Applied pharmacology to biotechnology

In 2001 the first draft of the human genome was completed.

There were two different studies, one private and one sustained by different states.

Basically now with the humane genome sequence the amount of new target for treatment with drugs would be enormously amplified.

In our genome there are 25000 genes. But we have a bigger number of proteins, thanks to the post-trasductional modification, and different splicing ecc.

In 2001 only 500 targets were known, now they are al lot of more, thank to the DNA sequence.

The drug targets now are divided in:

- Membrane Receptors 45%
- Enzymes 28% (is easier to inhibit than to activate) es: aspirin blocks COX-1
- Hormones and factors 11%
- Unknown 7%
- Ion channels 5% (lidocaine, a local anestetic, it blocks the propagation of action potential by blocking sodium channel)
- Nuclear receptors 2% (this receptors bind steroids)
- DNA 2% (some anti-cancer drugs)

There are also targets unknown which means we use drugs, but we don't know how it works. An example is a drug called dimetil-fumarate used in multiple sclerosis. This drug is a modulator of oxidative stress. It was discovered 1960, it was discovered as a cure for psoriasis. In this time the mechanism of action was unknow, until someone studied this molecule and find out that it could have some antioxidant properties, it reduces oxidative stress. In multiple sclerosis there is oxidative stress so the dimetil fumarate can be used also for this disease.

Do we use this drug target properly? We consider all the different situation in which the drug works? Are we use the drugs at the maximum of their possibility? Maybe not.

How can I decide if I'm using well or not a certain drug? For instance, I can consider some clinical data such as Adverse drug reaction.

ADR=Adverse drug reaction are the fourth cause of deaths in USA after miocardial infarction, stroke and cancer.

(We are talking about approved drugs)

The interesting thing is that most of these unpredictable deaths are due to individual variability in drug responses. So, a drug can kill someone but can save another one, on the basis of their genetic differences.

The specular aspect of this variability is the lack of efficacy of a given pharmacological treatment with exposure of the patient only to the side effects without any beneficial effect.

ADR also means therapeutic failure.

Example of a study: An ADR has been studied, and they create a diagram which correlates Annual incidence and patient age.

Until we are under 5 years old we are very fragile, probably because the immune system is not completely balanced.

After 60/65 we have hyperbolic explosion of ADR. When we are old we are more fragile than ever.

Another aspect of the study is the organ mainly involved in adverse drugs reaction. The skin is the most organs involved.

The individual variability is due to: age, gender (women are more exposed to having ADR because they have a faster metabolism and because in past there were not clinical studies on women), alcohol, smoke, diet, physio-pathological condition of the patients.

But the mainly variability has genetic basis (50%). This variability resides in differences (polymorphism) in the genes coding for the proteins involved in the response to a given pharmacological treatment.

There are differences in genes, demonstrated by Human genome project, all individuals are different. The only individual who are identical are twin merozygote.

Of course, there could be similarities in gene sequences, between the same family and ethnic groups for example, but every individual has his own sequence. So if we want develop a drug, we must remember this variations.

WHAT IS PHARMACOGENETICS? PHARMACOGENETICS is the discipline that studies genetic basis of the individual response to drugs

Pharmacogenomics is the same thing of pharmacogenetics.

The genes that influence the response to a given drug can be divided into three broad classes:

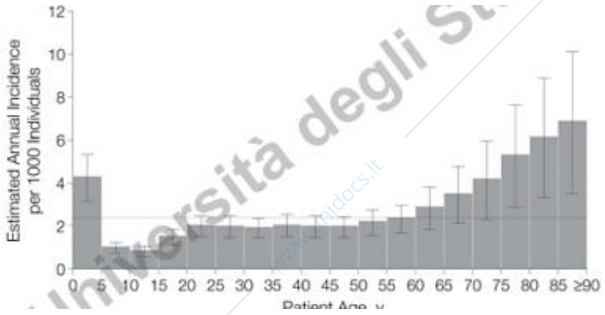
- Genes encoding the primary therapeutic target, such as receptors, enzymes, transporters
- Genes encoding proteins involved in drug metabolism
- Genes coding for proteins involved in the absorption, distribution, and excretion of the drug.

Polymorphism in these types of genes can affect the activity and the efficacy of the drug.

To be correct we can divide into two classes:

- Genes encoding primary targets
- Genes encoding protein involved in **pharmacokinetics.**

pharmacokinetics (how our body interact and modifying the drug, divided in absorption, distribution, metabolism, excretion)
pharmacodynamics (how the drug interacts with the



target and how it can modify our body)

A single gene because of polymorphism can be present in different allelic variants in the population. We say that a allelic variation is a polymorphism when is present more than 1% in population.

While mutations are present in a percentage lower than 1%

From a molecular biology perspective there is no differences between mutations and polymorphism. The difference is just in percentage.

There are different types of polymorphism, but we are interested in studying SNP's.

•SNPs (SINGLE NUCLEOTIDE POLYMORPHISMS)

Different people can have a different nucleotide or base at given location on a chromosome.

While mutations are present in a percentage lower than 1%

SNPs are classified in 3 distinct categories:

- SNPs of the coding regions(**cSNP**), in exons. This can potentially change the function of the protein. The simplest consequence of polymorphism is the substitution of a base. That is not always a problem due to de degeneration of our genetic code. So, with must distinguish between silent or not-silent polymorphism.
- Perigenic SNPs (**pSNPs**) affecting the regulatory regions at 5 ', the regions specifying the 5' and 3' untranslated mRNA, splicing junctions.
  The polymorphism can be in the sequence of the promotor, so we can have a highest or lower level of expression of the protein.
  pSNPs in the splicing sequence in 5' the mutation determinates that the sequence is not recognized by the splicing proteins causing a **intron retention.**
  If I have a pSNPs is in a 3' end, we have a phenomenon called **exon skipping.**
- SNPs found randomly (**rSNPs**) in the intergenic regions (microRNA , random DNA, ecc.)
  They can affect activity of the long non-coding RNA and so affect the activity of the protein they bind.

The polymorphism is present in our germinal cells, so we will transmit this to our children. So, it's something stable, not like mutations.

cSNPs:The obvious and simplest consequence of a polymorphism in exons is the substitution of a base. But our genome is degenerated, so it does not mean that it will affect the expression or the activity of the protein. In this case the polymorphism is called synonymous.

Sometimes it introduces an anticipate stop codon so at translational level I will have a shorter protein usually very unstable.

Sometimes we could have a non-synonymous polymorphism that doesn't change the function of the protein.

pSNPs: Single nucleotide polymorphisms in the perigenic regions. The polymorphism can be in the sequence of the promotor, so we can have a highest or lower level of expression of the protein.

Maybe the polymorphism is in the splicing sequence in 5' end the mutation. In this case its determinates that the sequence is not recognized by the splicing proteins causing a **intron retention.**

If I have a pSNPs is in a 3' end, we have a phenomenon called **exon skipping.** The protein in non-functional.

What about a patient with cancer? If a patient a cancer does a patient has two genomes. Its own genome and cancer genome.

These mutations can affect the response to chemotherapeutic drugs. Because maybe the cancer acquires the ability to exclude the drugs out of the cell. Can we distinguish the two genomes in terms of influence of drug response? Yes.

If I'm interested in how the tumour responses to the treatment, the tumour sensitivity is largely under the control of tumour genome.

If I'm interested the toxicity of the treatment, well the host genome is much more important than the tumour genome.

If I'm interested in drug availability, both the host genome and tumour genome are important in pharmacological response.

If we are talking about mutation accumulated in tumour that modifying drug response, we are talking about somatic pharmacogenetics.

The germinal pharmacogenetic means the polymorphism is present in all the cells of the body. We usually talk about this.

Gefitinib is a small inhibitor used for the treatment of lung cancer. It acts on EGF receptor. If the EGF receptor is mutated the drug does not work anymore. If the EGF receptor is normal Gefitinib will work.

Cetuximum is a monoclonal antibody directed against EGF receptor used in retto-colon cancer. But downstream the receptor there is a onco-gene which is responsible for the transduction pathways, and if it is mutated is always activated. So Cetuximim is usless, the signal is active due to the protocono-gene.

This was somatic pharmacogenetics.

Let's start with drug metabolism.

Drug metabolism is the field in which we have more information.

It is inappropriate to talk about drug metabolism because we have this mechanism before the drugs were invented. This metabolism is used to eliminate xenobiotics, and then **also drugs**.

The xenobiotics are very lipophile, so they can enter the cells very easily and that can create damage. Our body developed a mechanism that transform lipophile molecules into more hydrophilic molecules that are easily eliminable, and this is what happens with the metabolism of drugs.

Indeed, metabolism is not finalized to inactivate a drug, but to eliminate a drug.

Metabolism can be divided in two steps : the first in which the xenobiotic is modificated to be more reactive and the second in which the xenobiotics are coniugated to a molecules that make the xenobiotic more hydrofilic, and so more eliminable. Drugs are xenobiotics to us.

This is a typical curve you can observe a somminsitration of one dose of a drug.

The Cmax depend of what kind of metabolizer you are.

A polimorfism can influence the metabolism of drugs.

If the enyme does nor work anymore we will have a bad metabolism, a higher concetration of drug in our body and you could have ADR.

But if the metabolism is encreased, you have a lower Cmax and a lower efficacy of therapeutics effects.

These rules work for a drug. For a pro-drug it is the opposite.

The main enzyme involved in phase 1 is cytochrome. We have 80 genes encoding cytochromes.

Obv non all are involved of xenobiotics/drugs but the most variable are. Approximately 40% of drugs are metabolized by cytochrome.

In phase II there are other enzymes but the most important in UGTs, enzyme which is able to ad glucoronic acid to the substrate increasing the hydrofobicity.

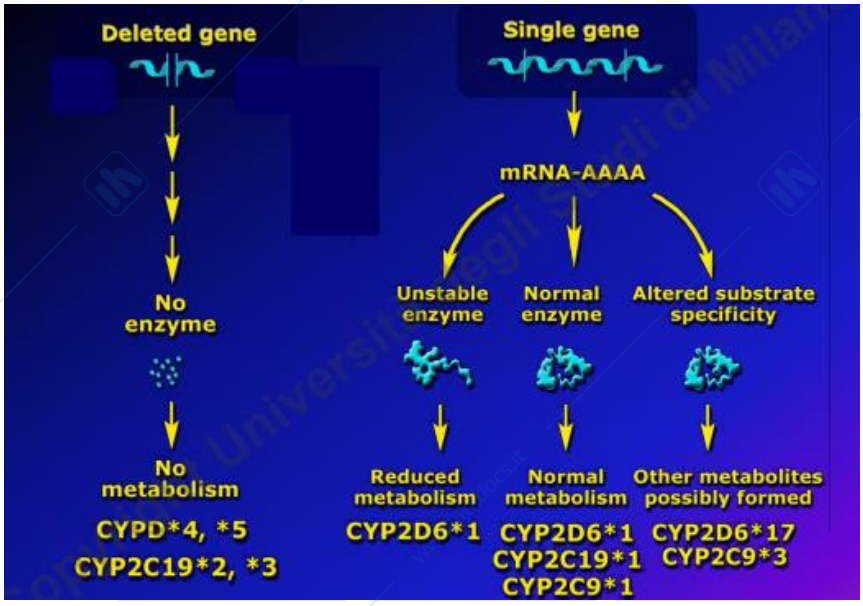
There are drugs that are metabolized just from phase I or phase II and there are drugs that are nont metabilized at all.

MOLECULAR MECHANISMS responsible for altered drug Metabolism in humans: How polymorphisms can affect the activity of cytochromes.

We can have normal metabolism, reduced metabolism or other metabolites possibly formed as increased metabolism.

It's possible to have a polymorphism which produce an enzyme not functional, so I don't have metabolism.

How can I understand if a polymorphism can impact metabolism? I can analyse ADR.



If the cytochromes do not work well, and I assume Warfarin, which is an enzymatic inhibitor used as anticoagulant, **the most**

important drug reaction will be magnified, in this case I got bleeding.

Many coagulation factors which are produced in the liver, are not in their active form. They must be activated by a post-traslation modifications.

They must be carboxylated to be in their active form.

The enzyme which is responsible for the carboxylation gamma-glutamic-carboxylase. Carboxylic group bind acids.

But gamma-glutamic-carboxylase needs a cofactor, the vitamin K. Reduced vitamin K is active but oxidized vitamin K is not. So I have to restore the reduced form. And so there is an enzyme called VKORC1, which does this work. Warfarin is an inhibitor of VKORC1.