Human pharmacogenomics: The development of a science

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Date received (in revised form): 6th July 2004

Abstract

Until about 50 years ago, the altering of a normal drug effect by a genetic deficiency was only rarely observed. Here, my discovery of the genetic variant of butyrylcholinesterase affecting succinylcholine action is described in some detail. Such discoveries led to the combination of the two older sciences, genetics and pharmacology, thereby forming pharmacogenetics. After the discovery of similar examples in the years that followed, pharmacogenetics expanded on the basis of two discoveries. First, the common occurrence of interethnic differences in drug response and, secondly, the fact that most pharmacological differences were multigenic. New methodologies brought a transition to pharmacogenomics; this included detection of clinically important genetic variants and has uncovered potentially new drug targets. The arrival of personalised medicine — where a patient's genes determine the choice of drug to be administered — can be hoped to gradually improve drug safety and efficacy. Efforts to reach this level of perfection are, however, dogged by uncertainties.

Keywords: *pharmacogenetics, butyrylcholinesterase, interethnic pharmocogenomics, personalised medicine, multigenic variation, history*

Introduction

Pharmacogenomics and pharmacogenetics are two closely related and overlapping sciences. It was only relatively recently that pharmacogenomics arose from pharmacogenetics, mainly reflecting technical improvements which are changing some outlooks, as will be described. The change paralleled the broadening of classical genetics into genomics.

Pharmacogenetics arose from the melding of the older sciences, pharmacology and genetics. The recognition that there are healing substances of the kind that we now call 'drugs' is certainly thousands of years old; Egyptian medical papyri contain numerous prescriptions.[1] Moreover, observations of human and animal inheritances were reported in early Greek papers.[2] Both pharmacology and genetics became recognised sciences only in the 18th century, however — pharmacology through the work of Claude Bernard and Oswald Schmiedeberg,[1] genetics through Gregor Mendel and Francis Galton.[2] Pharmacology and genetics remained separate sciences until 1931, when Sir Archibald Garrod anticipated the existence of pharmacogenetics in his book *Inborn Factors in Diseases*.[3] In 1949, J.B.S. Haldane predicted the occurrence of unusual reactions to drugs on the basis of biochemical individuality.[4]

In fact, by 1949, a few isolated cases of genetic differences in drug response had been seen. In 1932, the ability to taste the chemical phenylthiocarbamide (PTC) and the lack of it were shown to be inborn characteristics.[5] In 1937, Waldenström described drug-induced porphyria.[6] The genetic lack of atropine esterase in rabbits was demonstrated in 1943.[7] The Second World War revealed a haemolytic disease which was caused by the antimalarial drug primaquine only in American soldiers of African decent; its genetic basis was fully established after the war.[8,9]

Pharmacogenetics became an established science in the 1950s. First, there were further discoveries; for example, the genetic deficiency of N-acetyltransferase, the enzyme which destroyed the then revolutionary anti-tuberculosis drug isoniazid.[10,11] At about the same time, I discovered a genetic variation of butyrylcholine esterase (then called 'plasma cholinesterase' or 'pseudocholinesterase'), a variation which caused fatal responses to succinylcholine, a drug used during anaesthesia.[10] Secondly, in 1957, Motulsky summarised all then available data in a paper entitled 'Drug reactions, enzymes, and biochemical genetics', a paper sponsored by the Council on Drugs of the American Medical Association.[12] In 1959, Vogel published the word 'pharmacogenetics'.[13]

Pharmacogenetics: An early case study

By telling, in personal terms, the story of my discovery of a genetic variant of choline esterase, I hope to give an example which shows how a simple conclusion may arise from a combination of unforeseeable circumstances, and that such a conclusion may lead to a new scientific research area.

The discovery to be described was based on events in three cities: Berlin, Philadelphia and Toronto. The experience began my life-long involvement with pharmacogenetics and led me to participate in the further development of this area of study.

Berlin

The cholinesterase story had an indirect starting point after the Second World War in Berlin, close to my home. Nutrition was still generally poor, which often affected the state of people's health; local research[14] showed that low levels of nutrition lowered the concentration of some proteins. When a few people died from injection of the old — and generally considered safe — local anaesthetic procaine, a dietary change of a protein level was suspected. Procaine was known to be safe because, after its injection, it was usually quickly destroyed by an enzyme called procaine esterase.[15] My boss, the pharmacologist Dr Herken, suspected that poor nutrition in these patients had lowered their procaine esterase levels, thereby enabling the drug to kill them.

He asked me, a young investigator at that time, to study the effect of nutrition on procaine esterase levels. For this purpose, I was to use ultraviolet photometry;[16] using ultraviolet light, we hoped to be able to measure the level of procaine in the plasma. The production of ultraviolet light was difficult at that time in Berlin; the equipment required repeated attention by a physicist, and we were only able to measure the light intensity because the occupying US army had donated a photometer to Berlin University. My experiments showed that the method was able to measure procaine concentrations in human plasma, and thereby procaine destruction.

Some time after I had started this work in Berlin, my experiments were terminated. Sponsored by the US Unitarian Church, a group of American Scientists visited Berlin University, and one of the scientists (Dr Carl F. Schmidt) invited me to come for a year of study to his Department of Pharmacology at the University of Pennsylvania in Philadelphia. This was a wonderful turn of events, and shortly afterwards I travelled to Philadelphia.

Philadelphia

In the Department of Pharmacology in Philadelphia, I was kept busy participating in large cardiovascular experiments, and I also did some teaching. By talking with various investigators, I learned that the department owned a Beckman spectrophotometer, a little box on a bench top that could do the ultraviolet work that in Berlin had required a whole room and the help of a physicist. I was allowed to use this instrument when it was free, which was mostly at night. I was delighted to note that, with this spectrophotometer, I could measure the destruction of procaine, as I had done in Berlin. Playing around, I tried other chemicals and thereby found that the procaine-destroying enzyme also destroyed benzoylcholine, a substance known to be a substrate for plasma cholinesterase.[17] Thus, I had discovered a new method of measuring this cholinesterase activity, and my data indicated that procaine esterase was actually a cholinesterase. When I tried to publish this enzyme identity in a biochemical journal, the paper was rejected as useless, on the mistaken assertion that human plasma was known to have only one esterase (there are now known to be many!). The paper was published by a pharmacological journal.[18]

One day, a co-student introduced me to Dr Britton Chance, the famous head of the Johnson Foundation in Philadelphia. Dr Chance found my studies on plasma cholinesterase interesting and offered help whenever needed. He invited me to visit his foundation and to attend lectures. I thereby received instruction in enzyme kinetics, a field unfamiliar to many pharmacologists. This knowledge later led me to an important research decision.

My original invitation to study pharmacology in Philadelphia was for a year, but after a while Dr Schmidt invited me to become a permanent member of his department. I was happy to accept, however, this required a change to my student visa to an immigration visa. The simplest way to get this change was to leave the USA and to formally immigrate from Canada. I therefore prepared for this.

Toronto

A special experience made me aim for Toronto in Canada: Dr J.K.W. Ferguson, chief of the Department of Pharmacology in Toronto, attended a US meeting where I gave a talk. Afterwards, he invited me to visit him in Toronto whenever possible; thus, I hoped to immigrate to the USA from Toronto. When I met Dr Ferguson in Toronto, we had some interesting conversations and he invited me to take up a permanent position in his department. I accepted his invitation, partly because of an unusual but pleasant experience: while I was in Toronto, the future Queen Elizabeth and her husband visited the city; they rode in an open car, without safety guards, through the streets and the University campus, amidst friendly cheering by the crowds. This was in strong contrast to the ecstatic reception of General McArthur on his return to the USA from the Korean War; this elation reminded me of a Hitler reception which I had experienced as a youth in Germany. It was obvious to me that Canadians had a quieter mentality than many Americans, and I consequently felt that life in Canada would be better than in the USA — and better than it had been in Germany. When I wrote to Philadelphia, asking for Dr Schmidt's opinion, he wished me good luck in Toronto.

I noticed that studies of cholinesterase were being pursued in Toronto. While the discovery of cholinesterase in the early 1920s had earned Dr Otto Loewi, in Austria, the Nobel Prize, the Dutchman Bruno Mendel and his colleague H. Rudney, working in Toronto, discovered in 1943 that there were actually two different cholinesterases: one occurring in

red blood cells and in the central nervous system,[17] and the other only in the plasma. They called the one in red blood cells 'true cholinesterase' and the one in the plasma 'pseudocholinesterase'. True cholinesterase was obviously important, acting at nerve endings to destroy the transmitter substance acetylcholine. The function of pseudocholinesterase was a puzzle, and was still under biochemical investigation when I arrived in Toronto. I heard about this ongoing research and learned that the investigators were still using the traditional gasometric method to determine cholinesterase activity. This method was labour intensive and complicated, requiring precise measurements of CO_2 gas, which was liberated in minute quantities by cholinesterase action. I told the investigators that I had a much better method for measuring the enzyme activity and they asked me to demonstrate a comparison between the two methods.

I received blood donations from a number of students, measured their cholinesterase activity using both methods and found that the results correlated very well.[19] The biochemists were still not satisfied; as all of my tested students had esterase activity in the normal range, they wanted to know whether my test would properly indicate cases of low activity.

Dr Ferguson knew how we could find such cases. Certain patients with mental health problems were routinely given electroshock treatments, since there were no effective drugs at that time. The shock was produced by application of an electric charge to the skull, and thereby to the brain. A side effect of this treatment was sometimes an occurrence of severe muscle contractions, severe enough to break a bone in the patient. To avoid this side effect, patients received an injection of succinylcholine prior to the shock application; succinylcholine, a depolarising blocker of acetylcholine action, prevented the muscle contractions without affecting the mental benefit of the shock. The treatment usually required multiple shock treatments over the course of weeks or months.

A local physician knew patients in whom succinylcholine did not just act for a few minutes, as was usually the case, but who remained paralysed by it for about an hour. This prolonged action of succinylcholine was known to be caused by an impairment of plasma cholinesterase, which normally brought about the rapid breakdown of the drug.[20] Tests in a government laboratory had confirmed low cholinesterase activity in such patents. I therefore received blood samples from some of these patients so that I could test their cholinesterase activity using my ultraviolet method.

When I measured the enzyme activity via the destruction of benzoylcholine in these patients, I noticed that the reaction was strange. Usually, the disappearance rate of benzoylcholine followed a straight line until the reaction was finished, usually within a few minutes. In the plasma of the 'special' patients, however, the disappearance rate was not straight but curved; the initial reaction rate was normal but then gradually slowed down, so that it took a long time for the reaction to finish. I had learned enough enzyme kinetics[21,22] by then to realise that the enzyme–substrate affinity was low; that is, there was poor binding between enzyme and substrate. I showed that there were no enzyme inhibitors in the tested plasma. Thus, the reduced affinity could only have been caused by a structural change in the enzyme protein. Such a change was likely to have a genetic cause.

To test this hypothesis, I asked for an investigation of the relatives of the patients and thereafter obtained plasma from patients' parents. The destruction of benzoylcholine in these plasma samples was neither normal nor as abnormal as that in that of the patients. Further investigation took a long time, but I finally found a substance called dibucaine, which strongly inhibited the normal enzyme but hardly touched the patient's enzyme;[23] the esterase activity of the parent samples was partly inhibited. It was clear that the patients were homozygous, and the parents heterozygous, carriers of the enzyme variant, while the normal subjects had the wild-type enzyme. It was the clearest possible demonstration of the control of a phenotypic character by a structural, and readily measurable, variant of a gene. Very few equivalent human cases were known at that time.

I was excited by my finding, which indicated that a genetic variant altered a drug response. I wondered whether this was a unique observation or whether there were other such cases. I searched the literature and found some of the above-quoted case reports. I still considered the fact that a gene may affect a drug response to be an exciting but generally unknown truth. I decided to write a book about this topic. It took a few years and considerable effort; my book, entitled *Pharmacogenetics: Heredity and the Response to Drugs*, came out in 1962.[24] Further studies on this topic became the purpose of my scientific life.

The development of pharmacogenetics

The following years brought the recognition of many new pharmacogenetic examples.[25] For instance, there soon came the discovery of variable phenytoin hydroxylation, acetophenetidine dealkylation and paraoxonase activity. Of particular importance in the late 1970s was the discovery of the cytochrome CYP2D6 variation, an enzyme metabolising debrisoquine and sparteine now known to also metabolise and about 65 other drugs.[26] Of wide interest also was the finding of variable ethanol metabolism;[27] slow metabolism leads people to reduce alcohol consumption and thereby to less alcohol addiction. Many more examples could be cited, not only concerning drug-metabolising enzymes, but also variability of drug transporters and of receptors. Pharmacogenetics was on its way. The next, fundamental, discovery in pharmacogenetics was that of population differences.

Population differences

The frequent occurrence of population differences in drug responses represents an important aspect of pharmacogenetics. It was a strange coincidence that drew my early attention to this fact. Out of curiosity, students and colleagues in my laboratory studied the metabolic glucuronidation of amobarbital, an old and safe barbiturate drug widely used at that time. We happened to find that some members of a family could not metabolise this drug; we obviously had discovered a new genetic deficiency.[28] We therefore set out to discover whether this was a rare or a frequently occurring deficiency.[29] We asked our class of 140 students to volunteer to take the drug and then supply a urine sample. The laboratory tests showed average metabolism in most students, but seven samples had much more of the metabolite than did the others. I suspected a laboratory error and hoped to retest these students. I could only identify students by their allocated numbers and did not know their names. I gave a colleague the numbers of those to be retested; a few minutes later, he came back to me excitedly, telling me that the numbers I had given him all belonged to subjects with Chinese names.

Retesting of the suspicious urine samples showed that there had not been a laboratory error.[30] Furthermore, there were no other Chinese students in the class besides those retested. When I, of European origin, tested my own metabolism of amobarbital along with that of a south-east Asian colleague, our personal metabolic capacities correlated with those seen in the students. We therefore had strong evidence for an ethnic difference in drug metabolising capacity.

By coincidence, the genetic control of the metabolism of debrisoquine had just been discovered,[31] but because of our interest in pharmacogenetics, we also tested this in my laboratory. By chance, we noticed that students of Chinese origin metabolised debrisoquine significantly more slowly than those of European origin[32] — a difference later explained in Sweden by a genetic enzyme alteration.[33] It seemed to us that we had found a second ethnic difference in drug metabolism! I searched the literature, found more examples and published the available data.[34]

To date, many ethnic differences in drug response have been recognised. A recent survey enumerated 42 drug-metabolising enzymes that show differences between populations.[35] In addition, there are population differences between drug receptors and transporters. The differences are of two kinds: there are often differences in the frequency of a given variant between two populations, but, in addition, the kinds of variants may differ. In fact, the frequencies of given variants are more often different than identical in geographically separated populations.

It is interesting to consider the roots of such differences. There are some examples suggesting that the variation in a drug-metabolising enzyme becomes clinically important only when the drug is present — that is, it is immaterial when absent, the bearer of the variant being a perfectly healthy subject. In this case, the variant must represent a random mutation. In other cases, the variant may affect the health of subjects — whether or not a drug is present. For example, the haemolysis-associated deficiency in the primaquine-metabolising glucose-6-phosphate dehydrogenase (G6PD) enzyme[8,9] protects affected subjects against malarial infection by *Plasmodium*.[36] In consequence, G6PD deficiency occurs frequently in some tropical countries but is absent in central Europe. Genetic alterations represent fitness differences rather than randomness.

These pharmacogenetic data contradict statements, obviously politically influenced, that ethnically defined human populations are genetically indistinguishable; this not true. For example, Cavalli-Sforza and his colleagues tested 120 allele frequencies in 42 geographically separated human populations.[37] They found many differences, which they summarised by describing nine genetically distinguishable population clusters; Africans differed most from all others. Since an ethnic difference in a drug response may be clinically important, the possibility of such differences must be considered during the development of a new drug.

Multifactorial variation

As described, pharmacogenetics started with the recording of differences in drug metabolism or drug response due to an alteration in a specified protein, indicating variation in a particular gene. Most differences between individuals or populations, however, are not caused by a single genetic variant but by variations in, and cooperation between, many genes, usually contributed to by environmental factors. This general rule also applies to pharmacogenetics.

The differences between people may be caused by a number of variant genes which may interact and by variable expression of genes; environmental factors usually contribute to the variation. The expression of a gene may be affected by other genes, or by hormones, foods or drugs;[38] drug effects on gene expression have been known for a long time, as a result of finding that the application of a drug enhanced its metabolism. Thus, multifactorial variation is complex and its causes are usually unknown.

From a practical point of view, the fundamental question is often to discover the magnitude of the genetic and environmental contributions to a given variation, usually expressed in terms of heritability. The traditional way to answer this question is via a twin study, that is, a study comparing the within-pair differences in identical and fraternal pairs of twins. A twin study is the only means assessing the heritability of a disease or of any constant biological characteristic. In addition, pharmacogenetic differences have often been tested by twin studies.[39]

In this case, however, an alternative method is available.[40] Since a drug effect comes and goes, one can apply a drug two or more times to a given person and on each occasion

measure the drug response or metabolism. Since the person's genes are the same, any difference in response can only have environmental causes. By giving a drug twice to a group of people, one can compare the magnitude of the within-person and the between-person variations. Since the between-person variations will be caused by a mixture of genetic and environmental factors, the comparison reveals the magnitude of heritability.[40]

Pharmacogenetics and pharmacogenomics

As stated in the Introduction, the transition of pharmacogenetics into pharmacogenomics reflects technical improvements which have changed some outlooks. The development of methods that allow high-throughput screening of genes made it possible to assess the presence of variant structures in many genes at the same time.[41] Thus, one could look at the genome and not just the variation of one or other gene of interest. Looking at the genome[42] revealed the fact that only about 3 per cent of the DNA structures in it are traditional genes which function as protein producers. We still have to struggle with questions regarding the function of most of our DNAs. In other words, the traditional science of genetics widened into genomics and we now have two overlapping sciences which share much basic information.

'Overlapping' means that both sciences pursue the same aim; pinpointing genetic alterations which affect drug responses. The genomic methodologies may improve the rate of detection of such alterations, however. These methodologies permit, in addition, some previously almost impossible investigations, whose outcomes promise to strongly affect pharmacology and, thereby, pharmacogenetics.

Most common diseases are due to numerous, often interacting, genes, with environmental factors contributing to a greater or lesser extent, and genomic methodologies are used in the search for such disease-associated genes.[43] The detection of such a gene probably means that a new drug target has been identified; the target may be the gene itself or the protein encoded by the gene. Such a finding would stimulate the search for chemicals that fit the target and which thereby become new drugs which help to cure the disease.

This situation is often complicated by the fact that a somewhat different set of genes may cause the same disease in different people. In other words, there may be variations in the disease-contributing genes.[44] This could mean that different drugs may have to be used to combat the same disease in different people. Determination of which drug to use in which person will again require genomic screening.

Thus, pharmacogenomics may concern us with a kind of variation which was unknown and unsuspected at the time when pharmacogenetics ruled.

Personalised medicine

Since practically all drug effects are, to some extent, genetically controlled, personalised medicine means that the choice of drug for any individual will have to be determined mainly by that person's genes. This has many implications.

In the first place, in order to make any genetic test results clinically useful, they must be interpreted in terms of pharmacogenetics. Perhaps the future physician will be better trained in genetics than is currently the case; perhaps there will be pharmacogenetic specialists who will interpret the data for the physician; or perhaps appropriate computer programs can be developed which will provide the necessary information to the physician. But the most effective way to introduce pharmacogenetics into clinical medicine may be a change in regulatory and industrial policies; that is, to provide information on which (if any) genes are known to affect the action of any drug to be prescribed.[45]

Secondly, a number of legal and ethical questions have to be resolved. Knowledge of a person's genes may allow an assessment of state of health, disease probabilities and of probable life span — all items of interest to insurance companies. Is a person the owner of his or her genes, or of his or her genetic test results? Are there legal rules for the medical use of such results?

Thirdly, if the person's genetic make-up has to be established, who will bear the costs of the genetic tests? One can only hope that the costs will lessen as time goes on.

If these three problems are solved, the road towards personalised medicine is open. A first step could be assigning the patient to a genetically similar population group. Between-person differences tend to be smaller within a genetically defined population than in a random population. Before one knows a given patient's genes, his or her assignment to such a population could somewhat reduce the chance of encountering unexpected reactions. Thus, treating the patient as a member of a known population, even of a geographically or ethnically defined group, could be a small step in our efforts to create personalised medicine.

Even if one has located a gene which is changed in a given person, however, two problems remain. First, a given gene may be mutated in many different ways.[46] There may be an absence of a protein, a functional decrease or a change in properties, for example, affecting various drugs differently. This may cause difficulties. For example, cytochrome CYP2D6 metabolises debrisoquine in both a European and an African population, but an African variant metabolises debrisoquine but not metropolol.[47] Secondly, the expression, and thereby function, of a gene may be changed by gene interactions, hormones or environmental factors like foods and drugs.[48] Thus, identification of a functional gene still leaves uncertainties.

Whatever we do, now or in the future, creation of personalised medicine is a worthwhile aim, because it represents an effort to improve a person's chance of a healthy life;

however, personalised medicine will never be a truly reliable science. For example, environments may change and, more specifically, gene expression may change; all predictions based on gene structure therefore represent functional likelihoods but not certainties.

We will always have to live a life of probabilities.

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