Clinical Pharmacogenomics

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Outline

Germline Genomics

Candidate Gene Pharmacogenomics

- Drug Absorption
- Elimination
- Effect

Pathway Pharmacogenomics

Genome Wide Studies

Ten Drugs and Their Available Pharmacogenetic Tests December 2008

- Abacavir	HLA-B*5701
- Imatinib	BCR-ABL
- 5-Fluorouracil	DPYD-TYMS
- Clozapine	2 SNPs in HLA-DQB1
- QT-prolonging Drugs	Familion™
- Irinotecan	UGTIA1
- Azathioprine and Mercaptopurine	TPMT
- Warfarin	CYP2C9 and VKCoR
- Carbamazepine	HLA-B*1502

The Genomic Revolution

Photo of a cover of Nature magazine showing the title of an article in the magazine called The Human Genome.

Why Genomics?

The Genome Map is available on the web, to anyone, free.

The Human Hapmap is available on the web to anyone, free.

DNA is very stable

DNA can be amplified

Human Migration out of Africa

A map of the world is shown from Scientific American, July 2008 indicating dates and routes of migration out of Africa.

Microsatellites and Copy Number Variants

Diagram illustrating concepts of microsatellites, deletions, and duplications in the DNA sequence.

SNP Variability in The Human Genome
July 2008

- 2.85 billion base pairs
- ~22,000 genes
- 1.7% of the genome codes for protein
- 3.3% of the genome is as conserved as the 1.7% that codes for protein
- On average 1 SNP/1.2kb
- 10 - 15 million SNPs that occur at > 1% frequency
- ~450,000 SNPs in MCS (Multiply Conserved Regions)
- Copy number variations exist in 5-7.5% of the germline genome
- Most tumor DNA sequence is identical to that of the host
- **4-5% of the genome is in areas with high copy number variation**

SNP Variability In Exons

- ~150,000 SNPs in known exons
- 48,451 non-synonymous SNPs
- 1113 introduce a stop codon
- 104 disrupt an existing STOP

PharmGKB as a source of Candidate Genes and Pathways

Illustration of this computerized database.

PharmGKB Irinotecan Pathway

Illustration of this pathway.

UGT1A1 TA repeat genotype alters irinotecan neutropenia/activity

Graphic illustration

McLeod H. et al, 2003

Pharmacogenomic Journals, 2008

Shows photos of covers of issues of the following scientific journals:

Pharmacogenetics

Clinical Pharmacology & Therapeutics

The Pharmacogenomics Journal

American Journal of PharmacoGenomics

Pharmacogenomics

Hierarchy of Pharmacogenetic Information from Single Nucleotide Polymorphisms (SNPs)

SNPs that change clinical outcome (narrow top of pyramid)

SNPs that change drug response

SNPs that change pharmacokinetics

SNPs that change activity in vitro

Non-conservative amino acid changes

Non-synonymous SNPs in exons (broad base of pyramid)

Pharmacogenetic Principle 1: Value Decreases when Current Predictive Ability is High

Clinical value of a pharmacogenetic test is high for cancer chemotherapy drugs and low for beta-receptor blockers.

Meyer UA and Flockhart DA, 2005

Methods in Pharmacogenetics

There is a drawing of a down arrow along the left side of the following list:

SNP discovery:
- Candidate gene approach
- Pathway approach
- Genome Wide Arrays
- Next Generation Sequencing

Identification of gene and variants
Development of a genetic test for DNA variants
Correlation between genotype and phenotype
Validation
Application in Clinical Practice

Polymorphic Distribution

Chart showing population frequency and activity (Phenotype) and antimode.

The Value of Normit Distribution Plots: (an example is shown)

Population Distribution of CYP2C19 phenotype (an example is shown)

Flockhart et al: Clin Pharmacol Ther
1995;57:662-669

Skewed Distribution

A chart is shown indicating the population frequency and activity (Phenotype).

Example 1 of a Skewed Distribution: Heterogeneity in response to Inhaled Corticosteroids

A bar chart is shown indicating the Patients, %, and the % change in FEV, from baseline for the adult study, CAMP and ACRN.

Weiss ST et al. Hum Molec Genetics 2004; 13:1353-11359

Lessons

Germline genetic variation is a potentially valuable biomarket for many drug effects

Extremes of phenotype are often viewed as “discardable data”, but outliers (patients or events) should be viewed as important research stimuli

Drug effects on populations can obscure effects on individual patients. A significant proportion of people may be harmed by a beneficial drug.

Genetics and Drug Absorption

0.25 mg of digoxin po at steady state

Digoxin Cmax is higher in subjects with the TT genotype compared to the CC genotype for p-glycoprotein.

Eichelbaum et al, Proc Nat Acad Sci, 2000:March

Digoxin Transport across the Blood-Brain Barrier

Role of p-glycoprotein transport and passive diffusion illustrated schematically.

Genetics and Drug Elimination

Cytochrome P450 2D6

- Absent in 7% of Caucasians
- Hyperactive in up to 30% of East Africans
- Catalyzes primary metabolism of:
  - propafenone
  - codeine
  - β-blockers
  - tricyclic antidepressants

- Inhibited by:
  - fluoxetine
  - haloperidol
  - paroxetine
  - quinidine

CYP2D6 Pharmacogenetics

Frequency histogram for Debrisoquine/4-Hydroxydebrisoquine metabolic ratio in UMs, EMs, and PMs.

CYP2D6 Alleles

- 69 as of December, 2008

- 24 alleles have no activity

- 6 have decreased activity

- *1, *2, *4 and many others have copy number polymorphisms

- The *2 variant can have 1,2,3,4,5 or 13 copies i.e increased activity

Oligonucleotide array for cytochrome P450 genotesting

Microchip illustration

From: Flockhart DA and Webb DJ. *Lancet* End of Year Review for Clinical Pharmacology, 1998.

Paroxetine and CYP2D6 genotype change the plasma concentrations of endoxifen

Graphic illustration

Flockhart et al. 2003

CYP2D6 variant genotype and CYP2D6 inhibitors lower [Endoxifen]

Chart of variable plasma endoxifen levels as a function of CYP 2D6 genotype groups.

Methods

225 Charts were reviewed at each randomizing site to ascertain medication history

- Potent CYP2D6 inhibitors: Fluoxetine and paroxetine
- Moderate CYP2D6 inhibitors: Sertraline, cimetidine, amiodarone, doxepin, ticlopidine, or haloperidol
- Duration of coadministration: <1, 1-2, 2-3, 3-4 and 4-5 years

Statistics: Log rank test and Cox modeling

Lessons from CYP Pharmacogenetics

- Multiple genetic tests of one gene may be needed to accurately predict phenotype

- Gene duplication in the germline exists

- The environment in the form of Drug Interactions can mimick a genetic change

Bar charts showing Primary Cohort and Replication Cohort

***VKORC1* Haplotype and CYP2C9 Genotype changed Warfarin Dose**

Primary cohort: UW (N=185);
Replication cohort: Wash U (N=368).

All participants were Caucasian.

Rieder et al. N. Eng J. Med 2005;352: 2285-2293[

Phase II matters too: UGT1A1 TA repeat genotype alters irinotecan neutropenia/activity

Two bar charts are shown with the UGT1A1 genotype.

McLeod H. et al, 2003.

Thiopurine Methyl Transferase

Homozygous mutants are 0.2% of Caucasian Populations

Heterozygotes are ~ 10%

Homozygous wild type is 90%

- Metabolism of Azathioprine
- 6-Mercaptopurine

Thiopurine Methyl Transferase Deficiency

Frequency histogram for TPMT activity in 298 unrelated adults.

From: Weinshilboum et al. JPET;222:174-81. 1982

Examples of Human Receptors shown to be genetically polymorphic with *possible* alterations in clinical phenotype

G-proteins

Angiotensin II receptor and angiotensinogen

Angiotensin converting enzyme

Alpha-2 receptor

Dopamine D_4 receptor

Endothelial NO synthase

$5HT_4$receptor

2SNPs: 10 possible hapoltypes

Haplotypes

Diplotypes

Ying-Hong Wang PhD.
Indiana University School of Medicine

Observed β_1AR Haplotypes in Caucasians and African American Women (WISE study)

Frequency table

Terra et al. *Clin. Pharmacol. Ther*. 71:70 (2002)

Of 10 theoretical diplotypes, only 4 were present in the study population

Chart of haplotypes and diplotypes.

Ying-Hong Wang PhD, Indiana University School of Medicine

Diplotype predicts Beta-blocker effect

Graph of change in diastolic blood pressure as a function of diplotype.

Johnson et al. Clin Pharmacol & Ther. 2003,74:44-52.

Lesson: Diplotype *may* be a better predictor of effect than Genotype

A SNP that tags a Haplotype (tagSNP) may be an economical means of screening

Non-synonymous coding region polymorphisms in long QT disease genes

Illustration

Dan Roden MD, October 2003.

Pharmacogenetic approach to angiogenesis biomarker discovery

Essential Ingredients:

1).Genetic variability must have potential for biologic impact
2).Genetic variability must exist in drug disposition or destination
    -metabolizing enzymes/transporters/targets
3).Drug evaluated must be heterogeneous in outcome
    -mix of success and toxicity
4).Variability must be frequent
    -generalizability of results

Walgren et al. *JCO* 2005;23:7342-7349

Bevacizumab in breast cancer-**E2100:** a model of therapeutic heterogeneity

Stratify:

- DFI $\leq$ 24 mos. vs. > 24 mos.
- < 3 vs. $\geq$ 3 metastatic sites
- Adjuvant chemotherapy yes vs. no
- ER+ vs. ER- vs. ER unknown

Illustration

Bevacizumab increased grade 3/4 toxicity

Chart

Bevacizumab significantly improved PFS

HR = 0.60
Log Rank Test $p<0.001$

ORR
(measurable disease)
49.2% vs. 25.2%
P<0.001

Miller et al. **NEJM** 357:2666; 2007

Improvement in PFS/ORR did not translate into OS benefit

Chart of progression-free survival.

ORR
(measurable disease)
49.2% vs. 25.2%
P<0.001

Miller et al. **NEJM** 357:2666;2007

Detailed chart from Miller et al. **NEJM** 357:2666; 2007 with the following sentence across it.

We need something better!

VEGF -2578 AA & -1154 AA genotypes in combination arm outperformed control

Two graphs

The following was shown beneath the first graph.

Median OS

Control arm=25.2 mo
Combination arm=26.7 mo
Combination arm AA=37.0 mo

The following was shown beneath the second graph.

Median OS

Control arm=25.2 mo
Combination arm=26.7 mo
Combination arm AA=46.5 mo

Hierarchy of Pharmacogenetic Information from Single Nucleotide Polymorphisms (SNPs)

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Genome Wide SNP Arrays

Affymetrix 6.0 Gen Chip Arrays
- 906,000 SNPs
- 1.8 million genetic markers
- 946,000 copy number probes

Illumina Infinium Bead Chips

Genome-wide association study identifies novel breast cancer susceptibility loci

Nature May 27th, 2007

Chart

A snapshot of a webpage from Genome Research of an article published online before print November 22, 2006, 10.1101/gr.5629106

Methods

Genome-wide detection of human copy number variations using high-density DNA oligonucleotide arrays

By Daisuke Komura et al

Copy Number Variation screening:

"There is a decreased level of linkage disequilibrium between CNVs and SNPs, suggesting that SNPs are not an ideal surrogate for CNVs in association studies This implies that CNVs need to be assessed independently in whole-genome association studies. "

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An International Community of Genomic Analysts: http://dchip.forum5.com

Snapshot

Microarry Data Analysis Forums

Current Methods for PharmacogeneticTesting

By phenotype: metabolic probe drug or Western blot or Immunohistochemistry

By PCR with mutation-specific endonuclease

By PCR and allele-specific hybrization

By oligonucleotide chip hybridization

By laser lithography - guided oligonucleotide chip hybridization.

By rapid throughput pyrosequencing

Taqman probe screening

By genome wide SNP array

By rapid, robust and high throughput full sequencing

By including accurate quantitative tests of CNV.

Conclusions

Candidate gene pharmacogenetic testing is migrating beyond industry phase 1 trials into clinical practice

Multiple candidate gene /pathway testing has begun with warfarin

No germline genome wide patterns predictive of drug effect have yet become clinically useful

- Stay tuned!

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- Azathioprine and Mercaptopurine	TPMT
- Warfarin	CYP2C9 and VKCoR
- Carbamazepine	HLA-B*1502

Pharmacogenetics Websites

- www.pharmgkb.org
- The SNP consortium: http://brie2.cshl.org
- The Human Genome:
- www.ncbi.nlm.nih.gov/genome/guide/H_sapiens.html
- CYP alleles: www.imm.ki.se/CYPalleles/
- Drug Interactions: www.drug-interactions.com