Pharmacogenetics:

A SNPshot of the Future

Ani Khondkaryan

Genomics, Bioinformatics, and Medicine

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I. What is pharmacogenetics?

- It is the study of how genetic variation affects drug response and metabolism.

- The promise of pharmacogenetics is the optimization of drug therapy based on the individual patient's genetic profile.

Why is that important?

Most drugs act by interacting with proteins such as receptors, enzymes, and intracellular signaling proteins. Over the years, it has been shown that these proteins present genetic variations that can affect sensitivity to drugs.
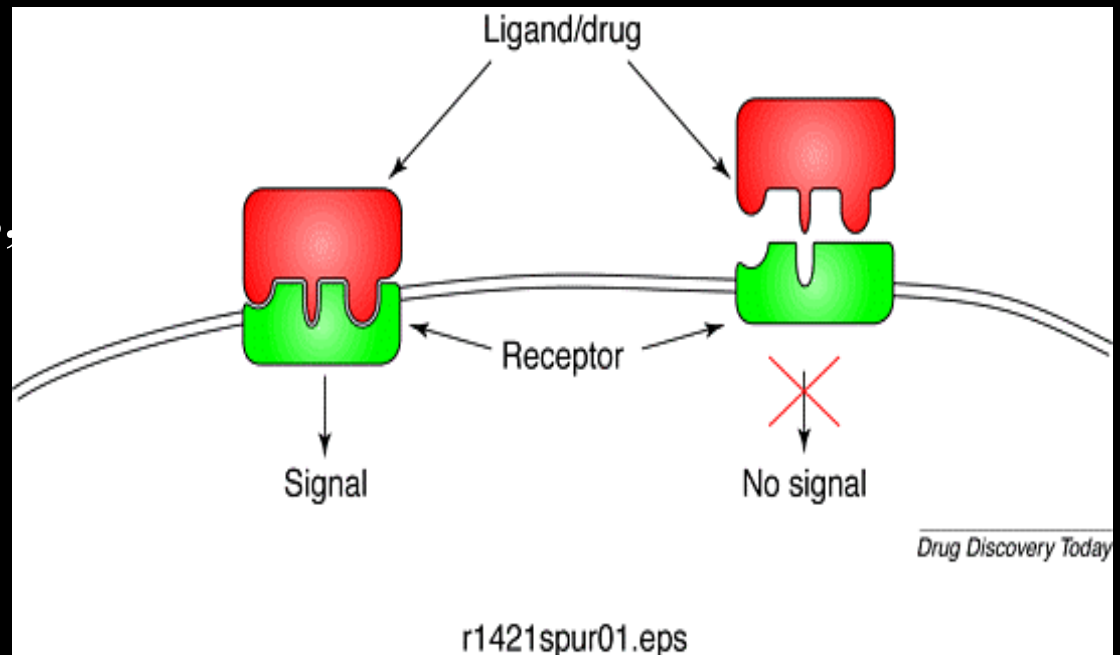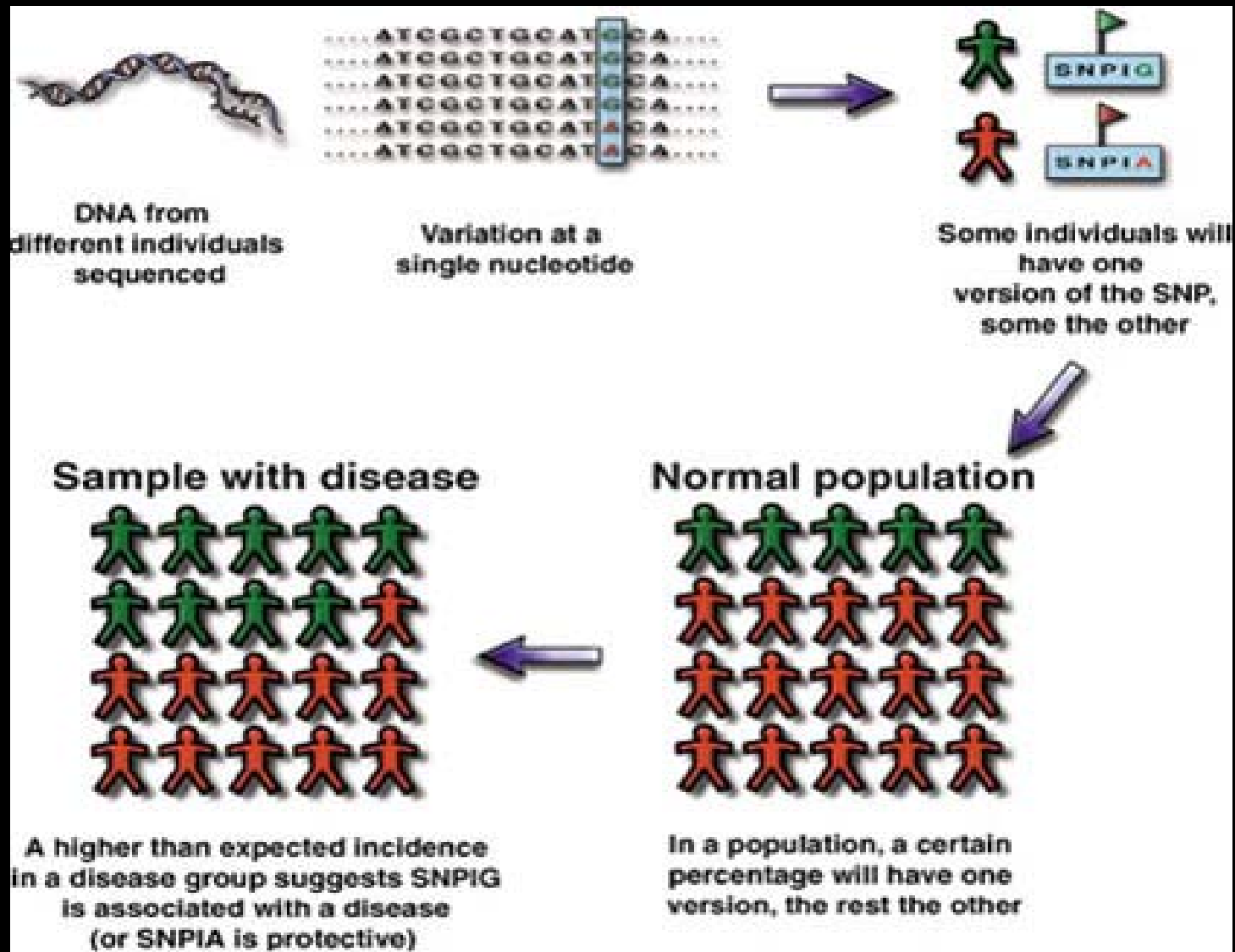

Major changes in protein structure can result from subtle variation in the genetic sequence encoding it.

How do these genetic differences of the patients affect the drug industry?

• most drugs do not work for all patients

• optimum dose requirements for many drugs may vary among individuals

• some drugs may even have adverse effects on patients

Two approaches to finding markers:

1. Genome-wide mapping approach

DNA from different individuals sequenced

Variation at a single nucleotide

Some individuals will have one version of the SNP, some the other

Sample with disease

Normal population

A higher than expected incidence in a disease group suggests SNPiG is associated with a disease (or SNPIA is protective)

In a population, a certain percentage will have one version, the rest the other

2. Candidate-wide mapping approach--an educated guess is made as to which of the 100 000 genes in the human genome are likely to be important in a disease.

This substantially reduces the number of loci needed, although it is likely that many genetic factors associated with a disease will be missed.

II. What are SNPs?

• SNP stands for <u>s</u>ingle <u>n</u>ucleotide <u>p</u>olymorphisms

• a SNP is a site of the DNA in which a single base-pair varies from person to person

• SNPs can serve both as a physical landmark and as a genetic marker whose transmission can be followed from parent to child

Polymorphism
"Poly" *many* "morphe" *form*

General population

94%

Single nucleotide polymorphism (SNP)

6%

# Why are SNPs the most useful markers for pharmacogenetics?

- SNPs are the most abundant type of DNA sequence variation in the human genome

- Most analytically straightforward class of genetic variants to catalogue in the human genome
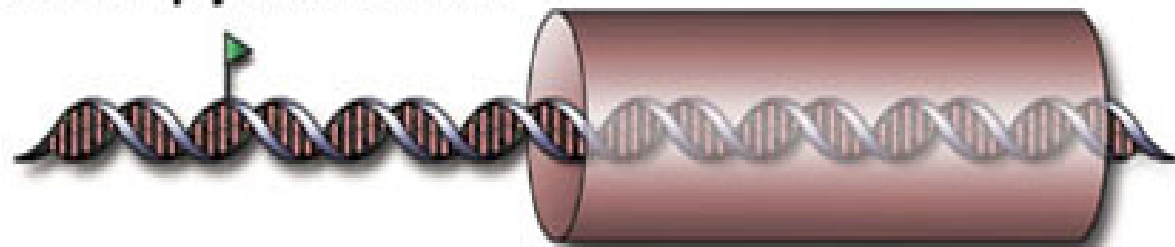
- SNPs have a low rate of mutation

Some more facts about SNPs:

• mean density of SNPs is approximately one per kb in the human genome

• low mutation rate per generation

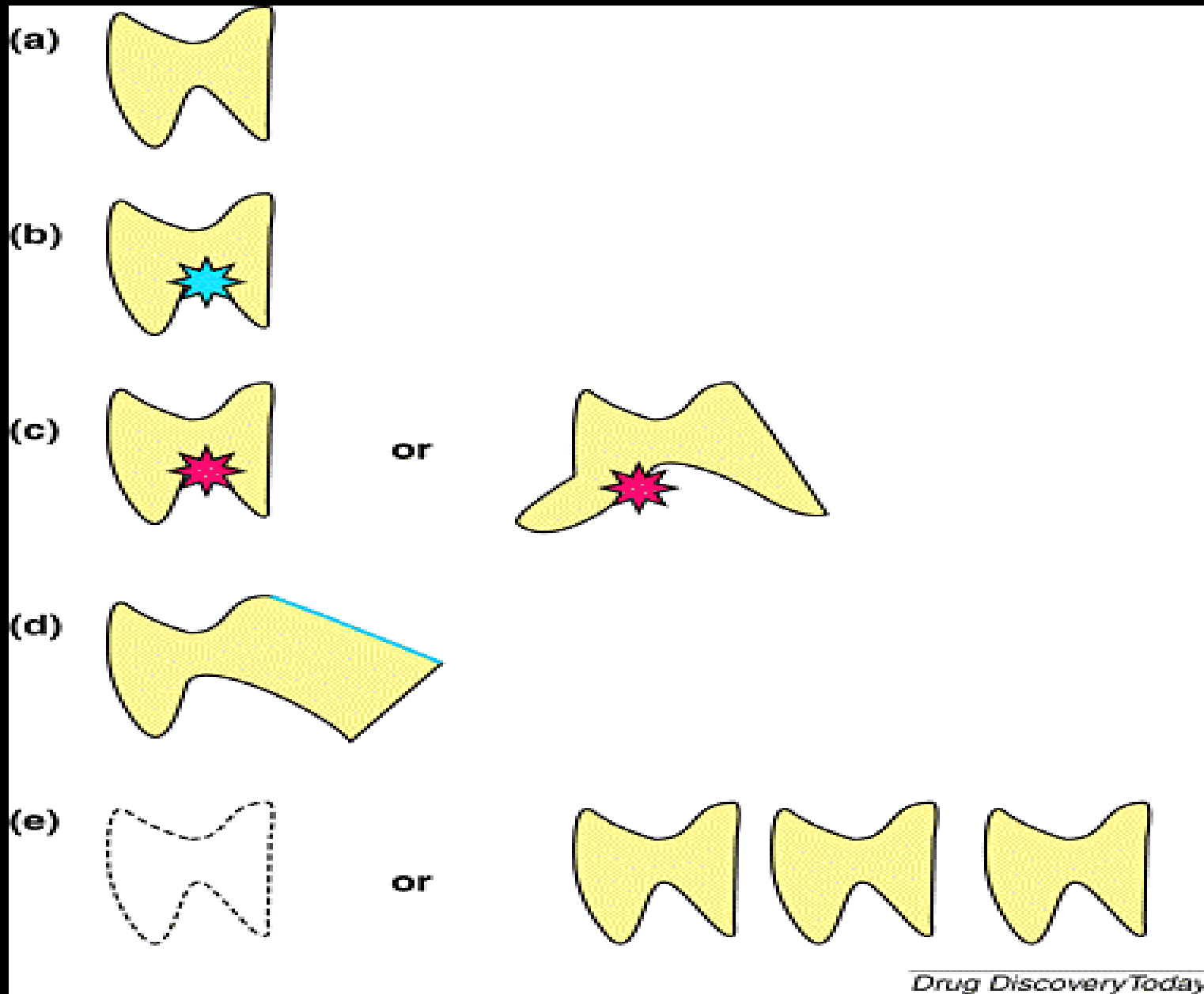• likely that a subset of SNPs are functionally important in complex disease traits

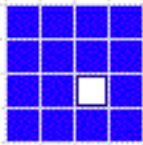A SNP may be part of a gene, and affect its function...
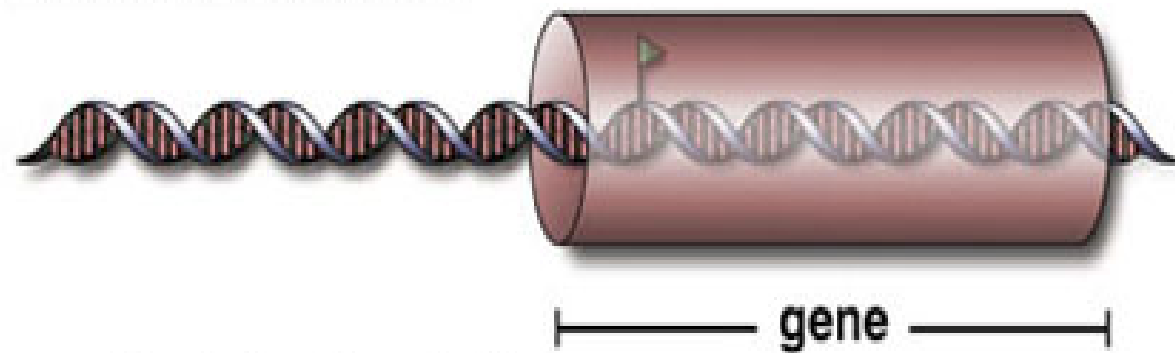
...or simply be close to it

gene

SNPs within coding regions (cSNPs):

Drug Discovery Today

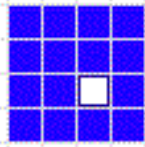APBiotech - AstraZeneca - Aventis - Bayer - Bristol-Myers Squib - F.Hoffman-La Roche - Glaxo Wellcome
THE SNP CONSORTIUM LTD
IBM - Motorola - Novartis - Pfizer - Searle - SmithKline Beecham - Wellcome Trust

SNP Consortium

http://snp.cshl.org/

- Non-profit foundation organized for the purpose of providing public genomic data

- Composed of the Wellcome Trust and 11 pharmaceutical and technological companies

APBiotech - AstraZeneca - Aventis - Bayer - Bristol-Myers Squib - F.Hoffman-La Roche - Glaxo Wellcome
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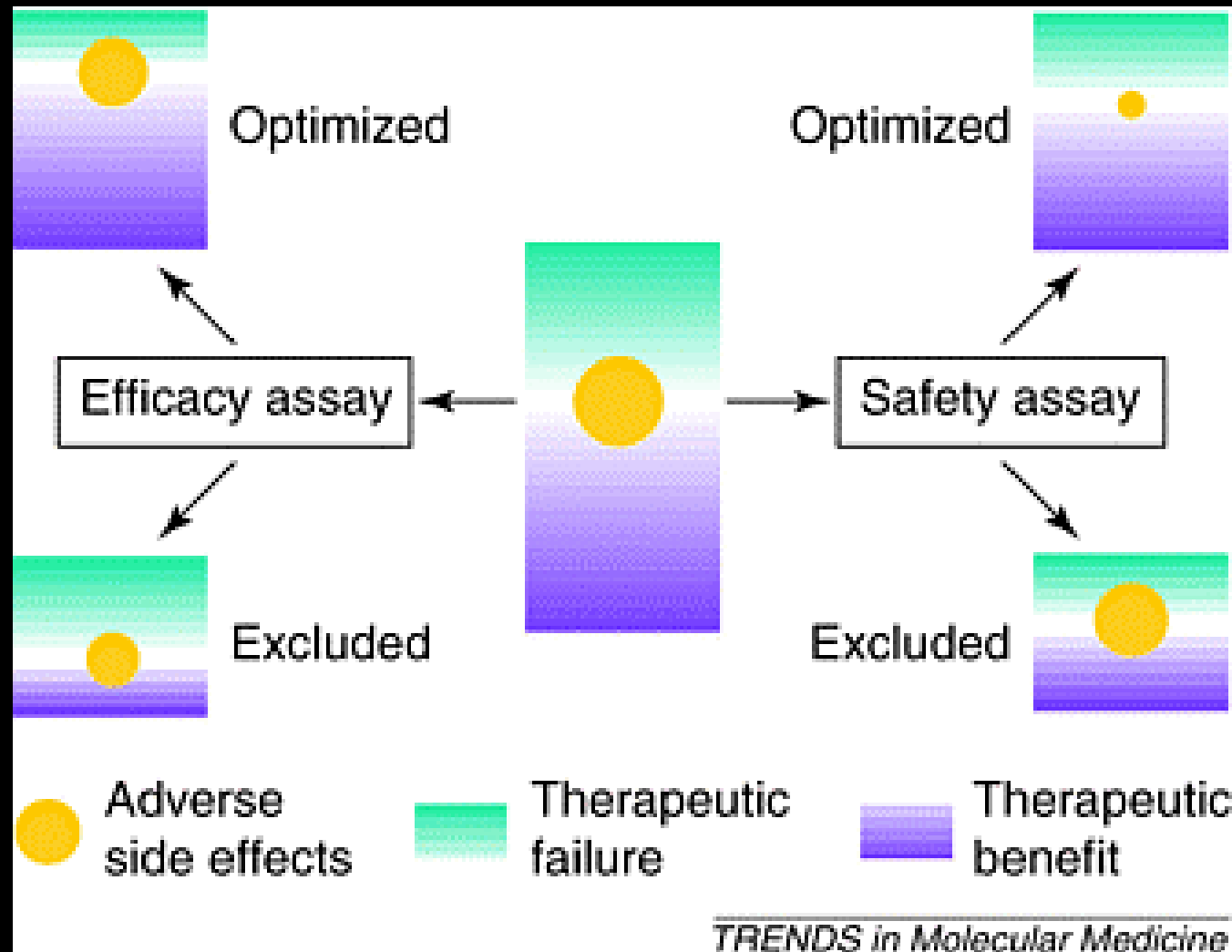
Project started in April 1999.

Mission:

1) to develop up to 300,000 SNPs distributed evenly throughout the human genome

2) to make the information related to these SNPs available to the public without intellectual property restrictions.

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III. How do SNPs affect the pharmaceutical industry?

• In preclinical development, being aware of the patients' SNPs can help pharmaceutical companies reduce the risk of failure due to variable efficacy.

• In the clinical trials, genotyping individuals can help determine the influence of these polymorphisms on drug efficacy

Pharmacogenetic assays will determine whether a patient is more or less well suited to the particular drug based on results from a genotyping assay. In some cases, there might be both efficacy- and safety-based assays.

Optimized

Efficacy assay

Excluded

Optimized

Safety assay

Excluded

Adverse side effects

Therapeutic failure

Therapeutic benefit

TRENDS in Molecular Medicine

More ways SNPs will help the drug industry:

• SNPs will identify candidates most likely to benefit from new medications being developed, and those likely to suffer adverse side-effects

 • Even medications that cause some people significant side-effects can be developed for a subset of patients who will derive therapeutic benefits from them

 • Physicians will be able to prescribe the most effective and safe medication for their patients

How are the SNPs used in the pharmaceutical industry?

Three major phases to pharmacogenetics as a process:

(1) SNP discovery

(2) SNP correlation

(3) SNP diagnostics

IV. Developing SNP markers:

1. Obtain DNA sequence surrounding SNP

2. Develop a PCR assay to amplify DNA segment containing SNP

3. Identify the SNP

4. Map the SNP to a unique location in the genome

5. Determine the allele frequencies of the SNP in the population

6. Develop a genotyping assay for SNP

A genetics researcher takes to the bench at the Wellcome Trust's Sanger Centre in Cambridge, England.

How it used to be done:

1. Construction of a small-insert random genomic library and obtaining DNA sequence data from these clones

2. Making PCR primers

3. Screening for polymorphisms

4. Genotyping assay for the SNP

5. Genetic mapping of SNP

Bottom line: expensive, took a long time

More recent strategies use sequence data or map data generated by other groups for unrelated purposes.

Some techniques are:

1) Screening sequence-tagged sites

2) Mining EST databases

3) Exploiting the human genome project

4) The anchored re-sequencing approach

1. **Screening sequence-tagged sites—screen physically mapped STSs by DNA resequencing**

Advantages: bypasses steps 1 and 2, and 4 if the STS is already mapped

Disadvantages: most STSs are very short, so a relatively large number of STSs have to be screened before a SNP was found.

2. Mining EST databases generated from cDNA libraries made with tissues from many individuals. Comparing redundant cDNA sequences derived from different libraries amounts to screening different individuals for polymorphisms.

Advantages: eliminates steps 1 and 3, and 4 if use a mapped EST

Disadvantages: First, the sequence data quality is highly variable and many of the apparent SNPs turn out to be the result of sequencing inaccuracies. Second, the cDNA clones are generated by reverse transcriptase, an enzyme with a high replication error rate. Third, ESTs are generally small, so SNPs will be found only in a fraction of the ESTs.

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3. Exploiting the human genome project

Overlapping regions between neighboring clones of two different libraries or diploid organisms can be compared for variations.
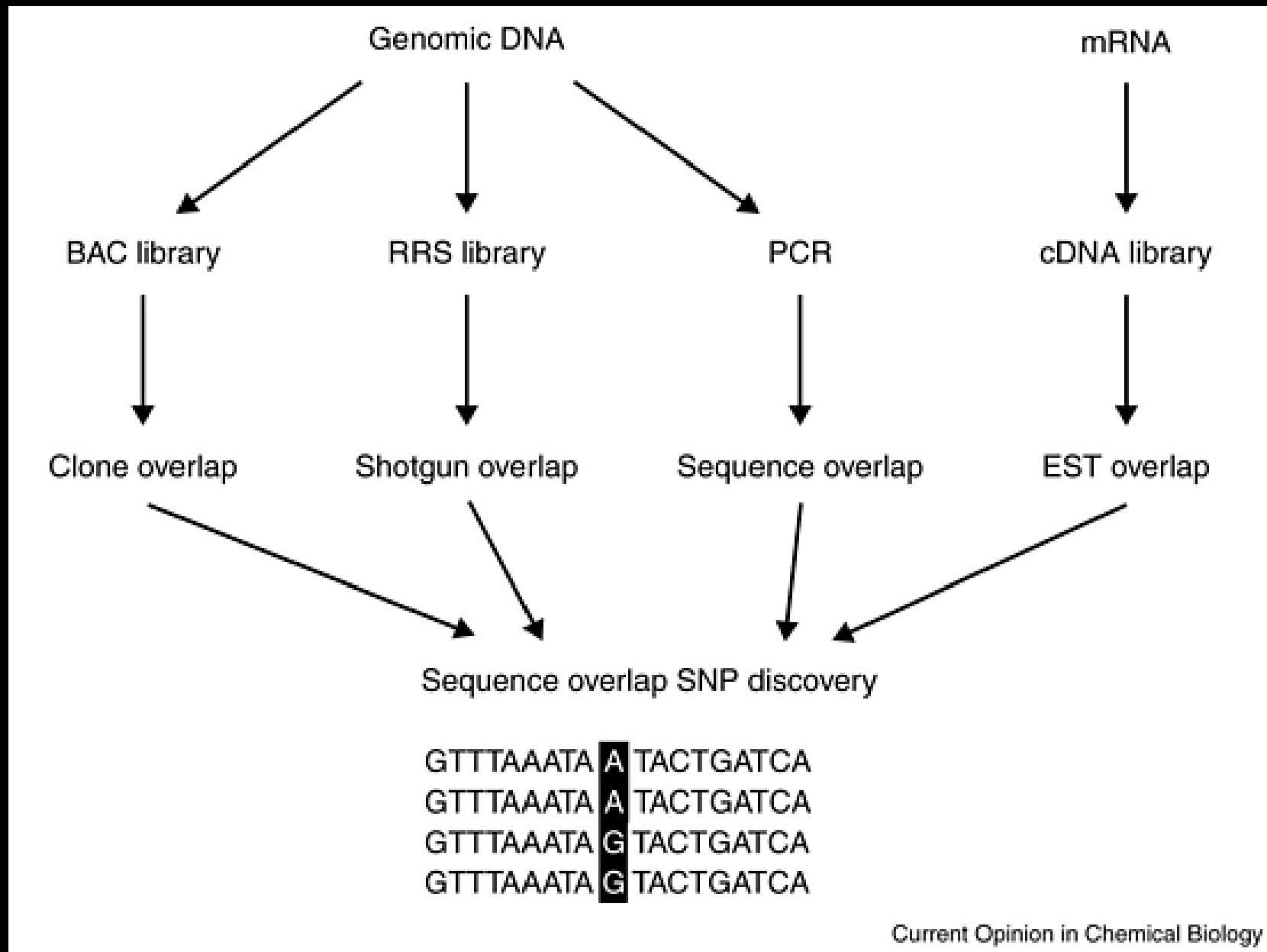
Precise mapping available.

Advantages: skips steps 1, 4, and 5;

highly efficient and cost-effective

4. The anchored re-sequencing approach—SNPs are identified by comparing relatively short but high quality genomic-sequences against the rough draft human-genome sequence

Advantages: skips steps 1, 4, and 5 (same as last approach)

Alternative methods for sequence-based SNP discovery.

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•   More than a million genetic markers in the form of single nucleotide polymorphisms are now available for use in genotype–phenotype studies in humans.

•   The focus of variation analysis is now shifting from the identification of new markers to their typing in populations, and novel typing strategies are rapidly emerging .
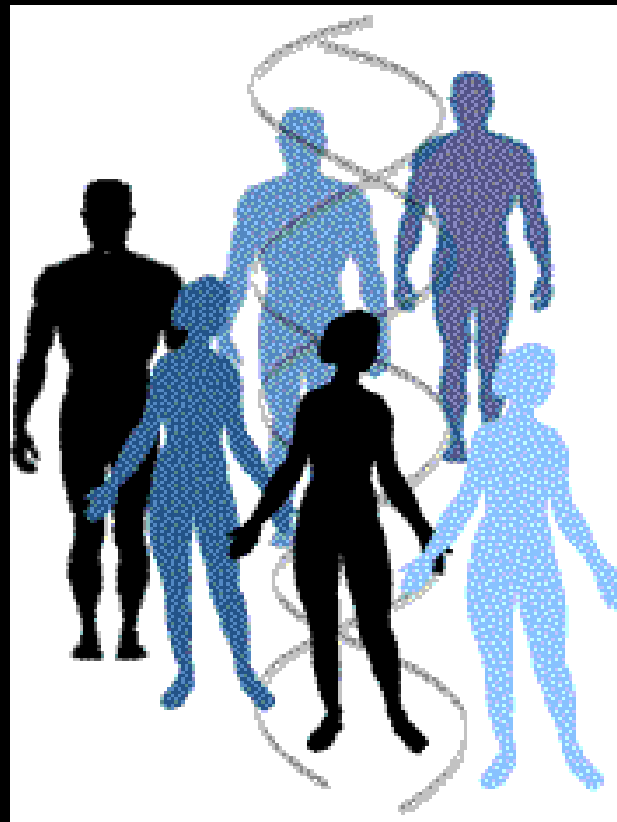
V. Future Challenges

1. DNA tests that can handle the massive number of genotyping assays in an efficient and inexpensive way must be developed

2. error detection

3. analytical methods must be developed to sort through the voluminous data produced both in the projects that are searching for disease genes and in genetic profiling projects

VI. Conclusions

- SNP libraries will shorten the disease-gene discovery process and initiate the era of personalized medicine

- Human geneticists will have at their disposal a super-dense genetic map to identify genes contributing moderate effects on complex traits

- Drug companies will be able to determine genetic profiles that will tell them whether an individual patient will benefit or suffer adverse side-effects when given a particular drug

- Physicians will be able to prescribe the most effective and safe medication for their patients.

Taken together, the current developments in academic, biotechnology and pharmaceutical genetic research present the most radical hope for significant change in patterns of medical treatment.

THE END!