Genetics Prior to the Human Genome Project

• focus was on relatively rare disorders caused by:
  • an extra or missing chromosome or part of a chromosome
  • dominant mutations within a single gene

• clinical issues handled primarily by medical geneticists and genetic counselors with occasional involvement of primary care providers
Genetics in the Era of the Human Genome Project

- a broadened scope concerned with more common disorders only partly attributed to gene mutations
- more than 9/10 leading causes of US deaths have a genetic component
- future practice of medicine will be transformed with a greater emphasis on disease prevention, diagnosis and treatment

"Genomics will revolutionize the clinical practice of medicine"

Needs for Practicing Physicians

- understand the basis of genetics and the application of genomic advances into improving health care
- includes understanding currently available and evolving technologies
  - how proteomic screens are useful in ascertaining the risk for several common disorders and may contribute to the prevention of disease
  - how molecular assays can be used for the diagnosis of disease
  - how pharmacogenetic profiles and microarray assays can provide information on the most effective drug treatment regimen while minimizing possible life-threatening side effects
The Human Genome

- composed of 3 billion DNA base pairs
- contains 20,000-25,000 genes, representing about 2% of total DNA
- each gene codes for an average of 3 proteins
- 98% of the genome consists of non-coding DNA (ncDNA)
- complete sequence finished in April 2003
- variations both within humans and between humans and other primates are attributed primarily to structural variations (deletions and segment replications)
- the HapMap Project—a comprehensive map of human genetic variation is aiding in the search for genes involved in disease

Genomics & Geriatric Psychiatry

- complex disorders present major challenges
  - multiple contributing genes (proteins) involved
  - symptoms not expressed until late in life

- complex disorders present opportunities to integrate information from multiple modalities, which may provide keys to understanding
  - structural and functional imaging
  - biomarkers in plasma and CSF
  - genomics and proteomics
Late-Life Depression and Subsequent Dementia

- structural and functional imaging (MRI, PET, DTI, MTI)
  - signals for responders and non-responders differ in several brain regions; decreased integrity of white matter tracks predicts poor responses to first-line antidepressants
  - individuals with LLD exhibit impaired myelin integrity in fronto-striate-limbic circuits
- large white matter lesion load in non-depressed individuals may be associated with an increased risk to develop depression
- studies investigating white matter hyperintensities in LLD have shown that increased white matter lesions predict the development of dementia

- individuals with late-onset LLD show greater thinning in both genu and splenium than those with early-onset LLD, indicating that the former have greater deficits in cortical connectivity, perhaps representing a population with a greater risk of developing dementia
- studies on plasma biomarkers have been rather unremarkable
  - increased risk of incident depression associated with lower B12 and folate and higher homocysteine
  - higher levels of IL-6, but not CRP may be associated with depression
  - individuals with depression had lower plasma Aβ42 and higher plasma Aβ40:Aβ42 ratios; those with high plasma Aβ40:Aβ42 ratios had greater memory impairment, visual-spatial ability, and executive impairment than those with low plasma Aβ40:Aβ42 ratios
Genetic Biomarkers and Late-Life Depression

• several genes may serve as potential markers
  • in patients with late-onset LLD, the L/L genotype (long variant associated with greater 5-HT reuptake) of the promoter region of the 5-HT transporter gene has been associated with smaller hippocampal volumes, suggesting that the L/L genotype may be a susceptibility marker for LLD (but results controversial)
  • BDNF polymorphism (Val66Met) associated with white matter hyperintensity and perhaps vulnerability to vascular injury, but data on LLD are meager
  • a role of ApoE4, a risk factor for Alzheimer's disease, in LLD is unclear

Genetic Biomarkers and Dementia

• imaging biomarkers
  • normal elderly who convert to MCI and AD have gray matter atrophy in anteromedial temporal and left angular gyri prior to clinical detection of dementia
  • presymptomatic mutation carriers of familial AD have disruption of white matter tracts connecting limbic structures
  • individuals with MCI who convert to AD show greater hippocampal activation with fMRI

• neurophysiological biomarkers
  • abnormalities in P600 and N400 in amnestic MCIs who converted to AD
• genetic biomarkers

• familial forms of A.D. (autosomal dominant with mutations related to the amyloid precursor protein or presenilin) represent a small proportion of patients

• the ApoE4 allele is implicated in sporadic A.D. and is believed to represent a major genetic risk factor for the disease

• recent studies have implicated polymorphisms in glutathione S-transferase gene in regulating the rate of cognitive decline in ApoE4 carriers

What do we know today?
The most applicable information today concerns variations in Pharmacogenomics or Pharmacogenetics --- how and why genetic variations among individuals affect:

• the capacity of individuals to metabolize drugs (pharmacokinetics) and

• the ability of drugs to have a therapeutic effect on the individual (pharmacodynamics).

How do DNA sequence variations affect an individual's response to drugs?
The Pharmacogenomics Era

• current approaches to develop blockbuster drugs are difficult and expensive
• pharmacogenomics can increase a product's market
• there are immediate clinical demands for pharmacogenomic products, which are finding mainstream success
• pharmacogenomic tools are being developed and used in mainstream medicine
• regulatory and reimbursement structures for pharmacogenomics are currently being written
• the first high-profile pharmacogenomics products are likely to be in oncology

PriceWaterhouseCoopers "Personalized Medicine: The Emerging Pharmacogenomics Revolution" (2/05)

The Questions

• Why are specific pharmacological agents effective for specific conditions in some patients, but not effective in others?

• Why do some individuals exhibit marked side effects and adverse reactions to a particular drug while other individuals do not?
Pharmacogenomics Involves A Two-Pronged Inquiry

Pharmacokinetics
What the body does to the drug (ADME)

Pharmacodynamics
What the drug does to the body (receptors, enzymes, uptakers)

Polygenic Determinants of Drug Effects

Pharmacokinetics

How the body handles drugs

Absorption
Distribution
Metabolism
Elimination

Critical for achieving therapeutic response and determining both side effect profile and drug-drug interactions.

Drug Transporters

• Drug disposition involves transporters among which members of the ATP-binding cassette family, such as p-glycoprotein, have been most studied.
  • functions in the energy-dependent cellular efflux of substrates
  • plays a role in the excretion of drugs and metabolites into urine, bile and intestinal lumen
  • at the BBB, p-glycoprotein limits the accumulation of many drugs in brain
  • encoded by human ABCB1 gene
  • both synonymous (no change in amino acid encoded) and nonsynonymous (causes amino acid change) SNPs identified that alter both the expression and function of the transporter
Drug Metabolism

• Differences in the activities of CYPs (cytochrome P450s) are affected by genetics, environmental factors, concurrent medications, gender, age, health, hormones, hepatic diseases, inflammation, nutritional status & more
• enzymes involved in both Phase I and Phase II reactions exhibit genetic polymorphisms, which can lead to increases or decreases in plasma drug levels, thereby affecting therapeutic responses and side effects
• classic example - bimodal distribution of isoniazid acetylation in 267 subjects 6 hours after an oral dose due to differences in 2 acetylation genes


Phase I and Phase II Enzyme Polymorphisms

Drug Metabolism

- CYPs of families 2 and 3 play major role in drug metabolism
- **CYP3A4**
  - present in very high abundance in liver; involved in the metabolism of more drugs than any other CYPs
  - however, its activity is affected more by environmental factors and concurrent medications than inherited variations
- **CYP2D6**
  - activity extensively influenced by inherited variations, as well as concurrent medications
  - more than 75 alleles identified that alter function and expression, altering therapeutic response to 'standard' doses of drugs
  - some ultrarapid metabolizers have been found to have multiple copies of the gene, from 0 to 13

Pharmacogenetics of Nortriptyline

plasma concentrations following a single oral 25 mg dose of nortriptyline in individuals with 0 to 13 copies of **CYP2D6**

Pharmacokinetics: The AmpliChip CYP450 Test

• approved by the FDA in January 2005 (still only one available on market)

• chip contains thousands of predetermined DNA sequences in 25 molecule long elements (oligonucleotides) at specific locations on the chip

• represents first microarray technology distinguishing 29 known polymorphisms in CYP2D6 and 2 polymorphisms in CYP2C19

Pharmacokinetics - CYP2D6

• 27 alleles and 7 duplications tested to characterize individuals into 4 phenotypes
  • ultrarapid metabolizers (UMs)
  • extensive metabolizers (EMs) - normals
  • intermediate metabolizers (IMs)
  • poor metabolizers (PMs) - do not express CYP2D6 (7% Caucasians; 2-4% African-Americans; 1-2% Asians)

• high prevalence of several reduced activity alleles leading to a greater percentage of IMs with low enzyme activity
  • CYP2D6*10 allele in Asians (50% allele frequency)
  • CYP2D6*17 and *29 alleles (30% each in certain African populations)
Drugs Metabolized by CYP2D6

<table>
<thead>
<tr>
<th>CV drugs</th>
<th>Antipsychotics</th>
<th>Antidepressants (most all)</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>carvedilol</td>
<td>aripiprazole</td>
<td>amitriptyline</td>
<td>codeine</td>
</tr>
<tr>
<td>metoprolol</td>
<td>haloperidol</td>
<td>clomipramine</td>
<td>dextromethorphan</td>
</tr>
<tr>
<td>propafenone</td>
<td>risperidone</td>
<td>desipramine</td>
<td>flecainide</td>
</tr>
<tr>
<td>timolol</td>
<td>thioridazine</td>
<td>duloxetine</td>
<td>mexiletine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fluoxetine</td>
<td>ondansetron</td>
</tr>
<tr>
<td></td>
<td></td>
<td>imipramine</td>
<td>tamoxifen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>paroxetine</td>
<td>tramadol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>venlafaxine</td>
<td></td>
</tr>
</tbody>
</table>

**Pharmacokinetics - CYP2C19**

- 3 alleles tested to characterize individuals into 2 phenotypes
  - extensive metabolizers (EMs)
  - poor metabolizers (PMs)
- (3-5% Caucasians and African-Americans; 13-23% Asians)

Drugs Metabolized by CYP2C19

<table>
<thead>
<tr>
<th>Proton pump inhibitors</th>
<th>Anticonvulsants</th>
<th>Antidepressants</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>omeprazole</td>
<td>diazepam</td>
<td>amitriptyline</td>
<td>cyclophosphamide</td>
</tr>
<tr>
<td>lansoprazole</td>
<td>phenytoin</td>
<td>clomipramine</td>
<td>progesterone</td>
</tr>
<tr>
<td>pantoprazole</td>
<td>phenobarbital</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pharmacokinetics: Current State of the Art

• alterations in ADME, particularly drug-metabolizing enzymes, affect
  • therapeutic drug responses
  • side effect profile / adverse events
  • drug-drug interactions
• knowledge advances about polymorphisms in the genes coding for the proteins involved these processes, in concert with further developments in technology, will enable patient screening to determine the best drug and most optimal dose for each individual

What about drug targets?

Pharmacodynamic Polymorphisms
Pharmacodynamics

• psychiatric disorders represent complex genetic diseases not characterized by a single, causative gene and not exhibiting simple patterns of inheritance

• susceptibility architecture is heterogeneous such that no single constellation of genes will be characteristic of all affected individuals

• it is also likely that the same causative allele may lead to variable phenotypes depending on genetic background


• genes involved are likely to represent a small increase in risk and are likely to be modified by other genes and the environment

• the '3-hit' hypothesis proposes that the interaction between genetics, early and late environmental factors leads to disease development

Schizophrenia

- genetic factors – top candidates
  - VMAT
  - NR2B subunit of NMDA receptor
  - β2 subunit of GABA-A receptor
  - D2 receptor
- environmental factors
  - maternal infections
  - malnutrition
  - marijuana use during early adolescence
  - stress
- developmental abnormalities
  - molecular alterations
    - DA system
    - GABAergic system
    - glutamategic system
- anatomical alterations
- functional alterations

What does all this mean and can we make sense of it?

It is important to remember that association does not establish causation.
Approaches to Identify Schizophrenia Susceptibility Genes

- twin studies demonstrate a genetic component representing > 50% risk
- genetic linkage studies
  - assume a single gene or gene region within a family is causative and apply techniques of genetic analysis to identify the genes
  - makes no assumptions about underlying neurobiology of the disease and assumes that a single effect can be identifiable in a complex genetic background
- candidate gene analysis using whole genome association studies
  - assumes that certain genes are involved
  - searches families for possible alterations in those genes

Schizophrenia Susceptibility Genes

- **COMT**
  - SNP in exon 4, a G-A substitution, leading to a val-met change (val159met)
  - val-COMT has lower enzyme activity than met-COMT
  - surprisingly, schizophrenia was associated more with val than met allele
  - in animals, KOs or COMT inhibitors led to increased DA in PFC & not striatum
  - evidence indicates that DA reuptake in PFC is in very low abundance & does not play a role in DA inactivatio

- Dysbindin (**DTNBP1**)
  - 17 SNPs identified
  - functions as a chaperone protein (trafficking & tethering) for several receptors & signal transduction proteins; expression is decreased in schizophrenic hippocampus
• Neuregulin 1 (NRG1)
  • 1200 SNPs identified
  • NRG1<sup>+/−</sup> heterozygote mice are hyperactive in novel environment and have abnormal PPI, the former ameliorated by clozapine
  • NRG1 signaling involved in both neuronal & glial function including development, neurotransmission and synaptic plasticity

• Regulator of G-protein signaling 4 (RGS4)
  • 13 SNPs identified
  • expression decreased in schizophrenic brain
  • RGS4 is a GTPase activator that desensitizes Gi/o and Gq, thereby negatively regulating some DA, Glu metabotropic and muscarinic receptors; RGS4 is also involved in neuronal differentiation and is under DA regulation

• Disrupted-in-schizophrenia 1 (DISC1)
  • first reported by Millar in 2000 in Scottish family in which >50% have suffered from schizophrenia or other forms of mental illness; translocations involving chromosomes 1 and 11
  • expressed in many tissues, but particularly in hippocampus and cerebral cortex
  • gene needed for normal brain development
  • blocking its function produces subtle abnormalities in structure resembling schizophrenia

DISC1 is a scaffolding protein, particularly affecting the cAMP and dynein-NUDEL signaling pathways.

- DISC1 binds to PDE4B, a phosphodiesterase that hydrolyzes cAMP, and inhibits PDE4B activity.
- When cAMP increases, it causes DISC1 and PDE4B to dissociate, resulting in increased PDE4B activity and increased dynein signaling pathway.
- DISC1 is critical for complexing with NUDEL and the MAP dynein at the centrosome.
- Dynein signaling regulates growth and development of the cerebral cortex.
- Decreased DISC1 alters neuronal migration and dendritic arborization in the developing cerebral cortex.

---

**Signaling Pathways in Psychiatric Disorders**

1. Signaling pathways are modified by hormones.
2. Signaling pathways underlie neuronal communication.
3. Psychiatric drugs have both short- and long-term effects.
4. Complex signaling networks underlie higher order brain function.
Signal Transduction Deficiency Hypothesis of Depression

- chronic antidepressants activate CREB which determines transcriptional activity
- P-CREB is down-regulated in fibroblasts from patients with major depression and in postmortem brain from suicide victims with history of major depression

Dendritic Branching in Hippocampus

a) normally innervated pyramidal neuron
b) stress leads to increased glucocorticoids which affect normal transcriptional mechanisms (via CREB) and decrease BDNF expression; stress also decreases dendritic arborization
c) antidepressants activate CREB, reversing effects of glucocorticoids, resulting in increased BDNF and increased arborization

Where do we go from here?

Integrative/Convergent Functional Genomics

• Why is it taking so long to unravel pharmacogenomics?
  • psychiatric disorders are polygenic with variable penetrance
  • phenotypic heterogeneity is not taken into account
  • gene-environment interactions & effects of environmental factors on phenotypic expression are not taken into account

• Convergent functional genomics (CFG)
  • appreciates both the strengths & limitations of animal model and human data
  • integrates multiple independent data from animal models with that from human genetic and tissue expression studies

CFG Approach Applied to Bipolar Disorder

- Methamphetamine model - in both humans and rodents
  - manic-like features in the activation phase
  - depressive-like features in the withdrawal phase
  - with higher doses and administered chronically, psychotic-like symptoms emerge
In Sum

- 2009 represents the most exciting time to use advances in knowledge and technology to maximize the therapeutic efficacy and minimize the side effects and adverse events associated with drug use for psychiatric as well as other disorders.

- Pharmacogenomics represents a truly translational approach to medicine and requires clinicians and basic scientists to work together and integrate findings from the clinic with those from the laboratory for the betterment of humankind.