babies with a devastating disease decided to try what was then nearly unthinkable: to eliminate a terrible genetic disease from the planet.

The disease is Tay-Sachs, a progressive, relentless neurological disorder that afflicts mostly babies, leaving them mentally impaired, blind, deaf and unable to swallow. There is no treatment, and most children with the disease die by 5.

The families raised money and, working with geneticists, began a program that focused on a specific population, Ashkenazi Jews, who are most at risk of harboring the Tay-Sachs gene. The geneticists offered screening to see whether family members carried the gene.

It became an international effort, fueled by passion and involving volunteers who went to synagogues, Jewish community centers, college Hillel houses, anywhere they might reach people of Ashkenazic ancestry and enroll them in the screening and counsel them about the risks of having babies with the disease. If two people who carried the gene married, they were advised about the option of aborting affected fetuses.

Some matchmakers advised their clients to be screened for the gene, and made sure carriers did not marry.

Thirty years later, Tay-Sachs is virtually gone, its incidence slashed more than 95 percent. The disease is now so rare that most doctors have never seen a case.

Emboldened by that success and with new technical tools that make genetic screening cheap and simple, a group is aiming even higher. It wants to eliminate nine other genetic diseases from the Ashkenazic population, which has been estimated at 10 million, in a worldwide screening.

Internet

"Carrier screening for cystic fibrosis, Gaucher disease, and Tay-Sachs disease in the Ashkenazi Jewish population: the first 1000 cases at New York University Medical Center"

By late 1993, the genes for cystic fibrosis and Gaucher disease and the mutations common among Ashkenazi Jews had been identified. In response to these advances, heterozygote screening for cystic fibrosis and Gaucher disease was added to the more than 20-year-old Tay-Sachs disease screening program at New York University Medical Center, New York, NY. … Patients and their referring physicians were informed about the new carrier tests. At the time of screening, patients could choose their tests (hexosaminidase A by enzyme analysis for Tay-Sachs disease or mutation analysis for cystic fibrosis and Gaucher disease). … The majority of Ashkenazi Jewish patients chose to have testing for all 3 diseases. If they previously underwent screening for Tay-Sachs disease, then they chose to undergo testing for cystic fibrosis and Gaucher disease. All carrier couples for each of these diseases went on to have prenatal testing. All mixed-marriage couples in whom the Jewish partner was found to be a carrier for Gaucher disease chose to have prenatal diagnosis. One fetus was identified as having cystic fibrosis. Since the program was initiated, no Ashkenazi Jewish baby has been born with any of these diseases at New York University Medical Center.

Kronn et al. (1998) *Arch. Intern. Med.* **158:** 777.