THEME

SAFER DRUGS TO MARKET

BY ANALYSING

LATEST DEVELOPMENTS

IN

- Pharmaco vigilance
- Drug Safety
- Risk Management

RECENT TRENDS IN CLINICAL TRIAL INCLUDING PHARMACOGENOMICS APPROACHES FOR DRUG DEVELOPMENT AND RATIONAL THERAPY

(DRUG SAFETY)

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DRUG DEVELOPMENT PROCESS

- Pre clinical study : In Animals IND
- Clinical study : In Human Beings

  Phase I (Safety)

  Phase II (Efficacy and safety)

  Phase III (Confirmatory)

  ……. Marketing approval

  (controlled study)

  Phase IV In Population

- Rare adverse drug reaction

- Chronic toxicity
- Use in special groups (Pregnant women, Children, Elderly)
- Drug interaction

PHARACO VIGILANCE

- Post marketing tool
- Process of
  - Monitoring medicines used in every day practice to identify unrecognised adverse effects
  - Assessing risks and benefits
  - Providing information's to users for safe and effective use

Pharmaco epidemiology safety study

- Observational study designed
- to assess the risk associated with drug exposure

international society for pharmaco

ADVERSE DRUG REACTIONS

Dose Dependent

types A, C & E

- **Augumented:**

  Predictable, known pharmacology (A)

- **Chronic:**

  Long Time Therapy (C)

- **End of use:**

  Due to stopping of Drug (E)

Dose independent

types B, D & F

Bizarre:

- Unpredictable/ Idio syncratic reaction, immune reaction (Anaphylaxis), toxicity from reactive metabolite, unknown pharmacology (B)

Delayed

- **Carcinogenicity/ Terato genicity,** occur after several years (D)

Failure

- Unexpected failure of treatment (F)

GENOME:

- Complete set of genes (A basic functional unit located on chromosome made up of DNA which control cell function and reproduction) in the chromosomes which give instruction for growth, development and function.
- Human Genome consists of 3.2 billion base pairs in 24 chromosomes.

DRUG TARGET AND PHARMACOGENOMICS:

- ✓ **In drug discovery: potential target for drugs identified which may be**

A. **Enzyme in pathway**
B. **Receptor**
C. **Transporter**
D. **Protein in signal transduction (transfer of genetic material)**
E. **Protein production in pathological condition (structural and functional changes because of diseases)**

AFTER HUMAN GENOMES ARE SEQUENCED

- No of drug targets: ~8000
- (2329 for antibodies and 794 for drug proteins)
- Based on ligand binding studty,399 molecular targets belonging to 130 protein families identified
- Targets show wide variations (polymorphism) so different activity
- It leads to inconsistent clinical result
- In most cases, variation in drug response is due to more than one gene mutation. This can provide new targets to evaluvate

GENETIC VARIATION

- Sequence Differences in the Same Gene Are Called Alleles
- Sequence Differences Can Be in Coding Regions, Regulatory Regions (i.e., Promoters, Enhancers), Introns, at 5' or 3' Flanking Regions, etc
- If Alleles Are Present in **>1%** of Heterozygous Individuals in a Given Population, the Variation Is Called a **Polymorphism**
- If Alleles Are Present in **<1%** of Heterozygous Individuals in a Given Population, the Variations Are Called **Sequence Variants**

GENETIC FACTORS AND DRUG EFFECTS:

Variation in genetic coding result in polymorphism of

a)Metabolizing enzyme

b)Receptors

c)Tronsportors

d)Combination of Alleles (One or more Variations of genes in chromosomes)

- ✓ **which affect drug response**
- ✓ **Eg: CYP2C9 is a metabolizing enzyme**
- ✓ **(GENE is CYP2C9& alleles are +2 and +3**

Normal, +2 and +3 has 12% and 8% activity of enzyme

Eg for (b):

- **Beta-2 adrenargic receptor shows polymorphism**

  One polymorph show decreased receptor activity than another which shows increased activity to salbutamol in asthma

- **Eg for (c) Serotonin transporter gene has two forms**

- ✓ **Short form (ss)**
- ✓ **Long form (ll)**

Short form show decreased activity with citalopram in children with depression

- **Eg for (d): 5 HTTLPR-s allele with CYP2C9*3 allele has massive depressive disorder.**

- ✓ These genetic variation can cause toxicity or failure in clinical trial
- ✓ Therefore patients are to be grouped by genotyping methods
  - ❖ Amplifluor
  - ❖ TaqMan methods
  - ❖ Haplotype mapping more than one genetic variation in chromosomes are studied to find out genetic basis of diseases

Cont…

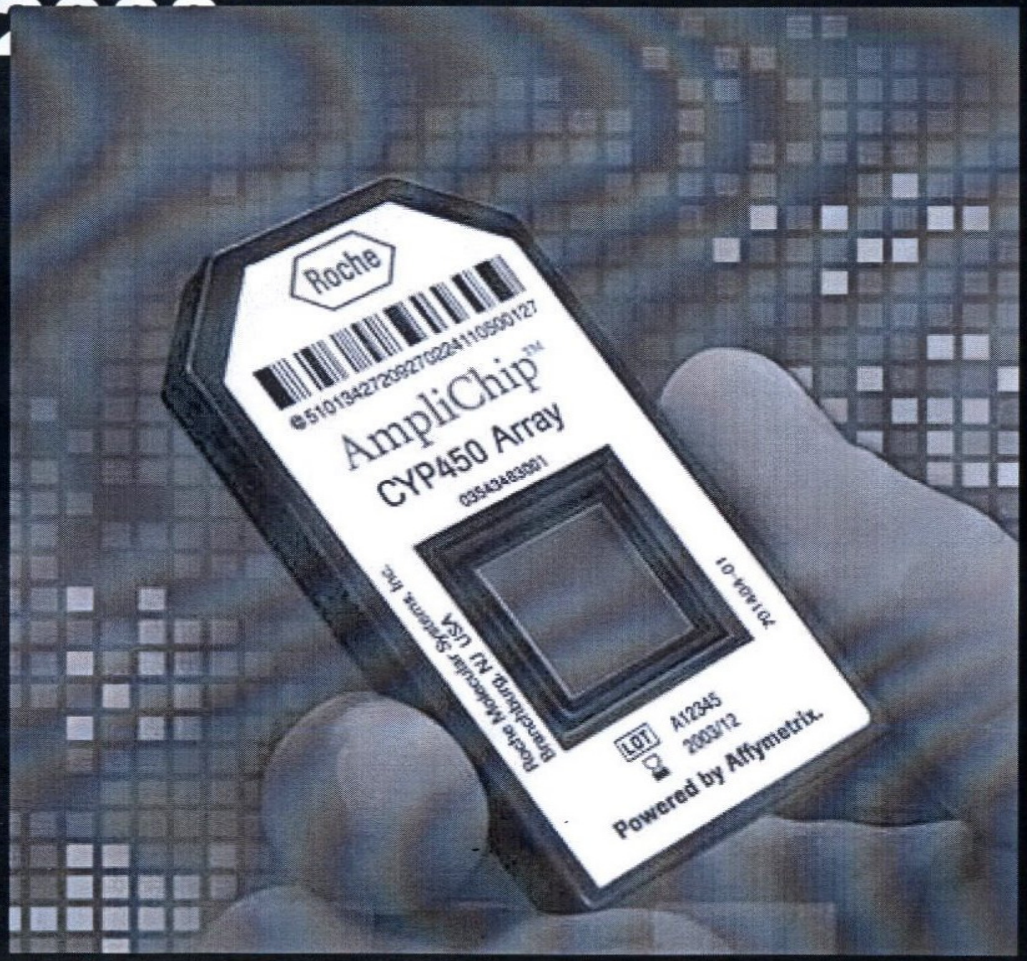
CLINICALLY relevant effects are documented in metabolism

Eg: Cytochrome P450 family (CYP) polymorphism.

CYP2D6 and CYP2C19 are valid biomarkers. FDA APPROVED Roche's Ampliclip to measure polymorphism of two enzymes.

AMPLICHIP CYP450 ARRAY –

17 With a Drop of Blood or Smear From Inside of Patient's Cheek, the Gene Chip Checks for 31 Variations in Two Genes (CYP2D6 and CYP2C19).



GENETIC BIOMARKERS:

BIOMARKER:

- ✓ **It is a characteristic measured, evaluated as an indicator of biological process, pathogenic process or pharmacological response to a therapeutic intervention**
- ✓ **Eg: Blood pressure, blood cholesterol level (linked with disease like heart attack)**

GENETIC BIOMARKERS:

- ✓ **It related to drug response**
- ✓ **Eg. (a)CYP enzymes, (b) certain genes of Her-2 gene in somatic cancer cells**

ICH DEFINITION:

PHARMACOGENETICS:

- ✓ **Influence of variation in DNA sequence on drug response (subset of pharma - cogenomics)**
- ✓ **Use of generic techniques in the study of drug ADME,response and pharmacological action.**

ICH DEFINITION:

PHARMACOGENOMICS:

- ✓ **Investigation of DNA and RNA characteristics related to drug response.**
- ✓ **Whole genome application of Pharmacogenetics.**

GENOTYPING Vs PHENOTYPING:

- **GENOTYPE: Is all or part of genetic make up of organism. It is in DNA molecule and inherited.**

  eg: Amplifluor, TaqMan method

- **PHENOTYPE: Is observable characteristic of organism produced by the interaction of genotype and the environment**
- **Biochemical and functional analycsis.**

GENETIC TESTING: DONE TO PREDICT

- ✓ Suspected or Risk of developing diseases

PHARMACOGENOMIC TESTING:

- ✓ It is used after the disease occurs
- ✓ Predict likely drug response

GENOTYPING METHODS: IDENTIFIES INDIVIDUAL DNA STRUCTURE

- ✓ It is easy, small sample of peripheral blood or buccal swab from patients less invasive

METHODS:

- ✓ Polymerase chain reaction (PCR)
- ✓ Allele specific PCR
- ✓ Fluorescent Dye based high throughput techniques
- ✓ Mass spectrometry, gene chip technology
- ✓ TaqMan allele assay (High Throughout Genotyping Method)

PHENOTYPE METHODS:

- Phenotype method:Observable characteristic of cell or organism through biochemical or functional analyses
- Drug are given to individuals
- Parent drug and metabolites are measured in urine, blood and other tissues.
- Metabolic phenotyping is influenced by age, nutrional, general health and concomitant medication
- It is invasive and time consuming

PHARMACOGENOMIC AND CLINICAL TRIALS:

- With Invitro methods, if the drug is metabolized by polymorphic enzyme, a decision can be made about continuation of clinical study
- These will help in selecting patiends with normal metabolizing enzymes; it will also help to prevent adverse effect
- Pharmacogenetic principles can be used for inclusion or exclusion criteria when metabolic path way known in clinical trial

PHARMACOGENOMICS IN PHASE-I

- **PHARMACOKINETIC STUDIES**

Pharmacokinetic includes ADME

Pharmacokinetic(PK) of drug is influenced by:

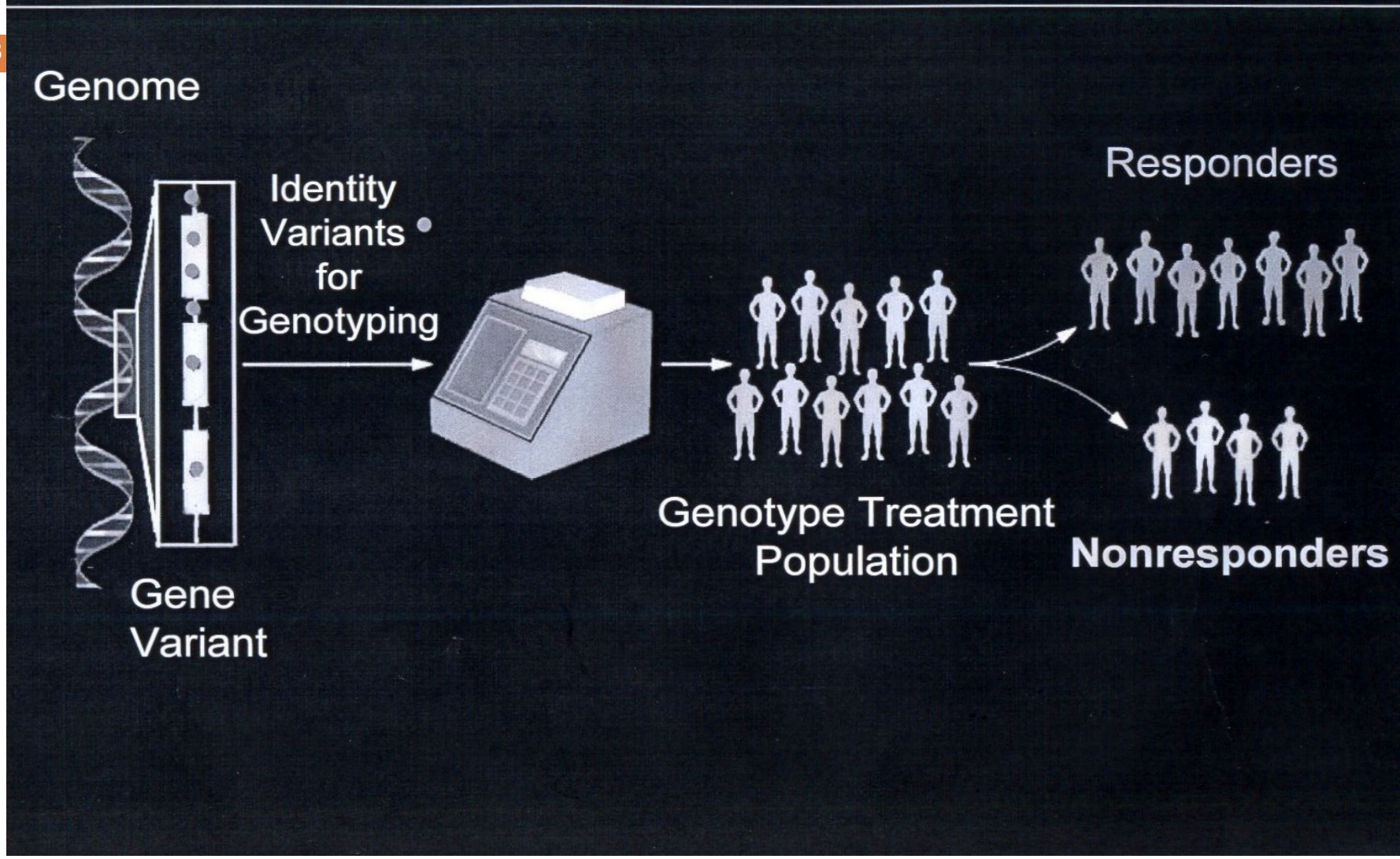
Intrinsic factor: Age, body weight, genetic make up etc

extrinsic factor: food interaction, tobacco, alcohol etc

Cont…

- **Phase-I study to be done with genetically preselected subjects.**
- **Genotyping is encouraged in phase-2 & 3 clinical study(genetic subpopulations))**
- **If significant differences occur between genetically defined subpopulations, this will have implications on different dose recommendation and labeling.**
- **For phenotype / genotype, suitable warnings/ contraindications are appropriate in the label**

IDENTIFICATION OF PHARMACOGENETIC VARIANTS



DRUG METABOLISING STUDIES:

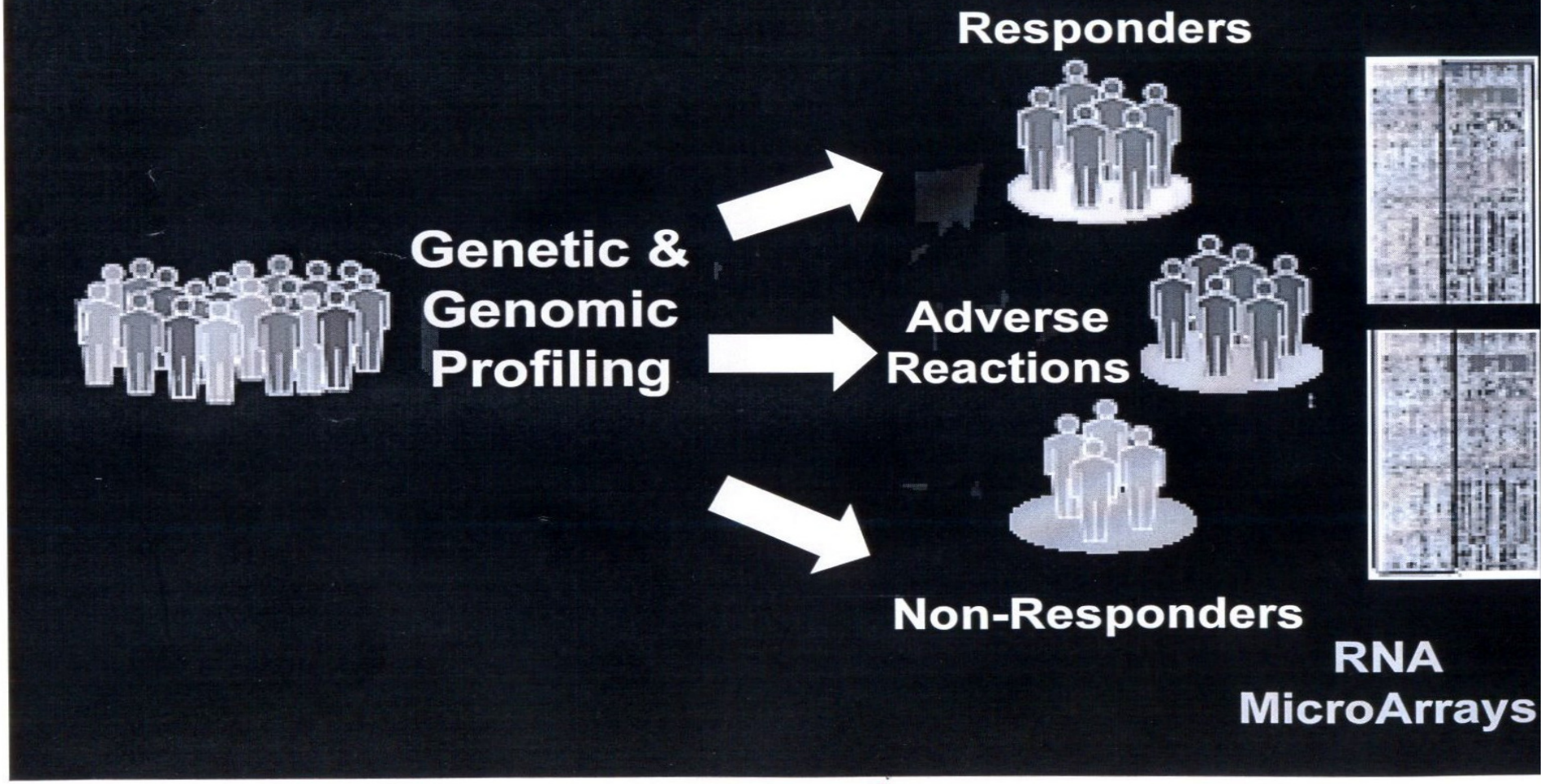
- If "Invitro screening" show drug is metabolized by one system, then invivo studies to be done with genotype/phenotyped subjects in phase-I trail

- Characterization of sub groups show different exposure to the drug and show different safety profile. When involvement of polymorphic genes has been verified "invivo studies" of effects of polymorphs on pharmacokinetics of drugs contribute to safety/efficacy/adverse events

PHARMACOGENOMICS IN PHASE-2/3 EFFICACY AND SAFETY STUDIES:

- In phase-1 pharmacokinetic programmes, it is necessary to investigate pharmacogenomic/pharmacokinetic relationship in patients and link this data with safety/efficacy results
- Where genetic factors are known or suspected, it is necessary to collect pharmacogenomic data in phase2/3 patient study.
- For selection, type of disease, level of genetic involvement in primary/secondary pharmacodynamics, availability of validated biomarkers, pharmacogenomic data also used
- Cancer cells has genetic defects. The genetic difference and pharmacogenomics are used in clinical development
- Primary = responder / non responder
- secondary= patient at the risk of adverse reaction

REFINING THERAPEUTIC DECISIONS & PREDICTING DRUG EFFICACY



If a drug is inactivated by metabolism, slow metabolism will increase exposure

DRUG	SLOW METABOLISER	FAST METABOLISER
Active drug (eg. Warfarin)	Good efficacy Accumulation of active drug can produce adverse reaction Lower dose may be needed	Poor efficacy Greater dose or slow release formulation may be needed
Prodrug (Eg. Codeine)	Poor efficacy Possible accumulation of prodrug	Good efficacy Rapid effect

METABOLISING ENZYME GIVE RISE TO FOUR PHENOTYPES

	Genotype	Phenotype
Poor metaboliser	No functional allele	Lack of functional enzyme: metabolism using different route
Intermediate metaboliser	One functional allele and one deficient allele or Two partly deficient alleles	Metabolism may be reduced
Extensive metaboliser	Two functional alleles	"Normal" metaboliser
Ultra- rapid metaboliser	Duplicated allele or multiduplicated functional alleles	Extremely high metabolic capacity

Allele: One or more variation of gene in chromosomes

PHARMACOGENOMICS: Correlates genotype and phenotype relevant to drug response

	Pharmacokinetics	Primary Pharmacodynamics	Secondary Pharmacodynamics
typical phenotype effects	Plasma levels	Efficacy	safety
Examples of genotype variables	Metabolizing enzymes (e.g, CYPs) Transporters (e.g, pGP, OATP)	Drug targets (eg. Receptors for growth factors)	Immune response systems (eg. HLA-B*5701) Channels (eg. HERG)

Genotype can be elucidated by studying phenotype using

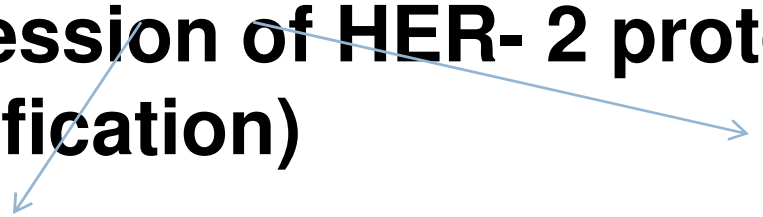
PHARMACOGENOMICS

- Pharmacogenomics Refers to the Investigation of Genetic Variants to Identify Those Involved in Effecting Drug Metabolism
  - Use Genomic Approaches to Identify Genes & Variants Involved in Drug Metabolism
    - Genome –Wide Association Studies
    - RNA Expression Arrays
  - Correlating Gene Expression or Single-Nucleotide Polymorphisms with a Drug's Efficacy or Toxicity

- Pharmacogenomics Is the Whole Genome Application of Pharmcogenetics

PREDICTION OF EFFICACY OF DRUG

- **Drugs designed with pharmacogenomic support have predetermind efficacy eg:Trastuzumab,monoclonal antibody against metastatic breast cancer.**
- **It is effective only in women so genetic over expression of HER- 2 protein (It is called genetic stratification)**



Clinical study those women

For therapy identify

POLYMORPHIC CYTOCHROME P450 GENES INVOLVED IN DRUG METABOLISM

Family	Gene	Alleles of Functional Significance	Drugs Metabolized (Selected)
CYP2	CYP2C19	↓ & 0 Activity Alleles	Antieplieptics, Antidepressants Antianxiety Drugs
CYP1	CYP1A2	↑,↓ & 0 Activity Alleles	Acetaminophen Antifungals Cocaine Codeine Cyclosporine A Daizepam Erythromycin Cholesterol-Lowering Statins Taxol Warfarin

POLYMORPHIC CYTOCHROME P450 GENES INVOLVED IN DRUG METABOLISM

Family	Gene	Alleles of Functional Significance	Drugs Metabolized (Selected)
CYP3	CYP3A4	↑ &↓ Activity Alleles	Caffeine Propranolol
CYP2	CYP2C9	↑,↓ & 0 Activity Alleles	Angiotensin II Receptor Blockers Nonsteroidal Anti- inflammatory Metronidazole Oral Hypoglycemics Warfarin
	CYP2D6	↑,↓ & 0 Activity Alleles	Antiarrhythmics Antidepressants Antipsychotics Beta-adrenergic blockers Narcotic Analygesics

SOME DRUGS WHOSE METABOLISM IS AFFECTED BY CYP2D6 VARIANTS

- Antiarrhythmics
- β-Adrenergic Receptor Blockers
- Neuroleptics
- Tricyclic Antidepressants
- Decongestants (Pseudoepherine)
- Importance: Drug Selection & Dose

PHARMACOGENETIC BIOMARKERS AS PREDICTORS OF ADVERSE DRUG REACTIONS*

Gene or Allele	Relevant Drug (Disease)	Specificity	% of Pts with an Adverse Drug Reaction**
TMPT (Mutant)	6-Mercaptopurines (Cancer)	Very Good	1-10
UGT1A*28	Irontecan Colorectal Cancer)	Good	30-40
(CYP2C9 & VKORC1	Warfarin (Anticoagulant)	Good	5-40
CYP2D6 (Mutant)	Tricyclic Antidepressants	Relatively Good	5-7
HLA-B*5701	Abacavir (HIV)	Very Good	5-8
HLA-B* 1502	Carbamazepine (Biopolar /Epilepsy)	Very Good	10 (in Asians)
HLA-DRB1*07 & DQA1*02	Ximelagatran (Anticoagulant)	Good	5-7

* Ingelman-Sundberg, NEJM 358,637,2008; * *Affected Caucasians Except Where Note

EFFICACY OF VARIOUS DRUGS

Class of Drug	% Refractory or Insufficient Response
Selective Serotonin Reuptake Inhibitors (Depression)	10-25
ACE Inhibitors (Hypertension, Proteinuria)	10-30
Beta Blockers (Cardiac)	15-25
Tricyclic Anti-Depressants (Depression)	20-50
HMG-CoA Reductase Inhibitors (Statins)	30-70
Beta 2-Agonists (Bronchodilators)	40-70

FREQUENCY OF SLOW NAT2 ALLELES IN DIFFERENT ETHNIC POPULATIONS

Population	Frequency (%)
Eskimo	23
South Pacific Islanders	35
Korean/Chinese/Japanese	37
North & South American Indians	50
African	71
Central & Western Asian	74
European	75
Egyptian	96

PHARMACOGENETICS: EXAMPLES OF ALTERED DRUG RESPONSE

Enzyme/Disease	Gene
• Glucose-6-PhosphateDehydrogenase Deficiency (G6PD Def)	G6PD
• ***N*-Acetylation & Tuberculosis**	**NAT2**
• Cytochrome P450 Enzyme – Drug Metabolism	CYP2D6
• Warfarin & Coagulation	CYP2C9 VKORC1
• Thiopurine S-Methyltransferase & Cancer	TPMT
• ACE Inhibitors, Antidepressants, Diabetes, Asthma, etc	

SOME AGENTS THAT CAUSE HEMOLYSIS IN G-6-P D DEFICIENT INDIVIDUALS

Acetanilid
Phenylhydrazine
Sulfanilamide
Sulfacetamide
Sulfapyridine
Sulfamethoxypyridazine (Kynex)
Salicylazosulfapyridine (Azulfidine)
Thiazosulfone
Diaminodiphenylsulfone
Trinitroluene

Nitrofurazone (Furacin)
Nitrofurantion (Furadantin)
Furazolidone
Furaltodone (Altofur)
Quinidine
Primaquine
Pamaquine
Pentaquine
Quinocide
Naphthalene

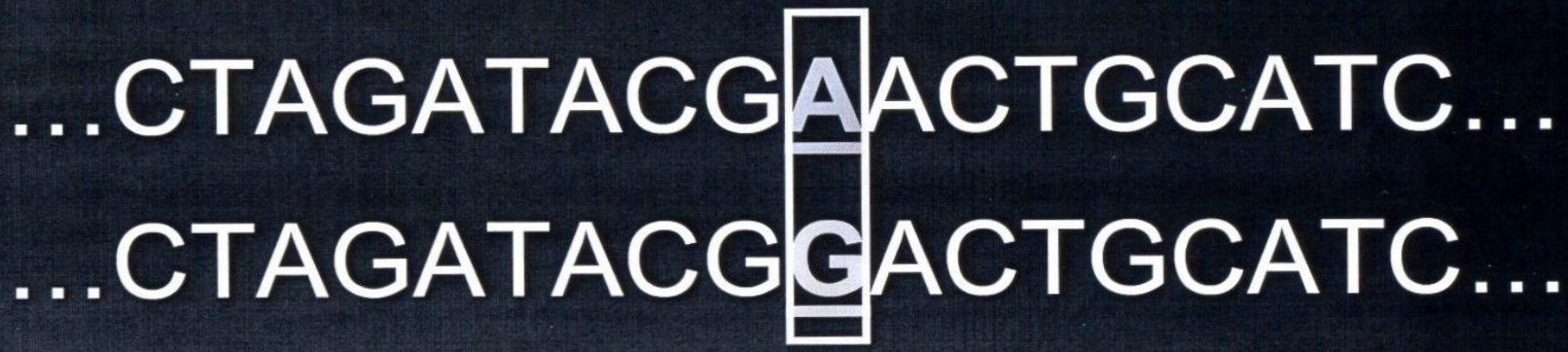
Neosalvarsan
Fava Beans

GENOME-WIDE ASSOCIATION STUDIES

- Age-Related Macular Degeneration
- Amyotrophic Lateral Sclerosis
- Asthma
- Atrial Fibrillation
- Bipolar Disorder
- Breast Cancer
- Celiac Disease
- Colorectal Cancer
- Congestive Heart Failure
- Coronary Artery Disease
- Crohns Disease
- Hypertension
- Late-Onset Alzheimer Disease
- Lung Cancer
- Memory
- Nicotine Dependence
- Obesity
- Parkinson Disease
- Psoriasis
- Prolonged QT Interval
- Prostate Cancer
- Rheumatoid Arthritis
- Restless Leg Syndrome
- Type 1 Diabetes
- Type 2 Diabetes
- Ulcerative Colitis

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)

A Single Nucleotide Polymorphism (SNP) Is a Nucleotide at a Specific Position in the Genome that Varies in Different Individuals

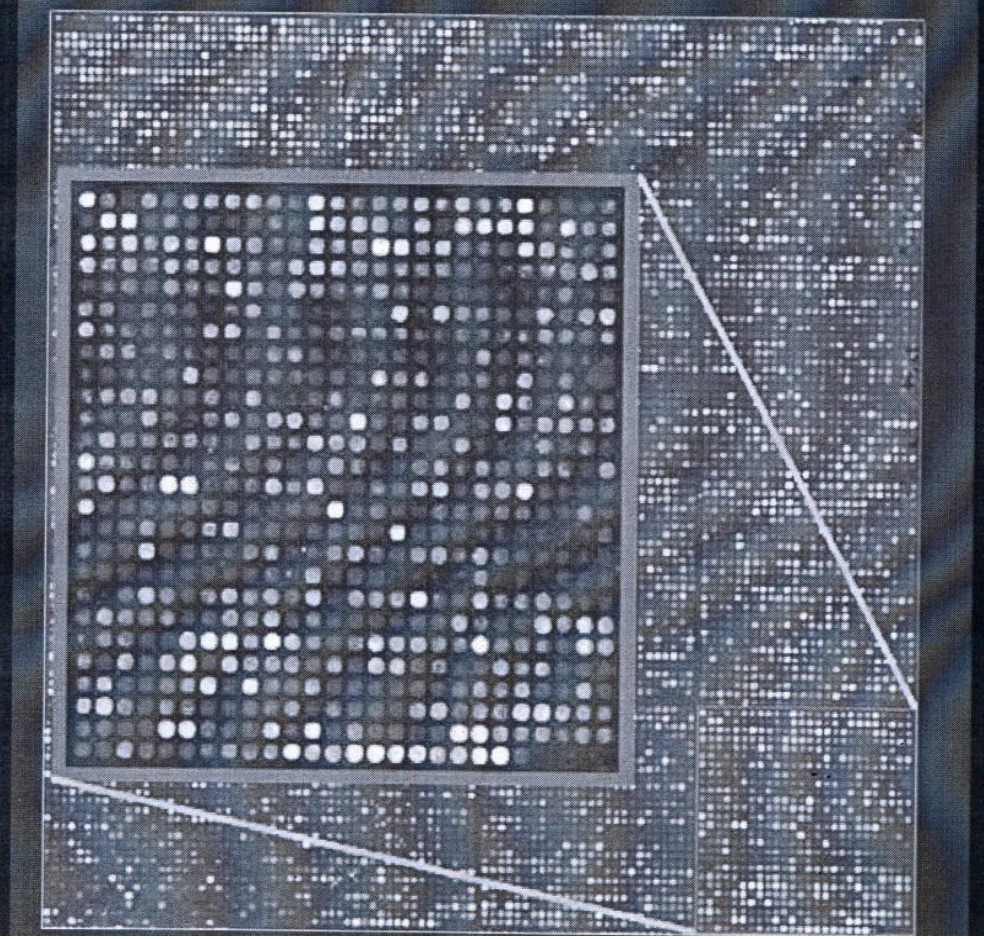


- Frequency: 1: 300 to 500 Nucleotides
- Widely Distributed Among All Chromosomes
- It is a Polymorphism when the Variant Occurs in at Least 1% of a Specific Population
- Useful for Linkage and Association Studies

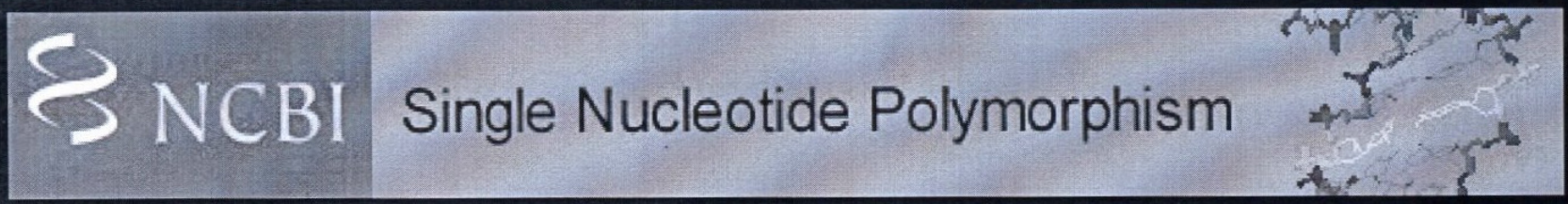
GENOME-WIDE ASSOCIATION STUDY

Case-Control Studies

DNA SNP MICROARRAY



SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)



http://www.ncbi.nlm.nih.gov/SNP/

31,000,000 SNPs in Database (2/08)

11,880,000 Reference SNPs

BENEFITS OF PHARMACOGENETICS

- **Improve Drug Choices:**
  - Each Year, ~100,000 Americans Die of Adverse Reactions to Medicine & ~2 Million Are Hospitalized
  - Pharmacogenomics Will Predict Who's Likely to Have a Negative or Positive Reaction to a Drug
- **Safer Dosing Options:**
  - Testing of Genomic Variation Improve Determination of Correct Dose for Each Individual
- **Improvement in Drug Development:**
  - Permit Pharmaceutical Companies to Determine in Which Populations New Drugs Will Be Effective
- **Decrease Health Care Costs:**
  - Reduce Number of Deaths & Hospitalizations Due to Adverse Drug Reactions
  - Reduce Purchase of Drugs Which Are Ineffective in Certain Individuals Due to Genetic Variations
  - Speed Up Clinical Trials for New Drugs

USEFUL RESOURCES AND DATABASES FOR GENETIC-BASED STUDIES

- Genetic Association Database: An archive of human genetic association studies of complex diseases. http://geneticassociationdb.nih.gov/
- Schizophrenia Gene Database: An archive of genetic association studies performed on schizophrenia phenotypes. http://www.schizophreniaforum.org/res/sczgene/default.asp
- Online Mendelian Inheritance in Man: A catalogue of human genes and genetic disorders. http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM&itool=toolbar
- Human Gene Mutation Database. A catalogue of published gene lesions responsible for human inherited disease. http://www.hgmd.cf.ac.uk/ac/index.php
- Human Genome Variation Database: A catalogue of normal human gene and genome variation. http://www.hgvbase.org/
- dbSNP: A catalogue of human single nucleotide polymorphisms. http://www.ncbi.nlm.nih.gov/projects/SNP/
- GeneSNPs: A database of polymorphisms in human genes that are thought to have a role in susceptibility to environmental exposure. http://www.genome.utah.edu/genesnps/
- PharmGKB: A database of pharmacogenomics research. http://www.pharmgkb.org/index.jsp
- GeneCards: A database of human genes that includes genomic, proteomic and transcriptomic information, as well as orthologies, disease relationships, SNPs, gene expression and gene function. http://www.genecards.org/

Kingsmore et al., Nature Reviews Drug Development 7:221, 2008

DRUG DOSE SELECTION

- Conventional method: Select a dose by trial and error

  Pharmacogenetic method: It replaces conventional.

  Dose decided by Pharmacogenetic status of patients. Eg: Oral anticoagulants Cancer

PHARMACOGENETICS WILL IDENTIFY TARGET POPULATION FOR THERAPY:

- Polymorphism in appolipoprotein, (APOE), Cholesteryl ester transfer protein (CETP), Stromelysin-1 and Beta-fibrinogen occur during progression of atherosclerosis and cardio vascular events. (biomarkers).
- People with the above polymorphism get maximum effect from NMG-CoA inhibitors than people without that.

PHARMACOGENOMIC DATA IN LABELLING:

- ✓ **Gives direction in identifying patients require dose adjustments for efficacy or prevent adverse effect (individualised medicine)**
- ✓ **US FDA approved pharmacogenetic labeling for warfarin and irinotecan for safety limitation**

LIMITATIONS

Genotype identification facility

- ✓ **Industry to develop methods of pharmacogenetic testing**

Cont…

- **Ethnic diversity need**
- **Cost of testing is high**
- **Educating doctors**
- **Biomarker estimation to be scientific**
- **US FDA has made mandatory submission for certain category others voluntary**

FDA RECOMMENDS WARFARIN LABEL REVISION TO INCLUDE INFORMATION ON CYP2C9 AND VKORC1 GENOTYPING

FDA U.S. Food and Drug Administration
U.S. Department of Health and Human Services

FDA News

FOR IMMEDIATE RELEASE
August 16, 2007

Media Inquiries:
Karen Riley, 301-827-6242
Consumer Inquiries:
888-INFO-FDA

FDA Approves Updated Warfarin (Coumadin) Prescribing Information

New Genetic Information May Help Providers Improve Initial Dosing Estimates of the Anticoagulant for Individual Patients

LECTURE SUMMARY

- All Drug Effects Vary From Person to Person & All Drug Effects Are Influenced by Genes
- Most Drug Responses Are Multifactorial
- Genetic Polymorphisms of Single Genes Affect Numerous Drug-metabolism Enzymes. Some Polymorphisms Result in Adverse Reactions or Inefficacy of Drugs at Usual Doses
- Genetic Polymorphisms of Drug Targets & Transporters Are Being Increasingly Recognized As Causing Variation in Drug Responses
- The Frequency of Variation of Drug Effects Varies in Different Ethnic Populations