Presidenza del Consiglio dei Ministri

ITALIAN NATIONAL BIOETHICS COMMITTEE

FROM PHARMACOGENETICS TO PHARMACOGENOMICS

21st April 2006

Premise

In the National Bioethics Committee plenary meeting of the 25th of October 2002, Prof. Luigi De Carli proposed the creation of a working group to update one of the most important documents published by the NBC in the first years of its activity and precisely in 1994: the document dedicated to the *Human Genome Project*. According to De Carli it was by now necessary to examine the problems of the so-called postgenomic era, and to shift attention from the study of the genome to that of the proteome. The Committee, unanimously, approved this proposal and asked Prof. De Carli to assume leadership of the working group, which was called *From Genomic to Proteomic*. The group convened for the first time on the 22nd of November 2002 and consisted of Prof. Amato, Prof. Bompiani, Prof. Caporale, Prof. Coghi, Prof. Dallapiccola, Prof. Di Pietro, Prof. Eusebi, Prof. Flamigni, Prof. Gaddini, Prof. Marini, Prof. Neri, Prof. Piazza and Prof. Silvestrini. By the second meeting on the 27th of February 2003, it was decided that Prof. Bruno Silvestrini should co-lead the group. The following experts, "external" to the Committee, immediately gave their generous availability to collaborate with the NBC: Prof. Rosalia Azzaro, Prof. Mario del Tacca, Prof. Antonio Leone, Prof. Luca Pani. Two hearings were made during the group's work, one with Prof. Andrea Mattevi on proteomic, on the 19th of June 2003, and one with Prof. Gerolamo Lanfranchi on bioinformatics on the 18th of November 2004. To all these colleagues go the Committee's most sincere thanks.

During the group's work, the two coordinators initially suggested the elaboration of two different documents: one on **pharmacogenetics**, a theme on which we have a considerable amount of already elaborated and fairly consolidated information, and another one on **proteomic**, a theme which instead has still uncertain boundaries and needs a more extensive study of different aspects regarding in particular bioinformatics and structural biology. However, in the following work it was decided to concentrate all energies on drawing up one single document, capable of including both of those aspects and which, starting with pharmacogenetics, would trace its evolution towards pharmacogenomics.

The document has therefore assumed the final title of *From Pharmacogenetics to Pharmacogenomics*. Having been presented to the Committee during the plenary meeting of the 21st of April 2006, it was unanimously approved. The reader will easily see how it starts with the new direction of genetic analysis, based on a thorough investigation of the genome, and integrated with the study of the interactions between genes and between genes and proteins, and how it highlights the new bioethical problems coming from the personalisation of care imposed by these new horizons of knowledge. We are, with the themes discussed in this document, facing new epistemological horizons, which cannot be reduced to slogans, however effective, like the well known *the right drug to the right patient in the right dosage*: what's at stake not only mostly escapes public opinion's understanding, but is generally ignored or not fully known by bioethicists themselves. Nevertheless these are problems that invest not only pharmacological research, but also and above all clinical practice and that, for this reason, require precise and objective ethical positions. In this document the NBC opens the way to this new thematic horizon, aware of having accomplished, once again, a well thought through pioneering work. The careful reader will notice, in addition, how the document discusses the movement between two different ideas, of relevant interest not only from a medical point of view but also from a bioethical and philosophical point of view in general. The first has been identified by the tendency to see the gene as an autonomous functional entity, able to predetermine, as well as to allow us to predict, the characteristics and behaviours which are the expression of it, whilst the second leads us to consider it as part of a complex, dynamic and highly variable system, subjected to the influence of the genome in its entirety, and of the

environment. Against this background, again emerges the problem of the boundary between those events that man is a victim of and those that he is able to influence, becoming their maker and therefore being morally responsible for them.

Prof. Francesco D'Agostino
President of the National Bioethics Committee

Synthesis and recommendations

Pharmacogenetics and pharmacogenomics have opened, studying from different and complementary points of view the genetic basis of the response to drugs, new perspectives on the personalisation of care, on the creation of drugs focused on a precise genetic target and on the production of some drugs using not only the traditional chemical synthesis, but also cells, tissues and transgenic animals. Although the response to drugs is influenced by many other factors as well, like the environment, diet, age, lifestyle, state of health or illness, there is still no doubt that the knowledge of the individual genetic characteristics can contribute to the creation of increasingly safer and more effective therapies.

From this, follow a variety of bioethical problems, which include the possible conflict between individual and collective needs and rights, the right distribution of the relative duties and benefits, the unknown outcomes and the risks of interventions which affect, in the wider sense, the course of life itself. The National Bioethics Committee has discussed this on various occasions, both in general and in particular terms, but feels the need to discuss them again in more depth in relation to pharmacological therapy.

For this reason a working group has been created, composed by internal members who are supported by external members, who, in the respect of the institutional tasks of this committee, proposes on the one hand to give a summative view of the topic so that it can be understood also by those who are not in the know, and on the other hand to give some direction for the purpose of the eventual predisposition of legislative acts too.

The first chapter aims to place pharmacogenetics and pharmacogenomics within the process that has transformed genetic in one of the great protagonists of the scientific and technological progress. With the theory of the evolution of the species, it has first outlined the existence of an overall project of life, to which man participates next to all the other living beings, and then with molecular biology has deciphered it, arriving in the end, with biotechnologies, at manipulating it and affecting its course. The relationship of man with nature, intended as a complete living system, has come up again in new terms, giving rise to a series of philosophical and religious, as well as practical, questions. In particular, the problem of the distinction between what is genetically predetermined and what takes form during the course of a life, due to the effect of the environment and of actions that, within the limit in which they are free, require a moral responsibility.

The second chapter is dedicated to the drug. With this term we intend an active ingredient, made up of substances usually able to have a variety of effects and uses dependant, as well as by their intrinsic properties, by the dosage, by the method of administration, by the circumstances and by the individual sensitivity, which is linked both to genetic constitution and to the other factors mentioned above. The term “medicinal” is instead linked to a specific therapeutic application of the drug, which can be of a preventive or a curative type. The marketing of the drug requires a registration with the health authorities and it is often connected with the acquisition of a patent, which ensures its monopoly for a certain amount of time.

Drugs act in two different but interconnected ways: either they act on the organism, giving it what’s necessary and enhancing its internal resources, or they substitute it, acting in its place. The first is typically the case for vitamins and vaccines, the second of antibiotics, of antipsychotics, of antihypertensive and, in the more general sense, of symptomatic agents. The genetic constitution has a critical role in both these types of intervention, but in different forms.

An additional distinction must be highlighted between drugs made of substances of natural origin and those made of artificial ones, synthesised for the first time by man. The

first ones are not intrinsically safer than the others, but have a history that involves a wealth of knowledge and experience capable of guiding their use. The second ones have a higher degree of uncertainty and potential dangers. Before being used they require, therefore, a rigorous and thorough trial.

An important aspect of the response to drugs is represented by the processes of pharmacokinetics and metabolism, terms which describe the absorption, the distribution in the tissues, the transformation in active or inactive metabolites and, finally, their excretion through urine, faeces, lungs, skin and other excretory organs. These processes have a genetic basis, but are also subjected to environmental factors, in particular with regards to dietary habits.

There are illnesses in which the genetic or, on the contrary, the environmental element prevails: for example, some genetic anomalies always translate in the same pathology, independently from external factors, as it happens, leaving aside the genetic constitution, in the case of vitamin deficiency and of the exposition to particularly virulent infective agents. In the majority of cases, however, genetic and environmental factors are strongly linked and they mutually affect each other. It follows that the same genetic characteristic can have, according to the environmental conditions, different implications, even opposite ones.

The chapter dedicated to the drug concludes with a mention of the pharmaceutical patent, which tends to enhance the value of the chemical innovation rather than of the therapeutic one. This problem, which, after having distorted research and the development of traditional drugs, risks to have a negative influence on biotechnological products as well, must be looked at in more depth and tackled on a legislative level.

The third chapter focuses on the genetic control of the response to drugs. After some clarifications about the meaning and the use of the terms pharmacogenetics and pharmacogenomics and some historical details, pharmacogenetics' methods of analysis are outlined in their evolution towards genomic and proteomic.

In their widest meaning, pharmacogenetics can be defined as a discipline which studies the genetic basis of the individual differences found in the response to drugs. Pharmacogenomics must not be seen as a simple updating of pharmacogenetics in relation to recent developments in the research on the human genome, but as a new approach, based on the analysis of the genetic variation extended to the whole genome in a dynamic system formed by a net of interactions between genes and proteins.

The research in genetics applied to pharmacology started at the beginning of the 1950s by Arno Motulsky and the term pharmacogenetics was introduced by Vogel in 1959. The conceptual premises, however, had already been advanced by Garrod at the beginning of the 1900s, with the studies on metabolic congenital errors and on the analysis of this illness' segregation within a family.

For pharmacological purposes, the most important stages of the following developments in biochemical and molecular genetics regard the control of the genes' activity, the introduction of DNA recombining techniques, the sequencing of genomes and the structural analysis of proteins, which marks the movement from genomic to proteomic. Between the most recent technical innovations, we mention the "microarray" of DNA and proteins, which allows the simultaneous, structural and functional analysis of a multiplicity of genes and genic products.

The genetic control of the response to drugs can be exercised at different levels: a) the drug's absorption; b) metabolism, transport and elimination; c) target's characteristics; d) adverse reactions. The genetic systems on which this control is based can have different levels of complexity, which go from the simple occurrence of the monogenic inheritance, often described as a simple Mendelian, to those made up of a variety of components that characterise the polygenic and multifactorial inheritance.

In the monogenic inheritance, genetic polymorphisms are of particular relevance, and they are represented by common mutations that can be found in at least one person in a 100, which shape differences in the genetic constitution of an individual or of a population. The minimal variation to generate a polymorphism is the change of a DNA base, which can have an effect on the function of a gene involved in the pathogenetic process or in the response to the pharmacological treatment.

The final effect on the phenotype of the genes controlling the characteristics associated to the pathology cured by a specific drug, or to the drug's metabolism, is a quantitative and qualitative variation, also in the pathological sense, of the response to the drug. The consequences can involve both the therapeutic action, as well as the negative effects, like secondary toxic reactions, intolerance and hypersensibility.

In defining the genetic basis of the response to drugs is necessary to take into account the margins of uncertainty due to a variety of conditions that can affect the validity of the genetic data. Some of the conditions to be considered are the variations of expressiveness and penetration of the genes responsible and the interactions with other genes.

An important component of the variability in the response to a drug is the environment. The environment can influence the expression of a genetic character the more strongly, the higher is the number of the genes involved. It can be presumed that, with the progress of knowledge, the genome's analysis, until now mostly focused on monogenic systems, could be extended to polygenic systems, which, although more strongly affected by the environmental component, ensure a better adaptation of the pharmacological therapy.

The first phase of the pharmacogenetics analysis consists of identifying and mapping the gene or genes which codify for the potential targets of the drug or those that are in some way involved in its activity. In the following phase we proceed to the isolation and cloning of the gene and, finally, to the DNA sequencing.

The study of the association between the variation of the genes and of the DNA and the response to drugs is made by using appropriate genetic analysis on a sample of subjects treated with the drug, who are compared to a control group (NBC, 1999).

The pharmacogenetics approach offers the double advantage of improving the treatment's efficacy with the choice of the most appropriate drug and of increasing its safety, avoiding the risk of adverse reactions.

The Human Genome Project identified new families of genes, which will be able to function as markers for the diagnosis of an increasing number of hereditary and acquired pathologies and, through their products, they will make available more targets for more and more selective drugs. The accumulation of the necessary information to define the genetic profiles needs the elaboration of data storing and management systems. This need is answered by bioinformatics, which is an area in rapid expansion and a precious work instrument.

Finally, we mention the logical development of pharmacogenetics and pharmacogenomics: genic therapy, the target of which is the gene that directs or regulates the synthesis of the proteic product.

This overall view shows the relevance and at the same time the enormous complexity of this topic. What emerges is a precise indication, in line with what previously discussed: to guarantee to pharmacogenetics and pharmacogenomics the necessary resources at every level, from basic research to practical applications, but without distracting attention from other opportunities which, if pursued in the appropriate manner, can give us more extensive and immediate benefits.

The chapter concludes with some examples which tend to further highlight the complexity of the topic. In particular, we must stress the importance of the psychological aspects, which substantially affect the outcomes of medical care.

The fourth chapter suggests a strictly bioethical reflection. We stress both the link between pharmacogenetics and pharmacogenomics, as well as the boost given to these two disciplines by the mapping and the sequencing of the human genome, remembering that the NBC has previously expressed a positive judgement on these developments, obviously for as much as they contribute to the welfare and health of man.

Although pharmacogenetics and pharmacogenomics studies have not yet led to extensive practical applications, the efforts to link the results of the pharmacological action to a person's genetic structure seems to deserve support also in relation to the unavoidable genetic singularity of each person. The judgement appears positive if we obtain – from this link – more therapeutic benefits and a reduction of the risk of adverse reactions, according to the principle of "beneficiality" and "non-harmfulness". This direction of research, however, must not lead to an exasperation of the "genetic reductionism", both as a line of thought, as well as a will to overcome the interrelations that human organisms – also in their physicality – develop with the environment.

We must identify those human illnesses which will be the primary object of research as well as scientific and economic effort. It would not be ethically right to turn our attention to pathologies for which effective and safe drugs are already available, for exclusively commercial reasons, overlooking those which have a limited epidemiological incidence, but stand out because of the high variability and uncertainty of therapeutic responses or by a high frequency of adverse reactions. The development of this branch of research can contribute, due to the characteristic of basing itself on the evaluation of very analytical parameters of living structures, to the knowledge and the correction of some imbalances, innate or acquired, existing between men with regards to their health. This development, if aimed at those who have most need of it, is inspired by principles of justice, with particular attention to the right to health care, which is one of medicine's fundamental principles.

With regards to the investigation on man, we refer to national and international criteria referring on one hand to the trial of drugs and, on the other hand, to genetic investigations, having as objective the care of both the ill patient and the healthy subject. In these conclusions, we don't deem necessary to further dwell on these criteria, either than for stressing that they concern the solidity of the scientific premises of each investigation, the evaluation of the relationship risk/benefit, free and informed consent; the care of privacy, the accurate preservation of samples and documentation, the obligation of sharing the outcomes.

From this, emerge some suggestions, which can be summarised as follows:

1. In a general sense, we stress the value of scientific and technological research as an instrument of progress and the moral obligation of ensuring adequate support for it, also in the law, in terms of human and economic resources;
2. Support of cutting-edge research must not compromise, however, the enhancement of already acquired scientific knowledge, which is simply waiting to be translated into practical applications;
3. With regards to pharmacogenetics and pharmacogenomics, genetic analysis should be considered, for those cases in which the link between genetic constitution and response to drug is certain, as one of the fundamental criteria of therapeutic choice;
4. We recommend that genetic analysis is kept in higher consideration also in drugs trials, as well as in medical and epidemiological research in general, in order to consolidate and extend knowledge in this sector;

5. We draw attention on a crucial, but little known, aspect of the patent: the excessive weight which it attributes to the chemical novelty, even to the detriment of the therapeutic benefit. This anomaly must be investigated and, eventually, corrected in the law;

6. Finally, we highlight that pharmacogenetics and pharmacogenomics are at a critical turning point in scientific and technological progress, which for the first time offers man the opportunity to directly intervene on the project of life. Consequently, it is of fundamental importance for the scientific community to promote information to the public that is not only correct, but also clear and accessible to all. Only in this way it will be possible to spread a climate of trusting and mutual collaboration, indispensable to achieve the hypothesised benefits from this important chapter of development of knowledge.

1. Preliminary considerations

After physics, genetics has asserted itself as one of the main protagonists of scientific, cognitive in general and technological progress. It has given strength to the idea that life is sustained by a unitary project in which all living beings participate, even if some have only reached the first pages, whilst man has arrived, in terms of individual and social organisation and complexity, further. It has clarified the molecular basis of this project and has allowed us to intervene in it not only indirectly, as it used to happen before, but with direct interventions, which allow us to take it apart, rebuild it, eliminate some traits and introduce others. Many foods are already produced today with GMO, which are genetically modified organisms, whilst cloning allows us to obtain genetically identical copies of living beings, even in species that put aside asexual reproduction millions of years ago, opting for sexual reproduction.

The benefits that medicine can achieve from this are evident. Genetic anomalies involved in hereditary diseases can be identified with increasing accuracy. In the case of somatic cells, they are already, in some cases, susceptible of correction. With regards to the genetic therapy of the germinal line, which would reverberate on the descendants, the National Bioethics Committee has in the past expressed the opinion that it could not be “suggested and accepted in the case of man for a variety of technical, scientific, social and, therefore, also legal and ethical reasons”; it did not exclude, in addition, that “the acquisition of new knowledge and the development of genetic engineering techniques could make it more targeted and safer” (NBC, 1991). The social, legal and ethical perplexities remain, but in the meantime the scientific and technical progress has been so fast, that it could soon be possible. In a more general sense, diagnosis and therapy, including pharmacological therapy, of all illnesses will improve.

The possible dangers are just as evident as the benefits. First of all we risk, in the enthusiasm given by the successes of research on the genome, to underestimate the pathologies linked to environmental factors and to not allow sufficient resources for the further study and control of them. We can distinguish two large classes, respectively originating from deficiency and from aggression. As these terms indicate, the first is linked to the lack of elements indispensable for the functioning of the organism: not only food, water, salt, air and sunlight, whose importance is known since ancient times, but also vitamins and other substances necessary for good health, some of which we have just begun to know the existence of. The second class depends instead on harmful agents: it includes not only infections, but also different types of tumours and acute and chronic poisoning. Often responsible for this are biological, chemical and physical agents, which, in different doses and circumstances, do not have a harmful, but a protective effect. For example, an excess of food, lipophile vitamins and the exposition to sunlight produce

damages as serious as those caused by their absence. To the previously known agents, new ones have been added, like prions and asbestos, but it is reasonable to believe that there are others, which have still not been thoroughly studied.

These pathologies are also partially affected by individual genetic constitution, but an investigation on their environmental causes would have, already in itself, a decisive influence on the population's state of health. For example, a document by the World Health Organisation states that a reduction in dietary excesses, together with the consumption of fruit and fish in substitution of animal fats, with an increase in physical exercise and the abolition of smoking could reduce cardiocirculatory illnesses by 75%, which in developed countries are the main cause of death and disability (WHO, 2002).

Unlike pathologies of environmental origin, hereditary pathologies are connected to genetic anomalies inherent to the organism from the moment of conception.

Up until now the problem has been tackled by trying to limit its spreading with genetic consultancy and prenatal diagnosis.

The developments on genetic research have opened the way to the correction of these anomalies in somatic cells and, in perspective, also germinal cells. Genetic constitution, however, often gives only one potentiality, which during a person's existence can develop into different psychophysical characteristics, at times even opposite. For example, at the basis of some mental illnesses are genetic traits apparently involved also in artistic, scientific and philosophical creativity (Jamison, 2002; Bogousslavsky and Boller, 2005). It follows that some genetic traits have positive ort negative characteristics not per se, but in relation to the environment and the circumstances. Before correcting them or erasing them forever we must study their significance in more depth and, when possible, enhance their positive aspects.

The problem of the multifactorial response to drugs is particularly evident with psychic processes, which often affect not only the illnesses' individual perception, but also its course.

There's nothing less fertile, in science, of the absolute certainties some believe to possess. If, in light of these considerations, we look at what's happened, in our century, in medicine, what we now know that we don't know can really help to understand the meaning of what we know today. In synthesis, we believe we can say that recognising the illnesses' etiological factors has become increasingly harder, the more the discovery of the recognisable ones reduced the field of the not-known. On the contrary, the knowledge of the pathogenic mechanisms, particularly those of illnesses of unknown etiology, have increased, feeding in many the hope of getting, through them, to the discovery of etiological factors. This progressive gap between the known and the unknown, regards the body's illnesses: what resists our ability to know refers essentially to etiological factors. This is a problem linked, in reality, to our ability to know, in other words, our techniques, the methods we use, and the meaning we give to the term "scientific". It is all this that we find, when we approach what we don't know, and it makes us stay in this situation.

The same complexity encountered in the use of the drug can also be found when we try to understand what this means for the patient, the doctor and the drug itself and we try to bring together all eventual acquisitions in this field, in the treatment.

In the last 20-30 years it has been highlighted, in particular in the USA, in England and also in other countries, that the importance given to "scientific" medicine, different from the non-scientific one because it is "evidence based", unlike the other one, is progressively growing. This fact currently influences the choice of treatment and it could also influence the direction of scientific research in the future.

The invitation to caution emerges also from the increasing awareness that the expression of individual genetic traits depends on the complex and variable interactions linking them to the genome's remaining part.

These considerations once again raise the problem of the relationship with the so-called “natural order of things”, intended as a whole living system, to which man is subject, as well as protagonist: subject, because he is overcome and dominated by it, protagonist because he also protects the project of life and contributes, both individually and through scientific and technological progress, to its development. In contrast to the other living beings with which he shares his part in the whole living system, man has the ability to study it, to find its fundamental laws and to extract from it the instructions and means necessary to satisfy his needs, having as guide not only instinct but reason as well.

The NBC has given its opinion, in various circumstances, on the developments and application of genetics and biotechnologies. It has done this both in a general way and with reference to specific problems, as for example genetic therapy, resources allocation, free and informed consent, trials on humans and animals, the privacy of personal data and the use of transgenic animals (NBC, 1991 a, b: 1992 a, b, c; 1993; 1994 a, b; 1995 a, b; 1997 a, b, c; 1998; 1999; 2000; 2001; 2005). However, it had not, so far, studied in sufficient depth the implications of those developments on pharmacotherapy, which, for good and bad, is one of the most important sectors of medicine.

The working group entrusted with the task of filling this gap feels the need, as a premise to ethical reflection, of illustrating the basic scientific facts: it has been proposed, in particular, on one hand to avoid unrealistic or excessive expectations, on the other hand to identify and clarify the possible applicative implications. The working group has felt the need, in context, to call attention to the big basic choices, faced by medicine, between care and prevention and, in particular, between medical interventions which mobilize and enhance the organism’s resources and those that support them externally, acting in its place.

The topic is complex, but we have tried to explain it in terms that are as much as possible accurate, but also understandable by all, including those who are not knowledgeable in this field, respecting the bioethical principle that “the obligation to share, with the authorities and the public, the correct use of resources and research outcomes has become mutual not only between science and politics, but also with regards to civil society” (Azzaro Pulvirenti, 2003).

2. The drug

Pharmacology is a discipline in rapid evolution, so it’s being articulated in increasingly specialised branches, but it is still founded on some simple notions which cannot be ignored, from whatever perspective and point of view we look at it.

.1 Definition and generalities

This is the first point we need to look at. The World Health Organisation proposes, in one of its documents (WHO, 1973), the following definition: “Any preparation able, when introduced in a living organism, to modify one or more functions”. It is a clear definition and it comes from an authoritative source, but it is so synthetic that it requires a detailed commentary, word by word.

“Preparation” can be used to describe an enormous range of substances, on their own or combined in a variety of ways, of molecular dimensions which can vary between few and thousands of units, organic or inorganic, natural or artificial. Their effects can be chemical, chemical and physical or merely physical, as in the case of some diuretics which boost, through a purely osmotic mechanism, the passing of liquids from one to another compartment of the organism.

The phrase “introduced in a living organism” presents the concept of the intrusion into the organism, which is crucial because the active ingredients of many drugs are substances ordinarily present in the organism, which regulates their concentration and their activity within fixed limits. The external introduction escapes this control, allowing us to get other effects, often drastically different from a quantitative, as well as qualitative, point of view. For example, hexogen adrenalin not only regulates heartbeat and other physiological functions, as it usually does, but it can resolve a heart attack or an anaphylactic shock, which are potentially lethal. In the same way, cortisone can have different effects from those of its endogen correspondent. Insulin, which normally regulates sugar levels, can induce a convulsive state similar to electroshock. With vitamins, the boundary between physiological and pharmacological effects is more uncertain. Generally, we consider as physiological effects those linked to their ingestion through food, and pharmacological effects those caused by a pharmaceutical preparation.

“To modify one or more functions” expresses the fundamental property of drugs, which translates in a wide variety of possible effects, due not only to their intrinsic characteristics of each drug, but also by the dosage, by way it is administered and, in a more general sense, by the methods and circumstances of use. The botulinic toxin, a poison capable of killing thousands of people in minimal quantities, is successfully used against the blepharospasm and other pathologies, as well as in cosmetics to clear facial wrinkles. 100 mg of acetylsalicylic acid, the active ingredient of the common aspirin, inhibits the aggregation of platelets and prevents thrombosis; 300-500 mg of it reduces the production of the chemical mediators of infection and alleviates headaches and other common aches (but can also erode the mucous membranes that cover the gastro-intestinal tract and other areas of the body, causing dangerous haemorrhages); 5000-8000 mg of it shows yet other effects, which allow its use in selfimmunitary pathologies, like rheumatoid arthritis. Each of these effects, desired and undesired, can involve a different biological substratum.

In conclusion, all the preparations that have the general characteristics abovementioned, can be called drugs, but assume one or more specific connotations according to how, on whom and where they are used. In particular, they become drugs when they are used “to prevent or treat human or animal diseases” (Council Directives, 1965).

.2 Two classes of drugs

Drugs usually connect to receptors located on the surface of or inside the cells, which are the link for or the site of its effects, but other and equally important processes exist. One is represented by the systems of specialised transportation, which let the drug overcome the biological barriers even when its chemical and physical characteristics would not allow it. In addition, there are effects which do not require the receptors’ intervention or specific processes. It is the case, already mentioned, of the movement of liquids due to an osmotic action.

Generally, only the drugs’ main properties are studied and exploited, although there are usually more than one. In addition, each of them causes a variety of effects, desired and undesired, which further complicate matters. We must also take into account the environmental effects on the organism, which in time can change the response to the drug. To the so-called “genetic certificate”, which suggests the prescription of drugs on the basis of individual characteristics, should therefore be added an “environmental certificate”, which is much more difficult to draw up because it is in constant, incessant evolution.

Drugs can be grouped into two fundamental classes, according to whether they support the organism externally, acting in its place, or whether they give it what it needs to function correctly and enhance its dormant properties. In the absence of a generally

accepted term, temporarily, those of the first type have been provisionally called “non-physiological” and the others are called “physiological” (Silvestrini, 1987). Non-physiological drugs are typically represented by antibiotics, analgesics and drugs used in the treatment of mental conditions and hypertension. Physiological ones are represented by vitamins and vaccines.

Physiological drugs do not require particular checks before being used because they have a correspondent in the organism’s composition and organisation. However, there are circumstances of use in which they cease to be so. This is, for instance, the case of hormones: they are physiological when they are used to cure illnesses caused by a deficiency; they aren’t, when they are used otherwise. In the second case, they require the same rigorous tests of non-physiological drugs.

A further distinction must be made between “natural” drugs, made up by substances present in the animal, vegetable and mineral world, and “unnatural” ones, also called “artificial”, which are substances synthesised for the first time by man. Chemical synthesis is not, from this point of view, relevant, because the same substance can be extracted by a natural or synthesised source, without this affecting its properties in any way. Natural drugs are not intrinsically safer than the unnatural ones. However, they have an advantage in their history which, if read carefully, gives us precious information on their therapeutic and toxic properties. If they are part of our dietary habits, the comparison between the health conditions of populations who consume them and those who don’t, gives us an indication of their effects. In this way it has been possible to identify illnesses linked to either a deficiency in particular substances, like vitamins, or to an excess of other substances, like lead and vitamins themselves.

Unnatural drugs are a recent invention. They are the fruit of human intelligence, but they are often created in laboratories that are far removed from the real conditions of medicine and life in general. Although the trials to which they are subjected before being used are severe, they always present a certain risk. Therefore, between the non-physiological drugs it would be wise to give precedence to the natural ones: unfortunately this simple rule is often not followed, also for patent reasons which will be discussed in point 5,

The drug is a material entity, with physical and chemical properties that explain its effects and allow its qualitative and quantitative determination. The functions of the organism are, however, influenced also by factors that available knowledge and technology let use measure only indirectly, through their effects. This is the case in psychotherapy and with regards to psychological factors in general, which can affect the course of the illness as much as and at times more than drugs. For example, rigorous clinical studies, carried out in “double blindness” conditions, show that not even the strongest antidepressant is effective for more than 6 patients out of 10, against a response to a placebo which rarely goes below the threshold of 3 patients out of 10. It can be inferred that, on the basis of a simple mathematical calculation, a pharmacological therapy carried out indiscriminately on all those suffering from depression would be useless, as well as potentially harmful, in 7 patients out of 10: in 4 because they do not gain any benefit from it and in 3 because a little psychological help would suffice, like the patient’s participation in a clinical trial, to allow him/her to overcome the morbid episode. Depression affects the mind, but these considerations also apply to physical illnesses. A personalised medical assistance would be preferable to a simply pharmacological one, but it is difficult and not all doctors are able to carry it out. Any reasoning about pharmacogenetics and pharmacogenomics that did not take into account these psychological implications would be incomplete.

.3 Two classes of illnesses

In the preliminary considerations we have mentioned the existence of two big classes of illnesses: mostly environmental, which are due to external causes, and mostly hereditary, which are inherent to the organism from the moment of conception. The first is subdivided in deficient and aggressive. The topic deserves to be revisited, referring it more explicitly to the opportunities intrinsic to the recent developments in genetics.

Environmental illnesses of the deficiency type are caused by the lack of substances indispensible for the functioning of the body: not only food, water, salt, air and sunlight, the importance of which has been known since ancient times, but also vitamins and other essential substances. We are just beginning to glimpse the importance of some of them. For example, recent epidemiological investigations suggest the protective role of some polyunsaturated acids found in food, called Omega-3, not only in some cardiocirculatory pathologies, but also in auto-degenerative and selfimmunitary ones, (Barberger-Gateau et al., 2002). Environmental illnesses of the aggressive type include infections, even prionic ones, as well as some forms of tumours and a variety of degenerative pathologies. The substances that support them are often the same ones that, in different quantities and circumstances, have a protective effect: this is the case of food, kitchen salt, vitamins A and D and the exposition to sunlight, as an excess of those is as fatal as their absence.

Medicine successfully fights against environmental illnesses since ancient times, when their causes were still unknown. It has succeeded in this task especially by protecting man by dangers taking hygienic and sanitary measures. A clear example of this can be found in the ruins of the biggest civilizations that have followed each other on the face of the Earth: sewage systems, aqueducts, canals for the flow of stagnant water, baths, rules for a healthy living. We must think, for example, about the Cloaca Massima in ancient Rome and about its aqueducts, the ruins of which still dominate many landscapes. In addition, we must reflect on the prohibition, recurrent in many cultures, of eating pork and molluscs, which can transmit dangerous diseases like cysticercoids, typhus and cholera. A similar measure is the boiling of coffee and tea, which is a simple and effective way of sterilising drinks, at the same time improving their taste. The same can be said about daily ablutions, imposed by some religions and carried out even in the desert, where water is precious. Even the religious norm of periodical fasting probably has a therapeutic value, which deserves a more in depth study.

As well as with hygienic and sanitary measures, environmental diseases have been fought using three categories of remedies, which act directly on the organism. The first includes invasive and non-invasive manual interventions, like surgery, medication of injuries, immobilisation of bone fractures, assistance during birth. A great amount of evidence of this can be found both in mummy skeletons and in written texts, like the Hippocratic Oath, which mentions the removal of stones. The second category involves psychological assistance to patients, which implies not only a relief of the symptoms, but also a stirring of dormant internal resources, capable of preventing illnesses or, if they manifest themselves, to concretely affect their course. The third category, which we will look at, includes drugs intended in the general meaning of this term, but used as medicines.

Their first traces go back to prehistoric settlements, where medicinal plants were stored in special areas, separate from the spaces destined to food. With few exceptions, like raw liver used by Hippocrates to cure exophthalmia and rickets, their effects remained of a symptomatic type until relatively recent times. The change happened with the first great, modern physiological remedies, represented respectively by vitamins and vaccines, which act strengthening the organism and mobilitating its dormant defences.

The biggest amount of the modern therapeutic arsenal, however, is made up of non-physiological drugs, represented in part by active ingredients extracted from traditional

remedies, in part by artificial molecules, that is, recently synthesised molecules. Some have merely symptomatic effects, like morphine, or at least effects limited to the manifestation of the illness, like hypertensive drugs and drugs used to cure mental illnesses, other intervene on the causes of illnesses, like anti-infective chemotherapy and antibiotics. Their common characteristic, the one that sets them apart form physiological drugs, is the newness of their composition and, in large part, of the functioning on the organism. As a consequence, they are distinguished by uncertainties and evident dangers, especially in the case of drugs that lack a natural history, from which to take information about their desired and undesired properties. After the thalidomide tragedy those drugs undergo preventive, severe trials, which however never fully guarantee their safety.

The knowledge of individual genetic characteristics will allow the improvement of both the efficacy and the safety of the entire arsenal available for the fight against environmental illnesses. Drugs will be personalised, adapting the dosage to the needs and to the response of each of us. It will also improve safety, because it will be possible to assess the risk of the undesired effects which have a genetic basis. New perspectives are opening also in drug research, particularly with regards to those drugs that can be used against infections and with regards to the two sides of their intervention: on the one hand against viruses, bacteria and protozoon, on the other hand in favour of the organism defensive mechanisms.

It would be a serious error to proceed without a strategy which takes into account the complexity of the problem, with particular regard to the difference between hygienic-sanitary measures and interventions on the organism, between care and prevention, between physiological and non-physiological, natural and artificial drugs. Without this strategy we risk not only wasting precious resources, but also to feed disillusion and scepticism, which would prevent us getting the benefits inherent to genetics and to its contemporary developments.

These considerations are applicable also to the treatment of the symptoms and, in a more general sense, of the manifestations of hereditary diseases, present in the organism from the moment of conception. Their eradication does not require pharmacological measures, which must be pursued with the caution dictated by an awareness of their biological significance. In fact, even those illnesses which cause disabilities capable of jeopardizing the quality and the duration of life, have merits that have remained in the course of natural selection because, in particular environmental circumstances or conditions, are useful for survival.

Caution is even more important in the case of hereditary diseases linked to genetic abnormalities that can have, according to environmental circumstances or conditions, favourable or unfavourable expressions. A case that illustrates this, but we could mention many others, is the one of the Pima Indians, from Arizona. For thousands of years they have lived as nomads, feeding on fruit, acorns and seeds, the availability of which is subject to strong variations from season to season, as well as from place to place. Therefore, those individuals having a particular gene, called “economiser”, have been naturally selected, as it allows the most efficient use of food and the storage, in the form of adipose deposits, of the excess energy. This ability has enabled the survival of the Pima’s ancestors, but it has become counterproductive with the overabundance of food. The same population, which in the past was in great physical form, today is exposed to obesity and to the diseases that it brings, starting with diabetes. The knowledge of these types of genetic characteristics is of the outmost importance in establishing the necessary amount of food and other essential elements on the basis of individual needs. Pharmacological problems are therefore connected to dietary ones.

In synthesis, we can state that, whilst some genetic traits have an undisputed pathological connotation, others have positive and negative implications which must be

carefully evaluated. This notion too, belongs to the wealth of fundamental knowledge which not only pharmacological research, but also genetic research, cannot leave out of consideration, whatever is the theoretical or applicative, ethical or merely technical perspective from which we look at it.

.4 Pharmacokinetics and metabolism

To achieve the desired effect, a drug must be present in its place of action in the appropriate concentration which, if the dosage and the method of administration are the same, depends on the quantity and the speed of absorption, on the tissue distribution, on the relationship between the free quota and the one linked to the proteins in the blood, on the transformation in active or inactive metabolites, on the excretion through urine, faeces, lungs or skin. All these processes have a genetic basis, which determines their quality and quantity. For example, some people transform certain drugs in inactive metabolites, others do not have this ability: consequently, a dosage that is therapeutic for the first ones can be toxic for the second ones. However, the response to a drug also depends on environmental factors, which can modify it substantially through the course of its existence.

To understand the importance of this last phenomenon we must go back to the abovementioned notion according to which the drug always represents, even if it is made up of physiological active ingredients, a true “intrusion”, to which the organism reacts with a biochemical and functional counter-adaptation. The first finds its most common expression in the “enzymatic induction”, which consists of an overproduction of the enzymes responsible for the drug’s metabolic transformation. If this transformation translates into an appearance of inactive metabolites, the drug progressively loses it efficacy. In the case of poisons this phenomenon is called mithridatism, from the name of an ancient king who, although he did not have any scientific knowledge, he used science to protect himself from enemies. On the contrary, if the abovementioned transformation translates into the appearance of active metabolites, the drug becomes progressively more active, until it reaches toxic levels. These same processes, respectively of metabolic activation or inactivation, happen also with food, some of which activates the same enzymatic systems as drugs. Therefore, regardless of the genetic basis, the response to drugs can change with time not only as an effect of their previous administration, but also according to dietary habits.

The functional counter-adaptation is instead linked to the activation of systems which have physiological effects opposite to those of the drug: for example, excitement versus sedation, hyperalgesia versus analgesia, bradycardia versus tachycardia, etc. This phenomenon happens with many drugs, but in this way they progressively lose their efficacy, although this is particularly evident with drugs used in the treatment of mental conditions, the effects of which have a mental aspect, allowing their conscious recognition. Therefore, this is typical of the so-called illegal drugs, or abused substances, and it explains addiction, dependence and, when suspended, abstinence (Silvestrini, 2001).

Consequently, more than the response to the drug, what are genetically predetermined are the processes that sustain it, but in the form of a potentiality that can expresses itself in a variety of ways during our lifetime, even ways that are opposite compared to the initial ones, as an effect of both preexistent pharmacological therapies and diet, as well as other environmental factors.

.5 The patent

The patent is the administrative certificate of the paternity of an invention and the right to benefit from it, in the respect of the rules of civil cohabitation. Its ethical relevance is mostly linked to the influence it has on the choices of scientific research.

The patent protects a concrete good, which is such because it satisfies a just as concrete need. Its object combines two elements which interlink and support each other: the abstract idea that is its basis and the material instrument that translates it into practice. The invention can reside in one or the other, but it has a patentable value in the second.

The pharmaceutical patent must be put in this frame, introducing to it a visible anomaly. The problem started in the 1800s, when scientists, who before simply extracted drugs from natural sources or reproduced them maintaining their original characteristics unchanged, began to create them ex novo. Following this, artificial molecules have multiplied and they have become part of the composition of the majority of objects of daily use, but at the beginning they were seen as an extraordinary enterprise, linked to creative abilities previously considered to be a divine prerogative. They have therefore given fame and prestige to those who created them and have been thought deserving of a special patent, called “of product”, which highlights the artificial molecule, independently from its practical value. There are also other pharmaceutical patents, like those “of use and procedure”, but they are weaker than the one of product and can be easily overcome.

The abovementioned anomaly means that the chemical novelty has become the main objective of pharmaceutical research, even disregarding the patient’s needs. The proliferation of the so-called “me-too”, repetitive drugs that have taken away a great amount of human and economic resources to other uses potentially more valuable, has its origin largely in this patenting anomaly. In addition artificial molecules are full of unknowns and dangers because, unlike the natural ones, do not have a history able, if carefully read, to guide their uses. As we have previously said, the thalidomide tragedy has pushed health authorities to impose toxicological texts increasingly more severe, which have slowed down the flux of artificial molecules re-evaluating, at the same time, the knowledge and the practical opportunities offered by nature.

The teachings we gain from this can be applied to any type of patent, including the biotechnological patent, as well as to scientific research in general: the value of human invention resides first of all in the benefits that can be achieved. The hope is that this lesson comes from reason, without waiting for it to be imposed by the brutal force of events, and that it translates in legal measures that change the current patenting system.

The extension to the biotechnology sector of the patenting monopoly, conceived and tested in accordance to the traditional industrial field, raises delicate legal problems. On this point, it has great importance to establish if biotechnological patents should be authorised following the classical model of the patenting discipline, according to which the inventor can claim the exclusive right to exploit all the possible future uses of the patented invention, or if the scope of the patent should be limited so that only the use declared in the patent can be claimed (“protection based on purpose”).

The need to adapt the invention patent’s traditional scheme to the specificities of biotechnological invention, in line with the objective of recognising to the patent’s owner a monopoly that does not exceed the real contribution of knowledge he/she has given to society, which would go beyond the function of the patenting institution, is felt especially with reference to: a) the intrinsic characteristics of the patentable matter, which is the biological element, living and self-replicating; b) the effect of blocking research, which would come from the possibility of stating, in the patent’s request, the characteristics of the biotechnological invention (for example, the physical, chemical or biological properties of a new microorganism) through a general formula, capable of understanding the multiplicity of applicative variations still unknown to the inventor him/herself and therefore susceptible of hindering future research and experimentation.

European law has accepted, with the directive of the European Parliament and of the Council n. 98/44 on the legal protection of the biotechnological invention, a solution of compromise, which, as such, seems at times to be contradictory and appears, in any case,

perfectible, to both the supporters and the detractors of the biotechnological patent . In fact, reading it for the first time, the object of the European monopoly on biotechnical invention seems to include only the procedures or products concretely developed by the depository of the patent's request, whilst future experimentation and, with it, the possibility of obtaining other patents of invention, should still be free and licit. In other words, the European directive seems to aim, first of all, to sanctioning exclusively the unauthorised marketing of the procedure or the product containing the patented biological element and not also the research of new uses of already known biological elements. This is the perspective of art. 8 of the directive, which however extends the protection given to the inventor through the patenting monopoly "to all the biological materials deriving from the patented one and having the same properties." Similarly, with regards to the invention's description, art. 13 disciplines in detail the request procedures for a patent and for accessing deposited material, simply establishing, however, that the industrial application for which the patent is requested must be concretely illustrated in the request.

Despite the caution of the European legislator, according to an approach that we could today call "bio-politically correct", numerous doubts about the real scope of directive n. 98/44 have been put forward in the past, especially with regards to the application of biotechnologies on man. We must remember, in fact, that art. 5 of the European directive establishes the absolute prohibition of patenting the human body in the various stages of its constitution and development, and of the mere discovery of its elements, including the partial sequence of a gene. However, this prohibition must be interpreted together with par. 2 of the same directive, in which is stated that an "isolated element of the human body", or otherwise produced through a technical procedure, including the partial sequence of a gene, can be a patentable invention even if the structure of such element is identical to that of a natural element. This specific, problematic aspect, has been recently revisited by the European Parliament, in their decision concerning patents of biological inventions, adopted on the 25th of October 2005, and it stated that "the directive allows the patenting of the human DNA only in relation to one function, but… it is not clear whether the field of the patent's application is limited to the aforementioned function or whether it can extend to other functions". Recalling in particular the patent given by the European Patenting Office with regards to the selection methods for human germinal cells , the Parliament invited this Office to "give patents on human DNA only in the presence of a concrete application and to limit the patent of invention to such application, so that other users can use and patent the same DNA sequence for other applications (protection based on purpose)". Finally the Parliament, after stressing that "no consideration regarding research can be more important than that of human dignity", invited the Commission to study if the interpretation of the directive founded on the so-called protection of purpose could be pursued through a recommendation to the member States or if it is necessary to amend art. 5 of the directive .

3. Genetics and drugs

.1 Definition and historical background

A variety of definitions of "pharmacogenetics" and "pharmacogenomics" can be found in literature. On the first there seems to be widespread consensus: "pharmacogenetics is the study of the effects of the genetic variations in the individual response to drugs, including safety, efficacy and interactions between drugs". As such, pharmacogenetics is aimed at developing personalised therapies.

However, there is no agreement on the definition of "pharmacogenomics". Some interpret it simply as an operative evolution of pharmacogenetics because of the

developments due especially to DNA sequencing, and therefore they define it as “the study of the genome and of its products (including the RNA and proteins) because such study is connected to the discovery and development of new drugs” (Pharmacogenetics Working Group). Others instead, identify a conceptual difference in comparison to pharmacogenetics: the source of the variations due to the response to drugs studied by pharmacogenetics is of a “structural” type and therefore it is an individual’s static and global characteristic, whilst pharmacogenomics studies a second source of variation which is “functional”, that is, linked to the expression of the genes in the cells and tissues. Whilst the first source is not specific to tissue, the second source is specific to tissue and therefore it is a dynamic and changeable variability factor, in response to endogen and hexogen stimuli (Consortium on Pharmacogenetics).

More in general, pharmacogenetics can be defined as a discipline “looking at the genetic basis of individual differences in the response to drugs”, whilst it is pharmacogenomics that has the task of transferring the new knowledge on the human genome to research, both in order to discover and develop new drugs and to identify new therapies. However, even in recent studies, pharmacogenetics is interpreted in both ways. The two terms in reality are not interchangeable, because pharmacogenomics is not only an updating of pharmacogenetics to the most recent advancements in the knowledge and analysis techniques of the structure and organisation of the human genome, but represents also a new approach to the study of genetic variations associated to the response to drugs. The analysis is extended to the whole genome and is carried out in a dynamic system formed by a net of interactions of genes and proteins functions. In this way, it is possible to obtain a better resolution of the targets and to further personalise treatments, adapting them to individual genetic characteristics. In addition, pharmacogenomics allows us to create drugs and treatments that are entirely new, starting with genic “constructions” of human origin, produced with genetic engineering techniques and inserted in guest cells of microorganisms or animals in an in vitro culture for the synthesis of proteins with pharmacological activity. This way, a new generation of re-combined drugs is born. In addition, chemical synthesis will be increasingly substituted by biological synthesis.

Pharmacogenetics is a sector of research since the 1950s with Arno Motulsky, who identified in genetic variations the origin of the individual differences in the response to pharmacological treatment. The term was introduced in medicine by F. Vogel in 1959, as a science that looks into the genetic basis of the variability in the response to drugs. However, the conceptual premises were expressed by Garrod who, at the beginning of the 1890s, with his essay on congenital metabolic errors and on the analysis of the segregation of such defects in certain families, founded human genetics.

Garrod guessed the existence of chains of biochemical reactions in the biotransformation of precursors and intermediates of the final products of metabolism and hinted that the different responses to medicinal substances and to infective agents could be linked to individual specificities in these processes. These studies of a mostly speculative character, inspired to the criteria of classic genetic analysis, opened the way to more experimental disciplines like biological chemistry, molecular biology and pharmacology. Important stages in the further developments of molecular genetics with regards to pharmacology has been the research on the genetic control of biosynthesis, which took us to the formulation of the hypothesis “a gene – an enzyme”, at the beginning of the 1940s; the definition of the DNA structure, the deciphering of the genetic code and the elaboration of the models of the genes’ regulating activity in the 50s and 60s; the introduction of the re-combined DNA techniques, thanks to the discovery of restriction enzymes and the finalisation of the techniques of DNA sequencing, in the 70s; the production of re-combined drugs in bacteria and the operative project for the determination

of the sequence of the entire human genome, in the 80s.One of the most recent technical innovations is the DNA microarray, which allows the simultaneous analysis of increasingly extensive series of genes in order to identify their mutations and to characterise their expression within different tissues, in normal and pathological conditions. From the analysis of genes and their interactions to that of proteins: the so-called “post-genomic” era, which will concentrate on proteomic, has started.

.2 Genetic control of the response to drugs

Individual differences in the response to drugs are commonly found in therapeutic practice and they can be attributed to a variety of factors, which are for the most part uncontrollable. The rapid progresses in molecular genetic analysis, cytological and formal, have allowed us to greatly reduce this margin of imponderability, leading to the identification of the components that are qualitatively and quantitatively definable. The genetic control of the response to drugs can be exercised at different levels: a) drug absorption, b) metabolism, transport and elimination, c) target characteristics, d) adverse reactions. The target definition is an essential phase in the creation of therapeutic instruments that are increasingly focused and effective. Genetic systems on which this control is carried out can have various degrees of complexity, which go from the simplest situation of the monogenetic inheritance, often defined as simple Mendelian, to systems made up of a variety of components, which characterises polygenetic inheritance. In the monogenetic or oligogenic inheritance, of particular relevance are the genetic polymorphisms, which shape situations of diversity in the genetic constitution of individuals or populations that can be characterised also as ethnical groups. What we define as polymorphic is a character and the gene that determines it, when that same gene presents itself in different variants with a frequency that significantly exceeds that of the rate of spontaneous mutation. For many genes this frequency is arbitrarily fixed to 1%. In a wider sense, the term polymorphism is applied to any variant DNA sequence.

We can estimate the possible differences in the DNA sequence of two individuals chosen randomly in the population, to 2-3 millions. The minimal variation that can generate a polymorphism is the change in a DNA base. Single nucleotides polymorphisms, the so-called SNP, are a systematic find extended to the whole genome, with the accumulation of sequencing data. The majority of SNP is devoid of genetic effect. The substitution of a single nucleotide can happen inside a gene of a codifying or non-codifying region, it is a regulating sequence near or far from the gene or outside the gene, in a non-codifying region. It must be remembered that more than 80% of the genome is made up by this last type of sequences formed by low or high repetition elements, assembled in arrangements of various dimensions. When the change happens in a sequence inside the codifying region of the gene, it can lead to the synthesis of abnormal proteins, whilst when it happens in a regulating sequence we have a variation in the quantity of proteins produced, with consequent imbalances in the function, very often defective. In this way, alterations in the DNA sequence can cause the loss or the change of the normal activity of a gene that manages the synthesis of a protein, directly or indirectly involved in the pathological process sensitive to the drug, in the mechanism of action, in the metabolism or in the transportation of the drug.

Polymorphisms for single nucleotides, when they manifest an effect, are often associated to an alteration in the activity of a protein. More extensive and complex forms of genetic variation that create polymorphisms are the deletions, that is, the removal of one or few nucleotides in the DNA sequence, duplications, that is, the addition of one or more supernumerary copies of a DNA segment, the repetition of short sequences of DNA in tandem in a variable number (VNTR), chromosomal micro-rearrangements. More

extensive chromosomal rearrangements and variations in the number of chromosomes generally are part of the chromosomal pathology.

A sequence variation that has no effect on the structure and function of a gene or of a regulating element involved in the response to a drug or in the genesis of an illness sensitive to a drug, can be equally instrumental in pharmacogenetics analysis as it can be used as marker. Its physical association with the genic variant, in fact, allows us to locate it and to study its hereditary transmission.

The final effect on the phenotype of genes that control characteristics associated to the pathology cured by a specific drug or characteristics linked to the metabolism of the drug, will be a reduction to a different level, until the complete absence of the response or an altered response. The manifestation regards both therapeutic effect, and adverse effects, like secondary toxic reactions, intolerance and hypersensitivity.

As typical examples of genetic polymorphisms influencing the drug's action, the ones regarding anti-cancer drugs must be mentioned. It has been proven that the genetic constitution of both the tumour and the patient, can influence the outcome of a pharmacological treatment. Therefore the maximum efficacy of an anti-cancer drug requires that it is made to fit not only the particular tumour and the phase of its development but also the individual genotype. The activity of enzymes degrading antiblastic compounds like 6-mercaptopurine, 6-tioguanine and 5-fluorouacile is extremely variable because of a variety of genic mutations; the consequent enzymatic deficit can determine serious systematic toxicities. This explains the close associations between genotypic variants for the drugs' metabolism and adverse reactions. A further element of complexity is given by the interaction between genes regulating the progression of the neoplastic disease and genes that modulate the effect of the anti-cancer drug.

In defining the genetic basis of the diversity in the response to a drug treatment, it is necessary to take into account the boundaries of uncertainty due to a series of conditions that can affect the validity of the genetic data. We must first of all consider that the correspondence genotype-phenotype is not a constant relationship: the lack of correlation can be due to variations in the expression and penetration of the genes that control functions inherent to the illness, the drug's operating mechanism or its metabolism. The expression measures the intensity of the character under analysis, whilst the penetration indicates the frequency with which the genic variant present in the individual manifests itself. Variables to be considered are the operating methods and times of the genes involved. We know genes variants which increase the probability of the onset of an illness or the sensitivity to pathogen agents; an important source of variation can be the beginning of the illness, which can be early or late. Another element of interference is the interaction of the genes identified as responsible for the pharmacological response with other genes, the allelic forms of which can vary from individual to individual. These interferences strongly limit the classic pharmacogenetic analysis confined to one or few genes directly involved in the response to drugs. In pharmacogenomics this limitation is considerably reduced, as the analysis can be carried out on an extensive amount of genes at the same time, since it studies their expression.

An important component of the variability in the response to drugs, is the environment.

The correlation genotype-phenotype and the dynamics of populations of characters with regard to their sensitivity to the drug's therapeutic and toxic effects, must be considered from a flexible, Darwinian point of view. This must take into account some effects that in the conventional evolutionist models have been often neglected. One of the most important ones is the so-called "niche construction", according to which living organisms, and the human species is no exception, not only can adapt to different environments, mostly through mutation and selection, but in part they also contribute to

their creation. Darwin himself realised that organisms can change the environment in a way that can affect their evolution, creating new selective pressures. In this perspective the link between organisms and environment is a two way relationship. The notion that the genes' action is outsides the organism's confines, has been referred to using the term "extended phenotype". This concept is supported by that of "phenotypic plasticity". There are many examples of niche constructions in animal species and especially in man. Culture must be considered as a niche that man constantly changes, whilst at the same time enduring its effects. Diet and the use of drugs are subjected to these feedback mechanisms (retroaction). A typical case is that of the tolerance to lactose in the adult, developed in European populations in the course of a few thousand years, which has followed from the "cultural" practice of consuming cow milk. These considerations highlight the difficulties in the use of genotypic data in the choices linked to pharmacological therapies.

Easily identifiable environmental factors are the interactions with other drugs, health conditions and the patient's lifestyle. The higher is the multiplicity of genes involved, the more the environment can affect the manifestation of a genetic character. A typical polygenic arrangement is formed by a higher number of genes of the same type with addictive action or of a different type, all concurring in determining the character; the inheritance of quantitative type causes a constant variation. The analysis of polygenic analysis is complex and genotypisation is problematic, as it cannot be done directly from known data, but through elaborate statistical methods. In the studies on the genetic determinism of the response to drugs, until now attention has been focused on monogenic and oligogenic systems. But it can be presumed that with the development of knowledge on the genome, analysis can be extended also to polygenic systems, with the consequent increase of selected classes of patients that can be subjected to the treatment and a better adjustment to pharmacological therapy.

.3 Methods of genetic analysis

The first phase of pharmacogenetic analysis consists in identifying and mapping the gene or the genes that are the potential targets of the drug or are in some way involved in controlling their activity. The conventional method identifies the phenotypic variation and from this goes back to the genic product and to the gene responsible. This is then located on the chromosomes through cytogenetic analysis techniques, formal genetic analysis, familiar studies and molecular analysis. With the same techniques, mutants characterisation can be carried out. In a following phase we proceed to the gene's isolation and cloning and finally to DNA sequencing. Data and materials extracted from these analyses are essential to the execution of pharmacological tests.

Genetic variations that can be inherited certainly have a determining role in human pathology. Family history is one of the most important risk factors for most illnesses, from cardiovascular ones, to cancer, to obesity, to autoimmune forms, to psychiatric disorders and to diabetes, just to mention the ones with the highest socio-sanitary effect. The identification of the "illness" genes and of their variants is an essential phase in the creation of preventive, diagnostic and therapeutic measures.

Until now, we have identified more than a thousand genes responsible for simple Mendelian hereditary illnesses, which are relatively rare, in which the genetic component is prevalent: In these cases the variation of a single gene is necessary and sufficient cause for the pathology's development. But the majority of common illnesses are due to the combined effect of a number of variations of the DNA sequence, to which must be added the interaction with environmental factors. Genetic studies on this type of illnesses are based on the investigation of families and populations. These methods' limitations depend

essentially on the reduced analysis capabilities, when the genetic component has a low incidence, and from the restrictions of the DNA regions that can be explored for the sequence variations. A complete analysis of an illness genetic determinism would involve the examination of all the genetic differences of a large sample of affected individuals and of control. This can be achieved only with the sequencing of the entire genome of each individual. An approximation to this theoretic objective can be a systematic analysis of all the known genetic variables extended to the whole genome, in different populations, to establish their role in the onset of illnesses. These studies are based on the connection between DNA's polymorphic variants, for single nucleotides (SNP) with specific alleles (alternative forms of specific genes). A particular combination of alleles along the chromosomes is called haplotype.

An international project finalised to the constitution of a data bank on the sequence variations generally found in the human genome, was undertaken in 2002 by a Consortium called HapMap (Haplotype Map). The aim was to give information that could guide the study of the genetic basis of illness. Recent is the publication, by this Consortium, of the data relative to a million polymorphic variants for single nucleotides, on a sample of 269 individuals belonging to four populations. As well as representing an inestimable resource for medical genetic and for the study of the structure, the function and the evolution of the human genome, with particular attention to recombining processes, the map of human haplotypes can be an extremely useful instrument for pharmacogenomics, as it can accelerate the development of the knowledge of those genome variations that determine the differences in the response to drugs.

The gene's identification and localisation in its variant forms can be achieved even without knowing the protein and the physiological mechanism involved, using the method called of inverse genetics, which "infers" the protein of the DNA sequence, once it has been located on the chromosome. The study of the association between genes variations and DNA and differences in the response to drugs, is done using appropriate genetic tests on sections of individuals treated with drugs, compared with control samples. This allows us to assign to a certain genetic constitution, within defined confidence boundaries, the type of response expected in therapeutic treatment. For a complete description of these tests' typology, of the conditions and the application criteria and of the related bioethical problems, we refer you to the NBC's document "Bioethical Trends in Genetic Tests" (NBC, 1999).

Pharmacogenetics' final objective is the use of knowledge, of methodologies and of genetic data to improve drugs' safety and efficacy. With regards to safety, the pharmacogenetics approach offers the advantage of avoiding inappropriate therapeutic treatments that can present risks for the patient due mainly to adverse reactions. Efficacy can be improved in two ways: a) with the choice of the most appropriate drug in use and with the planning of new drugs, operating on the basis of the data on the patient's genetic characteristics and on the illness's genetic variants; b) with the drug's prescription and dosage adjusted to the different metabolic capabilities linked to the patient's genetic constitution.

Genomics has added a new dimension to pharmacogenetic research. The genome project has allowed us to identify a great number of new genes, of which however we still don't know the function. Particular interest is therefore directed to functional genomic and especially to the techniques of genic expression profiles, through DNA microarray and proteic pattern analysis. The study of transcription profiles can be done through different types of comparisons: affected cells and normal cells, cells treated with drugs and non-treated cells, cells that respond to therapy and resistant cells. Experimentation can be conducted both in vitro, on cellular cultures, and in vitro in the patient's clinical trials. The transcriptoma's analysis with microarray allows us to classify genes in distinct groups that

contain already known genes and new genes. Very often the genes in these groups, co-regulated, share a specific biological function essential in the operating mechanism or in the transformation of a drug. Only the genome's systematic analysis can allow this type of studies, which are precluded or at least limited by classic pharmacogenetics. Protein analysis can use proteic microarray, mass spectrometry, nuclear magnetic resonance and, for structural studies, methods of X rays refraction. The definition of the exact three-dimensional configuration of a protein allows us to find the point in which the action of a drug could activate or deactivate the function.

Complementary technologies, which are part of functional genomic and proteomic, allow the simultaneous study of thousands of genes and proteins and the resolution of their structure with the possibility of recognizing markers for the diagnosis of an growing number of hereditary and acquired pathologies and of identifying new targets for treatment that includes increasingly selective drugs.

A recent estimate based on the complete sequencing of the human genome fixes to about 32,000 the number of human genes, a bit more that twice the amount of genes of the Drosophila melanogaster midge. Extrapolating data recently obtained in mice, this number could shrink further to 20,000, whilst it would be about 10 times higher than the number of a RNA transcript.

It is evident that the functional evolution of proteins in superior eukaryotes is more the result of a combinatory diversification of regulation nets than of a proportional increase in the number of genes. Understanding the connections between proteins and between genes within the net of cellular signals is a necessary premise for the selection of drugs' targets and this is the principle challenge awaiting pharmacogenomics.

The completion of the human genome sequence has created the conditions for the development of highly specific drugs, adapted to each patient's genetically determined individual characteristics.

Structural biology is one of the vanguard disciplines in the sector of molecular biology. In particular, bio-crystallography is, par excellence, experimental methodology that allows us to discover the disposition in space (in other words, the structure) of atoms constituting biological macromolecules, whether they are proteins, DNA, sugars or even an entire virus. Crystallographic analysis through X ray diffraction can be compared to a special form of microscopy. In the case of the most familiar optical microscopy, the sample (for example a cell) is illuminated with electromagnetic radiation, in the band of the visible, to wavelengths (350-800 nm) appropriate to resolving its fine particulars at the microscopic levels (the nucleus of a cell has typical dimension of about 500 nm). In the same way X rays, belonging to the electromagnetic spectrum with wavelengths near 0,1 nm, allow us to separately observe (resolve) the single atoms of a macromolecule, identifying their position with a precision of 0,01 nm in the context of the three-dimensional structure, for example of an enzyme. The potentiality of these methods within biological research, to study the structure and functionality of proteins, has been known since the 1930s. However, the lack of appropriate experimental support has limited its effective development until the beginning of the 1980s, when progress in biochemical and bio-molecular methodologies have provided analysable samples in significant quantities (tens of hundreds of mg) and to a high level of purity. In fact, these are essential conditions for the growth of proteic crystals (or nucleic acid) of dimensions and quality appropriate to bio-crystallographic investigations.

The attraction of bio-crystallography consists in its ability to reveal the extraordinary complexity of biological macromolecules. They are involved in all chemical reactions within living organisms. These reactions span from metabolism linked to energy production, to reactions causing the development of muscular force; from giving the ability of vision, to the mechanisms of immunitary defence; from the reproductive processes, to processes

connected to cerebral and nervous activity. Bio-crystallographic study of biological macromolecules, and of proteins in particular, has revealed the extreme complexity of these molecules, which have a multiplicity of functions associated with a strong structural variability. The knowledge of the functioning structure and mechanism of biological macromolecules does not have only a value in itself, as scientific knowledge. In fact, because of the central role of the molecules that are the object of bio-crystallographic study, this knowledge has an enormous applicative potential. It is not by chance that the "colossuses" of the chemical and pharmaceutical industry finance research groups in the bio-crystallographic field. The study of the structure of biological macromolecules can allow the development of compounds that change these molecules' functionality. These compounds can be used as drugs. The famous "cocktail" of drugs currently used in the cure of AIDS was born, in part at least, by the bio-crystallographic study of the proteins produced by the virus. In addition, knowledge of the structure can allow the use of proteins useful to chemical synthesis and to the production of substances that are of interest to industry. In the same way, currently in the developmental phase are technologies that allow the removal from the environment of noxious chemical compounds through the use of enzymatic proteins, capable of making such compound innocuous (the so-called "bioremediation").

In this context, structural genomic constitutes a new area of research within structural biology. It has the objective of studying the structure of biological macromolecules, proteins in particular, with semi-automated methods. In other words, to classic genomic, which led to the reading of the whole DNA of various living organisms (including man, the so-called "Genome Program"), is now added the possibility of deciphering the three-dimensional atomic structure of proteins codified from genes, the sequence of which has been obtained by genomic studies. Structural genomic is a worldwide initiative that however sees Japan and United States at the cutting-edge. Last year, the "National Institute of General Medical Sciences" (NIGMS) financed 7 pilot centres, the activity of which will extend for ten years. In the first five years, their aim is to define the technologies for the automation of crystallographic methodologies. In the second five years, it is foreseen that the use of the developed technologies will allow the determination of the three-dimensional atomic structure of literally thousands of human proteins and of other organisms, some of which are pathogens (for example the Mycobacterium tuberculosis, the etiological agent of TBC).

The pharmacogenomics approach does not only consent the personalisation of therapy but allows us to choose the best possible target for each patient. An approach to the research of new targets of therapy and to the development of new drugs, is that of chemical genomic, which integrates combining chemistry with the technique of transcriptional profiles, with proteomic, with informatics and with miniaturisation technologies. With this method the biological effect desired in a model system is researched through the screening of extensive collections of different compounds. A sign of activity is the starting point for the development of a new drug but also for the study of molecular mechanisms involved in the function under examination. In a way, the paradigm of genomic chemistry is a return to the empirical research of substances with pharmacological activity. Of growing importance in the rational development of drugs, are the methods of computerised stimulation and of analysis of the three-dimensional structure of the drug-receptor complex, through X rays diffraction. Crystallographic analysis furnishes a unique and irreplaceable approach for the construction and assembly of proteic molecules with enzymatic activity of interest to industry and pharmacology.

The accumulation of the necessary information for the definition of genetic profiles, for the application of appropriate algorithms and for their elaborations, makes it necessary to develop increasingly sophisticated systems for data depositing and management. For

this, bioinformatics represents an area in rapid expansion and an instrument of work necessary to pharmacogenomics.

From the last decade of last century, a new era in genetic studies has opened. In fact until then, it was in the tradition and in the possibilities of genetic research to tackle the study and the function of single genes. Thanks to the enormous technological progress of that time, new disciplines have been born, which allow us to tackle, decipher and analyse the global genetic information present in and expressed by groups of cells, tissues, and even an entire organism. Genomics aims at analysing the sequence and function of an organism's entire DNA; Transciptomics studies the fraction of the genome that is transcribed in RNA and determines the expression level of all transcribed genes. In the same way, Proteomics tends to identify and quantify all proteins, including also the different post-translation modifications they can undergo. Finally, Metabolomics tries to link all the information obtained with the abovementioned approaches, coordinating genes, transcripts and proteins in integrated functional nets. These experimental approaches are applied to single cells, to tissues, organs or entire organisms in the most diverse physiological or pathological conditions of embryonic development or differentiation.

At the same time and as support to these new disciplines, a new branch of informatics has developed, Bio-informatics, which applies programming and computer calculus to the management and interpretation of genomic data. For example, new languages, called BioLims, have been developed and applied to the management of genome sequencing projects: in fact, through these instruments, all the complex experimental passages necessary to deciphering a genome can be coordinated and controlled. BioLims can coordinate the work of the robotic instruments used to treat thousands of samples together with manual work or the experimenters' analysis, controlling the flux of information in experimental passages and recording the "history" of every sample. As the deciphering of a genome can be defined as the ordered reconstruction of a puzzle often made up of millions of pieces, we can easily imagine the essential advantages these informatics approaches have brought to genomics. Bio-informatics has in addition developed numerous instruments that allow us to predict, from the knowledge of the simple DNA sequence, the gene's function and regulation, the function and structure of the protein eventually codified, up to the presence of genes and proteins with similar functions in other species ("Gnomon" project). Informatics algorithms with which we can study the function of a DNA sequence completely in silico are evolving very rapidly and are often now a premise to finding experimental hypothesis of study on genic functionality. Other algorithms have turned out to be essential for the assembly of complete sequences of very big genomes, as for example BLAT, which allows the multiple aligning of strings of very long sequences.

The results of very numerous projects currently being carried out throughout the world in the field of genomic disciplines, in the great majority of cases, are put into public access databanks (GeneBank, EBI, GEO, SwissProt, etc.). These databases contain not only the information of gene and genome sequences obtained so far, but also information of functional genomics, like those extracted from the analysis of transciptomes and proteomes. This information is constantly integrated thanks to instruments of informatics analysis that have led to the construction of genomic-functional databanks in which, from a single information (the sequence of a transcript or the structure of a proteic pattern), we can easily go back to all the connected information in a process of data mining that is increasingly facilitated. A significant result of these new generations of databanks is that, for example, of the integration of data of global genic expression with those of proteomics, which will allow an increasingly complete view of molecular bases of cellular metabolism.

Even the functional analysis of genes has had a remarkable development in scale and technologies until recently used to study single genes, are now applied to hundreds of

genes at the same time. We only need to think about the silencing, destruction or genic super-expression projects on a large scale already carried out on model organisms (yeast, the Caenorhabditis elegans nematode, Drosophila melanogaster and the mouse) and the projects of systematic analysis of proteic interactions in vitro and in vivo carried out in yeast and in the cells of mammals. These projects are collecting a vast amount of functional data that must be interconnected, with specific bio-informatics instruments, to those already present in genomic databanks.

The exponential growth of the data produced by genomic disciplines allows us to imagine the increasingly important role of bio-informatics instruments. The number of genomes that are deciphered is in constant increase, due on the one hand to the use of the centres of genomic sequencing and on the other hand to technological advancements: recently, it has been introduced on the market an automatic sequencer able to complete the sequence of a bacteria genome of medium dimensions (4 megabases) in only 5 hours. In addition, it is thinkable that we can begin to tackle the complete sequencing of genomes of different individuals of the same species (personalised genomics). For example, a recent project by the National Cancer Institute USA, predicts the complete sequencing of the cellular genome of human tumours of different neoplastic evolution, to map, once and for all, all the variations, at the DNA level, that accompany the process of evolution of neoplastic cells. New and more advanced algorithms are therefore necessary to manage the quantity of genomic data that will accumulate. In order to effectively tackle specific research topics, it is increasingly necessary to start from the accumulated genomic data and the researcher cannot, seen their growing complexity, manage them manually, instead he/she will have to use specific bio-informatics tools, capable of organising this information in an integrated and finite manner. Only this way we will be able to tackle complex problems like the evolution of chromosomes and genomes in the scale of evolution, the relationship between the global genic expression in determinate pathologies and the profiles of genomic variations (SNP), as well as the prediction of molecular mechanisms involved in the regulation of complex cellular functions.

Because therapy's ultimate target can be considered the gene that directs or regulates the synthesis of the protein, gene therapy is the logical development of pharmacogenomics. The concept of therapeutic gene, which is now used in place of the restrictive concept of therapy of the gene, has widened the application field of genic therapy, which interests now a growing variety of pathologies, from hereditary ones to acquired ones, from monofactorial ones to multifactorial ones.

The impact of genomic on pharmacology is not limited to the innovation and development of drugs production, with predictions that are not easy with regards to its width and the times of realisation, but it certainly interests basic research in the current time. In fact, the discovery of new markers for the diagnosis and of new therapeutic targets for the treatment of hereditary and acquired diseases, in general contributes to increasing our knowledge of biological processes mechanisms that are at the basis of physiology and pathology.

.4 Personalisation of care

The systematic analysis of the genome has facilitated, also thanks to the discovery of single nucleotide polymorphisms (SNP) and to the development of the techniques for their identification, the research of genetic determinants involved in the susceptibility to illnesses. The investigations, until now mostly directed to the most common pathologies and to those with the strongest socio-sanitary impact, are carried out with a variety of approaches: studies of association on a large scale, like those regarding polymorphisms linked to general morbidity; research focused on genetic factors involved in specific

pathologies, like Parkinson disease; polymorphic mutations and variants of already known genes, like those linked to cerebral ischemia, to psoriasis, and to rheumatoid arthritis. Focus of the study are also the SNP haplotypes, typical of the genes for which we have proved or guessed an association with a particular illness, like Hirschsprung, Alzheimer and Parkinson, or with a syndrome, like the obsessive-compulsive one.

The study of the genes involved in the genesis and development of the pathological process offers the opportunity of both improving existing pharmacological therapy, and giving it new targets. According to a recent investigation, more than 500 products of known human genes have been identified as target for the drugs currently in use and it is predicted that such number can be increased, including also genes and genic products capable of functioning like therapeutic effectors, to 5,000-10,000. In fact, genes and proteins, as well as interacting with drugs, can also have a direct therapeutic function.

However, as observed in previous chapters, it must be stressed that individual differences in the response to drugs can depend on genes involved in the drugs' metabolism and transportation, as well as on their direct effects. In these circumstances, environmental factors can have a prevalent role. A typical case is the one of bio-transforming enzymes in the liver, belonging to the family cytochrome P450, the first of which was mapped in 1987: being equal the genetic determinants, a drug can be active or inactive in line with functional level of these processes, which depend on previous expositions not only to the drug in consideration, but also to structurally similar substances contained in food.

Despite the complexity of the problem, there is no doubt that genetics can give a precious contribution to pharmacological therapy, improving both its safety and its efficacy. This is demonstrated by the two examples listed below, the first regarding some drugs used in psychiatry, the second regarding the inhibitors of growth factors in oncology.

An example of the possible contribution of pharmacogenetics and pharmacogenomics to the personalisation of care is given by the so-called Malignant Neuroleptic Syndrome, potentially fatal, identified by the following clinical manifestations: hyperpiesia, muscular rigidity, akinesia, vegetative problems (irregularity of the heartbeat and of arterial pressure, sweating, tachycardia, arrhythmia) and alterations of the conscious state, up to shock and coma. The treatment of this syndrome consists of immediately suspending the administration of antipsychotic drugs and instituting an intensive symptomatic therapy aimed, in particular, to reducing hypothermia and correcting dehydration. As well as the antipsychotics haloperidol, clozapina, olanzapine, quetiapina, risperidone and ropinirole, have also been involved in this syndrome antidepressant drugs belonging to the tricyclic class.

The Malignant Neuroleptic Syndrome tends to manifest itself with higher frequency and gravity in patients who are carriers of a genetic anomaly caused by the dopamine receptor D2, which reduces the affinity for the ligando dopamine and it is responsible for a hypo-dopaminergic state that is gravely emphasised by anti-dopaminergic drugs, like antipsychotics. The genotype of the patients affected by this syndrome is characterised by the polymorphism of the gene that codifies for the dopamine receptor (DRD) and for the restriction enzyme TaqI A. This polymorphism translates in a reduction of the relative density and receptorial function. The frequency of the genotype A1 is much higher in patients affected by the abovementioned Neuroleptic Syndrome (93.3%) than in others (57.2%) (Suzuki et al., 2001).

This knowledge offers the opportunity not to eliminate, but to reduce the risk of a dangerous collateral effect of some drugs of psychiatric use, carefully evaluating the patient's genotype before starting the therapy.

Another example of the possible contribution to pharmacogenetics and pharmacogenomics to the personalisation of care, is given by the studies on the inhibitors

of growth factors, the receptors of which are frequently amplified by the tumoral pathology, so that it confers to it a more aggressive clinical outcome. The pharmacological targets more studied in solid tumors are erbB1 (EGFR or HER1) and erbB2 (HER2/neu), two proteins that belong to the erbB family. ErbB1 is a transmembranaire glycoprotein of 170 kD, that forms homo- (erbB1/erbB1) or hetero-dimers (erbB1/erbB2, erbB1/erbB3) with other members of the family following the link with EGF or other ligants. These include the transforming growth factor α (TGF-α). ErbB2, a tyrosine-kinase of 185 kd anchored to the cellular membrane. Although its ligando has not yet been identified, it is known that this protein is the preferential partner of heterodimerizations within this family. Cases of genic amplifications, mutations and over-expression of erbB members have been reported in numerous neoplases, including glyo-blastomeres, mammary, pulmonary, colonic, bladder and head-neck tumors. The inhibitors of the receptor tyrosine-kinase (RTK) gefitinib and erlotinib block the activation of the transduction system of the signal triggered by the erbB1's RTK, whilst cetuximab and trastuzumab monoclonal antibodies act respectively on erbB1 and erbB2. The treatment with these drugs has led to significant clinical responses in patients affected by pulmonary tumour "not in small cells" (gefitinib, erlotinib), cancer of the colon-rectum (cetuximab) and mammary neoplasia (trastuzumab). Resistance phenomena to EGF-RTK inhibitors and to monoclonal antibodies anti-erbB1 can happen in tumours that present the most frequent mutation of EGFR, that is, EGFRvIII (ΔEGFR or del2-7EGFR). This mutation is characterised by the deletion of the exons 2-7 in the mRNA of EGFR and codifies for an extra-cellular domain of bond for EGF truncated, with a constitutive activity, independent from the interaction with the ligando. Recent studies have demonstrated the existence of specific somatic mutations, regarding the genic portion that codifies for the domain tyrosine-kinase of the receptor EGFR.

The gefitinib, the trastuzumab and other growth factors inhibitors are indicated in around 10% of patients affected by pulmonary tumour "not in small cells", correspondent to those in which we can find the abovementioned mutations (Bussolati et al., 2005; Kobayashi et al., 2005).

This exposition has been purposefully filled with technical details in order to show the topic's complexity and the high specialisation of the researchers involved. In addition, we must take into account that the optimization of the anti-tumoral therapy that can be achieved with growth inhibitors translates not in a recovery, but in an increase in survival so far documented in a relatively modest percentage of patients with precise characteristics, which constitute a very small amount of the tumoral pathology.

The abovementioned examples give rise to some reflections of basic importance. On the one hand there's no doubt that genetic research opens a series of concrete perspectives to medicine, in particular with regards to the personalisation of care. On the other hand it absorbs an enormous amount of human and economic resources: we must avoid the risk that this might be to the detriment of other opportunities, which could give us quicker and more extensive healthcare benefits. We must think, as an example, to the campaigns against smoking, which largely manifests its carcinogen action independently from genetic individual characteristics; or, with regards to psychiatric pathology, to the shortages that exist in the support of mental patients.

4. Bioethical aspects

.1 General considerations

.1.1 Pharmacogenetic and pharmacogenomic research constitutes a concrete expression of the very strong impulse given to the whole field of genetic research since the mapping and sequencing of the human genome, announced on the 12th of February 2001.

First of all, we must stress the significance and cultural value, as well as the scientific value, of the new trend, defined in terms of functional and proteomic genomics.

The knowledge on the functioning of the genes that can derive from it, is in itself a source of progress that must be judged as ethically positive if it contributes to the human good. Many of those who commented on the achievement of the awaited goal of the publication of 90% of the human genome (I.M.G.S.C.; Nature, 2001; Venier J. et al., 2001) agreed in stating that, in itself, the knowledge of the genic sequence does not offer new perspectives in the interpretation of the functioning of the gene, both as single entity and as unity of genes, if we don't also know the products of already formed genes.

Having already began to research, for the past few decades and especially in the last few years, the nature, structure and special configuration of particular proteins in relation to the presence and activity of certain genes, single or associated, and of their mutations, we have started this new stage in the exploration of nature.

The rapid progress of analysis techniques with a high level of automatisation and of bioinformatics, as research that until a few years ago required a very long time, today can be carried out in a short time, has focused public attention on this field of research, boosting the investigation of ethical and regulating problems raised by research, both in its possible applications in clinical practice and in the supply of healthcare. This field of research is still in its infancy and therefore it is not possible to entirely predict its potential and its development: for this reason, however, it offers the rare opportunity of exploring in their entirety their ethical, social, legal and economic problems whilst the field is still at its developing stage and can be shaped early.

.1.2 We must immediately observe that this specific sector of genetic research does not present aspects involving the appeal to "ultimate ethical principles" and therefore, presumably, it is not susceptible of generating conflicting principles. Both the aim of the research (improving human beings' health and reducing costs, both in terms of pain as well as economically) and the methods used to pursue them, seem devoid of irreducible moral controversies and, in effect, there doesn't seem to be any position contrary to pharmacological and pharmacogenomic research in principle (if not those, thankfully in a minority, inspired by a preconditioned refusal of scientific innovation). We can raise a general question on global justice and it regards the morality of allocating considerable resources to a type of research the benefits of which will undoubtedly fall (and at least for a long period of time) only on patients who possess higher financial means, with the risk, therefore, of worsening the already existing inequalities in the access to medical care. Although interesting, and even crucial for a global cultural assessment of the most advanced trends of biomedical research, the topic goes beyond this document's limits. Finally, we must also observe that bioethical problems raised by this specific field of research are not qualitative different from those regarding the entire field of advanced biomedical research and therefore can be tackled with the application, and the eventual adaptation in the specific case, of well tried general principles and normative instruments expressed in numerous national and international documents.

.1.3 With regards to an ethical judgement on experimentation in pharmacogenomics, we maintain the general ethical considerations already codified by bioethical reflection on biomedical research and held as rules within national, international and European law.

With this premise, it seems possible to state:

a) With regards to the "specific" pharmacogenomic and proteomic research we can also refer to the rules on the research on the human genome, inspired to protection principles elaborated from the late 1940s until today – and by now presiding every investigation of the human being.

General and founding principles are enunciated in solemn documents, like for example the Convention on Human Rights and Biomedicine (1997) Oviedo and the relative “Research Additional Protocol”, and in addition in the “UNESCO’s Universal Declaration on the Human Genome and Human Rights” (1996) .

b) As well as these general and founding principles, there are a variety of derivations, expressed in documents (national or international, with a different legal authority) regarding also practical aspects of conducting research of a genetic character, which would apply also to extensions of research in the proteomic field.

.1.4 Because of what stated above, bioethical problems posed by pharmacogenetics in its development toward pharmacogenomics, regard the evaluation and assessment (both at the level of research and of applicative pitfalls) of the benefits and the costs/risks, aiming at identifying the most appropriate rules to maximise the first and minimise the second. From the analysis of relevant literature, benefits and risks can be so summarised:

a) Benefits

- understanding of the genetic bases of the mechanism of response to drugs
- development of new, more effective and safe drugs, and in a quicker and less costly manner, thanks to clinical studies of new conception
- a safer (in relation to adverse events) and more effective (with the possibility of distinguishing the non-responsive or slow and the quick responsive) use of drugs, with consequent savings in costs for the SSN
- Improvement in the post-market pharmacovigilance of drugs, which can also allow the protection of benefiting drugs that in some genotypes can have adverse effects (and that today are withdrawn from the market)

b) Risks

-`connected to finding, using and preserving biological finds necessary to research
- connected to the use of obtained information, to avoid discriminate uses
- connected to the inclusion and exclusion of clinical studies
- connected to the eventual worsening of the phenomenon of orphan illnesses
- connected to the introduction of pharmacogenetics tests insufficiently validated.

.1.5 Before moving on to a more specific analysis, it is necessary to put forward a general, although synthetic, reflection about the conceptual frame within which the assessment of the benefits-costs/risks must take form, in order to be accurate, balanced and therefore productive. However, such a reflection does not specifically regard the pharmacogenetic sector, but the entire field of genetic research, it is here aimed at highlighting some aspects, surfaced in the course of the debate, from the various interweaving of which could derive, at the same time, an exaggerated overestimation of the benefits: in both cases the conditions for an accurate assessment (and, therefore, for the elaboration of efficient public policies) are lacking and instead the wrong perception of what is at play in genetic research, of what we can realistically expect and of what we must carefully avoid, is fuelled and confirmed, also by the public.

From the point of view of distorted perceptions, we could expect a further push towards the so-called “radical genetic reductionism”, or “genetic determinism”, already widespread today in many environments, with the statement that each manifestation of the phenotype, and even in human behaviour, is linked to (and determined by) a particular genetic asset: in this case, in the reductionistic equation, in proteomics research only that particular protein should be substituted to the gene and the result would not change.

R. Lewontine, S. Rose and J. Kamin (1984) proposed a reflection on the negative consequences of a reductionistic formulation, which they identify at the scientific level in the wrong conception of biological processes, complex by nature; at the ethical-social level

in fatalism, in de-responsabilisation and, al the level of research policies, in the pursuing of the wrong objectives in the allocation of resources. The same author concludes that new knowledge derived from the study of the human genome, used in a correct and appropriate manner, offers the possibility of improving the quality of life in our society, but they must be taken away from the reductionist matrix and inserted in an integrated vision of human biology, in its individual and social dimension.

Although this is, in reality, a general theme that involves the entire field of genetic research, a mention of it, especially for certain cultural and public policies effects, can be useful also in our context, particularly because genetic reductionism, beyond its most general cultural aspects, could lead, in our specific field, to “miracle” and therefore unrealistic expectations with regards to the predicting power of pharmacogenetics tests: instead of seeing, more realistically, pharmacogenetic information as having probabilistic nature (from this specific problems, which we will see later, are generated) and of balancing it with other factors that influence the individual response to drugs, a reductionistic mentality could see this information as final and omni-comprehensive. The slogan that summarises pharmacogenetics’ ideal objective (“the right drug to the right patient in the right dosage”) is only a slogan, although captivating.

.1.6 It is important first of all to distinguish between genetic reductionism as research methodology and genetic reductionism as ideology. The first is simply the fundamental nucleus of the complex’s comprehension program, starting with the simplest, which is at the basis of molecular biology and would implicate the idea that living creatures’ essential properties could be interpreted by looking at the structure and functions of their macromolecules, capable of transmitting, replicating and reading genetic information, that is the radical innovation of molecular biology. Applied to the field under consideration, this idea implies that any phenotypic trait, both normal and pathological, can be brought back, completely or partially, to events connected to the structure and functioning of genes.

Naturally, it is still under discussion to what point it is possible to push the explanation model described above and in what measure (and when) biomedical research that adopts this model (including pharmacogenetic research) will be able to give rise to therapeutic options capable of affecting, in a statistically significant way, people’s health. There are a variety of different points of view regarding this. The most widespread opinion is that the impact in clinical studies of what is happening in the most advanced genetic research will be of such magnitude that it will bring a real revolution in the way we practice medicine. But more cautious scholars bring attention to how the results transferred to clinical practice, at least at the therapeutic level, are up to now more promised than realised and that, in any case, the entity of this revolution must not be emphasised because, for example, its impact on the way in which the most common multifactorial illnesses are diagnosed and cured will not be relevant, because the correlation between genotype and phenotype is in this case very weak and there’s no benefit in massively turning to genetics (Holtzman and Marteau, 2000).

It will only be the further development of research on the genome (which, as we will see, is affected by numerous scientific and social factors) to decide who is right. What we need to stress is that genetic reductionism as methodology must not be confused with genetic reductionism as ideology, which is a more complex cultural phenomenon and only partially linked, maybe even a bit paradoxically, to the success achieved through the analytical and reductionist model in the program of research pursued by molecular biology (S. Sarkar. 1998) . The basic idea is that genes will have such an important part in our life that they will cause a cultural and spiritual impoverishment which, in the old question of the relationship between nature and culture, would bring back the dominance of nature,

obviously with all the consequences this would cause – even, for example, in terms of the education or racist policies that could follow. The debate provoked by Wilson's thesis, for example, is well known, as is the new polemics on the old question of the genetic root of intelligence aroused by the publication of a book by Herrnstein and Murray (R.J. Herrnstein, C. Murray, 1994) . The old genetic determinism could be resurrected, that is, the 1920s 1930s trend to favour the genetic explanation not only for illnesses and the response to drugs, but for any type of "deviation" from social norm. Of course we all know that the development of molecular biology has defeated this old determinism: no serious scientist today would support the thesis "a gene, a trait" in a deterministic manner and without distinguishing, for example, between monogenic traits, multifactorial traits, etc. And however today – because of the success of molecular genetics and of its promises amplified by mass media always looking for a scoop (like: intelligence gene discovered) – we are looking at a kind of return to this determinism which, in popular culture, seems to have become a true mystique of the gene (D. Nelkin, S. Lindee, 2006) , with its liturgy and even its own relics, where genes seems to have the same importance of the soul in religion. With regards to this, we talk of a kind of "genetic essentialism".

It is however clear that genetic determinism and reductionism, just as the eventual clinical reductionism – which is only a bad way to practice any medicine, not only genetic medicine – are not the necessary and inevitable result of a molecular genetic research and therefore they should not be listed as some of the "risks" of such research. They are a problem with regards to how society perceives and receives the advancements of science and, therefore, a problem of cultural politics, of great importance especially in a country like ours, in which the process of circulation of scientific knowledge is slow. If this is true, the criticism of genetic reductionism as ideology implies the cultural commitment of preparing society to welcome and to be able to evaluate, in their right dimension, the results of genetic research, which does not need to be exalted in order to produce its positive effects. This is without a doubt a crucial duty, which public authority must undertake: otherwise we risk having instruments of great efficacy without however being adequately prepared to use them to our patients' benefit.

The intervention that can be hypothesised in order to reduce the distorted interpretations present in public opinion, seem therefore linked to the general increase of the knowledge about the functioning of both genes and the proteins regulated by them.

Much could be derived by the action of cultural organisations (public and/or private) aimed at facilitating the correct interpretation of messages; from technically exact information, sober and devoid of emphasis in indicating future possibilities, by both journalists and doctors.

A great responsibility must be attributed also to the researchers' professional transparency and propriety.

The UNESCO's "Universal Declaration on the Human Genome and Human Rights" (1996) in art. 13 states in the following terms what is must be done in the matter (responsibility ethics):

"The responsibilities inherent in the activities of researchers, including meticulousness, caution, intellectual honesty and integrity in carrying out their research as well as in the presentation and utilization of their findings, should be the subject of particular attention in the framework of research on the human genome, because of its ethical and social implications."

This does not mean disregarding the freedom of research and of the communication of ideas.

The focus on the ethics of research can have the function of a stimulus rather than an obstacle to scientific progress and its productivity pitfalls, as well as spreading in public opinion the positive image of a responsible science.

From this point of view, it was recently launched the Commission's Recommendation of March 2005, regarding the researchers' European charter and a code of conduct for the employment of researchers . This, although it recognises that its "final political objective is to contribute to the development of a European market of attractive, open and sustainable work for the researchers" (considering 8), it however recommends: "3. Member states, in elaborating and adopting their strategies… adequately take into account and inspire themselves to general principles and Charter regulations…".

It's important to stress that in this Charter, between "General principles and requirements applied to researchers" are expressly mentioned: "freedom of research , ethical principles , professional responsibility , spreading and enhancement of the results ".

To elevate the level of knowledge and awareness, both of the researchers and of the public, of the problems involved, is not an easy task to perform, but it is now unavoidable, as it is well illustrated in the action plan in "Science and Society" launched by the European Commission and by the European Parliament in the VI Frame Program (2002-2006) and also present in the VII FP (2007-2001).

We must add that some governments has already taken this task very seriously: for example, in the recent white Book by the English government, dedicated to the programmatic lines for concretising the potentiality of genetic research in the distribution of healthcare, chapters 4 and 6 are dedicated to illustrating the measures (and the relative investments) in the field of medical education and workers' training and in the field of the promotion of public awareness and trust . It is important to highlight that this white book is maybe, at least in Europe, the first document of a governing authority that, in the context of a clear awareness of the profound changes that genetics will produce in the medium term in the delivery of basic healthcare, dedicates considerable attention to pharmacogenetics: and not only at the informative level, but also allocating investments aimed at a specific field, that of pharmacogenetics for drugs already in use in the therapy of common illnesses, a sector that does not seems attractive for investments by private companies, but that is of great interest for people's health and can produce, already in the short term, considerable savings in pharmaceutical expense.

.1.7 As we said, ethical questions posed by pharmacogenetics are not qualitatively different from those posed by genetic research in general and therefore we can assume that the conceptual and normative instruments already elaborated for the last one, are applicable also to the first, naturally with the adaptations that might be necessary to better grasp this field's specificity. According to a trend by now widespread in literature, we can identify the ethical problems arising from pharmacogenetics with reference to three levels: a) basic research; b) applicative research (especially pharmacological); c) application in clinical practice. In all three levels – and, obviously, with a different importance in each on them – bioethical problems involve essentially the following points:

a. the correct management of acquired information;
b. problems relative to the informed consent, to privacy and to confidentiality;
c. ethical and social implications of stratification;
d. implications with regards to equality in the access to medical care.

.2. Basic research: ethical judgement on current knowledge

.2.1 At the general level of pharmacological research, the fundamental problem regards the control of the flux of information. Pharmacogenetic research aims at establishing the influence of the genotypic variability in the response to drugs, influence that – as mentioned above – is one of the factors (and certainly not the only one) determining individual response. To identify this specific factor, research needs to collect,

preserve and analyse DNA samples. This need is common to the entire field of genetic research and poses the same problems that are at the centre of the debate on Biobanks (a European picture of the debate on national legislations can be seen in Survey on Opinions from National Ethics Committee of Similar bodies, public debate and national legislation in relation to human biobanks, Brussels, 2002; cf. also Data storage and DNA Banking, Report of the European Society of Human Genetics, of June 2000, to be updated). Three regulating principles have arisen: obtaining the informed consent, protecting privacy and guaranteeing confidentiality. These are by now largely consolidated principles in the general sector of biomedical research and in the more specific one of genetic research, of which therefore we need to explain the meaning and the applicative modalities in relation to the specific field of pharmacogenetics, because of the specific characteristics of this research and, especially, because of the potentially enormous quantity of information that the research can collect and preserve.

.2.2 The topic of the informed consent must be analysed under three main aspects: i) its extension; ii) its content; iii) its new modalities. Taking for granted, obviously, the general principle that the obtaining of consent must be preceded by correct, ample and comprehensible information (particularly difficult task for a geneticist), even from the point of view of terminology, the first problem (the extension of consent) regards the times, uses and people authorised access. We can, for exposition reasons, identify two extreme hypotheses, in between which there are a variety of other hypothesis. The first (ample consent) is that in which the consent is equal to a sort of blank cheque that the subject gives to the researcher both in relation to the time limitations in the use of the samples, in relation to their use in other research connected or following the first one, and finally in relation to other potential sample users. Although advantageous for the aims of the research, this hypothesis is considered lacking from an ethical point of view because it contradicts the main objective of the informed consent, that is, to put the subject in the condition of evaluating the costs and the benefits of his/her participation to the research: no evaluation is obviously possible when it is not clear what research will be carried out, with what aims and by whom. We still need to discuss whether, as long as clearly illustrated, this hypothesis remains available to the subject, as it can be founded on the conscious desire to offer a contribution to the progress of biomedical research. However, even in this case – and in relation to the possible risks (see later) – it should be accompanied, because of its range, by a very high level of protection of confidentiality.

The other extreme is the hypothesis of a consent “restricted” to the use of the DNA sample for a limited time, for a clearly limited research and only for the researcher that requests is. This is, essentially, the model that has prevailed in the first attempts at adding a pharmacogenetic sub-protocol to the normal protocols of pharmacological experimentation. Its excessively restricted character is however negatively judged, especially because it would imply – should the development of research require an extension – a new procedure to obtain consent, with the relative costs. It is in any case possible, in between these two extremes, to hypothesise models capable of conciliating, at the maximum degree possible, the needs of the research and of guaranteeing the respect of an individual’s dignity and of the subject’s rights. Although in a field in such rapid evolution it is not possible to dictate rigid and uniform rules, it can be stressed the general principle that anything we ask the subject to consent to, must be explained in detail, with rigorous and understandable language in the consent form and it must be made the object of clear communication, open to any requests of clarification.

.2.3 Going on to the second aspect (the contents), literature stresses in particular the following points. First of all, an accurate and impartial illustration of the benefits (if there

are any) and of the risks of the participation to research. With risks, obviously we don't mean those linked to sample collection methodologies (really minimal), but those psycho-social following from the improper or unauthorised use of information. For this reason – secondly – the specific illustration of the procedures needed is necessary in order to safeguard privacy and confidentiality: not, therefore, only the simple reference to the regulations already existing with regards to this, but the explicit indication of the techniques used to preserve the biological sample and the information, the people that have access to it and for what aims, the person responsible for the entire procedure, etc. Thirdly, the research sponsor and the possibility that it will generate marketable results will have to be indicated. Finally, the consent must include the mention of the possibility that in the course of research information might be discovered, secondary to the aim of the research and potentially beneficial (but also involving psycho-social risks) for the subject: in this case, it will have to be clearly detailed what happens to this information, who assesses its credibility, who has the task of informing the subject and, obviously, if the subject needs to be informed or not.

.2.4 The topic of "secondary information" is often recalled in the debate as a potential risk of pharmacological research, but according to some it is overestimated: the possibility that a pharmacogenetic research might also discover information that can be linked to genetic illnesses or to the predisposition to genetic illnesses, is very low and it is a function of the technology that is employed. The trend is going towards using very selective and targeted genetic markers, which are not able to identify predispositions to illnesses or other secondary information. If this is true – the object of contention is this – then it would be improper to apply, to pharmacogenetic tests, the regulating instruments, more stringent and rigorous with regards to the guarantees for patients, created for the tests to diagnose genetic illnesses (or the predisposition to illnesses). Although it can be hoped that no improper burdens are imposed on a sector of research on the basis of the simple fact that it belongs to a "sensitive" field like genetic research, it is certain that the loosening of the limitations must be compatible with the guarantee and the protection of privacy and confidentiality. We can in fact hypothesise that, even in the most favourable research circumstances, pharmacogenetics' data can generate secondary information to which others could be interested in and have access to. These are some of those circumstances mentioned in literature: a) the genotype that influences the response to drugs plays a role in the predisposition and/or the evolution of a certain illness, or in the sensitivity in the dependency from drugs or other substances; b) a pharmacogenetic test can even bring information with regards to the non-paternity (a "slow acetylated" child cannot be the son/daughter of a father who does not even have one of the alleles responsible for this recessive trait); c) the mere fact of being declared non-responder to a certain drug or to a class of drugs can have consequences, when, for example, that drug is the only one effective against a certain illness: virtually, the subject is declared incurable.

.2.5 The third aspect (the new modalities) regards the topic of the "group consent", a topic that is increasingly under scrutiny in the investigations on ethical issues of genetic research in general (especially of the populationistic type). The main idea comes from the preoccupation that a certain individual, even if he/she has not actively participated (or has refused to do so) to research, could still receive from it a psycho-social damage (in the form of a stigmatisation or discrimination) consequent to being perceived as a member of the social group, easily identifiable, on which the research is conducted. We can hypothesise, for example, that pharmacogenetic research can find out if a social group identifiable on ethnic or racial bases (terms still used in common language) is non-responder to a certain drug: in determinate conditions, this could translate in

discriminations in the access to treatments, which, in the specific case, could be connected to prejudice towards an ethnical background and would involve all the individuals belonging to a group, even those who did not directly participate to the research. Obviously, it is well known that genetic variability within a group can be even higher to that existing between groups and therefore the problem is not scientific as much as of public perception and recalls, once again, the need for a profound work of education and information, which we have mentioned before. With regards to the point under examination, we cannot hypothesise that the very speculative expectation of an indirect damage consequent to research the subject has not consented to, can present a sort of veto from all those belonging to the group, that research should be conducted only on consenting individuals. We can hope – in special cases and when research regards groups that can be considered vulnerable – that obtaining an individual's informed consent must be preceded and accompanied by a correct sensibilisation and consultation campaign. Good examples are not lacking in connected sectors (for example, the screening campaign for the Tay-Sachs disease in Hebrews of Askenazi origin or that for thalassemia in Cyprus); and in any case it is a topic we must take into account, also in relation to the topic of the "shared" character of genetic information: everything that is discovered on an individual's genetic make-up gives information not only on that individual, but also on his/her relatives. The topic is not specific of pharmacology, but poses also in this sector a problem of management of information that gives rise to the need to develop genetic counselling.

.3 Protection of privacy

.3.1 There are already numerous regulations aimed at protecting individuals from the damages resulting from the improper or unauthorised use of genetic information (prohibition of discriminating on a genetic basis, also in the field of work and insurance: etc. quote). The procedures of protection and guarantee must be already carried out during the research and – as we said – they must be accurately described in the informed consent form. Even if we still don't have a complete uniformity in the terminology, generally we distinguish between three different procedures applicable to biological samples for the control of the flux of information: identifiability, codification (simple or double), anonymisation.

.3.2 With the first system the identity of the research subject remains identifiable throughout the course of the research. From the researcher's point of view, the advantage of the identifiability is that it allows the integration of genetic information with other medical information recorded or obtainable from the subject and this increases the data's reliability, as well as the possibility of using new data that had not been contemplated as important at the beginning of the study. From the subject's point of view, the advantage is that eventual interesting information with regards to the subject's care, can be communicated to him/her, in the abovementioned ways. This system's disadvantages derive from the low protection levels ensured: the eventual secondary information derived from research can be linked to the subject and this represents a source of not always easily controllable risk.

.3.3 The second system ensures a higher level of protection and has two variants. In the single code system an identification number links the sample to the subject. The code is only known to the researcher and can be used to identify the subject within the limitations and the cases mentioned in the informed consent form, where even other people that, in certain circumstances, can have access to the code, can be listed. Even higher is the level of protection ensured by the double code system, where the sample's

identification number and the subject's identification number are linked to a code known only to the researcher. This system is considered more appropriate to reconcile the needs of the research and the protection of the subjects' interests. It leaves the possibility of accessing other medical data, eventually necessary for the research, open and more controllable, without the researcher being able to go back to the subject's personal identity. However it has considerably higher costs in comparison to the other systems and poses the problem (crucial for the procedure's conformity to standards) of the identification of the organism qualified to hold the connecting code. With regards to this, in literature there is talk of the creation of "intermediary fiduciary organisms" on which to entrust the management of this task: but the issue could be more general and involve the entire system of collection, preservation and treatment of biological samples (the problem of biobanks). An additional and important problem is the fact that there are, already in existence, in public and private institutions, banks of biological material and information, the collection of which has been carried out using very different modalities.

.3.4 The third system involves the complete anonymisation of the sample and this naturally eliminates any possible risk linked to the malfunctioning of the previous systems, offering the subject the highest guarantees with regards to the eventual damages resulting from his/her participation to the research. Anonymous or subsequently anonymised samples are preserved in already existing biobanks and it is common practice to allow new research to be carried out on them, as long as they are approved by the Ethical Committee. This system presents some disadvantages. First of all, the impossibility of accessing other medical information about the subject reduces the value of the acquired pharmacogenetic data, which – as abovementioned – depends also on the comparison with other factors that determine the response to drugs. Secondly, it becomes impossible to communicate to the subject any eventual secondary information that could be useful for his/her health and/or care.

.4. From the planning to the development of new drugs

.4.1 One of the sectors in which research in pharmacogenetics could produce good results in a reasonably short time, is that of the research and experimentation of new drugs. The scientific aspects connected to this issue have already been examined, as also the ethical aspects linked to the collection, preservation and treatment of biological samples necessary to create a consistent database of genotype-phenotype correlations. To complete the picture, and in order to highlight other aspects that need clarification, we examine in synthesis the main implications of pharmacogenetics on the way in which base research and clinical studies are currently designed and managed.

A better understanding, from a genetic point of view, of the biological mechanisms that contribute to the pathogenesis of an illness can lead, on the one hand, to more efficient and safe treatments with already existing drugs and, on the other hand, to identifying new targets for new drugs.

With regards to the first aspect, there is already considerable evidence of the benefits that the knowledge of the pharmacogenetic profile can have in the personalisation of therapy with existing drugs. We must not however make the mistake of overestimating the impact of pharmacogenetics in this field. In many cases, the concretisation of such an impact would be extremely expensive and the benefits, from the patient's point of view, very limited, especially when adverse reactions are light or moderate and the illness can be treated with other drugs. In other cases, however, using pharmacogenetics can have undoubted benefits, capable of adequately making up for the higher costs. This is the case with regards to seriously disabling illnesses (like schizophrenia) or life-threatening ones

(like tumors), against which we have efficient drugs that however work only in a limited percentage of patients and/or have very serious side effects. However, the pursuing of these undoubted benefits could be hindered by the problem of the costs of the research for the elaboration of validating tests, especially with regards to drugs that are not covered by a patent anymore. According to some, the public authority should intervene in this sector, both indirectly (through the creation of incentives of various nature, like what is happening in the case of the so-called orphan illnesses), and directly, supporting research in public structures with finalised projects.

.4.2 Moving now on to the second aspect, pharmacogenomics will allow us to considerably increase the number of biological targets for the drugs (as already stated in the NBC document on Ethical Committees in 1999). These drugs will then have to undergo clinical experimentation and one of the issues under discussion is whether – and eventually which - changes in the current regulations should be introduced in order to respond to the needs of pharmacogenetics.

Many authors, for example, agree in believing that the application of pharmacogenetics to the clinical development of drugs will require substantial changes in the design of the studies (for instance, with a greater focus on phase II base studies in comparison to the current one and a considerable decrease in the number of samples in phase III studies) and therefore a re-examination of the ethical principles on which human trials are based.

It will be in fact possible to enlist in clinical trials only responsive subjects, excluding the unresponsive ones. The ethical basis for such an exclusion is in the regulations that currently control the carrying out of clinical trials: not to expose the subjects enlisted in the trial to unnecessary or excessive risks and in any case risks that are not compensated by any benefits: if we don't know that a certain individual does not respond to a certain drug, enlisting him/her means exposing him/her to an unnecessary risk, without any compensating benefits for him or the research.

We must however be careful that the exclusion is based on individual genetic information and not – for reasons of convenience – on the mere fact that the individual belongs to a group in which we know there's no response to a drug: as we stated with regards to the "group consent", such an exclusion could be perceived as discrimination and would not have a scientific basis.

.4.3 The nature and the scope of the needed changes in regulations also depend on new perspectives and the speed of their concretisation, linked to the influence of a variety of factors, not excluding financial ones. Currently, regulatory authorities (FDA and EMEA, for example) do not require the incorporation of pharmacogenetics in drug experimentation protocols in order to obtain the authorisation to market. It must however be highlighted that in November 2003 FDA issued the first Draft Guidance (updated in 2005) for researchers who intend to communicate pharmacological data obtained in the course of clinical experimentations. This voluntary regime could quickly change, as research continues. According to a recent inquest, within 5 years 50% of clinical studies will involve getting the participants' genetic data and, according to some authors, by 2014 all new drugs will have been obtained through procedures that will use pharmacogenetic analysis. These estimates are based on the speed of innovation in this field, thanks to bioinformatics, and to the relatively increasingly lower cost of pharmacogenetics tests, and they also take into account that, as a growing mass of information becomes available and is attainable, other factors could push in this direction. A first factor, for example, is connected to the fact – abovementioned – that pharmacogenetics, as well as improving the therapeutic efficacy of drugs, promises also to improve safety, reducing or avoiding adverse reactions. As soon

as the available data will allow us to make reliable correlations, both on the already existing drugs and the new ones, the pharmaceutical industry will be increasingly pushed – also in order to avoid being taken to court for negligence – to incorporate pharmacogenetics in the design of clinical studies and, consequently, in the instructions for the administration of the drugs produced. There is a lot of discussion about the reliability of these predictions: but in reality, what is under discussion is the speed of evolution, not its direction.

.4.4 A second factor is linked to costs. Also with regards to this, the assessments are quite variable and in any case speculative. The most widespread opinion is that, in the short and medium term, we must not expect a decrease of the costs in drugs' development and experimentation, especially – as we were saying – because phase II will require a definite increase in the amount of patients in order to ensure the possibility of identifying a relevant number of variables, whilst the decrease of the number of samples for phase III is still controversial. It is difficult to say whether in the long term there will be a change of direction, but it is plausible to think that, in any case, the application of pharmacogenetics will improve the quality and efficacy of the drugs development process. Today the development of a drug requires about 10-15 years and the percentage of molecules which are of potential therapeutic interest to reach the market is below 0.1%. Excluding other factors (including the convenience of the pharmaceutical industry), pharmacogenetics could increase these percentages and contribute in reducing the number of drugs that are then recalled from the market because of adverse reactions. Finally, in the current state of affairs, it is very difficult to hypothesise whether pharmacogenetics will translate in a lowering of the cost of drugs, even if undoubtedly, thanks to the better efficacy and safety promised by the "personalisation of therapy", it will have a positive impact on people's health and on the overall costs incurred by the health authorities. These results, however, will raise some problems with regards to guaranteeing equality in the access to medical care.

.5 The stratification problem: illnesses and orphan genotypes

.5.1 The slogan "the right drug to the right patient in the right dosage" is often recalled in this debate, as the ultimate objective of pharmacogenetics. Naturally, this is an idealistic objective, because it would mean the total stratification of the patients in subgroups, each of which could even contain a single individual. However, leaving this futuristic possibility to one side, there is no doubt that pharmacogenetic research is destined to stratify (some prefer to say: differentiate) in subgroups both the patients (on the basis of their profiles of response to drugs), and the illnesses, giving rise to a new "molecular taxonomy of illnesses", that is, the idea that certain illnesses, up until now considered a single condition, present in reality, from a genetic point of view, a more heterogeneous picture and therefore require differentiated treatments, or "made to measure" treatments for that single patient and with minimal side effects.

Essentially, today it becomes possible to give scientific basis to phenomena already known since before the development of pharmacogenetics: for example, the phenomenon of drugs that do not go beyond phase II because they do not demonstrate sufficient efficacy and/or safety to justify the passage to phase III and therefore they are abandoned; or the calculations of costs-benefits which influence (or determine) the inclusion or exclusion of a drug from the list of those offered by the health authorities; or even the phenomenon of drugs that are recalled from the market for the high number of adverse reactions registered (and of which, thanks to pharmacogenetics, we could suggest the recovery). The only difference is that before the advent of pharmacogenetics the incidence

of these phenomena and the relative evaluations, also in terms of public policies, could be ascertained only on a statistical basis and after the event, with the relative costs, especially with regards to pain, that this involves. Pharmacogenetics could allow us to prevent the occurrence of these phenomena and this undoubtedly represents a benefit: the real problem is creating the right conditions to pursue this benefit, avoiding adverse reactions in terms of justice and equality in the access to medical care, or even in terms of denying access to those genotypes who, we have ascertained, cannot metabolise a certain drug. This phenomenon could become more pronounced in the future for reasons connected to investment policies in pharmacological research by the pharmaceutical industry.

.5.2 Stratification in fact seems to be a relevant factor for the development of new drugs aimed at specific genetic traits in serious illnesses (like tumors) and potentially, as tests become less costly and more reliable in predicting the drugs' efficacy and safety, for the entire filed of pharmacological research. This trend is in itself undoubtedly beneficial, but pursuing it could be hindered by socio-economical factors and could in any case lead to unique consequences. Although this is not the place to look at this issue in more depth, it is known that in the last few years, also because of the economic scale that can be obtained, there is a concentration process in the pharmaceutical sector and in biomedical research in general, which has led to the creation of multinational colossuses with investment policies that are oriented (we say this without any negative undertones) to profit. Data shows that the profit of many companies (which also covers the costs of any research that did not have positive outcomes) derive from a relatively small number of "blockbuster" drugs, that is, drugs capable of reaching a vast population of patients. An excessive fragmentation of the market could go against the interest of pharmaceutical industry which, in order to reach the same quantity of patients, would have to develop different versions of the same drug or different drugs, with the consequent increase in costs. Even if, in literature, it is also suggested the possibility that small companies could find it convenient to concentrate on a niche in the market and that, in addition, patients could be induced to willingly pay more for a drug that is certainly efficient (other conditions being equal) and safer, costs would be prohibitive in any case and health authorities would be subjected to hardly bearable economic pressures. These are, obviously, possibilities that can currently only be hypothesised, but that should not be undervalued in order to already identify possible corrections. In the debate, two possible avenues are indicated.

.5.3 The first is that of a larger direct involvement of the public sector in financing research, particularly with regards to fields of great interest to people's health (and potentially susceptible of lowering the costs incurred by health authorities), but not attractive to private companies. Currently the percentage of public investments in the pharmaceutical sector is around 10% of the total and it is not realistic (although desirable) to expect that this percentage will increase considerably in a reasonable amount of time. However, it can be a good base for experimenting new forms of collaboration between public and private, which in pharmacogenetics has already produced a few interesting experiences (cf. Nature Medicine's editorial of May 2000 titled The need for private-public partnership). These experiences – which involve overcoming a mutual mistrust between public and private, and entail new forms of synergy and interexchange between academic research and private research – can be developed usefully and in this direction – with regards to biotechnologies in general – is also moving the European Commission with the document Life Sciences and Biotechnologies: A Strategy for Europe, Brussels, 2002.

The second avenue (not alternative, but complementary to the first) consists in creating conditions in which private companies find it convenient to invest and to continue

to invest in pharmacogenetics. The model is the one used to tackle the phenomenon of orphan illnesses. In the USA already exists, since 1983, the Orphan drugs Act (revised in 1994) and, in Europe, in 2000, the European Regulation on Orphan medicinal products was issued, which establishes the conditions (only partially super-imposable) in which an illness can be declared orphan and the relative drug chosen to enjoy its benefits (fiscal and of other nature: for example, the extension of the patent's validity). It is difficult to predict what changes (especially in terms of numbers) will be necessary to make to this model in order to apply it to pharmacogenetics. The model has been designed to tackle the exceptions to the current standard of research and development of drugs; if the exception, as a consequence of extensive stratification, becomes the rule, the whole system will have to be redesigned and nobody is able to predict its scope and costs.

.6 Pharmacogenetics and clinical practice

.6.1 Even if the situation described above is certainly not foreseeable in the near future, there is no doubt that pharmacogenetics is destined to have an increasing impact in clinical practice and in the issuing of healthcare, especially pharmacological care, where the personalisation of therapy could involve, in some cases, certain savings, not only economical but mainly of pain, in the short term as well. Certainly this impact will be realised in a very gradual manner, but it is already possible to foresee the problems it will create and therefore to study the best measures to tackle them. If we avoid the temptations of genetic determinism, such problems can be brought back to well tried principles and regulations, which only need to be perfected in order to allow a correct and fair management of these new potentialities. The most adequate starting point to identify these problems, is to understand what implications pharmacogenetics could have for doctors, patients and other potentially interested subjects.

.6.2 The first point to stress, is that the transferral of pharmacogenetic knowledge about the single patient to an improvement of therapeutic efficacy and drug safety, presumes the availability and administration of reliable and validated tests and therefore it involves the same problems of information and consent already tackled in the general case of diagnostic tests and in particular genetic tests. As abovementioned, some scholars have highlighted that pharmacogenetic tests produce data with an information content different from that of tests for the diagnosis of genetic illnesses (also in relation to the possible psychological and social pitfalls) and therefore they could require less stringent safeguard procedures. We can also see that there are distinctions to be made with regards to the tests' targets: one thing are tests conducted on mutated DNA in tumoral tissues (as in the case of administering Herceptin, which is expressly indicated only for tumors that present a specific somatic mutation); a different thing is the analysis of the patient's genotype, even for just pharmacogenetic aims. Apart from the already mentioned fact that such tests can directly or indirectly lead to "secondary information", it should be maintained that – also because of the sensitive character of genetic information and of the current common perception of hereditary phenomena – caution suggests to maintain, for pharmacogenetic tests on patients, the same standards currently applied to other genetic tests, especially in relation to the quality of information to be given. In any case, the speed and extension with which pharmacogenetics will be integrated in clinical practice depends on many factors, partially economic (we will discuss it later), partially educative and cultural. Many investigations highlight, for example, the gaps in medical training with regards to the entire genetic field and it is therefore clear that the topic, abovementioned, of the promotion of training and information will be crucial to turning these new instruments into clinical practice. However, as it is unrealistic to think that every doctor will become an

expert in the administration and interpretation of pharmacogenetic tests, it will be important to think about territorial structures as points of reference: and anyway the integration of pharmacogenetics in routine clinical practice will require the availability of “user-friendly” technologies, easily carried out, therefore creating the problem (discussed later) of the direct access to the consumer, without the mediation of a health professional.

Even hypothesising the rosiest outcome, the substantial help that pharmacogenetics will be able to give doctors and patients involves problems that must be reflected upon.

.6.3 As we have recalled, currently – and apart from special cases like Herceptin and a few other drugs recently produced – regulating authorities do not require, if not on a voluntary basis, pharmacogenetic information as part of the documentation to obtain the authorisation to market a drug and, therefore, the genetic profile is not part of the treatment instructions. It is plausible to think that this situation will change quite quickly, even if gradually, and this poses questions that are the object of debate. Professional ethics forces doctors to give patients the best treatment available for their illness and without a doubt pharmacogenetics is destined to change something in the standard procedure through which choices have been made up until now. Will the doctor be able to prescribe a drug specifically targeted at a certain genotype, if the patient refuses to undergo the test? And how can we evaluate the case in which the test result only allows a probabilistic interpretation with regards to the efficacy and/or safety? What can be considered an acceptable risk boundary, also in consideration of the fact that the drug could be the only available one for that illness? In literature the answers to these and other questions are very variable, but there is agreement in believing that these are not new questions and that, therefore, the answers can be found through a patient analysis of each single situation in the light of existing principles and regulations.

.6.4 We must also consider the fact that, as has already happened for many diagnostic tests and as it is also happening for genetic tests, the availability of “user-friendly” pharmacogenetic tests could increment the direct marketing of the tests to the consumer (and internet sites already exist). There is a general tendency to discourage the self-administration of genetic tests, but is it not at all clear what control instruments could be set up to manage this phenomenon, also because it is not sure that, at least in certain situation, a patient who demands direct access, that is, without the doctor’s mediation, to tests could not also advance some argument in support of his/her demand. For example, in a care system based on private insurance, the mere fact of undergoing a pharmacogenetic test could attract the insurance company’s attention on the individual. To avoid losing the advantages of better drug efficacy and safety, an individual could therefore use pharmacogenetic tests directly. Always with regards to direct marketing, we must also remember that, potentially, pharmacogenetic tests can expand beyond the medical field. We can hypothesise, for example, that they can be useful in setting up a dietary regime, or a regime of nutritional supplements etc.: all of this would without a doubt create a push for the market, which someone could exploit.

.6.5 Leaving behind all these possible situations and focusing on the medical field, we can conclude that, all considered, pharmacogenetics represents a positive prospect for the doctor and the patient. We can also add that it represents an interesting prospect also for society as a whole and for health authorities, because – after the initial phase in which considerable investment is required – a more efficient and safe administration of drugs can lead to substantial savings. The cost of “wasted” drugs and the cost of treating adverse reactions to drugs are quite high (DATI) and the hope is that pharmacogenetics will have

an effect on them. At this level, however – and always in the hypothesis of a widespread integration of pharmacogenetics in clinical practice – there could be problems (also not new) with the rationality of the allocation of scarce, or in any case limited, resources destined to healthcare: these are problems that have different scopes according to whether we are considering public, private or mixed health systems. If – as we hypothesise – future generations of drugs will have a pharmacogenetic basis and, therefore, the instructions for administration will require a test to identify the patients they can be administered to, health authorities could elaborate regulations that, having to answer to the overall logic of health economy, could be in conflict with the individual's interests. For example, a health authority could decide to allocate a very expensive drug only to patients qualified as "fully responsive" and to deny it to "little responsive" patients: the decision could be easily founded on common pharmaco-economic principles which, although trying – and this is the shared hope – to integrate equity considerations in the access to medical care, is strongly limited by aggregative macro-economic approaches, which are not sensitive to the individual distribution of benefits. But – as we were saying – this is not a topic specific of pharmacogenetic, even if it could be exacerbated as a consequence of a heavy integration of pharmacogenetics in healthcare.

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