ually to an early death. Others in the family will certainly want to know; and those who are wishing to produce a family of their own may want to take advantage of the possibility of detecting the presence of the abnormal gene in the early fœtus and electing to abort fetuses carrying the gene. Selective abortion of conception products carrying an autosomal dominant disease gene with a low spontaneous mutation frequency, such as Huntington's chorea, will, of course, result in the virtual elimination of familial disease of this sort; but the problems associated with screening, detection and implementing selective abortion in the absence of any treatment are formidable ones.

Genetic counselling is now being undertaken on a wide spectrum of inherited disease, not only in the case of inherited familial or age-related chromosomal disorders and single gene disorders such as Tay-Sachs syndrome, but also for other conditions which have a complex genetic or which have a major environmental component, e.g. certain neural tube defects. What stance should individuals and society take towards progressive genetic counselling in these cases? At the present time it is evident that programmes which are aimed at detecting heterozygotes for the gene that causes beta thalassaemia are successful in that in many countries there is a significant decrease in the birth rate of homozygous children suffering from thalassaemia major. In most countries the screening that is undertaken is on a voluntary basis, but in Cyprus, for example, where 20–30% of the population are carriers and 1–2% of children are homozygous and die an early death from thalassaemia, there is an obligatory screening programme at marriage, with the results only being divulged to the couple and with no pressure being brought to bear on whether the couple should or should not marry. Should screening for severe inherited diseases which are an emotional and financial burden on parents, the family and the community, be obligatory and required by law? If so, where does society draw the line, which diseases? Where does the law stand if a couple produces a child with thalassaemia major? Is it entirely their problem, or does their doctor, the obstetrician and the hospital involved in prenatal care have some responsibility, and can they be sued? In the USA there have been a number of cases of litigation against obstetricians and other physicians for not having informed Jewish parents of their risk of producing children with Tay-Sachs disease, most of which were won by the parents. There are other successful actions against physicians for incorrectly informing, or for not informing, carrier parents of their risks for producing children with other inherited diseases and for not screening for chromosomal anomalies in women of high maternal age who eventually produced children with Down's syndrome. In these cases the parents claim for what is called a 'wrongful birth', but there are also reports of successful claims for 'wrongful life' where, in California, but not New York State or the UK, individuals with a genetic disease essentially and successfully undertook litigation against their doctors for allowing them to be born!

From the viewpoint of society, rather than the individual, it is clear that in the future we have to pay more attention to the cost-effectiveness of screening programmes to detect genetic disease. It is already evident that the financial cost of looking after patients with various inherited conditions, e.g. thalassaemia major, Down's syndrome, etc., are far more than the financial cost of the screening programmes that aim to prevent the birth of such individuals. In the financial sense these screening programmes more than pay for themselves, but how do we weigh financial cost against the value of a life, or a potential life, and how do we evaluate quality of life in this context? These are major societal problems which we have hardly yet come to grips with, but which are going to assume ever increasing importance, and will require some answers, during this present decade.

Postscript

This introductory overview was written in April, 1984. Much has occurred since that time as perhaps exemplified by the fact that the number of cloned human genes reported at the Human Gene Mapping meeting in Helsinki held in August 1985 (249) was more than double the number (104) reported at the previous meeting in 1983. At the present time well over 1500 genes and DNA markers have been mapped to the human genome: an increasing number of important disease genes have been mapped, e.g. cystic fibrosis, or cloned, e.g. chronic granulomatous disease, and probes of these and other similarly inherited conditions are now being used in antenatal diagnosis. Rapid advances are being made in genetic aspects of normal and abnormal growth and development, as for example with the isolation of homeobox genes involved in the laying down of patterns of development, and the further isolation and characterisation of genes involved in normal growth and in neoplasia. Research in the overall field of the genetics of man is probably at its most exciting phase in its history, and is unquestionably proceeding at an enormous pace.

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Biochemical diagnosis of genetic disease

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Key words. Genetic disease; early diagnosis; abnormal metabolites; enzyme defects; gene mutation; prenatal prevention.

I. Introduction

The increasing interest in human genetics and in the early diagnosis, treatment and prevention of congenital handicaps can be explained by various factors. In most industrialized Western countries, but also in the People's Republic of China, congenital disorders have become the major cause of infant mortality and morbidity. In more and more countries young couples do not only want to limit the number of their children, but they also want as much certainty as possible that their baby will be healthy. During the last decades basic research in pathology, cell biology, biochemistry and genetics had led to a better understanding of the etiology of many congenital disorders. Also, much information has become available about the incidence, mode of inheritance and recurrence risk of chromosomal aberrations, Mendelian disorders and malformations which are probably due to a combination of genetic and environmental factors. In addition, considerable progress has been made with the early diagnosis of patients, carrier detection and prenatal monitoring for fetal abnormalities, which in turn offers new perspectives in treatment and prevention.

Some of the new developments such as fetal monitoring, newborn screening or early diagnosis by recombinant-DNA technology have received wide attention from professional health workers, ethicists and the general public. This has undoubtedly helped to create a greater awareness of risks for affected offspring and has stimulated the general interest of couples in genetic counseling. In this respect, a variety of socio-economic factors play an important role as well.

The overall incidence of congenital disorders among liveborns is of the order of 4-6% depending on the exact definition, the time and methods of diagnosis, the ethnic origin, geography and other factors[13]. Between 10 and 20% of the congenital disorders are due to a single gene mutation and at present more than 3400 different syndromes have been identified to be inherited according to the Mendelian pattern[32]. As is shown in table 1, most individual genetic diseases are quite rare although some of them occur frequently in certain populations[26, 56, 58].

The early diagnosis of genetic diseases is especially important because of the high (recurrence) risk and the fact that in some diseases physical and/or mental handicaps can be prevented only if treatment is started early after birth.

For a long time the only possible means of early diagnosis of genetic disease was careful observation of the child's development followed by clinical examination if something was thought to be wrong. The mode of inheritance had to be derived from family studies and sometimes from extensive pedigree analysis.

Table 2. Early clinical symptoms and signs which might be associated with a genetic metabolic disease

Acute neonatal illness	Later in development
Vomiting	Psychomotor retardation
Food refusal	Loss of acquired functions
Growth failure	Hepato (spleno) megaly
Ketoacidotic attacks	Coarse facial features
Convulsions	Skeletal abnormalities
Hyperventilation	Visual problems
Lethargy	Deafness
Coma	Mental retardation
Icterus	Neurological abnormalities
Hypo/hypertonia	Peculiar odor
	Skin abnormalities
	Metabolic acidosis/ketosis
	Hypoglycemia
	Hyperammonemia

Today, this is still the basis for the early diagnosis of about 90% of the syndromes known or suspected to be of Mendelian inheritance. During the last decades, however, the responsible protein defect has been elucidated in about 300 genetic diseases[13, 32, 49]. In some instances understanding of the biochemical basis has resulted in the development of a rational and successful treatment[5, 49, 53]. In all instances it allows an early diagnosis to be made, in principle independent of the appearance of clinical symptoms[13, 32, 49]. During the last decades the development of new analytical methods has significantly widened the scope of detection of biochemical abnormalities in body fluids, organ biopsies, blood cells and cultured skin fibroblasts. The following section (II) deals with the different levels at which a genetic defect can be delineated: clinical features, abnormal quantitiy or nature of a metabolite in blood and/or urine, a deficient enzyme activity in cell material, a decreased amount or absence of a specific protein, a further elucidation of the nature of the molecular defect and finally the analysis of the mutation at the level of DNA itself.

In some instances simplification and automation of biochemical assays has made large scale newborn screening possible[5] and this has successfully been used for the early treatment of certain genetic diseases.

Most genetic diseases, however, cannot be treated and the main emphasis has therefore been on prevention by early diagnosis of index patients, carrier detection and genetic counseling (III). The possibilitiy of prenatal

Table 1. Gene mutations in man

Autosomal dominant	Examples	
overall incidence 0.6–7/1000	– familial hypercholesterolemia	1: 500
	– Huntington's chorea	1: 5000
	– osteogenesis imperfecta	1: 25000
Autosomal recessive	– cystic fibrosis	1: 2500
overall incidence 0.9–2.5/1000	– phenylketonuria	1: 12000
	– mucopolysaccharidoses	1: 25000
	– Tay-Sachs diseases varies from	< 1:100000 among Caucasians to 1:3000 among Ashkenazy Jews
	– sickle-cell anemia varies from	1: 600 among American blacks to < 1:100000 in other populations
	– β-thalassemia varies from to	1: 150 in Cyprus < 1:100000 in other populations
X-linked	– Duchenne muscular dystrophy	1: 7000
	– hemophilia	1: 10000
	– all others together	1: 5000

monitoring and selective abortion in cases where the fetus is affected offers new perspectives for many couples at risk[12a-14, 33, 59]. As will be reviewed in section II, (enzyme) protein assays and more recently also DNA technology play an increasingly important role in these areas of clinical genetics.

Again, methodology is only one aspect and a variety of political, demographic, socio-economic, religious and psychological factors will ultimately determine whether and how the available methods of early diagnosis and prevention are being used. In the meantime fundamental research in molecular and biochemical genetics proceeds rapidly and before different societies have responded to one new development, the next is already there.

II. Different approaches towards early diagnosis

a) Clinical aspects

Because of the large number of different conditions due to a single gene mutation and the considerable heterogeneity within many syndromes it is impossible to even summarize the clinical features of genetic diseases. In some instances a mutation only causes a minor change in the molecular structure of a protein without affecting its function and many of these mutations will remain undetected. Many others, however, do affect to a greater or lesser extent the function of a protein. The clinical features in such genetic diseases will mainly be determined by the organ distribution of the protein concerned and by the function(s) it normally performs.

The pathology of some genetic diseases is restricted to one cell type or organ system, as in hemoglobinopathies, red cell enzymopathies or muscular dystrophies. Other diseases are of a generalized nature and are associated with metabolic abnormalities and dysfunctioning of various cell types and organ systems.

Clinical symptoms may occur shortly after birth or later during the child's development or even at (late) adult age[13, 32, 49]. In countries where infectious diseases and malnutrition are no longer a problem, congenital disorders, including genetic disease are a major cause of infant mortality, especially during the first year of life. Also, many genetic diseases are associated with chronic physical and/or mental handicaps. British studies showed that 5.2% of all children at age seven had some kind of impairment; 85% of these were of congenital origin[54].

During the last decade it has become clear that many genetic diseases may express themselves as different clinical entities. Most lysosomal storage diseases, for instance, may occur as a progressive severe infantile form, or a milder later onset juvenile form and sometimes even as an even milder adult form. Complementation studies after hybridization of cultured fibroblasts from patients with such different clinical variants suggest that they are based on different mutations within the same gene[16].

Various studies indicate that about 6% of all admissions to a pediatric hospital are because of a genetic disease. The average time spent in hospital is about twice as long as for patients with a non-genetic disorder. Several genetic metabolic diseases, especially aminoacidopathies and organic acidemias, may become manifest shortly after birth with symptoms of acute illness which may result in neonatal death before a diagnosis has been made. In such instances parents not only lose a child but they also cannot be provided with adequate information about the recurrence risk in case of a future pregnancy. It is therefore of great importance that adequate clinical and chemical investigations are performed in all newborns with symptoms which may point to a genetic metabolic disease (listed in table 2, left). In case of a life-threatening condition samples of urine and blood and sometimes tissue from an organ biopsy should be collected and stored as well as cultured skin fibroblasts. This permits biochemical analyses to be performed even after the death of the patient and sometimes a diagnosis can be established in retrospect. This is especially important in Mendelian disorders which have a 25% or 50% recurrence risk.

Some genetic metabolic diseases are characterized by intermittent clinical problems: episodes of acute illness with for instance fever, ketoacidosis, neurological abnormalities alternate with sometimes rather long periods during which the patient is symptom-free. In the long run, however, many patients deteriorate and eventually die because of infections and/or cardiorespiratory failure[13, 47, 49].

Many genetic disorders are not apparent shortly after birth and often the child develops normally during a period varying from several months to several years. Psychomotor retardation and especially the loss of acquired functions should warn the parents and doctors that a genetic metabolic disease might be involved. Table 2 (right) lists the most common features during later development which may point to a genetic metabolic disease. Again, expert clinical examination and biochemical analyses must be performed as early as possible. Several studies indicate that an unnecessary diagnostic delay may result in the birth of two or even more affected children in one family because the parents were not aware of their high genetic risk. In Duchenne's muscular dystrophy for instance, early diagnosis is possible at the age of 18 months – 2 years by careful clinical examination combined with creatine phosphokinase assays in venous blood. In practice, the average age at which the diagnosis of this X-linked recessive disease is made in the first affected child appears to be 5-6 years, at which time many parents have already given birth to another child, 25% of whom will be affected as well. O'Brien et al.[39] have pointed out that early diagnosis of the first affected child followed by proper genetic counseling could have prevented the birth of more than half of all further affected children.

b) Abnormal metabolites

More than 100 genetic diseases are at present known to be associated with a specific abnormality of the nature and/or quantity of one or more metabolites in blood, urine or other body fluids[5, 6, 18, 49, 53]. Such changes can be the result of different mechanisms. If an enzyme normally involved in the metabolism of low molecular components is defective, one or more pathways may be blocked. This may result in an excessive production of intermediary products, and of their metabolites via alternative pathways, whereas the normal end product will be absent. An example is the autosomal recessive aminoacidopathy phenylketonuria, which is associated with accumulation

of phenylalanine and its metabolites via alternative routes: phenylpyruvic acid, phenyllactic acid and phenylacetic acid, whereas the normal hydroxylation product tyrosine is deficient.

Excessive production of a metabolite may also be caused by increased activity of an enzyme; possible examples are the high uric acid level in gout and the high levels of various metabolites in acute intermittent porphyria.

Deficiency of an enzyme involved in the metabolism of macromolecules will sometimes also be reflected in the urinary excretion of accumulation products, as in various types of mucopolysaccharidoses and oligosaccharidoses. Most other lysosomal storage diseases, however, are not associated with abnormal metabolites in blood or urine and have to be diagnosed by demonstration of the responsible enzyme deficiency in leucocytes or cultured skin fibroblasts.

Finally, several transport defects, usually in the intestine and kidney, are known to be associated with excessive excretion or the absence of one or more metabolites. A specially interesting group of diseases are those with a deficient transport of a vitamin which acts as a cofactor in one or more enzyme reactions. An example is methylmalonic acidemia due to a disturbance of vit. B_{12} transport[47]. It is, however, also possible that a mutation alters the structure of an enzyme in such a way that it can no longer bind to its cofactor; the resulting metabolic and clinical abnormalitites may resemble those due to a vitamin transport defect. In cases in which a cofactor is involved in several metabolic pathways the pattern of abnormal metabolites may be quite complicated.

The most important groups of genetic diseases where the chemical analysis of metabolites in blood and urine plays a major role in the early diagnosis are the organic acidemias and the aminoacidopathies.

About 50 organic acidemias are known to be associated with abnormal urinary excretion of a non-amino carboxylic acid[5, 18, 53]. Clinically, metabolic acidosis in combination with vomiting, dehydration, hypotonia and failure to thrive in the neonatal period, is an indication for thorough chemical analysis. The same is true for acidosis in combination with developmental delay later in childhood; often clinical features are precipitated by some infection or other stress. The results of simple laboratory tests for blood, pH and electrolytes and simple urinary tests for reducing agents and ketoacidosis may give a lead to more sophisticated analyses. Gas chromatography is the method of choice for the analysis of organic acids. Additional mass spectrometry will enable exact identification of metabolites and can also be used for quantitation by using labeled components as an internal standard[6, 18].

More than 60 hereditary aminoacidopathies are characterized by abnormalities of amino acids and other components in blood and urine[6]. During the last decades there has been a remarkable development in analytical techniques and the number of identified diseases has increased logarithmically. Two-dimensional thin-layer chromatography or high voltage electrophoresis are useful approaches to the detection of abnormalities. A variety of (automated) cation-exchange column chromatography methods is available for the quantitative analysis of amino acids in body fluids. More recently, high-performance liquid chromatography (HPLC) has permitted a reduction of the time needed for analysis and with this technique isolation and further analysis of a separated compound is possible as well.

Chromatographic techniques are also useful for the identification of urinary purines, pyrimidines and oligosaccharides and hence for the biochemical diagnosis of various inborn errors of nucleic acid metabolism, mucolipidoses and sialidoses[13, 49]. Electrophoretic techniques are often used as a screening procedure for mucopolysaccharidoses but the ultimate diagnosis in these genetic disorders must be based on enzyme assays in cellular material.

In our own country there are six regional laboratories with expertise in the analysis of metabolites in body fluids. Each of these investigates about 1000 new patients per year and performs chemical follow-up studies in another 800 patients. Thin-layer chromatography, high-voltage electrophoresis or column chromatography are performed in 65–75% of the cases. In about half of them gas chromatography for urinary organic acids is carried out, and this is combined with mass spectrometry in about 40%. Of neonates with acute illness and children with psychomotor retardation or other symptoms, 4–5% turn out to have a genetic metabolic disorder (table 3). In addition a variety of non-genetic metabolic abnormalities are found in about 20% of the patients, but most of these disappear spontaneously or upon treatment of the underlying disorder.

Since Guthrie's bacterial inhibition test for phenylalanine in the early sixties, which made large-scale newborn screening for phenylketonuria possible, simple tests for a dozen of other metabolites have been developed[5] (table 4). Most of these require a few drops of blood on filter paper, other tests are performed on umbilical cord blood, peripheral blood or urine. The main purpose of large scale newborn screening is the early diagnosis of congenital disorders where physical and mental handicaps can be prevented only if treatment is started shortly after birth. In a number of centers newborn screening has also been carried out for diseases that cannot be treated, such as Duchenne muscular dystrophy. The argument that the diagnosis of an affected neonate offers the best approach towards timely genetic counseling of the parents has, however, been challenged by several experts. Finally, in a few centers several tests for metabolites have been added to existing screening programs with the purpose of collecting data about the incidence and course of certain genetic metabolic disorders[5].

c) (Enzyme) protein defect

In about 300 Mendelian disorders the responsible protein defect has been identified[13, 32, 49]. About two-thirds of these are enzyme deficiencies and one-third, abnormalities of a non-enzymic protein. Examples of the latter are hemoglobinopathies, coagulopathies, defects of the complement system, collagen abnormalities, and the LDL (low density lipoprotein) receptor defect in familial hypercholesterolemia[49]. Almost all inborn errors of metabolism due to an enzyme deficiency have a recessive mode of inheritance. Mutations affecting non-enzymic proteins are usually dominant. In reality the situation may of course be more complicated because a deficiency of an

Table 3. Chemical diagnosis of genetic disease (% of total)

	Metabolite studies in body fluids[1]	Enzyme assays in cell material[2]
Carbohydrate disorders	8%	17%
Mucopolysaccharidoses	15	14
Sphingolipidoses/ mucolipidoses	1	15
Aminoacidopathies	60	12
Organic acidemias	13	
Nucleic acid disorders	2	8
Erythrocyte enzyme deficiency		7
Other genetic diseases	1	27

[1] Based on the investigation of 3500 new patients and 157 genetic metabolic disorders found (4.5%). [2] Based on enzyme assays in leucocytes (40%), cultured fibroblasts (30%), specific tissues or cells (30%) of 1700 patients, 215 (16%) of whom were found to have a genetic (enzyme) protein defect.

enzyme involved in the synthesis of a structural protein may affect the structure and function of this protein and still be inherited as a recessive trait.

The level at which a genetic protein defect can be defined depends mainly on the localization and quantity of the protein and on the available analytical methodology. Hemoglobin, for instance, can easily be purified in large quantitiy and its presence in red blood cells makes it readily available, from patients with different types of hemoglobinopathies as well as normal subjects. The development of protein sequencing techniques and more recently of DNA sequencing has made it possible to determine accurately the amino acid sequence of normal and mutant hemoglobins. At present more than 500 different hemoglobin variants are known[32]. Most of these are point mutations resulting in a single amino acid substitution, others lead to an abnormal chain length or interfere with a correct interaction between the polypeptide chains[32, 38, 56].

The availability of restriction endonucleases and recombinant DNA techniques meant an important new perspective for the delineation of the different types of mutation. The thalassemias appear to be a very heterogenous group of diseases from the molecular point of view. Gene deletions, abnormal transcription, reduced RNA synthesis, abnormal RNA processing, instability at the RNA or protein level, posttranslational changes and point mutations which affect the function of the alpha or beta chain may all result in thalassemia[38, 56]. Sickle cell anemia, on the other hand, is homogenous from the molecular point of view (base substitution in the β-chain). As will be discussed in section III, molecular heterogeneity may complicate the pre-natal diagnosis of various types of hemoglobinopathies[1, 28, 38, 56a, 58, 59].

1. Deficient enzyme activity. For nearly all other genetic diseases the delineation of the molecular defect has not yet reached the same level of sophistication. Most enzyme protein defects, for instance, are identified on the basis of a deficient activity in the test tube towards an artificial or natural substrate. For diagnostic purposes this is usually sufficient and most countries now have one or more laboratories where organ biopsies, blood cells or cultivated skin fibroblasts can be analyzed[13]. For a correct interpretation of the analytical data it is preferable that the laboratory has sufficient experience with the particular types of assays and expertise with the metabolic pathways investigated.

Table 3 illustrates the collected data from 5 centers in the Netherlands. Out of a total of 1700 patients suspected to suffer from a genetic disease, in 215 (13%) an enzyme deficiency could be demonstrated. In many instances the patients were selected on the basis of clinical features and the results of metabolite studies discussed in the previous section. In other cases, for example in sphingolipidoses, there are no specific metabolite abnormalities in body fluids and hence the clinical features are the main criterion for selection. If possible, the ultimate diagnosis of a genetic disease should be based on the demonstration of the responsible (enzyme) protein defect, even in cases where metabolite abnormalities in blood and/or urine are present. The latter may be quite characteristic for a particular disease, but they are not an adequate basis for genetic counseling, carrier detection or prenatal monitoring of future pregnancies.

About 80 genetic enzyme deficiencies out of the total of 210 presently known in man[32] are also expressed in cultured skin fibroblasts[13]. Although the diagnosis of such index patients can usually be made by assaying leucocytes, a skin biopsy, followed by cultivation and storage of fibroblasts is imporant in all instances where parents, or in X-linked disorders other relatives as well, might consider prenatal monitoring of a future pregnancy. As will be discussed in section IIIc, the results of enzyme assays on fibroblasts of the index patient and of the heterozygous parent(s) are essential for a correct interpretation of biochemical analyses of fetal cells in a pregnancy at risk. In several countries cell repositories are available where skin biopsies can be cultivated and fibroblasts can be stored; also biochemically defined mutant cells can be obtained for research and diagnostic purposes[13].

The activity of enzymes involved in genetic disease may vary considerably among patients, heterozygotes and normal individuals. In cultured fibroblasts the activity of many enzymes varies with cell cultivation conditions, but even when these and the biochemical assay are standardized, large differences between cell strains may exist. Figure 1 illustrates such differences for two lysosomal enzymes, α.1–4 glucosidase and α-L-iduronidase; the former is deficient in glycogen storage disease II (Pompe's disease) and the latter in mucopolysaccharidosis I H (Hurler's disease) or I S (Scheie disease). In both instances there is marked variation among heterozygotes and normal individuals. Although the mean activity in heterozygotes is about half the control value, carrier identification at the individual level is often difficult. For most enzymes a similar situation exists in leucocytes; enzyme activities in organ biopsies sometimes show a less wide range.

Figure 1 also illustrates an interesting phenomenon as far as the relation between clinical features in patients and the (residual) enzyme activity is concerned. In all patients with the severe infantile form of glycogenosis II hardly any α.1–4 glucosidase activity can be demonstrated. In cells from patients with the milder juvenile form, however, there is some residual enzyme activity and this is still higher in patients with the adult form. A similar relationship exists for different variants of several other genetic diseases. That this is not a general rule is shown in the right-hand part of figure 1, where α.L-iduronidase activ-

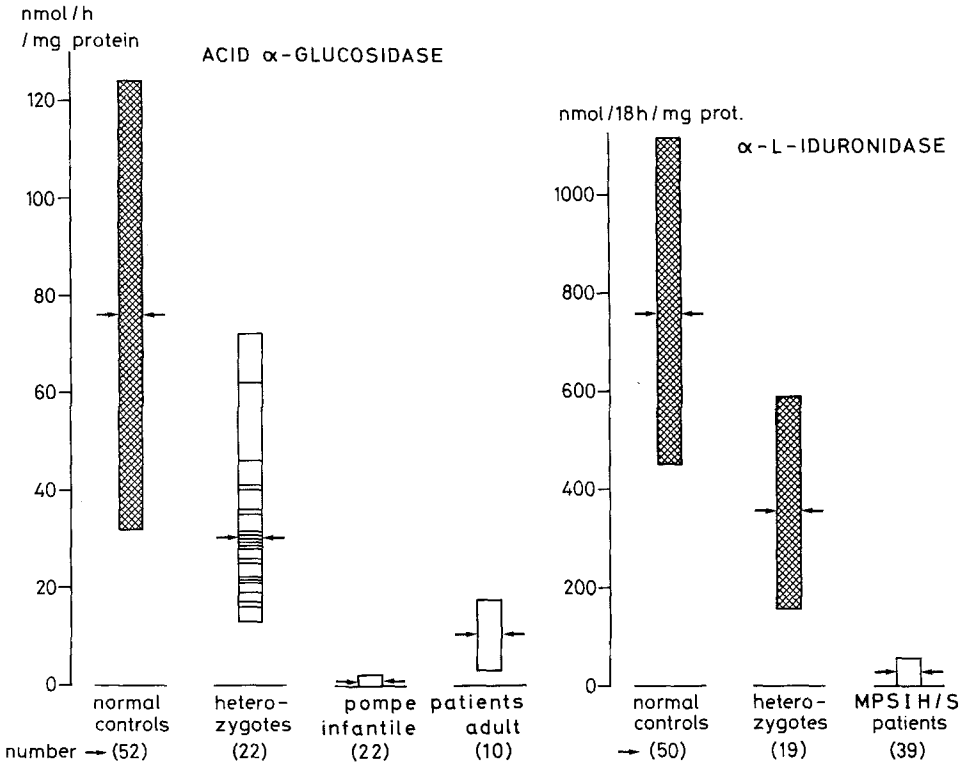

Figure 1. Examples of lysosomal enzyme activities in cultured skin fibroblasts derived from patients with different clinical variants of the same genetic disease. Left: acid α-1.4 glucosidase and right: α-L-iduronidase.

ity is completely absent both in patients with the severe mucopolysaccharidosis I H (Hurler's disease), which is associated with a variety of physical handicaps and mental deterioration, and in patients with mucopolysaccharidosis I S (Scheie syndrome), who only have moderate skeletal abnormalities and normal intellect.

In relating clinical features and enzyme activity it should of course be realized that the in vitro assay conditions in no way resemble the in vivo situation where enzyme and substrate interact at locally high concentration within a subcellular structure. More recently, several attempts have been made to mimic this situation by adding radioactive labeled substrate to living cells. After ingestion the metabolic fate of the substrate can be followed by isolating and separating the various metabolic products (fig. 2). In this way information can be obtained about the function of normal and mutant enzymes under in vivo conditions.

Of course, both this approach and the conventional test tube assay have the limitation that the enzyme activity is studied in one or a few cell type(s) only. Biochemical studies on organ cultures from patients with genetic disease may be of great value in understanding the pathogenesis.

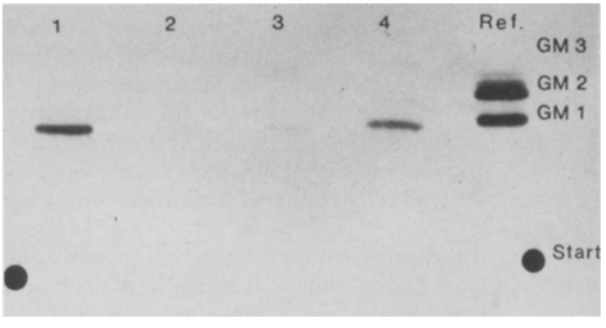

Figure 2. In vivo labeling of cultured fibroblasts. G_{M1}-ganglioside ^{3}H-labeled in the ceramide portion of the molecule (prepared in the Dept of Neurochemistry, St. Jörgen Hospital, University of Göteborg, Prof. Dr L. Svennerholm) is added to the culture medium and after incubation the various ganglioside fractions are studied by thin-layer chromatography. 1 = fibroblasts from a patient with G_{M1}-gangliosidosis, where G_{M1}-ganglioside accumulates intracellularly because of a total β-galactosidase deficiency. 2 and 3 are control fibroblasts, where the ingested radiolabeled G_{M1}-ganglioside is metabolized, and 4 = cells from a patient with a combined β-galactosidase/neuraminidase deficiency.

2. Defective post-translational processing. In recent years considerable progress has been made with the further identification of the nature of the enzyme protein defect in various inborn errors of metabolism. This has mainly become possible by the combined use of cultured mutant fibroblasts and immunological techniques. Cells are labeled with ^{3}H-leucine, treated with antibodies against the enzyme protein to be studied and after gel electrophoresis the various radioactively labeled molecular forms of the (mutant) enzyme can be studied[21, 22, 36]. Pulse chase experiments have been particularly informative in following the various post-translational modifications after the synthesis of an enzyme protein. In this respect much has been learned about the exact nature of the molecular defect(s) involved in various lysosomal storage diseases[3, 4, 21, 22, 24a, 46, 50].

Figure 3 illustrates at which different levels and subcellular sites a genetic defect may affect a lysosomal enzyme protein. In some variants of G_{M2}-gangliosidosis the gene defect results in the absence or reduction of mRNA and hence no synthesis or a reduced amount of precursor

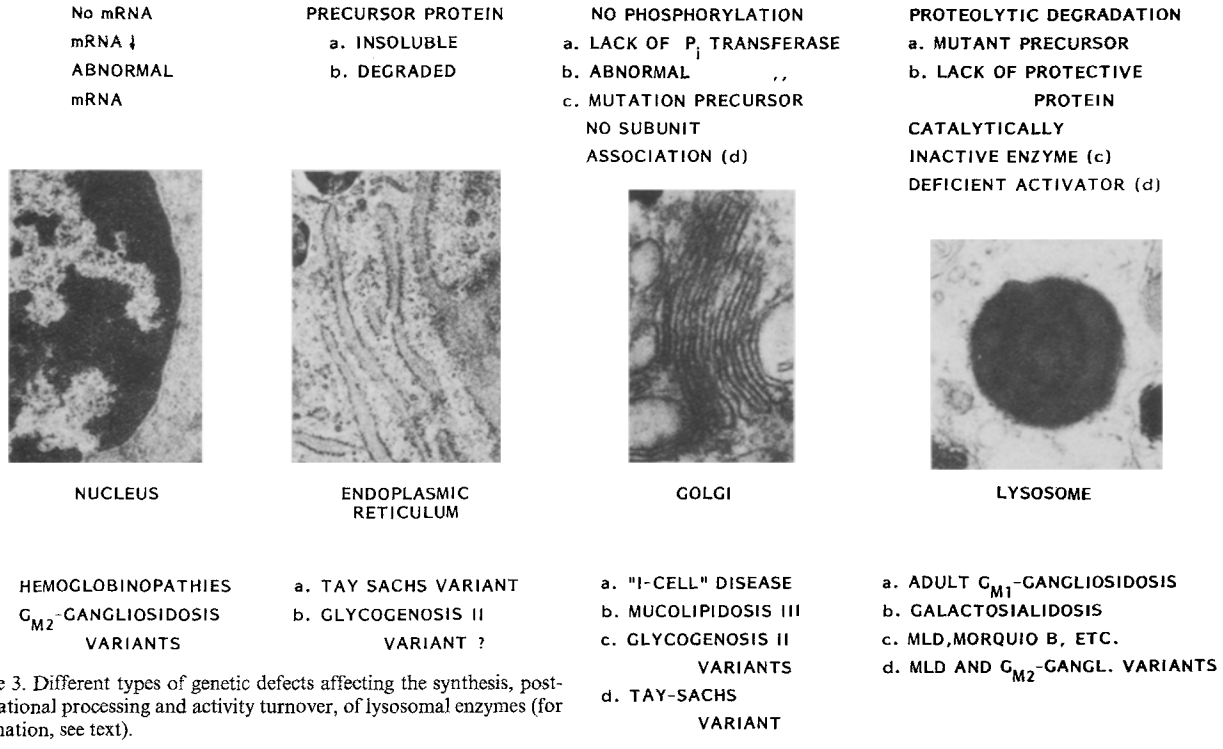

Figure 3. Different types of genetic defects affecting the synthesis, post-translational processing and activity turnover, of lysosomal enzymes (for explanation, see text).

protein is found[44]. In other variants of this disease[44] and in some patients with glycogenosis II[46, 50] precursor protein is synthesized, but it cannot detach from the endoplasmic reticulum and is therefore not further processed into mature, active enzyme.

The multiple lysosomal enzyme deficiency in fibroblasts from patients with mucolipidosis type II ('I-cell' disease) or type III was found to be due to a defect in the phosphotransferase which is normally required for the attachment of a mannose-6-phosphate recognition marker to the precursor forms of lysosomal (enzyme) proteins[23, 45]. When this marker is absent the (enzyme) proteins will not be compartmentalized within the lysosome but instead follow the route of secretory proteins[22, 36]. Secretion of precursor forms of lysosomal enzymes may also result from other mutations, affecting correct processing within the Golgi apparatus. Certain mutations of hexosaminidase, α-glucosidase and β-galactosidase interfere with the phosphorylation of the precursor forms of these enzymes and lead to a deficiency of these enzymes in patients with Tay-Sachs disease, glycogenosis II and G_{M1}-gangliosidosis respectively[24a, 44, 46].

A lysosomal enzyme deficiency may also be due to enhanced proteolytic degradation of precursor protein within the lysosome. This type of processing defect was found to be responsible for the combined deficiency of β-galactosidase and neuraminidase in patients with galactosialidosis[3]. The enhanced degradation is caused by a defective multimerization and complex formation of the two enzymes, which in turn is due to the genetically determined absence of a 'protective protein[3, 24, 24a, 51, 52].

A lysosomal enzyme deficiency may also be caused by a deficiency of an activator protein which is normally required for the binding of enzyme molecules to lipid substrate[31]. An example of this type of molecular defect is a variant of G_{M2}-gangliosidosis where hexosaminidase A is present but does not show any activity towards its natural substrate G_{M2}-ganglioside[9].

Finally, a large number of lysosomal enzyme deficiencies and other inborn errors of metabolism are due to a mutation which affects the molecular structure of mature enzyme in such a way that its function is impaired. In these instances a deficient activity is accompanied by the presence of cross-reactive material against specific antibodies[4, 13, 49].

Our insight into the exact nature of genetic defects which impair the normal post-translational processing of (enzyme) proteins will undoubtedly further increase when biochemical and immunological studies are combined with immunocytochemistry at the electron microscope level[11, 17] using cell material from patients with different types of genetic metabolic disease.

d) Gene mutation at the level of DNA

For many years complementation studies in lower organisms have been a useful tool to determine whether different gene mutations are involved in a certain metabolic defect. Also, in man, enzyme studies after hybridization of cultured mutant fibroblasts have contributed to the understanding of different clinical or biochemical features in patients with a genetic metabolic disease[16].

A more direct approach has become possible by the use of restriction endonuclease analysis and recombinant DNA technology[55]. The possibility of cloning (part of) a gene coding for a polypeptide permits the sequencing of this gene. This will give insight into the primary structure of the protein, which in turn might contribute to the understanding of its function[38, 49, 56]. The availability of a cDNA probe also permits a rapid screening of different mutant cells for the presence or absence of mRNA. In the former case the mutant gene must be isolated from DNA

Table 4. Experience with simple tests for metabolites in blood or urine in newborn screening*

Metabolite	Disease	Incidence among newborns
Phenylalanine	Phenylketonuria	1: 11300
T_4, TSH	Congenital hypothyroidism	1: 5000
Galactose	Galactosemia – transferase def.	1: 60000
	Galactosemia – kinase def.	1:150000
Leucine	Maple syrup urine disease	< 1:200000
Histidine	Histidinemia	1: 16000
Tyrosine	Hereditary tyrosinemia	< 1:200000
Methionine	Homocystinuria 1: 50000–	< 1:200000
(hydroxy)Proline	Hyperprolinemia	1:200000
Cystathionine	Cystathioninuria	1: 70000
Methylmalonic acid	Methylmalonicacidemia	1:100000

* Data derived from various contributions at the International Symposium on Neonatal Screening in Heidelberg (1980), eds Bickel et al.

in patient cell material and sequencing may elucidate the exact nature of the defect both at the level of DNA and that of mutant protein.

For diagnostic purposes DNA analyses are mainly of importance in prenatal monitoring for diseases where the responsible protein defect is not expressed in the available amniotic fluid cells or chorionic tissue (see III). Another application of DNA technology is in the (prenatal) diagnosis of genetic diseases such as Duchenne muscular dystrophy[3a, 10], Huntington's chorea[20] or cystic fibrosis, where the responsible protein defect is not yet known. In these instances polymorphic restriction fragments which are closely linked to the mutation may be demonstrable (table 5)[3a, 7, 8, 10, 20, 28, 37, 38, 56a, 57, 59]. Although this approach is interesting from a scientific point of view and may help in prenatal diagnosis of genetic disease, its application in health care is still limited. In many genetic diseases there is a large heterogeneity at the molecular level and for diagnostic purposes one type of DNA analysis will therefore not suffice. The necessity of extensive family studies makes the use of restriction fragment length polymorphisms impractical for large scale use.

Yet, there is no doubt that the development of DNA technology will further contribute to the early diagnosis of genetic disease. Table 5 summarizes which Mendelian disorders can already be diagnosed at the level of DNA itself either by direct demonstration of the mutation or indirectly by linked polymorphic restriction sites. In both instances much can be expected from the development of synthetic oligonucleotides[41, 59].

III. Prenatal diagnosis and prevention

Several follow-up studies after genetic counseling have shown that a considerable proportion of couples who have been informed about an increased genetic risk are deterred from pregnancy[13]. In the case of a Mendelian disorder, where the risk of a handicapped child is high, at least half of the couples refrain from (further) reproduction[12]. Yet this is a difficult decision, especially for couples who have not yet given birth to a healthy child. For many of them the possibility of prenatal diagnosis, and abortion if the fetus is found to be affected, is an important alternative. At present chromosomal aberrations, fetal sex (important in the case of a risk of an X-linked disorder) and some 80 inborn errors of metabolism can be diagnosed by analysis of fetal cells in the first or second trimester of pregnancy[12a–14, 33, 42, 59]. During the last fifteen years much experience has been gained with the sampling, cultivation and analysis of amniotic fluid cells. Most countries now have regional centers for prenatal monitoring and amniocentesis at the 16th week of pregnancy has become a safe and reliable procedure in experienced hands. In our own center 25–30 amniocenteses are performed each week and our total experience exceeds 12,000 prenatal diagnoses. The risk of maternal complications is negligible, the risk of fetal loss has decreased to 0.3% and in only 5 out of 1000 cases is repeat amniocentesis required because of a dry tap or a problem with amniotic fluid cell cultivation or analysis. After repeated puncture one or two weeks later a prenatal diagnosis could be made in all instances. Twin pregnancies do not produce problems when the two amniotic sacs are separated, which can be demonstrated by injection of a dye prior to the second puncture. The use of good ultrasound before and during amniocentesis is mandatory.

Compared to prenatal chromosome studies the biochemical analysis of fetal metabolic disease should be more centralized. The reason is that it requires expertise in (micro)chemical analysis of small numbers of cultured cells and the availability of adequate control cell material; also the number of requests for each of the genetic diseases is relatively small. Even in our own department, which is a reference center for the Netherlands and which receives many samples from foreign countries as well, only 5% of the total number of some 12,000 prenatal analysis involve genetic metabolic disease. Yet the number of affected fetuses detected in this category of pregnancies at risk (about 150) amounts to 21% of the total.

Table 6 gives an overview of the genetic metabolic diseases for which practical experience in prenatal diagnosis exists. Most of these disorders have an autosomal recessive inheritance, a few are X-linked recessive and only familial hypercholesterolemia is based on a dominant mutation. All diseases mentioned in table 6 are relatively rare and have an incidence among newborns between 10^{-4} and 10^{-5} except for Tay-Sachs disease and the hemoglobinopathies which may be very frequent among certain populations (table 1).

Table 5. Recombinant DNA technology and early diagnosis of genetic disease

Already applied	
α-Thalassemia (Eco R I)	Phenylketonuria (RFLP)
σ-Thalassemia (Eco, Hind III)	Hemophilia A (RFLP)
β^0-Thalassemia (Eco, oligonucleotides)	Hemophilia B (RFLP)
β^+-Thalassemia (direct analysis using Hph I, RSA I, Avr II oligonucleotides or by RFLP)	Duchenne muscular dystrophy (RFLP)
	Becker muscular dystrophy (RFLP)
Sickle cell anemia (direct analysis, using Dde I, Mst II, Cvn I, oligonucleotides)	Ornithine carbamyl transferase deficiency (RFLP)
α-Antitrypsin deficiency (RFLP)* oligonucl.)	
Application possible or in development	
Huntington's chorea	Cystic fibrosis
Retinoblastoma	X-linked mental retardation?
Polycystic kidney disease	Lesch-Nyhan syndrome

* RFLP, restriction fragment length polymorphisms closely linked to the mutation.

A few genetic metabolic diseases, such as argininosuccinic aciduria, glutaric aciduria I and II, methylmalonic acidemia, propionic acidemia, citrullinemia, tyrosinemia I and galactosemia can be diagnosed in utero by chemical analysis of specific metabolites in the amniotic fluid supernatant[13, 25, 42]. The use of gas chromatography combined with mass spectrometry (see also IIb) is especially useful in this context. The advantage of this approach is that a prenatal diagnosis can be established 1–2 days after amniocentesis. Disadvantages are that metabolite studies are an indirect method of demonstrating a genetic (enzyme) protein defect and that only a few diseases can be reliably detected in this way.

A much wider range of fetal metabolic diseases can be detected by biochemical analyses of cultured amniotic fluid cells (all those mentioned in table 6). The amount of cell material needed and thus the time of cultivation depends on the enzyme defect involved and on the sensitivity of the analytical method. In most instances a waiting period of 2–4 weeks has been necessary, but the use of micromethods usually allows a reduction of this period to 10–20 days[13].

For a reliable prenatal diagnosis it is a prerequisite that the precise biochemical abnormality for which the fetus is at risk is known. Also fibroblast cultures must be available from the index patient and the heterozygous parent(s) as well as control amniotic fluid cells which have been cultured under similar conditions as those of the pregnancy at risk (see IIc). So far nearly all prenatal diagnoses have turned out to be correct, either by the predicted birth of a healthy baby or by the demonstration of an enzyme deficiency in fetal tissues after termination of the pregnancy. The few mistakes were caused by inadequate knowledge of the variation of enzyme activities in relation to amniotic fluid cell type and cultivation conditions, the use of artificial substrate where natural substrate was needed or problems with the interpretation of high residual enzyme activities, which might be due to low heterozygous values as well. The overall results of prenatal diagnoses of genetic metabolic diseases have, however, been remarkably reliable in centers with sufficient experience.

Recently an important step forward has been made with the introduction of chorionic villus sampling and its direct cytogenetic and biochemical analysis (for reviews see 12a, 15, 59). In the 8th–10th week of pregnancy a 1.5 mm diameter polythene Portex catheter can be introduced vaginally, and under the control of a high resolution real time scanning ultrasound apparatus the tip of the catheter can be guided on to the chorionic plate. About 5–15 mg of chorionic tissue can then be aspirated and thanks to the pioneering work of Brambati and Simoni direct chromosome preparations of the fetal cells can be made[48, 59]. This permits rapid prenatal diagnosis of embryonal sex and chromosomal aberrations. If an abnormality is found, the pregnancy can be terminated by

Table 6. Inborn errors of metabolism which have been diagnosed in utero

	Diagnostic assay
Carbohydrate disorders	
Galactosemia	Galactose-1-phosphate uridyl transferase
Glycogenosis II	α-1.4-Glucosidase
Glycogenosis IV	α-1.4:α-1.4 Glucan-6 glycosyl transferase
Pyruvate carboxylase deficiency	Pyruvate carboxylase
Mucopolysaccharidoses	
Type I (Hurler)	α.L-iduronidase
Type II* (Hunter)	Sulfo-iduronide sulfatase
Type III* A (Sanfilippo)	Heparan sulfate sulfaminohydrolase
Type III B (Sanfilippo)	α-N-Acetyl-D glucosaminidase
Type IV (Morquio)	6-Sulfo-N-acetyl galactosaminide sulfatase
Type VI (Maroteaux-Lamy)	4-Sulfo-N-Ac. galactosaminide sulfatase
Oligosaccharidoses	
Sialidosis	Lysosomal neuraminidase
Galactosialidosis	β-Galactosidase + lysosomal neuraminidase
Mucolipidosis II ('I-cell' disease)	Multiple lysosomal deficiency
Mucolipidosis IV ('I-cell' disease)	E.M. Intralysosomal storage and possibly ganglioside neuraminidase
Fucosidosis	α-Fucosidase
Mannosidosis	α-Mannosidase
Lipidoses	
G_{M1}-Gangliosidosis	β-Galactosidase
G_{M2}-Gangliosidosis Tay-Sachs	Hexosaminidase A
G_{M2}-Gangliosidosis Sandhoff	Hexosaminidase A + B
Niemann-Pick disease	Sphingomyelinase
Gaucher disease	β-Glucosidase/glucocerebrosidase
Krabbe disease	Galactocerebroside-β-galactosidase
Fabry* disease	α-Galactosidase
Metachromatic leucodystrophy	Arylsulphatase A
Wolman disease	Acid lipase
Adrenoleucodystrophy*	Cholesterol ester fatty acids
Aminoacidopathies/organic acidemias	
Argininosuccinic aciduria	Argininosuccinase
Citrullinemia	Argininosuccinate synthase
Maple syrup urine disease	Branched-chain α-keto acid decarboxylase
Propionacidemia	Propionyl CoA carboxylase
Methylmalonic acidemia	
vit. B_{12} non-responsive	Methylmalonyl CoA apomutase
vit. B_{12} responsive	Defective cobalamin metabolism
Homocystinuria	Cystathionine-β-synthase
Cystinosis	^{35}S-Cystine chromatography
Isovaleric acidemia	Isovaleryl-CoA dehydrogenase
Glutaric aciduria I	Glutaryl-CoA dehydrogenase
Glutaric aciduria II	Fatty acid oxidation
Non ketotic hyperglycinemia	Glycine: serine ratio in fluid
Tyrosinemia I	Succinyl acetone in fluid
Nucleic acid disorders	
Lesch-Nyhan syndrome*	HGPRT Assay or ^{3}H-hypoxanthine autoradiography
Xeroderma pigmentosum	^{3}H-Thymidine autoradiography after UV radiation
Combined immunodeficiency	Adenosine deaminase
Other genetic disorders	
Sickle cell anemia	Endonuclease restriction site analysis of amniotic fluid cells or chorion
α/β-Thalassemia	Idem or hemoglobin assay in fetal blood
Menkes syndrome	^{64}Cu Incorporation
α_1-Antitrypsin deficiency	Endonuclease restriction site analysis using synthetic oligonucleotides
Congenital adrenal hyperplasia	17-α-Hydroxyprogesterone in amniotic fluid
Congenital nephrosis	Alphafetoprotein in amniotic fluid
Acute int. porphyria	Uroporphyrinogen-1 synthase
Cytochrome b_5 reductase defic.	Cytochrome b_5 reductase
Osteogenesis imperfecta	Proline incorp. in collagen
Hypercholesterolemia**	LDH Receptor, HMG-CoA reductase

*, X-linked inheritance, **, dominant, and all others are autosomal-recessive.

simple vacuum curettage within a few days after sampling at a time when the embryo is only 1–3 cm. The psychological burden for the parents is of course much less than in the case of amniotic fluid cell analysis and termination at the 16th–20th week.

After the demonstration that various enzymes which may be involved in inborn errors of metabolism are also expressed in chorionic tissue[15, 29, 48], several first-trimester diagnoses of genetic metabolic diseases have recently been made[12a, 15, 30a–c, 43, 59]. At the moment of writing practical experience with this new method has already been gained with 30 different metabolic diseases. The great advantage of this approach is that a result is available within a few days after chorion sampling at 8–10 weeks. Although chorion analysis seems very promising indeed and is likely to replace to a great extent amniotic fluid (cell) analysis, the abortion risk of chorion sampling and the reliability of its analysis must be thoroughly evaluated before definite conclusions are justified[59].

The combination of first-trimester chorion sampling and DNA analysis as described in the previous section (IId) has also permitted the rapid prenatal diagnosis of fetal hemoglobinopathies[12a, 19, 40, 56a, 59] and Duchenne muscular dystrophy[12a, 39].

The improvement of the methodology of prenatal diagnosis will undoubtedly result in a greater participation rate among couples at risk[59]. In the case of recessive Mendelian disorders, however, the diagnosis of the first affected child is usually the first indication that the parents have an increased genetic risk. It has been calculated that even if all these couples underwent prenatal monitoring of their next pregnancies, the birth of only 12.5% of all affected children could be prevented if the couples aimed at a total of two children[14, 35]. About 30% would be prevented if couples continued reproduction until they had two healthy children.

In the case of X-linked disorders the situation is more favorable because female carrier detection is often possible prior to the first pregnancy and hence the birth of a first affected child can be prevented.

The most effective approach towards prevention of genetic diseases for which no treatment exists, is of course large scale premarital screening for heterozygotes. If two partners are found to be carriers of the same mutation, genetic counseling and prenatal monitoring in the case of pregnancy will prevent the birth of affected children[2, 26, 27, 59].

Practical experience with such screening programs has been gained for Tay-Sachs disease among Ashkenazy Jews (carrier frequency about 1 in 20–30) in various countries, especially in the USA[26, 27]. Carrier screening for β-thalassemia has been successful in a number of Mediterranean countries such as Cyprus, where 1 person in 6 is a carrier[2]. Although encouraging results have been obtained it has also become clear that the availability of proper technology is only one aspect and that religious, socio-economic and organizational factors play a major role in the acceptance of the technology[34, 58, 59]. In view of the enormous differences among countries, populations and individuals it seems impossible to predict what will be accomplished in the future in prenatal diagnosis and prevention of genetic disease. Whatever differences exist, all countries, populations and individual couples will have in common that they want to give birth to one or more healthy child(ren). This fact, and the suffering involved in severe physical and mental handicap, will be a strong motivation for further studies on the early diagnosis, treatment and prevention of genetic disease. Sometimes progress is faster than expected; in other instances many years of basic research are required before a small step forward in health care can be made.

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