

New Perspectives in Inborn Errors of Metabolism: The Dubai Experience

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Introduction

Inborn errors of metabolism (IEM) comprise a large class of genetic diseases involving disorders of metabolism. The majority are due to defects of single genes that code for enzymes that facilitate conversion of various substances (substrates) into other products. In most of the disorders, problems arise due to accumulation of substances which are toxic or interfere with normal function, or to the effects of reduced ability to synthesize essential compounds. Inborn errors of metabolism are now often referred to as congenital metabolic diseases or inherited metabolic diseases, and these terms are considered synonymous.

Metabolism comprises the physiological and biochemical mechanisms by which foodstuffs are taken in by the body and converted from one form to another to provide energy for all the activities of the body. In its broadest sense, metabolism encompasses virtually every biochemical pathway and biophysical mechanism in the body and the resultant physiologic activities.

In a more limited sense, inborn errors of metabolism can be defined as disorders of the mechanisms by which specific major foodstuffs are converted to energy or cellular and tissue building blocks and final products and the mechanisms by which foodstuffs and products are degraded to be excreted.

In the strictest sense, inherited disorders of metabolism encompass a narrow spectrum of conditions that have been defined on a biochemical basis. Broad categories

include disorders of carbohydrate metabolism, disorders of amino acid metabolism, organic acidemias, lysosomal storage diseases, disorders of fatty acid metabolism, and mitochondrial disorders.

The term “inborn errors of metabolism” was coined by Sir Archibald E. Garrod about a century ago. He is known for the “one gene, one enzyme” hypothesis, which arose from his studies on the nature and inheritance of alkaptonuria. This was directly linked to autosomal recessive inheritance, according to Mendel’s rules of heredity. In 1909, Garrod published the first book on the topic, under the title “Inborn Errors of Metabolism” in which he described several other disorders, mainly albinism, cystinuria, and pentosuria (Garrod, 1909). Since then, a number of inborn errors of metabolism have been discovered. A book “The Metabolic and Molecular Bases of Inherited Disease”, comprehensively compiles a large amount of data and knowledge in this field with over 500 authors, and has almost 7,000 pages containing data on more than 500 inborn errors of metabolism (Scriver, 2001). Remarkable progress in the basic science and technology, and enrichment in the explanation of pathogenesis of inherited metabolic disorders over the last two decades have led to more focused initial clinical descriptions of these diseases. This knowledge has opened doors for new approaches in treatment of many, and genetic counseling for most of these disorders. Mucopolysaccharidoses illustrate a phenotype/genotype progress in the diagnosis and treatment of inborn metabolic errors. At the beginning of the last century only descriptions of the clinical phenotype of these patients were

available with no knowledge on the cause. These included coarse facies, dysostosis multiplex, frequent mental retardation, deafness, and hepatosplenomegaly. In 1957, Dorfman and Lorincz (Dorfman and Lorincz, 1957) reported increased excretion of acid mucopolysaccharides in the urine of these patients. Subsequently, the biochemical basis of the disease was revealed. Some years later with the introduction of electron microscopy, more insight was brought in to the field by identification of subcellular structures. This led to the definition of mucopolysaccharidoses as “lysosomal storage diseases”. 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 cell cultures of patients with mucopolysaccharidoses type 1 and 2 (Fratantoni et al., 1968). This was the first demonstration of lysosomal enzymes. In the last two decades, the molecular basis of this group of diseases was revealed and has recently contributed to the development of the enzyme replacement therapy. Over the next two to three decades, errors in glycolysis, the Krebs cycle, and adjacent pathways leading to accumulation of various substrates were elucidated by measurement of catalytic activities of these enzymes, although some of these errors are debated still. By the mid 1980s, techniques largely had switched from those of the biochemistry of intermediates and enzymes to the identification of mutations in genes. This was done by a large number of techniques that make use of DNA fragments (restriction fragment-length polymorphisms) so as to permit linkage mapping and gene sequencing. As a result, this breakthrough allowed both postnatal and prenatal diagnosis of these disorders as well as significant contribution to the development of

the enzyme replacement therapy (Theodoropoulou et al., 2001).

Only when countries in the rest of the world became able to afford similar equipment and similar research did we learn that many inherited disorders of metabolism are very widespread, even though rare in each population. The known incidence of metabolic disorders is often higher in one population than another, but few diseases are confined to a single race. Even the basic name of a disease can change as decades (and even centuries) pass. This is particularly true in the last century or two. What was “idiocy” in the mid-nineteenth century became subdivided into terms such as “amaurotic idiocy” which later became Tay Sachs’ disease, followed by “Tay-Sachs disease,” then “generalized gangliosidosis” then “generalized GM2 gangliosidosis,” “generalized GM2 gangliosidosis, infantile type with McKusick classification 230500,” and finally the current disease name, “severe deficiency of hexosaminidase A with infantile onset of symptoms.”

Advances in Diagnosis

Diagnosis of inborn errors has always been posing difficulty to physicians and non-specialized physicians. This is mainly due to the fact these disorders are relatively rare and most of them share common clinical manifestations. As timely diagnosis is the most important step in the treatment of these patients, there have been development and implementation of newborn screening programs for inborn errors. Newborn screening is the process of testing newborns for treatable diseases. Robert Guthrie is given much of the credit for pioneering the earliest screening for phenylketonuria in the late 1960s using blood samples on filter paper obtained by pricking a newborn baby’s heel on the

second day of life to get a few drops of blood. Congenital hypothyroidism was the second disease widely added in the 1970s followed by galactosemia, maple syrup urine disease and homocystinuria. The development of tandem mass spectrometry screening by Edwin Naylor and others in the early 1990s led to a large expansion of potentially detectable congenital metabolic diseases that affect blood levels of organic acids. The use of MS/MS has permitted several neonatal screening laboratories throughout the world to expand the spectrum of disorders to include fatty acid oxidation disorders and organic acidurias and to increase considerably the number of amino acidemias detected. Additional tests have been added to many screening programs over the last two decades but screening programs vary from country to country and have become a subject of political controversy. With the improvements of diagnostic methods, we shall be able to detect many more types which are frequently un/mis-diagnosed or add many more tests to screening testing panels. Use of Matrix-assisted laser desorption/ionization time-of-flight will certainly be a powerful tool in identification of proteins with altered structures, DNA screening procedures based on microarray technology will replace the current screening programs where a massive number of metabolic diseases can be detected in a very short period of time.

The field of inborn errors of metabolism is always expanding and recently there

have been several new types of disorders identified using new and even conventional technologies (Table 3.1).

New Therapeutic Approaches

Treatment of phenylketonuria in 1953 by H. Bicket by introduction of low dietary modifications was the beginning of era in the treatment of inborn errors. Subsequently, galactosemia, maple syrup urine disease, urea cycle, tyrosinemia, and fructosemia were treated in the similar way. Although diet deprivation has been successful in some cases, it has affected the long term outcome in some patients with galactosemia. Some studies have shown 40% incidence of developmental delay and 80% incidence of ovarian failure in girls with galactosemia who were treated with galactose-free diet (Waggoner DD et al, 1990). In some diseases like biotinidase deficiency, cobalamin defect and homocystinuria, pharmacological doses of vitamins and some co-factors have proven to be useful.

The more than 40 different storage disorders cumulatively affect 1 in 5,000 live. Since lysosomal enzymes are expressed ubiquitously, many organ systems are often affected. Generally, the pathophysiology of a particular disorder is related to the degree of substrate accumulation in a cell or tissue type determined by substrate synthesis and degradation rates and the sensitivity of the cell or tissue type to the stored substrate.

Table 3.1. Recently discovered types of IEMs

DISEASE	MANIFESTATION
Cholesterol biosynthesis defects (Smith-Lemli-Opitz syndrome)	Monogenetic cause of malformation
Congenital disorders of glycosylation (CDG syndromes)	A board spectrum of hemostasiological, and neurological multi-system disorders
Defects in leukotriene synthesis	Increases the frequency of inherited metabolic diseases
Creatine deficiency syndromes	Causing mental retardation and other neurological symptoms
2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency	Organic aciduria
2-methylbutyryl-CoA dehydrogenase deficiency	Organic aciduria

Type I Gaucher disease was the first and only genetic disorder that could be treated effectively with enzyme replacement therapy (ERT). Since then, treatment has become available clinically for Fabry Disease and Mucopolysaccharidosis Type I (also known as Hurler, Hurler-Scheie, or Scheie syndrome), Mucopoly-saccharidosis Type II (also known as Hunter syndrome), Mucopolysaccharidosis Type VI (also known as Maroteaux-Lamy), and Pompe disease (also known as acid maltase deficiency or glycogen storage disease type II). Until today, more than 3,000 patients with Gaucher have been effectively and safely treated, with the alpha-glucosidase produced by recombinant DNA technology (Grabowski et al., 1998; Mistry, 1999). Similar efficacy has been demonstrated in patients with Fabry disease using alpha-galactosidase recombinant DNA technology (Desnick, 2001). Enzyme replacement therapies have been successful in preventing or reversing the somatic manifestations of some disorders by taking advantage of the cell's efficient receptor-mediated uptake systems (Brady, 2003; Kakkis et al., 2001). Lysosomal enzymes carry oligosaccharide structures that enable their recognition by plasma membrane receptors, which deliver the enzymes to lysosomes. Thus far, enzyme replacement therapy has proven unsuitable for treatment of the nervous system in storage diseases, because the blood-brain barrier precludes entry of proteins administered systemically. To circumvent this obstacle, strategies are being attempted that include direct introduction of gene transfer vectors or genetically modified cells into the nervous system (Snyder et al., 1995; Taylor and Wolfe, 1997; Brooks et al., 2002).

Transplantation of hematopoietic stem cells, from either bone marrow or cord blood, has been utilized over the past

several years as a potential treatment for lysosomal storage diseases. Significantly, it is the only treatment to date that has been shown to prevent or retard neurodegeneration in storage disease patients (Krivit et al., 1998). Liver transplantation has been shown to be life-saving in some patients with late-stage Wilson disease. Research studies in a mouse model of metachromatic leukodystrophy (MLD) has demonstrated that transplantation of hematopoietic stem cells transduced with lentiviral vectors carrying arylsulfatase A prevents neurological disease as determined by the absence of sulfatide storage and neuropathology, and the preservation of neurological functions. Optimal prevention of CNS deterioration in MPS is possible only when BMT occurs before onset of neurological manifestations, generally at less than two years of age.

In addition to enzyme replacement therapy, some other treatment regimes including chaperone therapies and substrate inhibition therapy are currently under development for many lysosomal storage diseases. Various gene therapeutic techniques (*in vivo* and *ex vivo* technique) have been developed in order to administer the gene that is defective in a patient to the bloodstream or directly to the brain in order to overcome the blood-brain barrier. In limited trials, some enzyme has been produced by transduced cells, but enzyme production does not appear to be sustained and therefore does not result in a permanent cure. It is anticipated that transduced cells would not have a proliferative advantage over uncorrected cells. Furthermore, it is unlikely that metabolic cross-correction would occur as only small amounts of enzyme are secreted into the circulation (Brady, 2006). Recent studies have shown feasible and effective cell and gene therapy in a murine model of Gaucher disease *in vivo* (Enquist et al.,

2006). This could be a promising tool in the continued efforts toward development of safe and efficient cell and gene therapy for other IEMs.

Inborn Errors of Metabolism and the Human Genome Project

The human genome at one time was estimated to have 70,000-100,000 genes. New data from the Human Genome Project suggest this number may be closer to 30,000. Many of these appear to code for proteins produced in the brain. Data from the Human Genome Project has surely been useful in identifying mutations in the thousands of genes that must underlie inherited diseases of metabolism. Genetic data also will be useful in identifying mutations and polymorphisms that predispose to many of these diseases. This has provided a better understanding of these diseases and has been a step forward in offering new approaches in patient's treatment. Alexander disease, a neurodegenerative disease affecting the white matter of the brain, could be diagnosed only by brain biopsy or on autopsy. However, recent discovery has led to the diagnosis of the disease by gene analysis. In other words, gene analysis in many diseases has provided a more safe and convenient tool to confirm a tentative diagnosis without resorting to old invasive diagnostic procedures. The examples include mutational analysis in children with hereditary fructose intolerance, rather than liver biopsy or gene screening in patients suspected to suffer from Duchenne muscular dystrophy rather than muscle biopsy technique. Moreover, gene analysis has resulted in the improvement of various diagnostic procedures, mainly prenatal and pre-implantation genetic diagnoses which can promise birth of a healthy child for carrier parents. Although genotype-

phenotype correlation is significant and can be used as a prognostic factor in some diseases, yet there remain a large number of situations where the phenotype cannot be explained by genotype. Even with the extensive amount of work done within human genome project many questions regarding this fact remain unexplained, including some in inherited diseases of metabolism. The function of some post-transcriptional/translational factors, modificational activities, molecules interactions and synthesis is yet to be explained (Scriver, 2001). In a sense, the development of experimental animal models by the Human Genome Project has resulted in a better understanding of both pathophysiology and therapeutic options for many inborn errors of metabolism. Not only the disease causing mutations for inherited metabolic disorders have been identified, but rather a large number of polymorphisms which may still carry some risk for the development of disease in the long run is considerable.

The Dubai Experience

Due to an increasing number of high clinically suspected cases of inherited metabolic diseases in Dubai, in 1997, the Unit of Metabolic Genetics was established in the Genetics Department of Al Wasl Hospital, Department of Health and Medical Services. In the beginning, our services were only confined to the population of Dubai, but very recently, we have started providing service to various parts of the United Arab Emirates (UAE) with the four-fold aim to:

- diagnose affected patients,
- detect heterozygotes,
- perform prenatal diagnosis following amniocentesis or chorionic villi sampling, and
- explore research and development

opportunities into improved methods for diagnosis of metabolic diseases.

The number of diagnosable diseases has been increasing rapidly year by year. Advanced Laboratory diagnostic techniques using cell culture, radio-isotope studies, biochemical and molecular analyses, Gas Chromatography/Mass Spectrometry (GCMS) have become an important part of our routine laboratory services in diagnosing various metabolic genetic disorders. Since 1997, the Unit has received over 500 samples suspected

of some type of inherited metabolic disorder, and we have been successful in diagnosing 31 various types and total of 64 cases of IEMs, the majority being reported for the first time in the UAE (Table 3.2). An accurate diagnosis of IEM relies heavily on a large fund of knowledge on the part of experienced clinicians because of the large number of disorders, a high index of suspicion and access to expert laboratory services. This is critically important for subsequent appropriate treatment of these patients which may differ depending on the underlying enzyme defect.

Table 3.2. Inherited metabolic disorders discovered in Dubai during 1997-2006.

IEM	DEFECT	NO. OF CASES
I Carbohydrate metabolism		
1 Pompe disease	Alpha-glucosidase	3
II Amino acid metabolism		
2 Maple syrup urine disease	Branched chain ketoacid dehydrogenase	2
3 Homocystinuria	Cystathionine –beta-synthase	
III Organic acid disorders		
4 Methymalonic acidemia	Methylmalonyl CoA mutase, B12	7
5 3-OH-3-M-glutaic aciduria	3-OH-3-Me Glutayl CoA lyase	4
6 Holocarboxylase deficiency	Holocarboxylase synthelase	1
7 Branched keto-thiolase deficiency	2-methyl-3-hydroxybutyryl CoA dehydrogenase	2
8 Propionic acidemia	Propinyl CoA Carboxylase	3
9 2-OH-glutaric aciduria	Glutaryl CoA dehydrogenase	2
10 Glutaric aciduria type 1	Glutaryl CoA dehydrogenase	1
11 Canavan disease	Aspartoacylase	1
12 Multiple carboxylase deficiency	Multiple Carboxylases	1
13 Ketone utilization disorder	Beta-ketothiolase	1
14 Ethylmalonic aciduria	Short chain acyl CoA dehydrogenase	1
15 Pyruvate carboxylase deficiency	Pyruvate carboxylase	2
16 Pyroglutamic aciduria	Glutathione synthetase	1
17 Isovaleric acidemia	Isoraleryl CoA dehydrogenase	2
IV Fatty acid oxidase diseases		
18 MCAD (Medium chain acyl-CoA dehydrogenase deficiency)	Medium chain acyl-CoA dehydrogenase	2
V Lysosomal enzyme defect		
<i>a) Mucopolysaccharidoses</i>		
19 MPS type I	Iduronidase	1
20 MPS type II	Iduronate sulphatase	2
21 MPS type VI	N-acetylgalactosamine-4-sulphatase	1
<i>b) Glycoprotineoses</i>	(Arylsulphatase B)	
22 Beta-mannosidosis	Beta-Manosidase	1
23 Alpha-fucosidosis	Alpha-Fucosidase	1
<i>c) Lipid disorders</i>		
24 GM1-gangliosidosis	Beta-Galactosidase	5
25 Tay Sach disease	Hexosaminidase A	4
26 Sandhoff disease	Hexosaminidase B	4
27 Gaucher disease	Glucocerbroadase (beta-glucosidase)	4
28 Nieman-Pick disease	Cholesterol esterification	1
29 Krabbe disease	Galactocerberocidase	2
VI Vitamins metabolism disorder		
30 Biotinidase deficiency	Biotinidase	1
VII Membrane transport disorder		
31 Fumaric acid	Fumarase	1
Total		64

Recommendations

Study of inborn errors of metabolism (IEM) in UAE is in its infancy. The majority are clinically diagnosed since there are only a few clinicians and scientists with expertise in IEM, shortage of well-equipped laboratory facilities throughout the country, and well-organized plan. There is much to be done. The knowledge of the international academic community should be transferred to physicians and other medical staff, and implemented in health systems in different parts of the country; satellite metabolic centers and appropriate regional metabolic network should be planned, systemically established and well maintained; and extended newborn screening programs for metabolic diseases must be initiated as a preventive tool. Implementation process of such a scientific and technological development will be lengthy but will greatly benefit the patients and the community in the long run.

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