Predictive Genetic Testing – New Possibilities in Determination of Risk of Complex Diseases

Catarina Quinzii, Francesca Belpinati, Pier Franco Pignatti

Section of Biology and Genetics, Department of Mother and Child, Biology and Genetics, University of Verona, Verona, Italy

Predictive genetic testing offers the possibility to statistically determine the risk of inheriting a complex phenotype by establishing an individual’s genotype for metabolic polymorphisms. Here we discuss the conditions under which a predictive test may be offered to a patient and the problems connected with it. Examples of predictive genetic testing for multifactorial diseases and drug responses are given. We describe in detail the association of the C677T polymorphism of methylenetetrahydrofolate reductase gene with hyperhomocystinemia and folate levels, as an independent risk factor for cardiovascular disease, and the association of a polymorphism of the promoter of the 5-lipoxygenase gene and the response to leukotriene inhibitors in asthma. Prospective development of genomic medicine and its use in the study of complex traits will hopefully bring significant benefit to the population and enhance the prevention and therapy of common diseases.

Key words: asthma; cardiovascular diseases; genetic screening; genome, human; genomics; hereditary diseases; hyperhomocystinemia; mutation; pharmacogenetics; polymorphism (genetics)

Rapid progress in molecular genetics and human genomic research leads to faster gene identification and to the development of simple and reliable genotyping methods. It also offers the possibility of genetic prediction of each individual’s susceptibility to disease and drug responsiveness (1). It is, therefore, predicted that genomic medicine (2) will join the mainstream medical practice in the near future (3). We here delineate such prospective advances, propose some general requirements for predictive testing, indicate a few specific examples, and suggest directions for further development of the field.

Common Diseases

Based on studies of inheritance patterns in families and twins, it is thought that most frequent diseases in economically developed countries have a genetic component, ie, cardiovascular diseases, osteoporosis, hypertension, allergies, diabetes, Alzheimer disease, obesity, and tumors.

These diseases also have non-genetic components; therefore, the main task of researchers is to determine the relative roles of genes and environment in the determination of the cumulative risk of developing these diseases.

Predictive Testing

Conditions

A predictive genetic testing can be proposed if the following conditions are met:

a) The test must be available. As the knowledge of gene relations to diseases increases, the number of possible tests is bound to increase as well.

b) The risk is ascertained. This is now true only for a limited number of examples; different studies may show different values and the risk ratio can vary between very small and large value compared with the general population.

c) There are indications for individuals or groups of people to be selected for testing. It is not advisable to test everyone for everything.

d) The patient must benefit from testing. There must be ways to reduce the risk of a disease, e.g., a change in life-style and diet, or the enactment of specific therapeutic measures.

Problems

Various problems connected with predictive testing are the following:

a) Technical. Many genes may be related to the risk of disease development, and several different mutations in these genes may be present, with different degrees of involvement. A polymorphism may be un-
related to the risk, whereas another polymorphism in the same gene may be related to risk modification.

b) Medical. Genetic counselling may be difficult in respect of risk estimates, and certainty about the effect of the mutations in that particular individual may not exist. The role of environmental factors may be difficult to determine for the individual at risk. There may be several preventive and therapeutic measures to consider and their practical effectiveness and acceptability to the individual may vary. We must emphasize that the polymorphism is present at different frequencies in the normal population and it is not per se an indication of disease.

c) Ethical. Genetic information should generally be confidential and released only to the individual tested, after an informed consent has been obtained. Nevertheless, other members of the family may wish to receive that information as they may share genetic determinants with the tested individual. Generally, third parties should not receive the information.

Some Examples of Genetic Risk Modification for Common Diseases

Although examples of predictive testing are already available, more research is necessary to detect other common polymorphisms in the population that may be associated with the phenotype and to confirm isolated literature reports in independent studies. Some examples of polymorphisms possibly involved in common disorders are given in Table 1. For some of these disorders, possible interventions that affect the carriers of the mutation might be considered, e.g., avoidance of oral contraceptives in carriers of the factor V of Leiden mutation or the G20210A prothrombin mutation to decrease their risk of deep venous thrombosis and cerebral vein thrombosis (4), tamoxifen therapy in carriers of BRCA mutations to decrease mammary and ovarian cancer risk (5), or avoiding oral contraceptives in carriers of the factor V of Leiden mutation or the G20210A prothrombin mutation to decrease their risk of deep venous thrombosis and cerebral vein thrombosis (4), tamoxifen therapy in carriers of BRCA mutations to decrease mammary and ovarian cancer risk (5), or modifying dietary intake of calcium in carriers of the vitamin D receptor polymorphism to improve bone density (6).

Cardiovascular Diseases

The diseases affecting the cardiovascular system are the first cause of suffering and death in economically developed countries. Cardiovascular diseases are related to age, sex, familial history, cigarette smoking, obesity, hypertension, physical inactivity, diabetes, hypercholesterolemia, increased levels of low density lipoprotein-bound cholesterol, decreased levels of high density lipoprotein-bound cholesterol, and other factors. The percentage of risk attributable to either genetic or environmental causes can be estimated by epidemiological studies. Some of the risk factors are mainly environmental, as smoking or diet, whereas others are more genetic, as lipoprotein levels (7). Many different genes may be involved in development of cardiovascular disease, such as genes related to lipid or homocysteine metabolism, or to hypertension, blood coagulation, leukocyte adhesion, inflammation, etc. At the 50th anniversary of American Heart Association, it was indicated that the identification of genetic and environmental factors involved in cardiovascular diseases may stem the disease epidemic by the implementation of primary prevention measures in individuals with subclinical atherosclerosis or with no atherosclerosis but with multiple risk factors, or in individuals at low risk (8). A specific example of a gene/environment correlation in determining genetic risk modification in cardiovascular disease is given below.

Methyltetrahydrofolate Reductase and Hyperhomocysteinemia

There is a general interest in increased plasma homocysteine as a risk factor for coronary artery disease, especially since many cases of mild hyperhomocysteinemia can be easily corrected by folic acid supplementation (9). The folate-related methyltetrahydrofolate reductase enzyme, which participates in remethylation of homocysteine to methionine (Fig. 1), is important in preventing homocysteine accumulation. A point mutation in the methyltetrahydrofolate reductase gene (C677T) renders the enzyme thermolabile and less active. Homozygosity for the C677T mutation is common (10-18% of Caucasian population) and generally associated with increased homocysteine levels. Extensive investigations on this mutation as a candidate genetic risk factor for coronary artery disease have yielded conflicting results and a recent meta-analysis has been negative (10). However, this meta-analysis has been criticised because of insufficient data and the pronounced genetic and nutritional heterogeneity of the populations included (11). Our preliminary results also failed to detect any association between the methyltetrahydrofolate reductase C677T itself and coronary artery disease (12). However, we disclosed a gene/environment interaction of great potential interest for both patients with coronaropathy and those without it, i.e., that low folate status influenced homocysteine levels not only in TT but also in CT heterozygous subjects (Fig. 2). This prompted us to study a larger sample of patients. We found a graded interaction between this common mutation and folate status. Since genotype-specific thresholds can be estimated, we

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Table 1. Examples of possible genetic predisposition to multifactorial diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Mutation</th>
<th>Relative risk</th>
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<tbody>
<tr>
<td>Venous thrombosis</td>
<td>FV, VDR</td>
<td>Leiden, DR 3-4</td>
<td>8-20 (with oral contraceptives)</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>HLA, Apo E</td>
<td>Various</td>
<td>60-85% lifetime risk</td>
</tr>
</tbody>
</table>

*Adapted from: Strachan and Read, Human Molecular Genetics, BIOS Scientific ed., 1996.

*From ref. 4.

*From ref. 5.
found that subjects with folate levels below genotype-specific threshold values sufficient to determine hyperhomocysteinemia had a mild but significant risk of coronary artery disease (13). Hyperhomocysteinemia and low folate is present in 10% of CC homozygotes, 35% of heterozygotes, and 57% of TT homozygotes (Girelli et al, in preparation). Our findings, if confirmed, may be useful in designing future strategies to reduce the individual’s risk of coronary artery disease.

Pharmacogenetics

Individual variability in response to drugs can affect treatment efficacy, producing a diminished or increased response to a drug or even toxicity, which causes harmful adverse reactions. Efficiency of commonly used antipsychotic drugs can be as low as 40%, or 60% for antihypertensive drugs. It is estimated that in Great Britain 1/15 hospital admissions are due to adverse reactions to drugs (14).

There are several possible causes of the variability in the effects of drugs. They include pathogenesis and severity of the disease, the interaction among drugs, patient’s age, sex, nutritional status, renal and hepatic functions, concomitant diseases, and hereditary differences in drug metabolism or drug targets (15). Major pharmaceutical industries have recently proposed the use of pharmacogenetics for better individual specific drug efficacy and safety, effectively summarizing the idea in the slogan “the right medicine for the right patient” (16,17). Predictive genetic testing in pharmacogenetics may have distinct ethical, legal, and social implications, compared to predictive genetic testing for complex diseases. Usually, there is no stigma of a disease attached to the pharmacogenetic testing and the families of the patients may not necessarily be involved. Some inherited differences in drug effects have been known since the 1950’s, such as relation between prolonged muscular relaxation after suxamethonium and inherited plasma cholinesterase deficiency, hemolysis after antimalaric therapy and the level of erythrocyte glucose-6-phosphate dehydrogenase (G6PD) activity, peripheral neuropathy from isoniazide and inherited differences in acetylation of that drug (15).

Several other instances of pharmacogenetically effective genes have been found; the best known are common polymorphisms in drug metabolism genes and of some receptors and transporters, as well as in other drug-targets (15,18,19). Table 2 gives some examples of clinically relevant genetic polymorphisms influencing drug metabolism and drug effects. About 6-7% of the white population are slow metabolizers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Medications</th>
<th>Drug effect linked to polymorphism</th>
</tr>
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<tbody>
<tr>
<td>Drug-metabolism enzymes (from ref. 15)</td>
<td>Debrisoquin hydroxylase (CYP2D6)</td>
<td>Betablockers, antidepressants, antipsychotics, codeine, debrisoquin, dextromethorphan, and others</td>
</tr>
<tr>
<td>Thiopurine methyltransferase</td>
<td>Thiopurine methyltransferase</td>
<td>Mercaptopurine, thioguanine, azathioprine</td>
</tr>
<tr>
<td>Drug targets</td>
<td>Cholesteryl ester transfer protein (CEPT)</td>
<td>Pravastatin (anticholesterol)</td>
</tr>
<tr>
<td></td>
<td>β-adrenergic receptor (B2AR)</td>
<td>Albuterol (β, agonist)</td>
</tr>
</tbody>
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due to a defect of CYP2D6 (debrisoquine hydroxylase) of the cytochrome P450 group, the main enzyme in drug metabolism. For drugs with a restricted therapeutic range, e.g., tricyclic antidepressants, the slow metabolizers will develop a citotoxic plasma concentration of the drug after the administration of a standard dose. Also, in these individuals codeine might not be activated by CYP2D6 and therefore show no analgesic effect. The lack of inactivation of mercaptopurine, thioguanine, or fluorouracil by the enzyme thiopurine methyltransferase is present in rare cases (about 1 in 300 individuals) and produces increased toxicity of the drugs (15). A common polymorphism in the cholesteryl-ester transfer protein interpheres with the activity of statins in reducing morphism in the cholesteryl-ester transfer protein and increases toxicity of the drugs (15). A common poly-
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in drug metabolism. For drugs with a restricted thera-

cytochrome P450 group, the main enzyme
involved in the biosynthesis of leukotriene A4. Leu-

Table 3. S-lipoxygenase (ALOX5) – 147Sp1 promoter poly-
morphism and response to a leukotrien inhibitor ABT-761, an experimental derivative of Zileuton (22), in asthma

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. (%) of patients</th>
<th>Response ( % FEV1 variation)</th>
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<tbody>
<tr>
<td>3/5 repeats</td>
<td>64 (56)</td>
<td>+19</td>
</tr>
<tr>
<td>5/3, 4, 6 repeats</td>
<td>40 (33)</td>
<td>+23</td>
</tr>
<tr>
<td>3, 4, 6/3, 4, 6 repeats</td>
<td>10 (9)</td>
<td>-1</td>
</tr>
</tbody>
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without the 5-repeat allele are insensitive to ABT-761, an experimental drug derived from the commonly used leukotrien inhibitor, Zileuton (22).

Complex Inheritance and Multiple Genetic Testing

Several genes may be involved at the same time in the same individual in determining multifactorial diseases or drug response, e.g., a gene for drug metabolism together with a gene for a drug receptor (15), or a gene for Apo E and a gene for presenilin in Alzheimer disease (23). Therefore, the study of genetic interactions, as well as the study of other environmental factors as discussed above, will be relevant for multiple risk determination in a given individual. The analysis of several genetic determinants is important at the research stage, when genes and mutations possibly involved in the phenotype should be identified, and at the practical medical stage, when two or more mutations involved in risk modification may have to be tested in a given individual. The development of multiplex genotyping tests involving techniques of nucleic acids hybridization on solid supports may prove to be very useful, allowing rapid automated analysis of several samples at a time. Among other methods, reverse dot-blot technique or DNA chips for complex phenotype assessment have been proposed. For example, we used nucleic acids hybridization on solid supports for multiplex testing of 487 patients with coronaropathy and 261 without coronaropathy for polymorphisms related to cardiovascular risk assessment. Fifteen genes involved in various aspects of cardiovascular physiology (lipid metabolism, hypertension, homocysteine metabolism, thrombosis, and leukocyte adhesion) were tested. We found preliminary evidence of an association between coronaropathy and a common promoter polymorphism in the of Apo C III (apolipoprotein C III) gene (24). Another possibility is multiple genetic testing using DNA chips, as recently indicated for hypothetical pharmacogenetic risk assessment in acute lymphocytic leukemia drug therapy, screening (15). DNA chips may also be useful for the future development of tests involving single nucleotide polymorphism-linkage disequilibrium (SNP-LD) patterns to identify genetic profiles associated with an adverse drug response during the development of a drug or initial post-marketing surveillance phase (17).

Conclusion

Will the mapping and sequencing of the human genome have an impact on common disease prediction and treatment? In a recent paper “Will genetics revolutionise medicine?”, Holtzman and Marteau (25) indicated possible problems that might derive from the incomplete penetrance of genotypes for common diseases, the limited ability to tailor treatment to genotypes, and the low magnitude of risks conferred by various genotypes for the population at large. The effects of each single gene in complex traits have to be defined and genotypes with major effects found, before it becomes possible to genotype inter-

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References


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Correspondence to:
Pier Franco Pignatti
Department of Mother and Child
Section of Biology and Genetics
University of Verona
Strada Le Grazie 8
37134 Verona, Italy
pignatti@medgen.univr.it