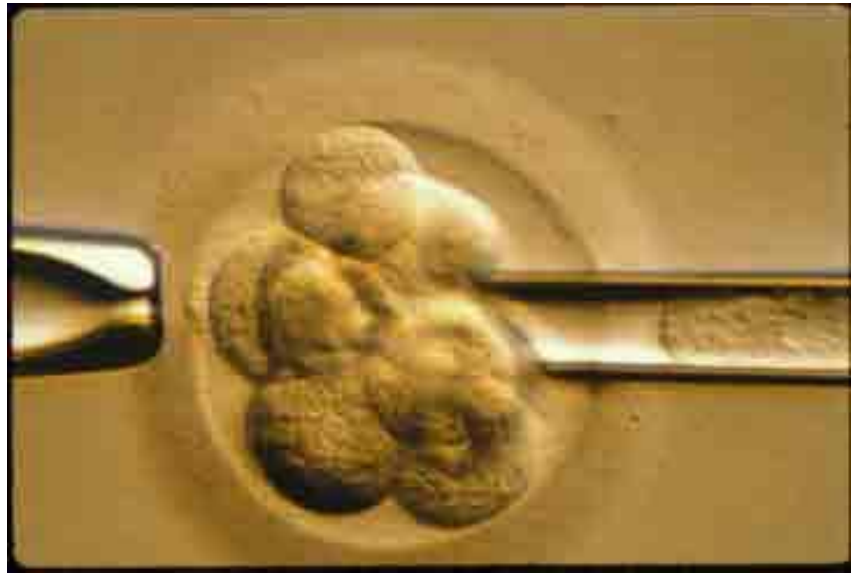
Pre-implantation Genetic Diagnosis (PGD)

Genetic analysis of a single cell from an eight-cell embryo done in conjunction with in vitro fertilization (IVF) to improve the chances of a “normal” pregnancy.

- Polar body
- Blastomere
- Trophectoderm



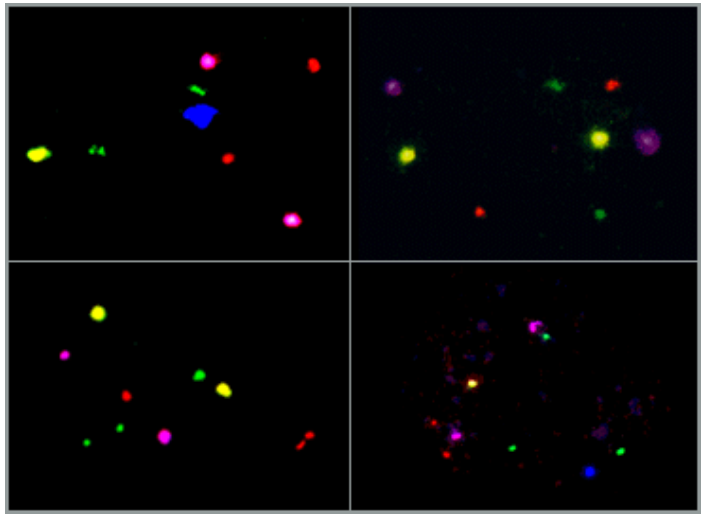
- Remove a single cell from the 6-8-cell embryo using a fine glass needle to puncture the zona pellucida and aspirate the cell

FISH

using fluorescent probes specific for each chromosome. These allow number and size of each chromosome to be checked.

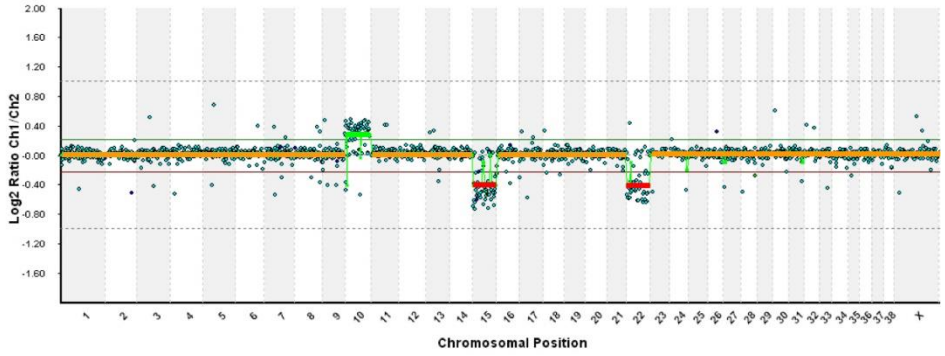
- useful for identifying aneuploidies (incorrect chromosome numbers) and translocations
- procedure destroys the tested cell
- limited number of chromosomes can be checked simultaneously; some abnormalites undetectable

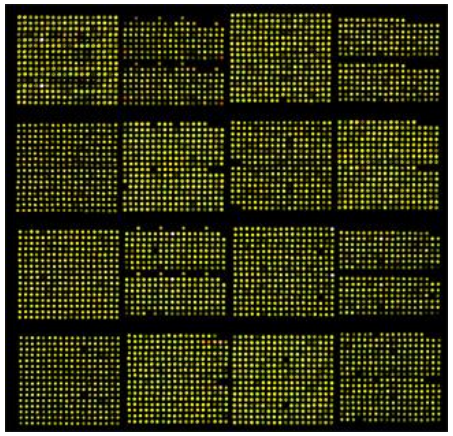
- Aneuploidy is the most frequent cause of spontaneous abortions.



genetic testing (PCR or gene chips)

- Array CGH (aCGH)





- PGD of monogenic diseases

based on indirect diagnostics using STR markers

direct diagnostics - sequencing

Limitations of PCR-based tests:

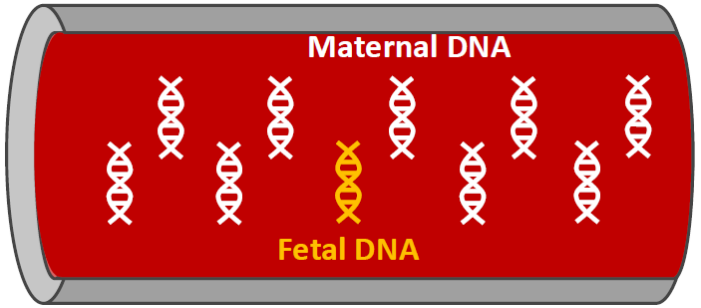
- Both alleles may not amplify equally, leading to misdiagnosis or inconclusive results

ADO (alelic drop out) – amplification of one alelle under the detection limit. Reasons unknown but influenced by cell lysis, PCR conditions, target DNA for sequencing, PCR product size

- PCR-based tests only detect disorders at target loci; other mutations may exist elsewhere

Cell free fetal DNA

- Short segments (<200 base pairs) of fetal DNA circulates in maternal plasma
- Origin is primarily **placenta**
- Placental cells undergoing apoptosis



- Reliably detected >7 weeks
- Increases throughout pregnancy (10-22 weeks constant)
- Cleared within hours of birth
  - Short half life (16 min), undetectable by 2 hours postpartum

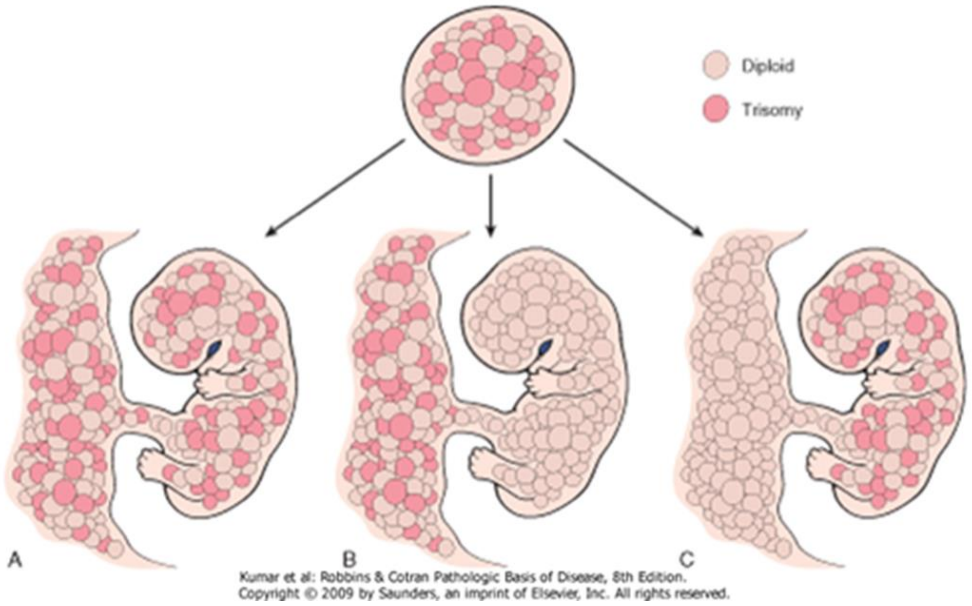
- Both cell-free ***fetal*** and cell-free ***maternal*** DNA circulate in maternal plasma.
- Cell-free fetal and maternal DNA circulate in maternal plasma as relatively short fragments (150-200 base pairs) and represent the entire genome.
- Fetal DNA comes primarily from the placenta.
- Maternal DNA comes primarily from maternal blood cells.
- Fetal DNA is 5-25% of the total cell-free DNA (~10% on average).

Cell free DNA Clinical Applications

- Sex Determination
- Single gene disorders – paternal origin
- Isoimmunization: noninvasively determine fetal Rh type
- **Aneuploidy: detect abnormal ratio of a particular chromosome;**
- **Does not detect nontargeted aneuploidies**

- Next generation sequencing
- Real-time PCR
- Many platforms and methods

- Why cell free fetal DNA fails?
- Confined placental mosaicism



Pharmacogenetics: From DNA to Drug Treatment

- Pharmaco***genomics***
  - The science of how genes affect the way people respond to drugs
  - How genes affect…

    …the way our body processes drugs (pharmacokinetics)

    …the interaction of drugs with receptors (pharmacodynamics)

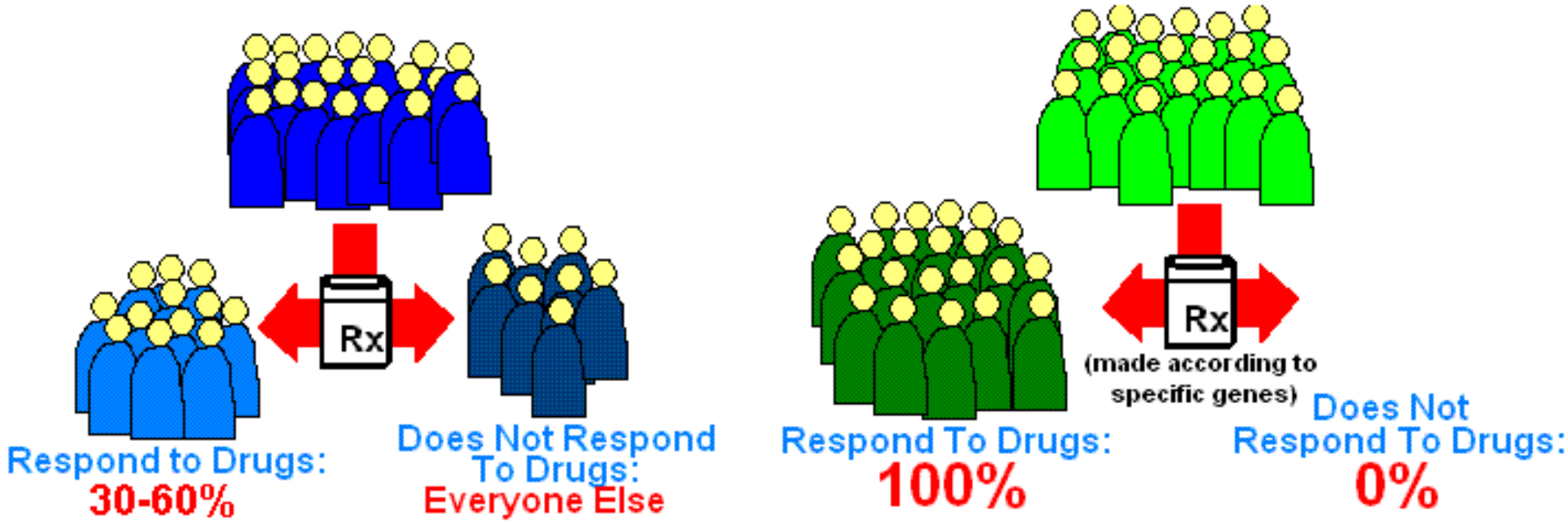
    …the treatment efficacy and adverse side effects

- Pharmaco***genetics***
  - A subset of ‘pharmacogenomics’
  - The study of how ***inherited variation*** affects drug response and metabolism

Why is this a good approach?

- Drugs can be dangerous
  - Many people have severe adverse reactions to drugs
  - Many people respond to drugs at different doses
  - Many drug treatments are horribly unpleasant, painful
- Drugs are expensive (to take and to make)
  - Ineffective drugs are a waste of money to take
  - Drug development needs to account for response variability
- Genetics provide *a priori* information
  - Genetics don’t change (except in cancer)
  - Genetics can point to the *cause* not just the symptom

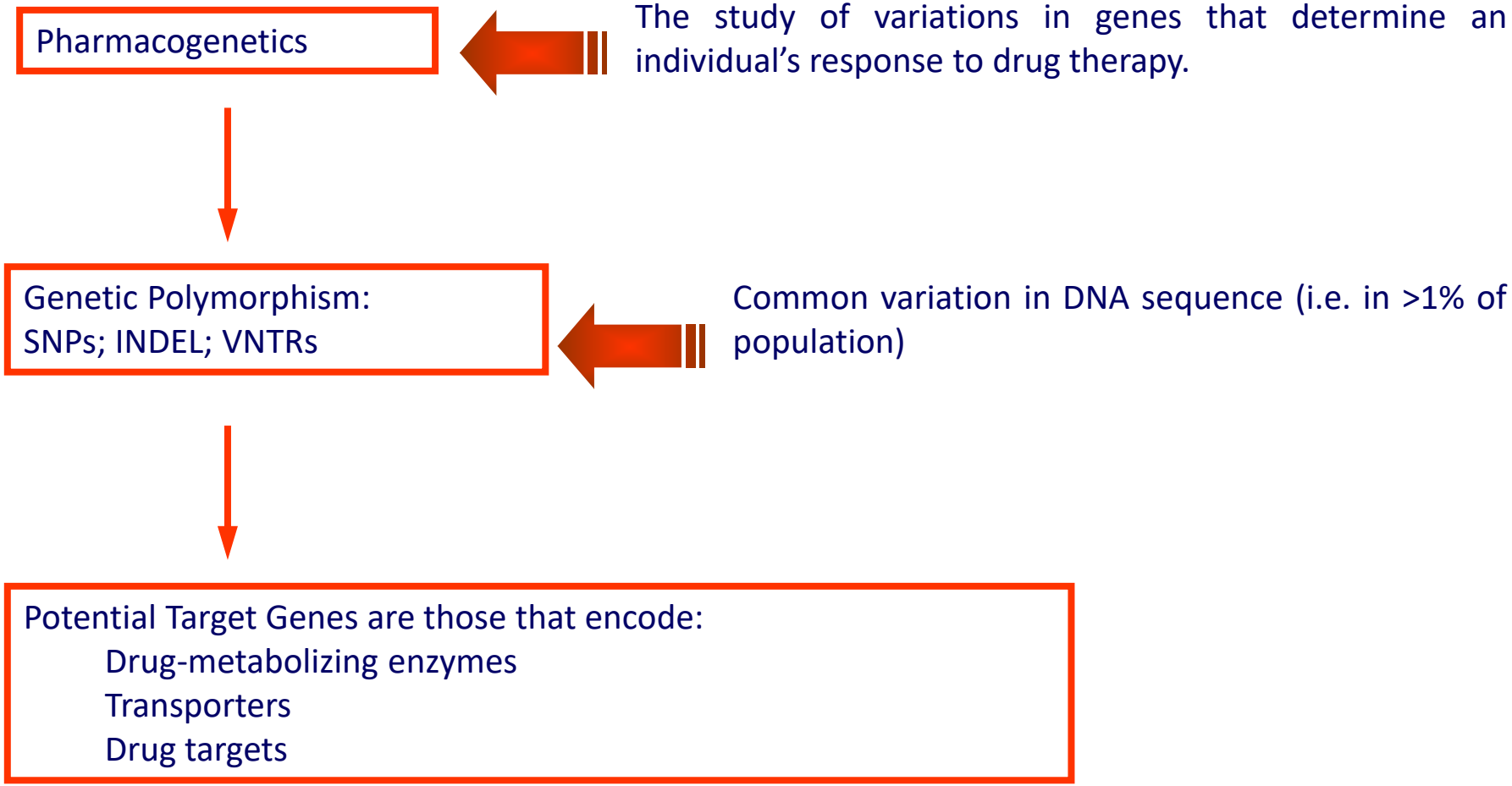
TODAY versus TOMORROW

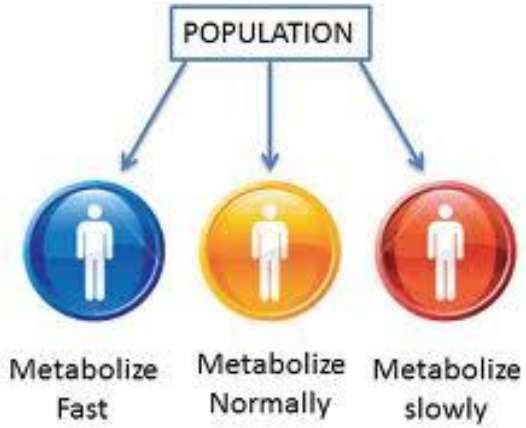


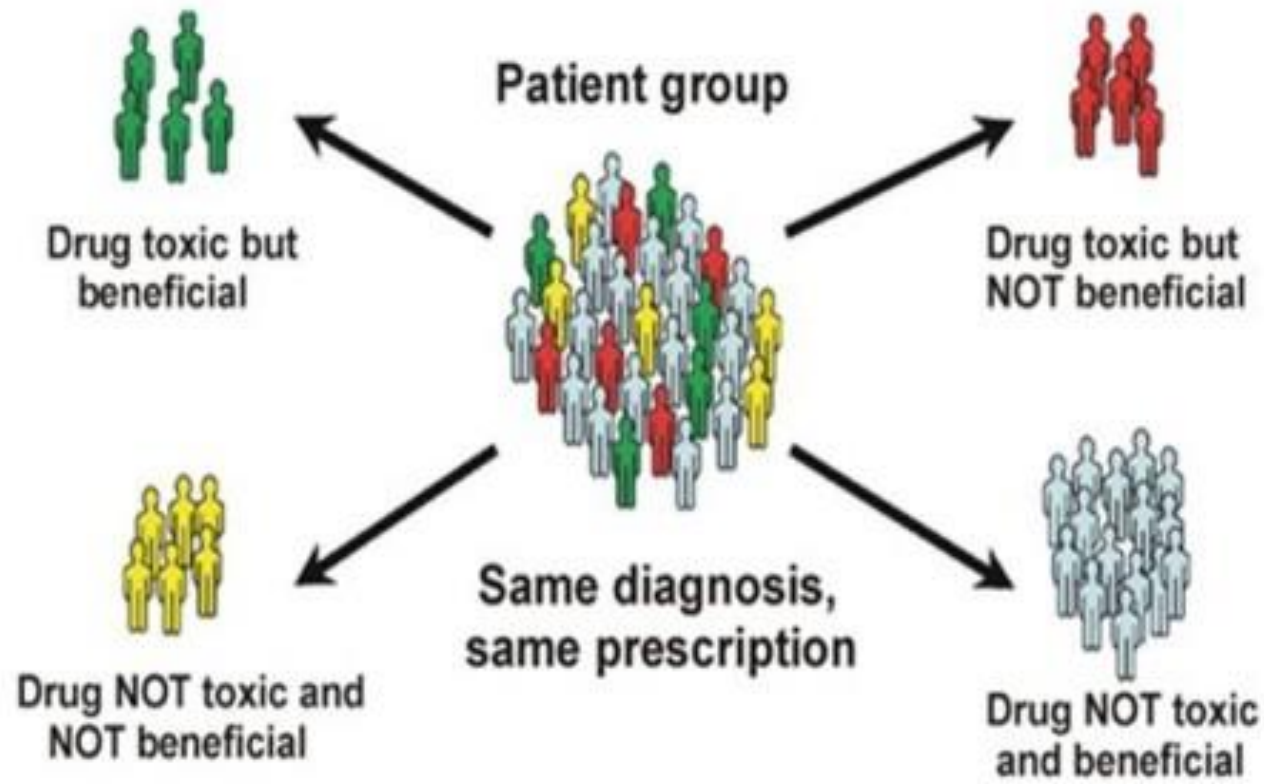
The Goal of Personalized Medicine

- The Right Dose of
- The Right Drug for
- The Right Indication for
- The Right Patient at
- The Right Time.

Courtesy Felix W. Frueh

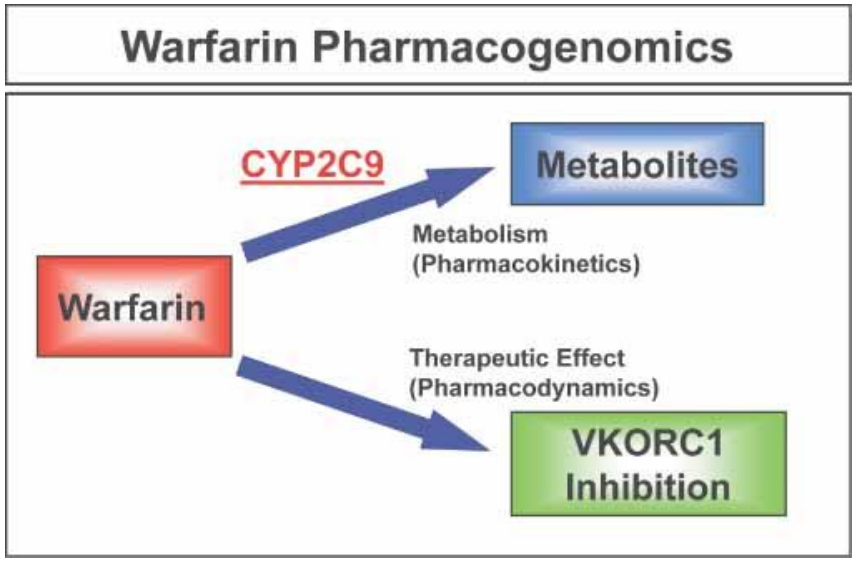

Pharmacogenetics
The study of variations in genes that determine an individual's response to drug therapy.
Genetic Polymorphism:
SNPs; INDEL; VNTRs
Common variation in DNA sequence (i.e. in >1% of population)
Potential Target Genes are those that encode:
Drug-metabolizing enzymes
Transporters
Drug targets

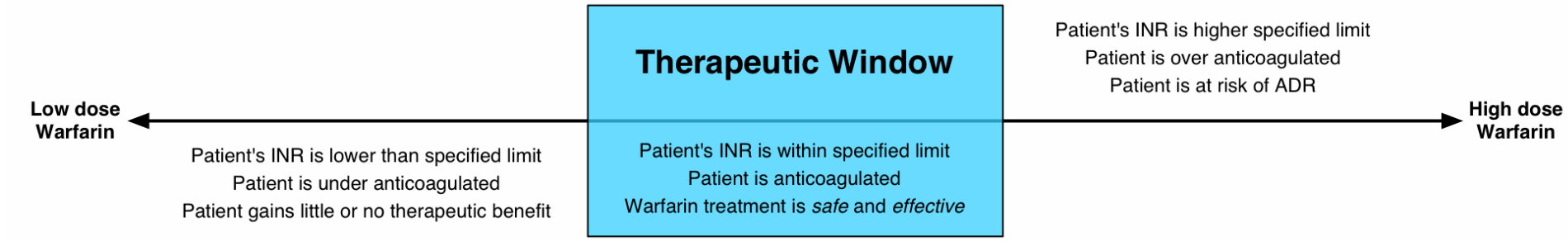

POPULATION
Metabolize Fast
Metabolize Normally
Metabolize slowly


Patient group
Drug toxic but beneficial
Drug toxic but NOT beneficial
Same diagnosis, same prescription
Drug NOT toxic and NOT beneficial
Drug NOT toxic and beneficial

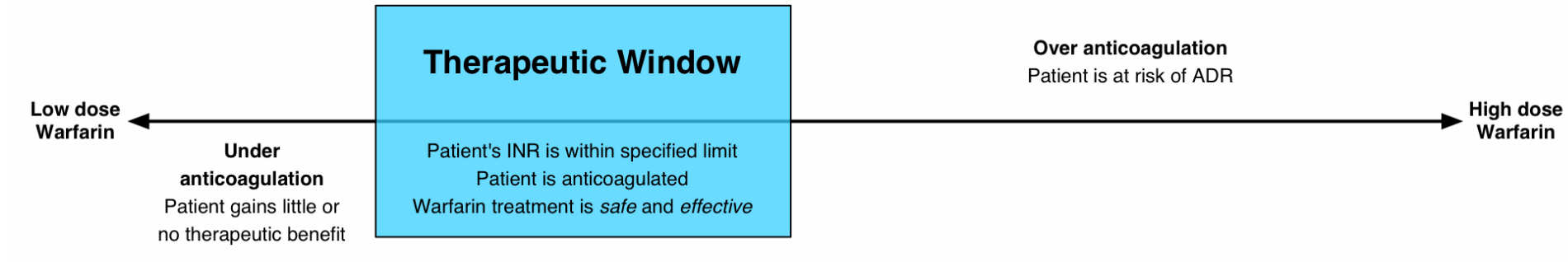
Warfarin: A dosage story

- Most widely used anticoagulant in the world
- Prescribed doses vary widely (1-40mg / daily)
- Therapuetic index is very low
  - High risk of bleeding early in treatment
- Two genes involved in metabolism: *CYP2C9* and *VKORC1*

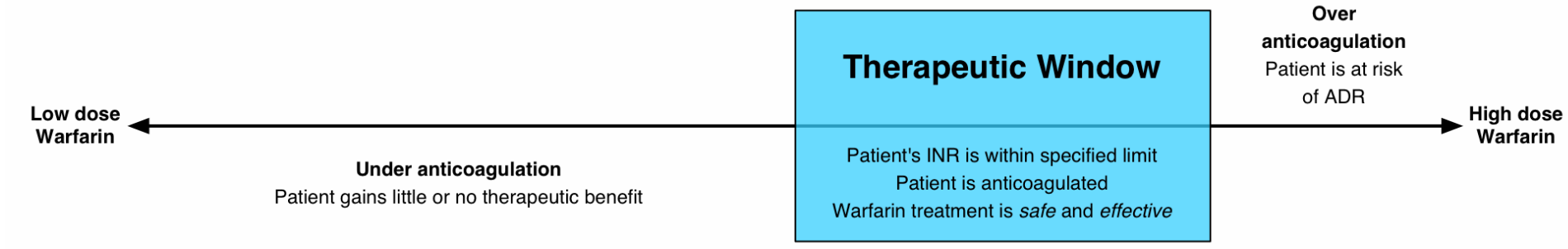




Homozygous wild-type *CYP2C9* and *VKORC1*

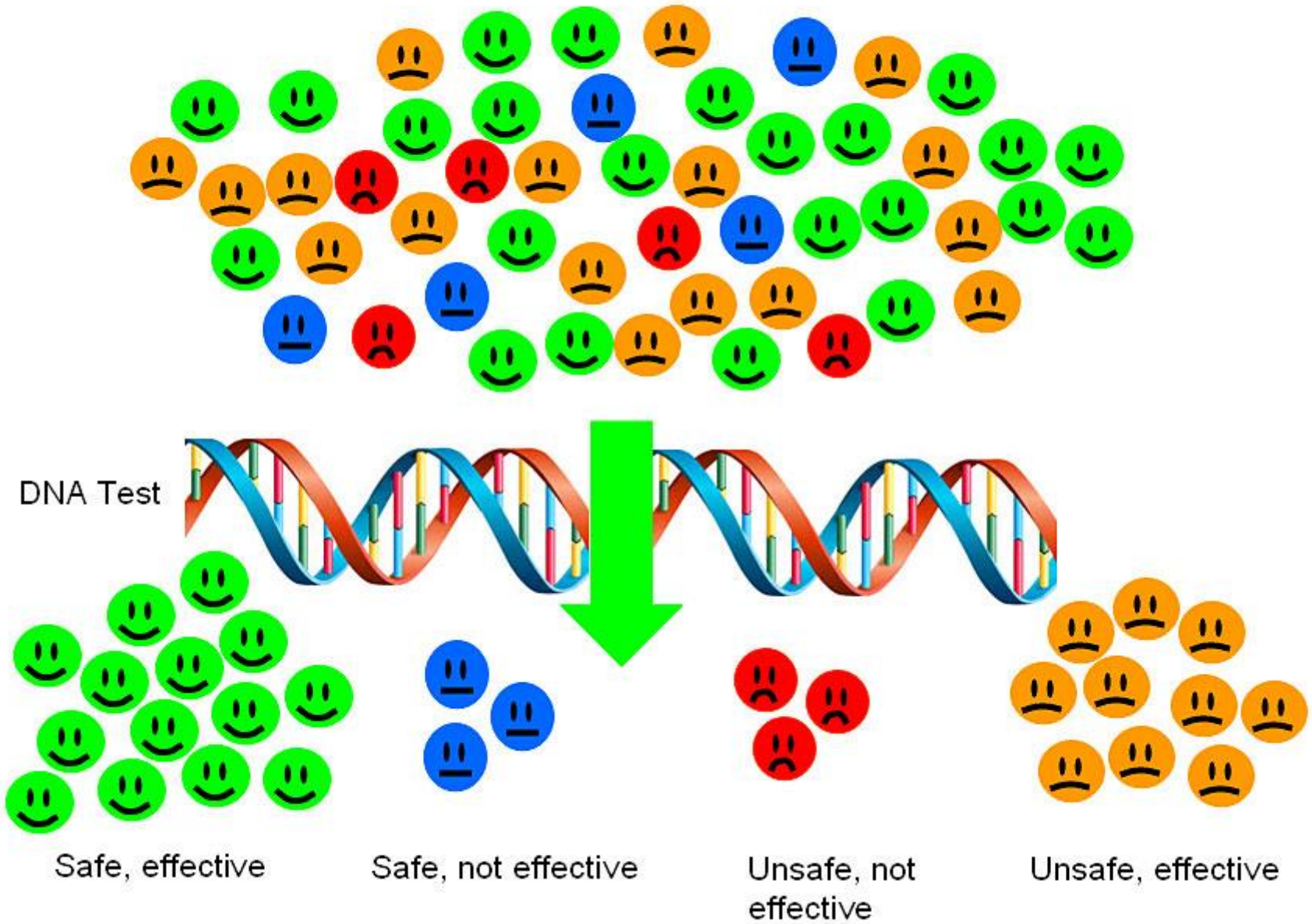


Carrier of *CYP2C9* mutant allele



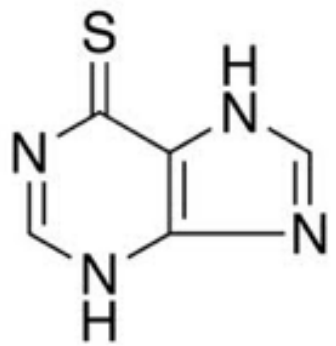
Carrier of *VKORC1* mutant allele

Your DNA Affects Your Response to Drugs

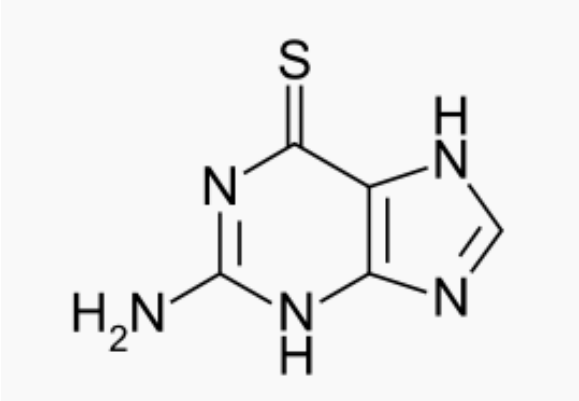


Purine Analogs

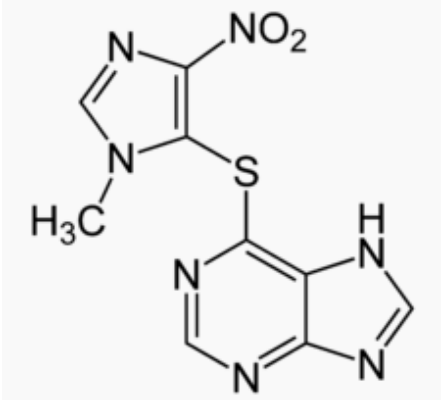
- 6-mercaptopurine, 6-thioguanine, azathioprine
- Used to treat lymphoblastic leukemia, autoimmune disease, inflammatory bowel disease, after transplant
- Interferes with nucleic acid synthesis
- Therapeutic index limited by myelosuppression
  (treatment limited by immune suppression side effect)



6-mercaptopurine



6-thioguanine



azathioprine

Codeine and Cytochrome P450 CYP2D6

- Codeine is a commonly used opioid
  - Codeine is a prodrug
  - It must be metabolized into morphine for activity
- Cytochrome P450 allele CYP2D6 is the metabolizing enzyme in the liver
- 7% of Caucasians are missing one copy of the Cytochrome P450 CYP2D6 gene
  - codeine does not work effectively in these individuals