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Gene Analysis and the Clinical Chemist¹⁾

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Summary: Knowledge of gene analysis methods and concepts will be important to the clinical chemist in the near future. Currently most gene analyses must be performed by indirect techniques, using polynucleotide probes hybridizing close to or on the disease gene but not on the position of the mostly unknown gene mutation (restriction fragment length polymorphism analysis). The sensitivity and specificity of such assays are affected by biological and methodologic factors, and are being continually improved. Preventive medicine is a promising area for gene analysis which will possibly fit well into the domain of clinical chemistry. The application of nucleotide hybridization analysis in tissue matching for organ transplantation, and in the detection and differential diagnosis of malignancies is in its early stages. A very promising, and rapidly emerging, technology is the direct detection and differentiation by gene probing of bacteria and viruses in medical microbiology. Guidelines for the ethical problems of gene analysis already exist within the field of medical ethics.

Introduction

Modern methods of gene analysis can be applied to many fields of medicine. This presentation attempts to assess the impact of gene analysis on clinical chemistry, as seen by a clinical chemist. The discussion focuses on three topics:

- (1) methodological characteristics,
- (2) some of the applications of gene analysis, and
- (3) the public debate about the impact of gene analysis on the individual and society.

Methodology

Several aspects of polynucleotide hybridization technology need to be further developed for routine use in clinical chemistry. The main goals for this development are to increase the specificity, sensitivity, and speed of analysis, to make the use of radioactive labelling unnecessary, to automate analysis, and to increase the cost/benefit ratio.

Table 1 reviews the different procedures of hybridization technology. Some subheadings have been added under the "Southern blot" technique to demonstrate the improvements in cleavage and separation of DNA/RNA. The Southern blot technique is addressed in figure 1, which depicts in a very simplified way the two basic forms of application:

direct analysis, with probes hybridizing directly on, the position of a gene defect, and

indirect analysis, with probes hybridizing in a position close to, but not directly on, the gene defect itself. The latter is called restriction fragment length polymorphism (RFLP) analysis, because the length of the DNA fragment visualized with the probe differs depending on the presence or absence of a gene mutation.

Today only a few gene defects can be analyzed with direct probes; indirect restriction fragment length polymorphism methodology has to be used for the majority of tests (8). This technique, however, only provides statistical evidence: a positive or negative test result is based on the establishment by analytical and biostatistical methods of a reliable correlation

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Tab. 1. Gene analysis: Procedures (1–4).

Procedure	Example
In situ hybridization	Chromosomal localization
Dot blot technique	Semiquantitative detection of extracted DNA
Colony hybridization	Dot blot for bacterial cultures
Plaque hybridization	Dot blot for viral cultures
<i>Southern blot</i>	RFLP, point mutations, deletions, etc.
Conventional restriction enzymes	(Homogeneous technique)
“Universal” restriction enzymes (5)	(Separation of high molecular weight DNA)
“Oligomer restriction” (6)	
Pulsed field electrophoresis (7)	
Northern blot	Gene detection with m-RNA

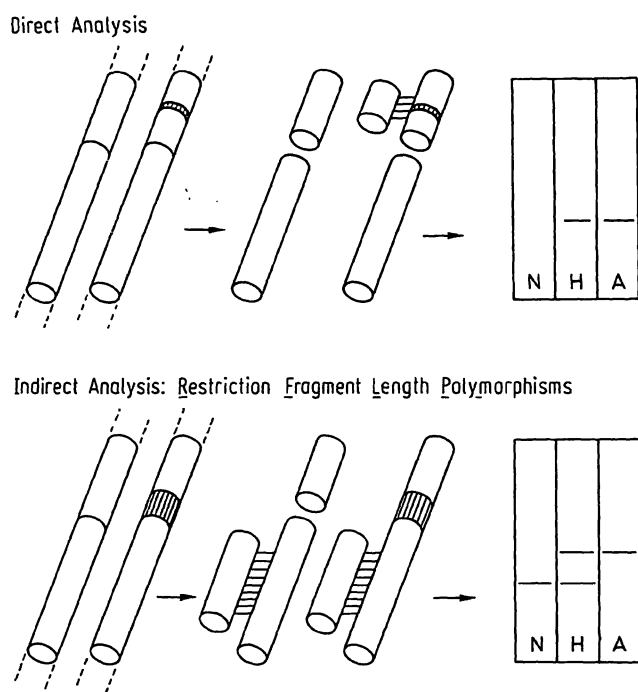


Fig. 1. Gene analysis: “Southern blot” technique. Schematic representation of a gene section with cleavage sites for restriction enzymes. *Direct analysis*: A probe is available binding on the gene mutation. After cleavage, on the separation gel a band will be detected in the samples from homozygous and heterozygous persons. *Indirect analysis*: A probe is available binding in some distance from the gene mutation. When the mutation eliminates the restriction cleavage site, the probe will mark a band of higher molecular weight in the separation gel. Normals show a band of lower molecular weight, homozygous patients show a band of higher molecular weight, heterozygous carriers show both bands. N = normal person, H = heterozygous carrier, A = affected homozygous patient, = gene mutation

(“linkage”) between appearance of a defined restriction fragment and a gene defect (1, 9). This is usually achieved by moving the probes stepwise closer to the gene defect (“gene walking, gene jumping”) until a “direct” probe has been found (10).

The clinical chemist is basically interested in judging the validity of diagnostic information obtained with gene analysis methods. Both biological and methodological factors influence the sensitivity and specificity of the results. Some examples of *biological factors* affecting the validity of test results are described below.

- *Association of disease with the marker gene chosen*: This affects the risk prediction for diseases. If HLA gene polymorphisms are used to predict the risk for type I diabetes, the sensitivity is only about 30%, because the disease and the marker gene are only loosely associated (11).
- *Penetrance of gene defects*: This affects the risk prediction for disease manifestation. Penetrance is the percentage of gene-defect carriers with manifest disease. The sensitivity of predicting cancer risk for specific organs by testing for (activated) oncogenes is about 50% because most of the tissue located oncogenes have a penetrance of this order of magnitude (13). The sensitivity of predicting the risk for *Huntington's* disease by testing for a direct *Huntington* marker would be nearly 100%, because this disease has an almost absolute penetrance (14).
- *Genetic constellation of the family*: The linkage of diseases with genetic markers depends upon the genetic constellation of a family; it is “family specific”. Assessment of the risk for genetic diseases with restriction fragment length polymorphism markers is only possible after the linkage has been established by testing as many members of the patients family as possible (15).
- *Linkage of marker genes with gene defects*: This affects the error probability of gene analysis. The problem has already been addressed with regard to restriction fragment length polymorphism

methodology. The linkage depends upon the distance between the gene defect and the marker position. The greater the distance, the greater the probability of a crossing over during the meiotic phase of cell division, resulting in uncoupling of the marker from the defect position. The unit of measure for this distance is the Morgan: one centi-Morgan is equivalent to about one million base pairs, and this distance is — with notable exceptions — equivalent to a crossing over probability of 1%, which means an error probability of 1% (16). For instance, the *Huntington* marker of *Gusella* (1983) binds at a distance of 5 c-Morgan from the gene defect. Therefore the sensitivity of a positive test with this marker is 95% (14). A good marker should be positioned at a distance of less than 0.2 c-Morgan from the gene defect, which translates to a sensitivity of 99.8% (9). The sensitivity can be improved considerably by using two probes binding on both sides of a gene defect.

Some *methodological factors* that have significant effects on the sensitivity and specificity of hybridization assays are the choices of technique and labelling system, degree of separation attained, length of the DNA used as a probe, and the nucleic acid selected for hybridization. Most of these problems are self-evident to the clinical chemist, so only two of them will be addressed further.

- The signal-generating label system used is directly responsible for the assay sensitivity. Table 2 shows a few examples of the large variety of labels published. The standard label is ^{32}P , resulting in an absolute sensitivity of 0.5 pg DNA. In recent years, great efforts have been made to develop non-radioactive detection systems. With the most elaborate enzyme-labelled biotin-avidin systems, a sensitivity of 1 pg DNA is routinely obtained. It is only a matter of time until non-radioactive labels will be available that give equal or better sensitivity than the ^{32}P label.

- Assay sensitivity and specificity can be greatly enhanced by hybridization with nucleic acid species other than chromosomal DNA. Table 3 summarizes the variants used in gene analysis. In clinical microbiology, for instance, the use of probes for RNA in bacterial ribosomes makes it possible to detect bacteria with greater sensitivity. r-RNA is present in higher concentration than chromosomal DNA: up to 5000 copies per cell.

Tab. 3. Gene analysis: Optimization of hybridization.

Goal: Increasing sensitivity		
Probe	Analyte	Example
DNA	DNA	Kinetics of association: precipitants
n(DNA)	DNA	Several probes
DNA	m-RNA	Increased stability of binding
DNA	r-RNA	Higher copy number (19)
Goal: Increasing specificity		
Probe	Analyte	Example
DNA	m-RNA	In situ: gene activation (20)
DNA	p-DNA	Antibiotics resistance (21)
		Enterotoxin genes (22)

Table 4 surveys the variety of sample materials that can be used for hybridization assays. It is interesting that modern cell sorting equipment has made it possible to isolate fetal cells from the mother's blood cells (1 fetal cell in 2.5 million blood cells). This technique will possibly facilitate prenatal screening procedures.

Tab. 4. Gene analysis: Sample materials.

Sample materials	Application (examples)
Blood	Infection: bacteria, viruses
Blood cells (nucleated cells)	Genetic diseases
	Malignant diseases
	Identity
	Exposure
Tissue cells	Genetic diseases
	Tissue typing (transplantation)
	Disease predisposition
	Oncogenes
Tissues	In situ hybridization:
	Papilloma virus infection
Fibroblast cultures	Genetic disease carriers
Sperm cells	Identity
Hair roots	Identity
Feces	Infection: viruses
Fetal cells	Genetic diseases
	from amniotic fluid
	from chorionic villi
	from maternal blood
Chromosomes	Genetic research: gene mapping

Tab. 2. Gene analysis: Labeling of probes (3, 17, 18).

Radioactive labels	Absolute sensitivity
DNA — ^{32}P	0,5 pg
DNA — $^3\text{H}/^{35}\text{S}$ (In situ hybridization)	
Non-radioactive labels	
DNA — Fluorochrome	100 pg
DNA — Enzyme	
DNA — Antibody — Enzyme	
DNA — Hapten — Antibody — Enzyme	
DNA — Biotin — Avidin — Enzyme	1 pg
DNA — Luminol — Activator — DNA	?
DNA — Biotin — Antibody — Gold	(In situ hybridization)

Application of Gene Analysis

Application of the hybridization technique should be restricted to areas in which it has definite advantages over classical techniques such as analysis of gene products by enzymatic or immunological assays. Some instances in which hybridization assays should be applied are:

- (1) if therapy depends upon test result,
- (2) if test accelerates diagnosis,
- (3) if no gene product is known
- (4) if there is no concordance between DNA and gene product,
- (5) if differential diagnosis via the gene product is too complicated or inadequate, and
- (6) if the test result is more accurate than with other techniques.

This list will certainly be extended in the future.

The applications for DNA/RNA probe techniques are expanding rapidly. Table 5 gives a — necessarily incomplete — list of such applications. A few of these applications which are of special interest for the clinical chemist will be discussed in some detail.

Prenatal and carrier diagnosis of genetic diseases is no topic of this review. It in principle is connected with genetic counseling and therefore is a domain of the human geneticist.

Tab. 5. Gene analysis: Areas of application.

Area	Application
<i>Human genetics</i>	
Genetic diseases	Prenatal and carrier diagnosis (15, 25, 27)
Preventive medicine	Environmental diseases (14, 28)
Occupational medicine	Preventive toxicology (14, 25)
Pharmacogenetics	Chemotherapy (61)
Malignant diseases	Diagnosis, differentiation (29)
Transplantation	Organ matching (26)
Forensic medicine	Human linkage (23, 24)
Heterologous insemination	Donor testing (25)
Genetic research	Cytogenetics (30, 31)
<i>Detection of pathogens</i>	
Infectious diseases	Bacteria, viruses (32, 33, 34)
Parasitic diseases	Diagnosis, therapy monitoring (35)

Preventive medicine is an interesting application for which clinical chemists should investigate gene analysis. The goal of this investigation is to detect persons at risk for certain diseases by pinpointing gene defects. Some of the diseases for which DNA probes are available are e.g. atherosclerosis (36), alcohol intolerance (37, 37a), and emphysema (38). Gene

analysis would be particularly useful for identifying individuals at risk for the environmental diseases of developed countries and for diseases for which no adequate biochemical markers are available. In this connection, an intensive search for the location of genetic defects for diseases as multiple sclerosis, *Alzheimer's* disease, manic depression, and diabetes is in progress.

It should be realized that environmental diseases have a multifactorial etiology. In addition to genetic predisposition, factors such as the lifestyle of the individual have a significant influence on the development of these diseases. The problems of screening programs, which are well known to the clinical chemist, also apply to preventive genetic analysis. The task is not only to identify the carrier of a genetic defect, but to identify carriers of the defect who are prone to develop disease. The measure for this probability is the "relative risk". It equals the percentage of defect carriers with disease manifestations, divided by the percentage of defect carriers without disease manifestations. Thus, a predictive value of a positive test in excess of 0.9 can only be obtained in patient samples in which the prevalence of defect carriers is greater than 10% and with markers indicating a relative risk of more than 100 (39). In addition, increasing knowledge about the heterogeneity of genetic defects (see e.g. l.c. (12)) leads to a further complication in defining individual "genetic predisposition". Therefore, the value of preventive genetic health screening will be restricted to carefully selected risk groups.

A widely discussed example is *occupational screening*: the attempt to assess the risk for employees ("hyper-susceptibility") by detecting genetic predispositions for occupational hazards, e.g., exposure to chemicals, air pollution, or extreme stress situations. Some chemical companies, as well as some government agencies in the U.S., have been utilizing such tests. However, as has been pointed out with regard to preventive health testing, the problems of multifactorial etiology and statistical significance apply to occupational testing. In addition, the genetic markers tested are not necessarily associated with susceptibility, and no cost/benefit analyses have been performed. Accordingly, the responsible authorities have stopped performing these tests in most cases. Intensified ecogenetic research is necessary to provide a firm basis for such activities (14, 25, 40).

A field of great promise is *pharmacogenetics*. The possibility to determine the "genetic phenotype" of patients regarding their response to drugs certainly will be an important step in optimizing chemotherapy.

It is of considerable interest to the work of the clinical chemist to note how laboratory testing in the field of *malignant diseases* will be influenced by the hybridization technique. It is difficult to predict the extent to which gene analysis will advance the use of biochemical techniques in cancer management. However, in some cases the use of DNA probes has already been proven to have a definite advantage in the detection or differential diagnosis of cancer, as shown in table 6.

Tab. 6. Gene analysis: Diagnosis of malignant diseases.

Immunoglobulin synthesis (29, 41)

Southern blot analysis with probe against chromosomal immunoglobulin genes
Detection of malignant B-cell clones
Diagnosis of lymphomas

Receptors (29)

Southern blot analysis with probe against T-cell receptor gene
Diagnosis of acute lymphoblastic leukaemias

Oncogenes (1, 41–43)

1. Chromosomal translocations
Detection in situ, restriction fragment length polymorphism in development
2. Gene amplification
Southern blot analysis with probe against chromosome 13 fragments
Diagnosis of retinoblastomas

- The detection of malignant B-cell clones is important in the differential diagnosis of non-*Hodgkin* lymphomas. Today, DNA probes are available for particular regions of chromosomal immunoglobulin genes. New restriction patterns are constantly being generated as a consequence of intrachromosomal gene rearrangement; the cell-specific IgG molecule is a different one in each new B-cell clone. Normally, these clonal immunoglobulin species cannot be detected by restriction analysis, because the fraction of each new B-cell clone is too small to be detected. However, if a malignant clone increases to constitute more than 1% of the total clone population, its band in the hybridization assay becomes prominent.
- Detection of chromosome translocations is of definite value in the differential diagnosis of leukaemia induced by activated oncogenes. For example, proof of the translocation of *sis* and *abl* oncogenes between chromosomes 9 and 22 is a definitive marker in the diagnosis of chronic myelocytic leukaemia. Today testing is done by cytogenetic techniques (including DNA probes), but probes for use in the restriction fragment

length polymorphism technique are being developed. The problem is that in the process of translocation the points at which chromosomes break vary over a distance of about 25 kilobases; this leads to some ambiguity in the evaluation of restriction fragment length polymorphism patterns.

A straightforward application of hybridization assays is the detection of foreign RNA and DNA to diagnose *bacterial and viral infections* in humans. Accordingly, much effort is being spent to develop routine techniques for use in medical microbiology. Table 7 summarizes the infectious diseases for which probes or test kits are available. The analytical problems of this methodology are threefold:

- Low sensitivity of assays (the detection limit for bacteria today is about 10^3 cells; for viruses it is 10^4 – 10^5 genome copies) (29, 34);
- Radioactive labelling must still be used; and
- Preconcentration, e.g. culturing, of samples is necessary.

Tab. 7. Gene analysis: Infectious diseases (1, 3, 17, 33, 34, 44).

Disease/Organism	Probes	Test kits
1. Bacterial Diseases		
1.1 <i>Enterobacteria:</i>		
<i>E. coli, Salmonella</i>	+	+
<i>Yersinia</i>	+	
1.2 Non- <i>Enterobacteria:</i>		
<i>Campylobacter</i>	+	+
<i>Legionella, Bordetella, Vibrio</i>	+	
<i>Streptococci, Staphylococci</i>	(+)	
2. Sexually transmitted diseases		
Herpes simplex virus, types I and II	+	+
<i>Neisseria gonorrhoeae</i>	+	(+)
<i>Chlamydia</i>	+	+
AIDS	(+)	
3. Viruses		
3.1 Viruses in blood		
Hepatitis virus A	+	(+)
Hepatitis virus B	+	+
Hepatitis virus non-A/non-B	+	(+)
Cytomegalovirus, adenovirus, rubellavirus, <i>Epstein-Barr</i> virus	+	+
3.2 Viruses in stool		
Rotavirus	+	
3.3 Viruses in tissues		
Papillomavirus	+	(+)
4. Parasites		
Malaria	+	

It can be anticipated that these methodological shortcomings will be resolved within a short time.

An example of the use of DNA probes in medical microbiology is their application in the diagnosis

of bacterial infections. In addition to increasing the sensitivity and speed of analysis, emphasis has been placed on developing more specific assays. For instance, by using probes for plasmid DNA, evidence can be obtained about the antibiotic resistance patterns (21), or about specific auxotypes (32). In addition, by using probes specific for toxin genes, evidence can be generated about the ability of *E. coli* to produce endo- and exotoxins.

Implications for the Clinical Chemist

Because of the many areas to which hybridization assays can be applied, the clinical chemist is interested in learning what impact these new techniques might have on his laboratory, and when.

Table 8 summarizes the predictions of several professional market analysts. It shows that the hybridization technique will have a strong impact on medical microbiology within the next few years and will complete or replace some of the classical bacteriological and viral culture techniques and some of the immunological methods that have just appeared on the market (45). Within 5 years laboratories are foreseen to routinely use hybridization assays rather than immunological methods for tissue typing and as a replacement for the analysis of gene products in screening for genetic disease. By the late 1990's hybridization assays hopefully can be routinely used to diagnose cancer.

Table 8. Gene analysis: Market trends.

Estimates for worldwide market (Millions US \$)					
Year	1985	1986	1988	1990	2000
Total	2	15	50	250	650
Infectious diseases	2	25	45	100	400
Transplantation	—	—	5	20	50
Genetic diseases	—	—	1	25	100
Cancer	—	—	—	1	100

Sources: Frost & Sullivan
Robert S. First
Drexel Burnham Lambert
Clinica

Public Debate

In the course of the controversy about the merits and risks of modern biology, the public has been made aware that gene analysis is a technique which could be used to infringe upon the basic rights of the individual. In many ways this debate has become irrational and emotional (14, 28, 46, 47). For exam-

ple, the use of gene analysis in occupational screening could be considered preventive medicine, but it could also be used for genetic exploration of employees or to bar certain classes of employees from holding certain positions.

To identify aspects of the controversy on which the clinical chemist must take a position, it is necessary to look at the arguments rationally. Table 9 shows some points that must be considered in evaluating the impact of gene analysis on the individual and on society. As can be seen, arguments have been presented for and against these points.

Tab. 9. Gene analysis: Areas of contradictory opinion (14, 25, 28, 46, 48, 49, 50, 51).

Individual rights	<ol style="list-style-type: none"> 1. Preventive medical examinations do not violate human dignity 2. Knowledge of predisposition before manifestation of disease may violate the individual's rights
Labour	<ol style="list-style-type: none"> 1. Responsibility of employers for welfare of employees 2. Restriction of individual rights of employees
Society	<ol style="list-style-type: none"> 1. Burden for support of "protected handicaps" 2. Duty to care for diseased and disabled
Minority groups	<ol style="list-style-type: none"> 1. Protection of the individual 2. Protection of the group (equality right)
Data protection	<ol style="list-style-type: none"> 1. Duty to protect citizens 2. Confidentiality 3. Right to informational self-determination

One example is the area of "social aspects." It is valid to ask about the extent to which society should carry the burden for groups with "protected handicaps." An argument could be made in favor of large scale prenatal and preventive screening; an equally valid counter-argument is the duty of society to care for its disabled and diseased, which is even included in the German constitution (52). Another example is the area of "data protection." The duty of the authorities to protect citizens from harm would lead to a system of disease prevention coupled with some degree of social pressure and handling of personal data. This is counterbalanced by the right to self-determination, an argument based on the rights of personality that denies to authorities any right to acquire or utilize personal data. At present there is no consensus on the arguments about gene analysis. As a result, various groups have formulated policies about how gene analysis should be handled (53, 54).

The clinical chemist searching for guidelines for his involvement in gene analysis should particularly consider ethical questions concerning the analysis and interpretation of the human genome (46, 49, 50, 51, 55, 56). These questions include the rights of individuals as opposed to the rights of society, and protection of the ill and disabled. Arguments concerning "genetic health" are irrelevant because the effects of genetic counseling, as the experts agree, will not change to a significant degree the gene pool of our population. According to professional authorities in the field of ethics, it will not be necessary to formulate special "gene ethics," because all problems can be solved by means of existing medical ethics (56). Rules that the clinical chemist can follow in his work include adherence to the responsibility principle of ethics. This means that he has to consider and to take the responsibility for the possible consequences of his work (50, 51, 57). It is considered necessary to attain consensus of the scientists engaged in gene analysis by involving the medical ethics commissions (48, 52).

An objective and detailed consideration of ethical problems in gene analysis was provided by the 1983 report of the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research (58). The commission advocates that five conditions be met to ensure the use of gene analysis to benefit the individual. These conditions are confidentiality, autonomy (individual choice),

knowledge (instruction), well-being (usefulness via well organized programs), and equity (equal access of all groups). However, the Commission also believes that overemphasis on individual rights may cause harm to other persons, e. g., children or the disabled. In such cases, it may be necessary to perform low-risk procedures.

Another set of recommendations, also covering the implications of gene analysis, was developed in 1985 by the International Conference on Bioethics in France (59). Meanwhile, the report of the Enquete-Kommission "Chancen und Risiken der Gentechnologie" (61) has been published, which contains a detailed analysis of the situation in Germany. Special reference to the situation of the clinical chemist will be given in the document "The Role and Responsibilities of the Clinical Chemist" which is being prepared by IFCC (60).

In spite of all questions and problems connected with the application of gene analysis to human beings, in an educated society it could lead to an improved quality of life, as the President's Commission foresees (58):

"In sum, the fundamental value of genetic screening and counselling is their ability to enhance the opportunities for individuals to obtain information about their personal health and childbearing risks and to make autonomous and noncoerced choices based on that information."

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