Genetics for Epidemiologists: Application of Human Genomics to Population Sciences

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A Genome-wide Association Study of Crohn Disease*

- GWAS examining 300,000 SNPs in 547 patients with Crohn Disease and 928 controls.
- Confirmation of two genes previously associated with CD (IL23R on Chromosome 1, CARD15 on Chromosome 16).
- Region of Chromosome 15 (p13.1) identified with multiple SNPs having significant associations with CD.
- Replication of 5p13.1 association in 1266 CD cases/559 controls and in 428 trios.
- SNPs located in gene desert.
  - Associated with expression of a prostaglandin receptor gene (PTGER4) located 270 kb proximally.
  - Speculated to control regulation of PTGER4 gene.

Course Overview

• Goal: To familiarize epidemiologists and population-based investigators with recent developments in the theory and methods of human genetics and genomics.

• Learning Objectives

• Format
  • Eight lectures
  • Case studies
  • Discussion
  • Webcast
NHGRI Catalog of GWAS (www.genome.gov/gwastudies/)

• All publications reporting genome-wide association studies (beginning March, 2005)
  – Platforms with density of at least 100,000 SNPs
  – Identified by literature searches, media, HUGE Navigator

• Data Presented
  – Citation
  – Disease/trait
  – Sample sizes
  – Chromosomal region
  – Gene
  – Association
    • Strongest risk allele
    • Odds ratio per copy
    • Risk allele frequency
    • P value of association
Genetics for Epidemiologists

Lecture 1. The Biologic Basis for Analysis of Gene Variants

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Lecture 1: Learning Objectives

• Provide an overview for the course.
• Review the structure and function of the human genome.
• Discuss patterns of inheritance.
• Describe types of genetic variation as potential causes of disease.
• Introduce online informatics resources which describe genotype and phenotype of human genetic variants.
# Genetics Vs. Genomics

<table>
<thead>
<tr>
<th>Genetics:</th>
<th>The science of inheritance (Bateson, 1905)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic Code:</td>
<td>DNA structure and function (Watson and Crick, 1953)</td>
</tr>
<tr>
<td>Genomics:</td>
<td>The field within genetics concerned with the structure and function of the entire DNA sequence of an individual or population (Roderick, 1986)</td>
</tr>
</tbody>
</table>
## The Challenge: Finding Genetic Variants Affecting Human Health

<table>
<thead>
<tr>
<th>Category</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomes</td>
<td>46 (22 pairs of autosomes, X, Y)</td>
</tr>
<tr>
<td>Genes</td>
<td>20,000-25,000</td>
</tr>
<tr>
<td>Base-pairs</td>
<td>3,000,000 kilobases (haploid)</td>
</tr>
<tr>
<td>Variants</td>
<td>99.9% of bases are identical between all people</td>
</tr>
</tbody>
</table>
“According to these figures, Simmons, your department has lost another No. 2 Double N -- and I want you to find it!”

Structure of Human Genes: Potential Sites of Gene Variation

- Exons
- Introns
- Regulatory Elements
  - Promoters
  - PolyA Tail
  - Enhancers
  - Silencers
  - Locus Control Regions
The Genetic Code

- Sequences of four DNA bases (A,T,C,U) are translated in triplets called **codons**, each encoding an amino acid.
- 64 codons (4X4X4) constitute the genetic code.
- Some of the 20 amino acids will be encoded by more than one codon.
- Three stop or nonsense codons designate termination of translation of mRNA.
# Mutations

<table>
<thead>
<tr>
<th>Class</th>
<th>Mechanism</th>
<th>Frequency</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome</td>
<td>Chromosome segregation</td>
<td>1/25-50 cell divs.</td>
<td>Trisomy 21</td>
</tr>
<tr>
<td>Genome</td>
<td>Chromosome rearrangement</td>
<td>1/1700 cell divs.</td>
<td>Cancer Cells</td>
</tr>
<tr>
<td>Gene</td>
<td>Base-pair mutation</td>
<td>1/1000 base-pairs</td>
<td>SNPs</td>
</tr>
</tbody>
</table>
# Types of Mutations: Single Nucleotide Substitutions

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silent (Synonymous)</td>
<td>No effect on amino acid sequence.</td>
</tr>
<tr>
<td>Missense (Nonsynonymous)</td>
<td>Alters amino acid sequence.</td>
</tr>
<tr>
<td>Nonsense</td>
<td>Encodes termination codon.</td>
</tr>
<tr>
<td>Termination</td>
<td>Destroys termination codon and affects adjacent gene.</td>
</tr>
</tbody>
</table>
Types of Mutations (Indels): Deletions and Insertions

• Simple: those involving a short segment of DNA and only 2 alleles.
• Short tandem repeat polymorphism: 2-4 nucleotide repeat units repeated 5-25 times, affecting many alleles.
• Variable number tandem repeats: 10-100 nucleotide repeat units repeated hundreds of times, affecting many alleles.
• Copy number variants: 200bp-1.5Mb segments of DNA affecting a few alleles.
Mendel’s Principles of Inheritance

• Segregation: The pair of alleles for any given trait separate and only one allele passes from each parent to an offspring; which allele passed is random.

• Independent Assortment: Traits encoded by different pairs of alleles are inherited independent of each other, unless genetically linked.
Mendelian Traits Listed in Online Mendelian Inheritance of Man (OMIM), as of July, 2007

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Known</th>
<th>Gene Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal</td>
<td>1932</td>
<td>1954</td>
<td>3386</td>
</tr>
<tr>
<td>X-Linked</td>
<td>177</td>
<td>133</td>
<td>310</td>
</tr>
<tr>
<td>Y-Linked</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>26</td>
<td>0</td>
<td>26</td>
</tr>
</tbody>
</table>
Patterns of Inheritance

- **Mendelian Disease**: Condition (phenotype) caused almost entirely by a single major gene, in which the disease is manifested in only 1 (recessive) or 2 (dominant) of the 3 possible genotype groups.

- **Common disease, Common Variant**: Common conditions (phenotype) attributable to a limited number of allelic variants which occur in 1-5% or more of the population.
Autosomal Dominant Inheritance

- Phenotype appears in every generation (unless new mutation).
- Any child of an affected person has a 50% risk of inheriting the trait.
- Male-to-male transmission is present.
- Phenotypically normal family members do not transmit the phenotype to children.
- Male:Female occurrence is usually equal.
Complete & Incomplete Dominance

**Complete dominance**: Phenotypes are indistinguishable in heterozygous or homozygous state.

(Example: Huntington’s Disease)

**Incomplete dominance**: Phenotypes are more severe in the homozygous than in the heterozygous state. (Example: Familial Hypercholesterolemia)
Autosomal Recessive Inheritance

- Phenotype, if it appears in one family member, is seen only in the sibship of the proband.
- Male:Female occurrence is usually equal.
- Parents of affected child are asymptomatic carriers of the gene variant.
- Consanguinity is increased in families with recessive disorders.
- Offspring of two heterozygous parents have the following risks: 25% affected, 50% carrier, 25% noncarrier.
X-Linked Dominant Inheritance

- Both male and female offspring of female carriers have 50% risk of inheriting the phenotype.
- No male-to-male transmission.
- Number of females affected > number of males.
- All daughters of affected males are affected but none of their sons.
- Severity in females modified by X-inactivation (Lyonization)
- Example: Vitamin D resistant rickets
X-Linked Recessive Inheritance

- Only males are affected.
- Affected males are related through carrier females.
- No male-to-male transmission
- Unaffected males do not transmit.
- All daughters of affected males are carriers.
- Examples: Hemophilia A, red-green color blindness
Y-Linked Inheritance

- Only males affected
- All sons but no daughters of affected men are affected.
- Not X-linked since male-to-male transmission occurs.
- Sex-limited, because trait does not pass through unaffected females.
Mitochondrial Inheritance

- Matrilineal inheritance
- All children of affected females are affected.
- Numbers of affected males and females are equal.
Exceptions to the Rules of Inheritance

- Sporadic mutations
- Genetic heterogeneity
- Nonpenetrance
- Variable expressivity
- Late onset conditions
- Sex-limited/sex-influenced phenotypes
The Common Disease-Common Variant Hypothesis

SNP1 in Exon 1 of GeneA
SNP1 in Exon 3 of GeneA
SNP2 in Exon 3 of GeneA
SNP2 in Exon 2 of GeneB
SNP, Reg. Element, GeneA
Environmental Exposures

Disease
Susceptibility Variants Associated with Systemic Lupus Erythematosus in Women*

- Case-control study of 720 women with SLE and 2337 control women.
- 317,501 SNPs assessed genome-wide
- Two replication studies with 1846 female cases and 1825 female controls.
- At least 17 SNPs associated with SLE at P<2xE-7

*International Consortium for SLE Genetics. Nature Genetics 1/20/08
## Logistic Regression Model of Independent Contributions of Markers Associated with SLE*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>OR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKY</td>
<td>3p14.3</td>
<td>1.27</td>
<td>9.2E-07</td>
</tr>
<tr>
<td>HLA region</td>
<td>6p21.33</td>
<td>1.82</td>
<td>4.5E-17</td>
</tr>
<tr>
<td>HLA region</td>
<td>6p21.32</td>
<td>1.40</td>
<td>2.8E-12</td>
</tr>
<tr>
<td>IRF5/TNP03</td>
<td>7q32.1</td>
<td>1.61</td>
<td>1.7E-14</td>
</tr>
<tr>
<td>KIAA1542</td>
<td>11p15.5</td>
<td>0.78</td>
<td>1.3E-07</td>
</tr>
<tr>
<td>ITGAM</td>
<td>16p11.2</td>
<td>1.70</td>
<td>1.9E-18</td>
</tr>
</tbody>
</table>

C statistic = 0.67; 15\% of heritability explained

*Int. Consort. for SLE Genetics. NatGen 1/20/08
Bioinformatics Resources in Human Genomics

Online Mendelian Inheritance in Man (OMIM)

- Catalog of human genes and genetic disorders.
- Concise information on most human conditions having a genetic basis.
- Pictures illustrating the condition or disorder
- Full citation information, linked to PubMed
- OMIM Numbering System
- OMIM Maps: cytogenetic location of genes and diseases.
GenBank
NIH Genetic Sequence Database

• Annotated collection of all publicly available DNA and protein sequences
  – >80 million sequence records
  – >80 billion bases

• Concise description of the sequence
  – Scientific name and taxonomy of organism
  – Bibliographic references (PubMed)
  – Features provided by submitter (e.g. biologic function, mutations and modifications, secondary or tertiary structure)
RefSeq: Database of Single Reference Sequences

- Each molecule in sequence (DNA, RNA, Protein).
- Nonredundant.
- Linked to nucleotide and protein sequences in GenBank.
- Updated by NCBI staff and collaborators to reflect current sequence data and biology.
- Review status indicated on each record.
dbSNP: Database of Single Nucleotide Polymorphisms

- 6.2 million validated human SNPs and counting
  - Up to 10 million SNPs likely exist
  - Over 200,000 SNPs within genes
- Relates genes to specific diseases.
Conclusions

• 1900-present: Human genetics, Mendelian disorders
• 1953-present: Molecular genetics, structure and function of the human genome.
• 1980-present: Identification of genetic variants and candidate gene studies
• 2003-present: Sequencing of the entire human genome and genome-wide association studies
Questions?

Realities of New DNA Sequencing Technologies...
Pearson TA, Manolio TA. How to interpret a genome-wide association study. JAMA 2008; 299: 1335-1344 (Includes a glossary of terms frequently used in GWAS).