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Therapeutic approaches to genetic disorders

Mohammad S. Zaghloul[1], Solaf M. Elsayed[1] and Heba Alla A. Hosseny[2]

[1]Genetics Unit, Department of Pediatrics, Faculty of Medicine, Ain Shams University, [2]Department of Genetics, El Glaa Educational Hospital

ABSTRACT

Although prevention is the ideal goal for genetic disorders, various types of therapeutic management are available. Such management approaches depend on the nature of the defect, how well it is understood at the genetic and biochemical levels and the practical feasibility of correction. In some conditions certain management is now tailored to the specific genotype. The patient being treated may be the fetus, the infant, the child or the adult. Treatment methods used in genetic disorders may involve surgical, cognitive/behavioral, pharmacologic, dietary, envairomental avoidance, transfusion, plasma exchange, enzyme, behavioral, cell, or gene therapy. Some have been developed on the basis of knowledge of the defect in the gene and its product, whereas others are empirical or aimed at controlling or mediating signs and symptoms without care.

Key Words:

Stem cell therapy, gene therapy, fetal therapy, PKU embryopathy, chaperons.

Corresponding Author:

Mohammad S. Zaghloul
E-mail: mszsalem@hotmail.com

INTRODUCTION

The vast majority of genetic defects are attributed to deficient or defective production of structural proteins, of regulatory proteins, or of catalytic enzymes which are the gene products of structural genes. Albeit, a considerable proportion of genetic diseases results from defective regulation of functions of structural genes, which is a major task exerted by regulatory genes. This defective regulation can happen at any point and can affect any stage of gene function and comprises a quite large spectrum of variable pathogenetic mechanisms. Examples of these mechanisms include disturbed chromatin remodeling, deficient or defective synthesis and or functions of micro - or small- RNAs, defective regulation of mRNA processing, splicing and turn over, defective monitoring of post-translation modifications due to e.g. defective chaperon actions, defective regulation of cell cycle dynamics that commonly results in development of tumors, defective regulation of morphogenetic mediators with consequent diversion towards teratogenesis and development of congeniral malformations. The list is too long and includes a wide variety of different mechanisms all of which result in pathogenesis and development of a large number of genetic disorders due to defective regulatory functions exerted by regulatory genes over structural genes.[1]

In view of the aforementioned simplified scheme of gene function, the list of therapeutic approaches to genetic disorders seems endless, at least theoretically. Currently, there are too many therapeutic approaches to genetic diseases targeting either the underlying pathogenetic mechanism (s) or aiming at reducing the side effects and deleterious consequences of these diseases secondary to altered pathophysiological states[2]. These therapeutic approaches include a wide variety of options including dietary management of inborn errors of metabolism through restriction of offending substrate (e.g phenylketonuria, maple syrup urine disease) and removal of toxic products[3], drug therapy for e.g. hypercholesterolemia, congenital adrenal hyperplasia and convulsive disorders[4], replacement therapy of abnormal or deficient proteins or enzymes (e.g. hemophilia and many storage disorders), cell-tissue-organ transplantation (e.g for many disorders like tyrosinemia and polycystic kidney disease), surgical intervention for genetically-determined congenital malformations and late developing surgically correctable complications and gene therapy approaches that comprise innumerable varieties of techniques aiming at correcting molecular defects leading to genetic diseases at nearly all known pathogenetic targets of these defects (e.g. exon skipping for Duchenne muscular dystrophy).[5]

The Choice of the best therapeutic approaches to a certain genetic disorder is determined mostly by both the underlying pathophysiological mechanism (s) and the resulting deleterious complications of the defect. For instance, genetic metabolic errors caused by deficient and or defective production of enzymes necessary for metabolism of ingested food substrates (proteins, carbohydrates, lipids and other nutrients) are characterized in general by accumulation of the ingested stuff in blood. In most cases, metabolic by-products of the accumulating substrate also attain high levels secondary to the metabolic block. Percolation of the accumulating substances to cells, tissues and organs follows with subsequent pathogenesis of toxic effects exerted on cells and tissues not acquainted to the presence of these substances in such high concentrations, hence the development of clinical manifestations and disease complications, e.g. neurotoxicity exerted by hyperphenylalanenemia in PKU and by hyperammonemia in urea cycle defects, cataract formation due to accumulation of the the sugar alcohol galactitol in galactosemia and the multi-organ toxicity observed in tyrosinemia type I due to the effects of the mitochondrial toxin succinylacetone which is a decarboxylation product of succinyl acetoacetate, a compound derived from the tyrosine catabolic intermediate fumarylacetoacetate. Fumarylacetoacetate itself induces mitotic abnormalities and instability in the genome[6]. Taken together, these data form the basis for a unifying hypothesis regarding the development of hepatocellular carcinoma in children with hereditary tyrosinemia.

In addition to the accumulation of the ingested stuff and its metabolic intermediates and/or its final metabolites, deficiency of the products, produced in normal amounts in normal conditions, results with consequent deleterious complications determined by the missing physiological and metabolic functions of these products. For instance, young women suffering from PKU give birth to very severely malformed children, a condition known as PKU embryopathy that comprises microcepha-

ly, mental retardation, hypotrophy and cardiopathy, unless they strictly take up the specific diet, until the PHE level has lowered down to normal, before the beginning of gestation. Serotonin deficiency, which is a feature of PKU, probably underlies the development of PKU embryopathy in view of its morphogenetic role in normal embryogenesis.[7]

Therapeuric approaches to genetic disorders include:

1- Substrate restriction/Removal approch:

Therapeutic approaches to most genetic inborn errors of metabolism include a comprehensive handling of all aspects of the metabolic defects and include dietary restriction of intake or removal of the offending substrate from diet e.g. restriction of protein intake in PKU and urea cycle defects and removal of galactose and fructose from diets of infants affected with galactosemia and fructosemia, respectively.[8]

Removal, degradation or detoxification of the accumulating toxic metabolites represents an indispensable and integral part of particularly in management of acute decompensation states of metabolic intoxication errors as well as in similar genetic diseases due to storage of ingested food constituents or storage of metabolic intermediates or final metabolites. Examples of this approach include use of sodium benzoate or sodium phenylbutyrate, oral lactulose and hemodialysis/peritoneal dialysis in states of hyperammonemia[9], use of plasmapheresis in Refsum disease, use of iron chelation in thalassemias and many other conditions.

2- Substrate addition/Replacement approch:

Supplementation of deficient gene product (s) represents the most proper therapeutic approaches for genetic diseases due to mutations resulting in deficient or defective production, or complete absence, of the gene products whether these are proteins, enzymes or other effector components. This approach is the oldest conventional approach adopted for this category of genetic defects and is still in practice. Treatment of hemophilia with anti-hemophilic globulin[10], treatment of hypothyroidism with L-Troxine, treatment of short stature due to growth hormone deficiency with GH and treatment of diabetes mellitus with insulin are just few examples.

3- Supplementation of the deficient or defective enzyme:

It seems the logical and effective physiological therapeutic approach for inborn errors due to deficient production of the enzyme, its production in a defective configuration or failure in targeting the synthesized enzyme from the cytosol to its final destination, e.g. the lysosome or the peroxisomes, as is the case in lysosomal storage disorders and peroxisomal storage defects, respectively. Though supplementation of deficient proteins seems a straightforward approach, supplementation of enzymes is a little bit more complicated due to the intra-cellular and intra-organellar localization of most enzymes.

Enzyme replacement therapy (ERT) is a therapeutic approach in which the specific enzyme that is inactive or absent in affected individuals is replaced

with synthetic functional enzyme preparation. ERT has been successful for the treatment of Type 1 Gaucher disease[11], Fabry disease, Hurler disease and most recently, has received approval for Pompe disease. ERT is also effective in the non-neurological symptoms of Mucopolysaccharidosis Types I, II IV and VI, Pompe and Niemann-Pick B, but has not yet proven to be beneficial in storage diseases that primarily affect the central nervous system because the replacement enzymes do not efficiently cross the blood-brain barrier.

However, the invention of recombinant enzyme and protein, synthesis technology by genetic engineering has made a revolutionary breakthrough in genetic therapies and marked achievements have been done in this respect and the list of genetic disorders amenable to treatment via this approach is regularly expanding (Table 1). Research trials are in active progress for synthesis of recombinant enzymes for other genetic errors due to enzyme deficiencies like storage diseases and many others.[12]

Table 1: Examples of protein produced biosynthetically using recombinant DNA technology[2].

Protein	Disease
1. Insulin	Diabetes mellitus
2. Growth hormone	Short stature due to growth hormone deficiency
3. Factor VIII	Hemophilia A
4. Factor IX	Hemophilia B
5. Erythropoietin	Anemia
6. Alpha Galactosidase	Fabry disease

In mild conditions, provision of enzyme co-factors or activators, mostly vitamins, in pharmacologic doses, might result in restoration of effective enzyme activity with remarkable improvement. This approach underlies the well established use of thiamine in maple syrup urine disease, of pyridoxine and vitamin C in homocystinuria, of vitamin B12 in methylmalonic aciduria and many other metabolic errors[13] (Table 2).

Table 2: Examples of vitamin – responsive inborn errors of metabolism[2].

Disease	Deficient vitamin Or coenzyme
1-Homocystinuria	B6
2- Methylmalonic Acidemia	B12
3- Propionic acidemia	Biotin

The finding that post-translational modifications defects of many enzymes and proteins are attributable to deficient or defective functions of restorative chaperones, a subfamily of heat shock proteins, secondary to mutations affecting one or more of the chaperon genes family, have opened a wide and promising therapeutic prospective[14]. This approach aims at either provision of these chaperones or correction of the genes producing them as a new modality

for combating this pathogenetic mechanism and treatment of diseases caused by this pathophysiological alteration.

Pharmacological chaperones are small molecules that specifically bind to and stabilize the functional form or three-dimensional shape of a misfolded protein in the endoplasmic reticulum (ER) of a cell. When misfolded because of a genetic mutation, the protein (or enzyme) is unable to adopt the correct functional shape. This misfolded protein is recognized by the quality control system in the cell and destroyed, leading to decreased amounts of enzyme that gets transported from the cell's ER to the cell's lysosome, hence, reduced enzyme activity. The binding of the chaperone molecule helps the protein fold into its correct three-dimensional shape. This allows the protein to be properly trafficked from the ER and distributed to the lysosome in the cell, thereby increasing enzyme activity and cellular function, reducing substrate and stress on cells.[15]

4- Organ-tissue-cell replacement and transplantation:

Replacement (transplantation) therapy is a wide term that encompasses too many varied applications. The concept of replacement therapy is a logic one based on the known pathogenetic mechanisms of most genetic disorders. Insufficient production of a gene product or its production in a defective way can be corrected by replacing the deficient or the defective product with a normal natural or synthesized one. This reasoning demarcated the narrow-scale use of the concept in hormone replacement therapy for endocrinal disorders, the use of immunoglobulin preparations for humoral immunodeficiency states, the use of anti-hemophilic globulin for hemophilia, the use of recombinant human enzyme preparations for many enzyme-deficient conditions and similar allied disorders.

Extension of the concept of replacement therapy to a wide-scale applications resulted in a revolutionary turn in medical practice. Thus, a whole organ might be replaced as in organ transplantation. In tissue transplantation, a part of an organ is replaced with a healthy one like cardiac valve transplantation, cornea transplantation and bone grafting. Cell infusion or replacement, like whole or fractionated blood transfusion or stem cell therapy is, in a sense, a transplantation technique. However, in stem cell transplantation the goal is to offer affected patients with a life-long chance for these cells to replace defective cells, damaged tissues, or failing organs.

Despite remarkable achievements in this field, chronic allograft rejection, the side effects of the long-term immunosuppressive treatment and organ shortage are still the major obstacles to achieving long-term survival. Precautionary measures needed to ensure success of replacement therapy stem mostly from fears of rejection of transplanted, infused or injected foreign material. However, advances in immunogenetics allowed for better prognoses by refining selection of compatible donors and expected advances in genetic manipulation techniques aiming at prediction or detection of rejection at its earliest stages[16], nullifying foreign antigen processing and detection and induction antigen-specific tolerance would surely

lessen the risk of rejection and related complications to a minimum. [17&18]

One major complication facing organ transplant recipients is the requirement for life-long systemic immunosuppression to prevent rejection. The advent of immunosuppressive drugs participated actively in ensuring the success of transplantation therapy; however, their use implies potential major side effects to exposed patients which are associated with an increased incidence of malignancy and susceptibility to opportunistic infections. Many gene therapy techniques have been advented to reduce and obviate these risks. Gene therapy has the potential to eliminate problems associated with immunosuppression by allowing the production of immunomodulatory proteins in the donor grafts resulting in local rather than systemic immunosuppression. Alternatively, gene therapy approaches could eliminate the requirement for general immunosuppression by allowing the induction of donor-specific tolerance. Gene therapy interventions may also be able to prevent graft damage owing to nonimmune-mediated graft loss or injury and prevent chronic rejection.[19]

Another major challenge to transplantation approaches is failure of transplanted organ, tissue, or cells to maintain their own survival. Enhanced apoptosis of transplanted organs or tissues is a common complication of transplantation therapy. Furthermore, some of the immunosuppressive drugs currently in clinical use might exert their activity at least in part through effects on apoptotic pathways. From the available data, it can be inferred that apoptosis contributes to the outcome after organ transplantation, being involved both in graft rejection and in transplantation tolerance[20]. Many genetic therapies were tried to lessen and obviate this risk with promising success, for instance, the use of heme oxygenase-1 gene transfer to prolong survival of cardiac allograft[21] and use of anti-apoptotic genes for prolonging survival of pancreatic islets transplantation.[22]

5- Pharmacological therapy:

Like most other diseases, pharmacological or drug therapy of genetic diseases occupies a pivotal role in current and expected, management of most of these diseases. The spectrum of this therapeutic approach is very wide and encompasses a very long list of both pharmaceutical chemicals and pharmaceutical biological preparations. Chemical drugs used widely in management of genetic disorders include anti-epileptics for genetically-determined seizures, lipid lowering drugs for genetic hyperlipidemias, Nitisinone (NTBC) for treatment of Tyrosinemia type I, hydroxyurea and piracetam for treatment of sickle cell anemia, trimethyl glycine (Betaine) for treatment of homocystinuria (Fig. 1), the iron chelator deferoxamine mesylate for treatment of thalassemias and similar chronic hemolytic conditions and too many other genetic disorders.

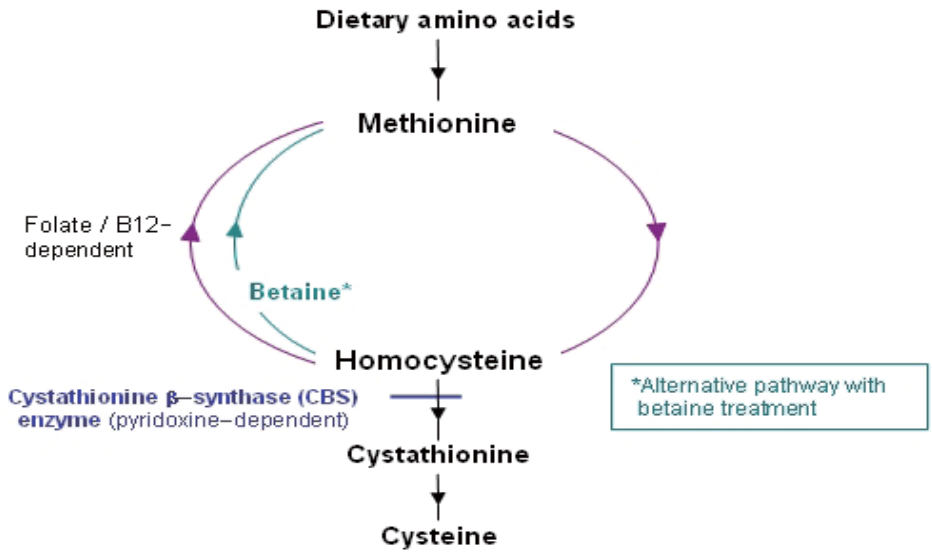

Fig. 1: Methionine metabolic pathway.

Similarly, pharmaceutical biological preparations have a major role in treatment, alleviation and prophylaxis of genetic diseases and their complications. Examples of these preparations include: Use of immunoglobulins in treatment of humoral immunodeficiency states, use of megavitamins therapy in management of many metabolic errors like B1 in maple syrup urine disease, B6 in homocystinuria, Folic acid for prevention of neural tube defects and use of α-tocopherol in Ataxia with vitamin E deficiency (AVED)[23]. Additional examples include the use of other synthetic vitamins, hormones, enzymes and proteins for treatment of genetic diseases due to defective or deficient production of these biological active and essential components.

6- Surgical intervention:

Surgical intervention for reatment of birth defects and possible developing defects in genetic disorders plays a very important role in management of these diseases. Without such an approach, the life of patients with surgically-correctable congenital malformations would have been a real misery. The same guidelines applied for these procedures in non-genetic diseases apply also for genetic diseases. The list of congenital defects amenable for surgical correction either for radical cure or for alleviation of sufferings and prophylaxis against worse downhill progression is too long, examples of candidate conditions include congenital heart diseases, congenital urinary tract obstructions, congenital neural tube defects, congenital bone dysplasia and malformations, congenital defects of external genital organs and many congenital ocular and auditory malformations.

Fine surgical intervention, also, is practiced for many genetic defects with considerable success. For instance, treatment of genetically-determined

intractable seizure foci by bipolar electro-coagulation of functional cortex[24], use of isolated or combined fine laser treatment for ocular visual defects and dysplastic conditions of the cornea, the retina and the choroids, implantation of smart micro chips for peripheral vaso-occlusive and myocardial diseases and the use of robotic surgery for more safe and effective surgical management of many malformation disorders reveal the role of fine or micro-surgical procedures in this therapeutic approach.

7- Fetal therapy:

Fetal therapy, or treatment of genetic diseases or malformations of the fetus during intra-uterine life, represents a crucial approach to avoid progression of such diseases or malformations to a hopeless situation if left without intervention till birth. Surgical intra-uterine management of some fetal conditions either via open fetal surgery or minimally-invasive fetoscopic surgery have an important role in treatment of many fetal malformations. Intervention in hydrocephaly, many types of spina bifida, congenital diaphragmatic hernia, urinary tract obstruction and some congenital heart defect illustrates few applications of this approach in fetal therapy.

Fetal drug therapy and prophylaxis also has a crucial role in treatment and / or prevention of a large number of genetic fetal disorders. Examples of such applications include use of corticosteroids prevention of external genital masculinization in female fetuses with 21-hydroxylase deficiency syndrome[25], biochemical amelioration of methylmalonic acidemia and biotin-responsive multiple carboxylase deficiency, control of fetal cardiac arrhythmias with Amiodarone[26] and treatment of fetal fetal goitrous hypothyroidism with L-Thyroxine.[27]

8- Gene therapy:

As the term implies, gene therapy aims at offering radical treatment of genetic diseases via correcting the underlying pathogenetic mechanisms of these diseases at the gene level. Based on our knowledge of these mechanisms, a wide variety of techniques have been theorized. Unfortunately, quite few of them are worthy of trial. With the exception of the success of classic viral-based gene therapy for malignant melanoma of the skin[28] and the success of stem cell therapy for some selected genetic disorders, the way to safe and successful gene therapy is still very long.

Current trials of gene therapy techniques comprise many different approaches. Some of them target the deficient or defective gene function by trying to compensate for the lost function by offering normal counterparts of the diseased gene to affected cells or tissues through e.g. gene transfer mechanisms. Other techniques target the whole affected cells by offering normal cells with normal whole genomes, like the case in stem cell therapy. In between these two extremes of the spectrum, too many other techniques are being tested with hopeful expectations. These approaches aim at targeting underlying pathogenetic mechanisms at all possible stages, for example by manipulation of post-transcriptional effector molecules like mRNA by micro- or small- RNAs, manipulation of the translation machine in the cytosol by signal transducers or by manipulating post-translational events by chaperones. Such techniques

include, for instance, the use of the ability of hammerhead ribozyme to induce site-specific cleavage of RNA to down-regulate the expression of mutant alleles[29], the use of RNA interference technology (RNAi) for control of underlying defects in movement disorders[30], the use of nanoparticles, instead of viral vectors, as gene carriers to target cells for many diseases, e.g. lung cancer where nanoparticles are used to target cancer cells with dual tumor suppressor genes[31], the induction of molecular chemerism as a potent regulator of cell functions to alter cell behaviour e.g. induction of B-cell and T-cell tolerance for preventing graft rejection and prolonging survival of transplanted organs[32,33] and use of pharmaceutical molecular chaperones to correct post-translational modifications defects which underlie the pathogenesis of a considerable number of common and serious genetic diseases like Alzheimer's disease, Parkinson's disease, Huntington's disease, Creutzfeldt–Jakob disease, cystic fibrosis, Gaucher's disease and many other degenerative and neurodegenerative disorders.[15]

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