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REVIEW

# Inborn Errors of Metabolism at the Turn of the Millennium

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Great progress has been made in the field of hereditary metabolic diseases since the beginning of the past century, when metabolic disorders were not really understood and could only be clinically described. Due to the development of basic sciences and advances in technology, we gained insight in the biochemical and molecular basis of hereditary metabolic diseases. It opened possibilities for their treatment, and also led to the discovery of more metabolic diseases, so today, there are more than 500 "inborn errors of metabolism" known. Although each of these diseases is quite rare, as a group, however, they affect about 1-2% of newborns and therefore pose a significant health problem. The realization about 50 years ago that some hereditary diseases are curable if timely diagnosed led to the introduction of newborn screening in most countries. Modern technologies in this field allow early diagnosis of more than 30 inborn errors of metabolism. Nevertheless, to diagnose most patients correctly, both selective screening involving teamwork and proper use of current technology are required. In addition to considerable development of diagnostic possibilities, the past decade was marked by advances in the therapy of inborn errors of metabolism. A number of clinical trials are currently underway, promising new and more effective approaches in the treatment of these patients. Thus, the field of inborn errors of metabolism at the beginning of the new millennium continues to be a scientific challenge to modern medicine.

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The term "inborn errors of metabolism" was introduced by Sir Archibald E. Garrod about a century ago (1). His hypotesis that autosomal recessive inheritance, according to Mendel's rediscovered rules of heredity, would explain the occurrence of alkaptonuria phenotype in the population, was based on his investigation of cosanguinity and distribution of the cases within the families. Garrod also realized that the manifestations of alkaptonuria were "congenital", present at birth, and not explicable by environmental factors. In 1909, he published the first book on the topic, under the title "Inborn Errors of Metabolism" (2), in which he described albinism, cystinuria, and pentosuria, in addition to alkaptonuria. From that time on, a number of inborn errors of metabolism have been discovered. A book "The Metabolic and Molecular Bases of Inherited Disease", which traditionally collects actual knowledge in this field, grows thicker and thicker with each edition. Thus, its 8th edition, published in 2001, and written by over 500 authors, has almost 7,000 pages containing data on more than 500 inborn errors of metabolism (3).

With the progress of basic science and technology over the past century, initial clinical descriptions of inherited metabolic diseases have been gradually enriched with explanations of their pathogenesis. This knowledge has opened up possibilities for effective treating of many, and genetic counselling for most of these diseases. The illustrative example of the progress in the diagnosis and treatment of inborn metabolic errors are mucopolysaccharidoses. At the beninning of the last century, there were only descriptions of the clinical phenotype of these patients – coarse facies, dysostosis multiplex, frequent mental retardation, deafness, and hepatosplenomegaly. The cause of the disease was unknown, and it had remained unknown until 1957, when Dorfman and Lorincz (4) reported increased excretion of acid mucopolysaccharides in the urine of these patients and thus revealed the biochemical basis of the disease. At about that time, the introduction of electron microscopy and ultracentrifuge technics allowed insight in subcellular structures. As a consequence, the mucopolysaccharidoses were defined as lysosomal storage disorders. The next step forward was made by the development of cell cultures, allowing Neufeld and Fratantoni in 1968 to identify "corrective factors" in cell culture media, which could correct the biochemical defect in patients with mucopolysaccharidosis type 1 and 2 (5). This was the first demonstration of lysosomal enzymes. Soon, the measurement of catalytic activities of lysosomal enzymes allowed both postnatal and prenatal diagnosis. In the last two decades, the molecular basis of this group of

the diseases was revealed and has recently contributed to the development of the enzyme replacement therapy.

Each of the inherited metabolic diseases is rare, but since their number is rather high, as a group they affect 1-2% of all live newborns. Taking into account only diseased children or hospitalized population, this proportion is even higher. Therefore, inborn errors of metabolism represent significant burden for public health, particularly in the countries where infectious diseases and pathology related to inappropriate pregnancy and delivery care are adequately suppressed. The mentioned estimations were calculated from the incidence of "classical" inherited metabolic diseases, such as aminoacidopathies, organic acidurias, fatty acid oxidation disorders, various storage disorders, disordes of mitochondrial energy production, neurotransmitter defects, peroxisomal disorders, defects of purine and pyrimidine metabolism and some others. But, there have been new groups of the inherited metabolic diseases described recently, such as cholesterol biosynthesis defects (e.g., Smith-Lemli-Opitz syndrome) as important monogenetic cause of malformation syndromes (6), congenital disorders of glycosylation (CDG syndromes) causing a broad spectrum of hemostasiological, endocrinological, and neurological multi-system disorders (7), and defects in leukotriene synthesis (8), which further increase the frequency of inherited metabolic diseases. Newly discovered inborn errors are also the reflection of novel powerful diagnostic techniques. *In vivo* nuclear magnetic resonance (NMR) spectroscopy of the brain has contributed to thedefinition of creatine deficiency syndromes, a newly discovered group of disorders causing mental retardation and other neurological symptoms (9). NMR spectroscopy of body fluids may prove even more promising method in diagnostics of both known and novel inborn errors of metabolism (10). Even "old" techniques still contribute to the discovery of new inborn errors of metabolism. Two recently described organic acidurias from the "well-known" isoleucine degradation pathway, the 2-methyl-3-hydroxybutyril-CoA dehydrogenase deficiency (11) and the 2-methylbutyryl-CoA dehydrogenase deficiency (12) were both revealed by "classical" gass chromatography and mass spectrometry. Hence, the field if inborn errors of matabolism is permanently and quickly increasing, both by its size and our knowledge.

### Human Genome Project and Progress on the Molecular Level

Unmeasurable progress in the understanding of inborn errors of metabolism has been made due to the rapid development of molecular medicine over the last two decades. The majority of genes, whose mutations cause inherited metabolic diseases, have been mapped and sequenced, and the disease-causing mutations have been found. This step forward in understanding these diseases has also offered new approaches in the management of the patients. For some diseases, molecular analysis is the only diagnostic tool. For example, until recently, Alexander disease, a neurodegenerative disease affecting the white matter of the brain, could be proven only by brain biopsy (characteristic Rosenthal fibers) or on autopsy. The recent discovery has made its diagnosis possible by gene analysis (13). For many other diseases, gene analysis is not the only, but the most convenient way to confirm the tentative diagnosis. For example, it is less aggressive to analyze the gene for a common mutations than to perform liver biopsy in children suspected to suffer from hereditary fructose intolerance, or to screen the dystrophin gene than perform muscle biopsy in patients suspected to suffer from Duchenne muscular dystrophy. Particular diagnostic benefit of gene analysis is recognized in cases of diseases caused by a single mutation, ie, hereditary hemochromatosis, which is mainly caused by the C282Y mutation (14). Gene analysis has also significantly improved the whole spectrum of methods leading to prenatal diagnosis and offered the identification of healthy heterozygotes, which helped significantly genetic counseling.

When molecular genetics was introduced into medicine, a widely held belief was that knowing the genotype at a particular locus would allow predicting the corresponding phenotype, and improve counselling and treatment. It is now clear that it was a rather naive belief. Although genotype-phenotype correlation is considerable in some diseases, there is still a large number of examples were the phenotype cannot be explained by detected mutations. Moreover, it has become clear that, beside environmental factors and mutations of the affected gene, many other factors influence the phenotype. The huge work done within human genome project has raised, but has not answered yet, many questions regarding fact that, even with known genotype we are not able to explain the phenotype variations in the majority of monogeneic disorders, including inborn errors of metabolism. The role of numerous factors affecting post-transcriptional events (including transport of RNA, protein synthesis, folding, degradation, etc) and their interrelationships are still unclear (15). Therefore, it is not surprising that "genome" is being replaced by "transcriptome", "proteome", "complexome", and "metabolome", reflecting the acceptance of the complexity of biological processes as a reality to be intensively studied in the future (16). In that sense, the Human Genome Project has also significantly contributed to the research of both pathophysiology and therapeutic options for many inborn errors of metabolism, ie, by the development of "knock-out mice" and other animal research models.

The extensive study of genes involved in inborn errors of metabolism has revealed not only the disease-causing mutations, but also many "neutral" mutations (without an apparent effect on phenotype). However, significant number of such "benign" mutations and polymorphisms still carry some risk for the development of disease in the long run or in the association with variations of other genes, thus contributing to complex, ie, multifactorial diseases. An illustrative example is the C677T polymorphism of the methylentetrahydrofolate reductase gene. This polymorphism is associated with diminished enzyme ac-

tivity leading to hyperhomocysteinemia, which is a known risk factor for early-onset vascular disease. The polymorphism frequency varies among populations e.g., in some western populations it can be found in about 38% of alleles (17). Since this can significantly influence the community health, and the adverse effects of the polymorphism can be supressed by folic acid supplementation, the mentioned and other polymorphisms, and the whole homocysteine metabolism, have become the focus of many research groups studying the early vascular disease.

The study of many genes involved in the development of inborn errors of metabolism has revealed spectra of mutations differing in the frequency and presence of particular mutations across different populations. Phenylketonuria is a good example. The disease is caused by over 400 mutations in the phenylalanine hydroxylase gene. Among them, less than 10 comprise more than three quarters of the total number of alleles. The frequency of particular mutations varies from population to population, reflecting the ethnic background and migrations in the past. For instance, in the Mediterranean region, there is a significant difference in the frequency of common phenylketonuria mutations despite small distances between countries. The most common mutation in Croatia, R408W, was found in 38% of alleles. It is of Slavic origin and its frequency reflects the migration of Croats in 7th century from southern Poland, where the mutation is very frequent, to the area that Croatia occupies today (18). On the other hand, the mutation is found only in about 3% of alleles in Italy, which is a neighboring non-Slavic country. Phenylketonuria alleles also document the "out of Africa" evolution of *Homo sapiens*, with independent appearance of phenylketonuria in oriental and Caucasian populations (19, 20). Obviously, the mutations tell us much about the history of the people as well as diseases.

### Diagnostic Progress

Inborn errors of metabolism are relatively rare and represent pathology rather unknown to primary care physicians and non-specialized pediatricians. Since early recognition of these diseases is a prerequisite for their favorable outcome, timely diagnosis has always been the most important step in their adequate management. Unfortunately, they have always been largely underdiagnosed. Slow progress in the diagnostics of inborn metabolic diseases, following gradual development of biomedical technology, has been jolted a few times by implementation and further development of neonatal screening. This diagnostic program covers the whole newborn population by use of laboratory test adequate for mass screening at acceptable cost. This way we can search for the inherited metabolic diseases that are relatively frequent and amenable to treatment, but whose clinical diagnosis is usually delayed. The history of the development of newborn screening is given in Table 1.

Due to technological development, the remarkable concept of neonatal screening is nowadays extended to early detection of more than 30 inborn errors of metabolism. The tandem mass-spectroscopy (MS/MS), introduced a few years ago in several Western countries, is a method by which amino acids and acylcarnitines can be analyzed in 600 samples daily. It allows early detection of most fatty acid oxidation defects, several organic acidurias, and some aminoacidopathies (as shown by the group from Heidelberg, Germany; Table 2). The combined frequencies of the detected disorders, in addition to conventional screening, amount to about 1:3,000. Similar results were reported for the Bavaria region in Germany (21). Beside offering the data on incidence of various inborn errors of metabolism, the screening results revealed that some diseases such as 3-methylcrotonyl-CoA carboxylase deficiency, previously thought to be rather severe, could be completely asymptomatic. Also, some cases of medium-chain acyl-CoA dehydrogenase deficiency were so mild that could not be diagnosed by usual biochemical tests (including specific loading tests), with the exception of enzyme assay (22). Since the application of MS/MS in the re-

**Table 1.** History of the neonatal screening

| | |
|---|---|
| 1953 | First successful treatment of phenylketonuria (H. Bickel) |
| 1961 | Introduction of "Fölling-Windeltest" in Germany (H. Bickel) |
| 1961 | Development of microbiological inhibition assay ("Guthrie Test") for screening for phenylketonuria (R. Guthrie) |
| Early 1970's | Extension of neonatal screening for galactosemia, maple syrup urine disease, homocystinuria |
| 1978 | Congenital hypothyroidism |
| 1987 | Biotinidase deficiency |
| 1994 | Switch from "Guthrie Test" to enzymatic micromethods (Heidelberg) |
| 1997 | Congenital adrenal hypoplasia (CAH) |
| 1998 | Introduction of ESI-MS/MS[a] = beginning of Extended Neonatal Screening (Heidelberg) |

[a]ESI – electrospray ionization; MS/MS – tandem mass-spetroscopy.

**Table 2.** Results of the newborn screening by tandem mass spectrometry in Heidelberg, Germany from February 1998 to April 2001 [a]

| Inborn error[b] | Positive | Prevalence | Recall rate (%) |
|---|---|---|---|
| PKU/MHP | 23/38 | 1:5,774 | 0.06 |
| MSUD | 2 | 1:107,000 | |
| NKH | 2 | 1:107,000 | |
| UCD | 1 | | |
| MCAD | 15 | 1:14,266 | 0.04 |
| SCAD | 6 | 1:36,000 | |
| VLCAD | 1 | | |
| CPT I, II, SCD | 3 | 1:70,000 | |
| IVA | 3 | 1:70,000 | |
| MMA/Cbl | 2 | 1:107,000 | 0.14 |
| GA 1 | 3 | 1:70,000 | |
| 3-MCC | 4 | 1:43,000 | |
| Overall | 103 | 1:2,077 | 0.30 |

[a]The number of samples analyzed was 214,000.
[b]PKU/MHP – phenylketonuria/mild hyperphenylalaninemia; MSUD – maple syrup urine disease; NKH – nonketotic hyperglycinemia; UCD – urea cycle disorders; MCAD – medium-chain acyl-CoA dehydrogenase deficiency; SCAD – short-chain acyl-CoA dehydrogenase deficiency; VLCAD – very long-chain acyl-CoA dehydrogenase deficiency; CPT I, II, SCD – carnitine palmitoyltranspherase deficiency type I, carnitine palmitoyltranspherase deficiency type II, systemic carnitine deficiency; IVA – isovaleric acidemia; MMA/Cbl – methylmalonic acidemia/cobalamin deficiency; GA 1 – glutaric acidemia type 1; 3-MCC – 3-methylcrotonyl-CoA carboxylase deficiency.

search and diagnosis of inborn errors of metabolism is far from being exhausted, it will probably allow us to search for some other diseases in the future. The extended newborn screening in Austria, starting in autumn 2001, will include screening for creatine synthesis disorders (S. Stöckler-Ipsiroglu, personal communication). In addition, some other metabolites, as sterols and bile acids, can be analyzed and used to detect patients with corresponding biosynthesis defects (23). In the future, new powerful techniques might further increase our diagnostic abilities. Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry, for instance, has a potential for rapid and reliable identification of altered proteins, whereas DNA microarrays, or gene chips, might allow DNA-based screening in a highly parallel and comprehensive manner.

Since great majority of inborn errors of metabolism have not yet been included in the extended screening program, it is estimated that most patients are still un(mis)diagnosed, despite the improved diagnostic methods. Therefore, selective screening, based on well-educated physicians ensuring appropriate selection of patients, and properly used current technology still remain of utmost importance for the diagnosis to be made in most patients.

### New Therapies

The therapy of inborn errors of metabolism has also been changing, in concert with the improvement of diagnostic methods. In 1950s, initial therapeutic nihilism was replaced with dietary modifications that could positively influence the course of the disease. This era started with low-phenylalanine diet in children with phenylketonuria, which was introduced by H. Bickel in 1953. Subsequently, several other inborn errors, such as maple syrup urine disease, urea cycle defects, galactosemia, fructosemia, and tyrosinemia type 2, have been managed in the similar way, ie, with substrate deprivation strategy. Pharmacological doses of vitamins have been useful in cobalamin and biotin metabolism defects, some patients with homocystinuria, and some others. Avoiding fasting is the therapy for defects in ketogenesis and glycogenolysis. Although such measures usually have dramatic beneficial effects, the long-term outcome still has to be assessed. The example is 40% incidence of developmental delay and 80% incidence of ovarian failure in girls with galactosemia who were treated with galactose-free diet (24).

Beside the mentioned groups of the diseases, the majority of other inherited metabolic diseases, with rare exceptions, were not amenable to treatment. Lysosomal storage disorders were in particular hopeless and the outcome was generally determined by the natural course of the illness. About 20 years ago, transplantation became an option in the treatment of some of metabolic diseases. Bone marrow transplants can correct the metabolic defect in some patients with mucopolysaccharidoses (particularly type 1), metachromatic leukodystrophy, Niemann-Pick disease, and some other, mainly storage diseases and immunodeficiencies. Liver transplantation has been life-saving in patients with late-stage Wilson disease, $\alpha$1-antitrypsin deficiency, Crigler-Najjar syndrome type 1, and some other progressive liver disease, and liver-kidney transplantation in patients with hyperoxaluria type 1. The major progress in the last decade has been made with the introduction of enzyme replacement therapy. It was initially applied in patients with visceral type of Gaucher disease and until today more than 3,000 patients have been effectively and safely treated, lately with the $\alpha$-glucosidase produced by recombinant DNA technology (25,26). According to the results of clinical trials, similar efficacy and safety of recombinant $\alpha$-galactosidase A substitution can be expected in patients with Fabry disease (27). Clinical trials have also demonstrated its efficacy in the patients with Pompe and Hurler disease (28,29). Results of preclinical and early-stage clinical trials in some other lysosomal diseases are encouraging as well, as are therapeutic experiments in animal models. Unfortunately, one of the main problems in enzyme substitution therapy remains crossing the blood-brain barrier in diseases with brain involvement. Other possible future options for the patients with lysosomal storage disorders, but also with other inborn errors of metabolism, are the inhibition of substrate synthesis (currently investigated for glycosphingolipidoses), liver repopulation, chaperon-mediated enzyme enhancement, transplantation of stem cells of various specificity, and gene therapy (27,30-32). All these are currently subjects of intensive research.

### Organizational and Social Issues

Scientific and technological development described above has benefited greatly to the patients suffering from inborn errors of metabolism. However, there is still much to be done. First, the knowledge of academic community should be transferred to physicians and other medical staff, and implemented in health systems in different countries. Nowadays, in the era of computers, this process has become much easier due to numerous recommendations and information, or even projects, available on Internet, permanent professional e-mail round tables, on-line editions of books and journals, free-access databases (like McKusick's catalogue), etc. In the implementation process, regional specificities like funds, local pathology (particularly in isolated communities and societies with high consanguinity rate), and religious and geographical features should be respected. Accordingly, specialized metabolic centers and appropriate metabolic network should be established and properly maintained. Unfortunately, novel diagnostic and therapeutic possibilities, such as extended newborn screening or enzyme therapy, are relatively very expensive and still unreachable dream for many countries, where no screening programs or even no health care system exist. Hopefully, the scientific progress will be followed in parallel by the social, political and economical progress, as a necessary prerequisite for giving the science its full sense.

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