Human disease genes summary

1. Goals: discover the basis for disease, understand key processes, and develop diagnostics and cures.
2. Finding human disease genes -- OMIM
3. Sickle Cell Anemia
4. Inheritance and linkage
5. RFLPs and chromosome "walking"
6. Huntington’s disease -- Scientific suicide
7. Future

Some examples of single-gene diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Molecular and Cellular Defect</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle cell anemia</td>
<td>Abnormal hemoglobin causes deformation of red blood cells, which can become lodged in capillaries, also confer resistance to malaria.</td>
<td>1/825 of sub-Saharan African origin</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>Defective chloride channel (CFTR) in epithelial cells leads to excessive mucus in lungs.</td>
<td>1/2596 of European origin</td>
</tr>
<tr>
<td>Phenylketonuria (PKU)</td>
<td>Defective enzyme in phenylalanine metabolism (phenylalanine hydroxylase) results in excess phenylalanine, leading to mental retardation, unless restricted by diet.</td>
<td>1/50,000 of European origin</td>
</tr>
<tr>
<td>Tay-Sachs disease</td>
<td>Defective hexosaminidase enzyme leads to accumulation of excess sphingolipids in the neurons of neurons, impairing neural development.</td>
<td>1/1000 Eastern European Jews</td>
</tr>
<tr>
<td>Huntington’s disease</td>
<td>Defective neural protein (huntingtin) may assemble into aggregates causing damage to neural tissues.</td>
<td>1/50,000 of European origin</td>
</tr>
<tr>
<td>Hypcholesterolemia</td>
<td>Defective LDL receptor leads to excessive cholesterol in blood and early heart attacks.</td>
<td>1/322 French Canadians</td>
</tr>
<tr>
<td>Duchenne muscular dystrophy (DMD)</td>
<td>Defective translation protein dystrophin leads to impaired muscle function.</td>
<td>1/3000 males</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>Defective blood clotting factor VIII leads to uncontrolled bleeding.</td>
<td>1-2/10,000 males</td>
</tr>
</tbody>
</table>

Common?
Find disease genes

At OMIM (Online Mendelian Inheritance in Man)


This database catalogs human genes and genetic disorders. The database contains textual information and references. It also contains links to MEDLINE and sequence records in the Entrez system, and links to additional related resources.

Gene finding 1. Sequence candidate genes or proteins

Sequencing Hb5 proteins revealed a single change: Glu6Val in the β chain. Fiber formation (R) at low [O₂] causes sickling of RBCs (center).
Inheritance

Three common inheritance patterns of human genetic diseases.

<table>
<thead>
<tr>
<th>Autosomal dominant. E.g. Huntington’s disease</th>
<th>Autosomal recessive. E.g. Cystic fibrosis (CF)</th>
<th>X-linked recessive. E.g. Duchenne muscular dystrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. M++  F++  M^D+ + F++</td>
<td>2. ?+/ CF/CF</td>
<td>X/Y</td>
</tr>
<tr>
<td>2. F++</td>
<td>3. ?/ CF/CF</td>
<td>X+X</td>
</tr>
<tr>
<td>3. F++</td>
<td></td>
<td>X+Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X/Y</td>
</tr>
</tbody>
</table>

□ = Male (XY)  ○ = Female (XX)

Autosome: not a sex chromosome
X, Y: sex chromosomes

Linkage--Recombination during meiosis separates genes

1. Genes on different chromosomes assort independently
2. Genes on the same chromosome are linked
3. This linkage is not absolute

Markers separated by 1 centimorgan have a 1% chance of being separated in meiosis.
1 centimorgan corresponds to ~750,000 bp in humans!
Gene finding 2. RFLP analysis

Look for restriction fragment length polymorphism (RFLP) that correlates with the inheritance pattern of the disease.

Fig. 9-46. Three alleles of a RFLP on chromosome 5 in 14 individuals in 3 generations. Each lane corresponds to the individual above it.

Can a gene be located by RFLP linkage?

A “crazy” approach:

1. Collect DNA from 100s of related individuals with and without the disease.
2. Establish their pedigrees without errors.
3. Digest their DNA with various restriction enzymes.
4. Probe Southern blots with RANDOM probes.
5. Look for an RFLP that is inherited with the same pattern as the disease.
**Linkage mapping requires large patient populations**

Markers separated by 1 centimorgan have a 1% chance of being separated in meiosis.
1 centimorgan corresponds to ~750,000 bp in humans!

For a “fully penetrant”, single-gene disease:
Linkage of a RFLP to a disease in 99/100 patients implies the RFLP may be within 750 kbp of the disease mutation.

In practice, many more patients are needed to get reliable linkage statistics.

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**Jim Gusella commits “scientific suicide”**

1980: Gusella starts his first faculty job at Massachusetts General Hospital with the aim of finding an RFLP marker for Huntington’s disease.

No one had ever found an RFLP marker for an unmapped disease gene.

The approach was to screen for RFLPs using random human DNA probes. As many as 300 probes might be needed to cover the genome.

At the time, there were two RFLP markers mapped in the entire human genome. The largest accessible HD family had 27 members—too few to establish tight linkage.

David Botstein, an originator of the RFLP concept, estimated it would take 10 years to find a marker linked to the HD gene!
More patients: HD families in Venezuela

1952: Biochemist and physician, Dr. Americo Negrette diagnoses Huntington’s disease at Lake Maracaibo in Venezuela.

1963: Negrette published Corea de Huntington: Estudio de una sola familia a través de varias generaciones (Huntington’s Chorea: Study of a Single Family Through Several Generations)

1972: Dr. Ramon Avila-Giron, a student of Negrette’s, attended the Centennial Symposium on HD in Columbus, OH. He showed the 146 participants from 14 countries a startling 20-minute, black-and-white film of several communities around Lake Maracaibo ravaged by HD.

Patient advocacy: funding to collect DNA in Venezuela

1981: Nancy Wexler leads a US/Venezuelan project to define pedigrees and collect blood samples from HD families in the towns on Lake Maracaibo in western Venezuela.
--Genetically isolated
--Large families
--High HD incidence
--All cases are believed to arise from a single “founder” individual who settled in the area in the 1870s.
Linking genotype and phenotype

March 10, 1983: “...The meeting room in the modulo takes on a slightly carnivalesque atmosphere as people from the barrio drift in, children darting underfoot, staring over shoulders, while the adults shoo them outside, where they peer through the doorway or huddle at the windows. ... Taped around the walls of the room is the pedigree chart, a computer-generated system of lines, circles and squares, like a Mondrian mural, that traces the relationships of all the local families with Huntington’s... Alice Wexler, Mapping Fate

Lake Maracaibo, Venezuela

Half fishing village, half urban slum, San Luis hugs the shore of Lake Maracaibo on the sandy southern outskirts of the city, unremarkable except for Huntington’s disease, which haunts almost every home... Today is a “draw day”, when those who have come earlier for a neurological exam will return to give blood and a small skin sample. ... The “draw” takes place on the other side of the modulo, in a closet-sized, air-conditioned examining room. Fidela Gomez, an emergency room nurse who lives in Miami but was born in the Canary Islands and grew up in Argentina, is key to this operation. She is so quick-witted and skillful with the needle that she draws blood before anyone knows what is happening. Fidela clips a tiny square of skin from someone’s arm and draws the six tubes of blood while bantering in rapid-fire Spanish, slowly shaking each dark red tube before handing the it over to an assistant, who applies identifying labels and packs them gently into a Styrofoam box.
The 12th probe, G8, is linked to HD

April 1983: Ginger Weeks, a technician in the Gusella lab at MGH, developed a new human DNA probe. The probe comprised a unique 17.6-kb fragment in the human genome. G8 showed an RFLP in HindIII-digested DNA. The RFLP gave a 65:1 chance of being linked to the HD gene in an Iowa family of 27 members.

July 1983: G8 revealed a 10^6:1 chance of being linked to the HD gene in an analysis of RFLPs in a pedigree of 75 individuals from Lake Maracaibo.

November 1983: Results reported in Nature, Gusella appears on the Today Show.

1984-1992: 6.2 Mb of DNA from the short arm of chromosome 4 is cloned and mapped.


HD gene: Ten years after

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Huntington’s disease gene

3144-amino-acid neuronal protein. Expansion of microsatellite in Huntingtin gene causes the disease. 28 or fewer CAG repeats: normal. Individuals with HD usually have 40 or more repeats. A small percentage of individuals, however, have a number of repeats that fall within a borderline region.

<table>
<thead>
<tr>
<th>No. of CAG repeats</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;28</td>
<td>Normal range; individual will not develop HD</td>
</tr>
<tr>
<td>29–34</td>
<td>Individual will not develop HD but the next generation is at risk</td>
</tr>
<tr>
<td>35–39</td>
<td>Some, but not all, individuals in this range will develop HD; next generation at risk</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Individual will develop HD</td>
</tr>
</tbody>
</table>

A similar strategy was used to find hundreds of “disease genes”, including the gene for Huntington’s disease.
Lessons

1. Strategy is to establish genetic and physical linkage (phenotype <-> RFLP or sequence).
2. Example of basic research solving a medical problem.
3. Number of genes not infinite.

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