Genetic aspects of inherited metabolic diseases

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As a group, inherited metabolic diseases are commonly considered rare disorders. However, some are exceptionally common in specific human populations. Different approaches are therefore required to manage this heterogeneous group of conditions. In this article, the genetic aspects of inherited metabolic diseases are described. These aspects include genetic mechanisms, diagnosis, genetic counselling, screening, prenatal diagnosis, and gene therapy.

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Introduction

It is customary to refer to inherited metabolic diseases (IMDs) as rare disorders. However, of the 2000 annual referrals to the Clinical Genetic Service in Hong Kong, almost half were IMDs.[1] Of these, the majority were due to glucose-6-phosphate dehydrogenase deficiency (G-6-PD), a common disorder found among southern Chinese. Hence, as a group, IMDs are not uncommon, but some are over-represented, while the majority are individually rare. Much has been learnt about the genetic implications of these diseases and a genetic approach should be devised for their management. In this article, I discuss the genetic mechanisms, diagnosis, genetic counselling, screening, prenatal diagnosis, and gene therapy for IMD.

Genetic mechanisms of inherited metabolic diseases

Since the pathogenesis of IMD has been alluded to in this seminar (see related articles by Low and Pang), the present discussion will centre on two aspects—the molecular basis of IMDs and their inheritance patterns.

Molecular basis

Since much has been written about normal gene structure and expression,[2] these will not be discussed here. It should be noted, however, that most IMDs result from single gene defects. Their underlying basis may be either single base pair substitutions, deletions or insertions in the DNA of the individual genes. Alternatively, amplifications of nucleotide repeat sequences have been found to be responsible for diseases such as fragile X syndrome[3] and myotonia dystrophica.[4]

It is important to note that genes can be either nuclear or mitochondrial in origin, the distinction of which lies in their different inheritance patterns. As a direct consequence of these mutations, errors occur in the process of gene expression. The end results of these mutations are either the decreased production of the proteins for which these genes are coded, or the production of structurally abnormal proteins. With IMDs, the proteins of major concern are either enzymes, for example, lysosomal enzymes; or structural proteins, such as collagens and fibrillins.

Inheritance patterns

While autosomal recessive (AR) is the most common form of inheritance pattern, other forms are not uncommon. As shown in Table 1, of the 280 conditions that are classified as IMD,[5] 189 (67%) are of AR inheritance, and 60 (21%) are of autosomal dominant (AD) inheritance. This compares with 6% each for X-linked recessive and mitochondrial inheritance. The classification of IMD presented in Table 1 has been criticised for an apparent lack of consistency in that some conditions are grouped together under common accumulated metabolites, while others are grouped under certain subcellular organelles. However, this classification does offer quick reference to the groups of diseases under discussion, and has been most commonly employed. Table 1 shows that the AD pattern is

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Table 1. Inheritance patterns of inherited metabolic diseases*

Metabolic disorders	Autosomal recessive	Autosomal dominant	X-linked	Mitochondrial
Carbohydrate	13			
Amino acid	35	3	1	
Organic acid	43	2	1	
Lysosomal	41	2		
Peroxisomal	9	1	2	
Mitochondrial				17
Purine/pyrimidine	8	4	4	
Porphyrins	1	6		
Collagen	8	15	3	
Steroid	9	2		
Lipid	5	7		
Metal	9	3	2	
Red blood cell	8	15	1	
Total	**189**	**60**	**14**	**17**

* Source: Holton JB. The inherited metabolic diseases. 1994.[4]

more commonly found in some of these groups, for example, in disorders of porphyrins, collagen, lipids, and red blood cells.

On the other hand, mitochondriopathies are a unique group of IMDs. Since mitochondria are far more abundant in ova than in spermatozoa, these diseases are maternally inherited.[6] However, because the mitochondrial genome is much smaller than the nuclear genome, this group of disorders accounts for a small proportion of IMDs. Similarly, since the X chromsome only occupies 5% of the human genome,[7] X-linked diseases are also less common than AR- or AD-inherited diseases. Yet, they are quite ubiquitously represented throughout the major groups of IMD. As mentioned earlier, other patterns of inheritance do exist. The association of unstable nucleotide repeat sequences with diseases and the phenomenon of genomic imprinting are notable examples.[8] However, these play only minor roles in IMD.

Genetic counselling

For genetic counselling of IMDs, three issues should be considered: the significance of proper diagnosis; the concept of genetic heterogeneity; and the awareness of variability in clinical presentations of diseases. In the following discussion, our local experience will also be described.

a) Diagnosis

All counselling must commence with a proper diagnosis. For IMDs, most patients are diagnosed at the neonatal, infantile, or childhood stages of development, although adult manifestation of disease is not uncommon for certain categories of IMD. Since many of these conditions may not be familiar to most clinicians, a high index of suspicion needs to be developed. For example, during the neonatal period, IMD may be erroneously diagnosed and managed as sepsis. Similarly, young adults may receive repeated surgery for "sebaceous cysts," which are subsequently diagnosed as xanthomas resulting from familial hypercholesterolemia.[9] The diagnostic process is not confined to the proband; other family members should also be examined and investigated for carrier status. For example, when the proband is a neonate whose investigations have not been completed before death, and no tissue sample is available for further investigations, the parents will be most concerned to know whether they are carriers of the deleterious genes.[10]

b) Genetic heterogeneity

This term refers to the occurrence of similar clinical presentations as a result of mutations involving either one gene, at different alleles, or mutations at different gene loci. The former are referred to as allelic, and the latter as non–allelic mutations. Glucose-6-phosphate dehydrogenase deficiency offers a good example of

the former. In this condition, allelic mutations at one single gene locus on the X chromosome can give rise to variation in the clinical presentation and biochemical abnormalities. Conversely, patients with mucopolysaccharidosis, and some forms of oligo-saccharidosis, may present very similar clinical features. Yet, these features may result from many different types of enzyme deficiencies, and hence, different gene mutations. Besides being sources of diagnostic confusion, genetic heterogeneity is also of importance in clinical management. For example, in homocystinuria, characterised by a deficiency of cystathionine-beta-synthase, some cases are associated with pyridoxine dependence, while others are not.[11]

c) Clinical variability
Apart from genetic heterogeneity, other factors can contribute to clinical variability that are of importance for genetic counselling. Three of these are discussed.

X-linked diseases
Although female carriers of deleterious X-linked genes present less commonly with clinical problems, they do manifest under certain circumstances to the same degree as affected hemizygous males.[12]

The occurrence in these females can be explained by the presence of homozygosity or double heterozygosity for gene mutation, extreme lyonisation or the presence of mutation in a female subject with only one X chromosome.

Altered penetrance and expressivity
These can occur in AD or X-linked inherited diseases. For example, unstable trinucleotide repeat sequences have been described as the underlying molecular pathology in fragile X syndrome.[3]

Although normal subjects have a small number of these repeats, carriers can harbour an expanded number of copies in the form of a premutation. Variability in the number of these copies can account for differences in clinical expression. When the number of copies further increases, the full-blown phenotype will manifest. These genetic phenomena are also found in myotonia dystrophica and other neurological diseases.[13]

Mitochondrial diseases
Clinical variability in mitochondriopathies are well known. Since each cell contains many mitochondria, some harbouring mutations and others not, whether the mutations will manifest as clinical diseases will depend on the proportion of mitochondrial gene mutations in the cells. This applies to both male or female subjects affected by these mutations. On a different note, it should be recognised that they are maternally inherited diseases, and would not be passed via affected males.

The need for a local register

The collation of data on local experience is hampered by not having a register for IMDs. In the Clinical Genetic Service, a total of 222 families with IMD were counselled from September 1981 to July 1988.[14] For the purposes of research and planning of health services, it is advisable to set up a register for IMDs in Hong Kong.

Genetic screening for inherited metabolic diseases

When individuals have an IMD, they and their family members will be managed by a clinical approach that includes diagnosis, treatment, and genetic counselling. This works particularly well with most IMDs, which are of low frequencies in the population. However, other conditions are so abundant and severe that the alternative approach of population screening is warranted. Criteria for screening diseases are well-established and should be seriously considered before any screening programme is started.[15] The following discussion concentrates on the history of IMD screening and the development of filter paper technology. It also includes a description of commonly screened diseases and our local experience.

Origin of screening
The following quote is from an article by Robert Guthrie: "It began with our second child, John. He is mentally retarded."[16] Originally in cancer research, Guthrie was stimulated by the death of his son to enter into research on mental retardation. Using the simple but elegant principle of competitive inhibition, developed from his earlier bacterial assay works for cancer patients, Guthrie was able to perform phenylalanine assays from filter paper discs impregnated with neonatal capillary blood. This methodology was subsequently employed for screening newborn infants for phenylketonuria on a massive scale. In the following decades, other types of analysis were introduced, allowing more IMDs to be screened. Hence, while the phenyalanine assay was pivotal to the development of newborn screening, Guthrie had always considered the filter paper blood specimen to be his most important contribution. It was termed filter paper technology.[17]

Table 2. Screening tests for inherited metabolic diseases

Disease	Metabolite screened for	Laboratory tests
Phenylketonuria	Phenylalanine	Bacterial inhibition assay Fluorometric assay
Galactosaemia	Galactose, galactose-1-phosphate GALT enzyme	Bacteriophage inhibition assay Beutler test
Maple syrup urine disease	Leucine	Bacterial inhibition assay
Homocystinaemia	Methionine	Bacterial inhibition assay
Biotinidase deficiency	Biotinidase	Semi-quantitative colorimetric assay
Congenital hypothyroidism	Thyroid stimulating hormone	Radioimmunoassay Enzyme-linked immunosorbent assay Immunofluorometric assay
Congenital adrenal hyperplasia	17-hydroxyprogesterone	Radioimmunoassay Enzyme immunoassay Fluoro immunoassay
Ghncose-6-phosphate dehydrogenase deficiency	G-6-PD enzyme	Colorimetric assay Beutler test
Haemoglobinopathy	Haemoglobin phenotype	Electrophoresis Isoelectric focusing High-performance liquid chromatography Immunological testing Molecular technique
Cystic fibrosis	Immunoreactive trypsinogen	Radioimmunoassay Enzyme immunoassay Mutational analysis
Dyslipidaemia	Apolipoprotein A-1 & B	Enzyme immunoassay Radial immunodiffusion Enzyme-linked immunosorbent assay Radioimmunoassay

Filter paper technology

There are several merits associated with employing filter paper for the collection and transport of neonatal blood samples. For primary screening, blood collection can be performed by primary health workers via a simple heel prick, thereby avoiding venepuncture. When properly air-dried, the papers can be mailed to distant laboratories under any climatic conditions. At the laboratory, the filters can fit into the automatic and computerised processes. They can also be stored for long periods, allowing re-investigation and acting as sample banks for alternate investigations. This methodology is far superior to other forms of sample collection, and has

been most commonly employed for neonatal screening of IMDs.

Commonly screened inherited metabolic diseases

Although screening methods are available for many IMDs, screening programmes are practical for just a few. Table 2 lists the more commonly screened conditions, either on a whole population basis, or for at-risk groups.[18] Most centres screen for phenylketonuria since it is the prototype of neonatal screening. From the mid-1970s, congenital hypothyroidism (CHT) has been incorporated as a routine part of neonatal screening, although a mere fraction of CHT patients suffer from an IMD. Homocystinuria, galactosaemia, maple syrup urine disease, and biotinidase deficiency are screened in selected centres. Congenital adrenal hyperplasia, dyslipidemia, Tay-Sachs disease, and Niemann-Pick disease are screened for in specific populations.

Local experience

Neonatal screening has been conducted in Hong Kong since the early 1980s. As more than 95% of the population is Chinese, the majority from the southern provinces of China, G-6-PD deficiency is a prime target for screening. In the past, hyperbilirubinemia resulting from deficiency of this enzyme was an important cause of mortality and morbidity.[19] In 1984, a territory-wide screening programme was started by the Hong Kong government. Cord blood was employed as the screening sample, since this method of blood collection fitted well with the existing routines. So far, more than 10 000 families have been counselled. Deficiency of this enzyme was ascertained in 4.47% of male and 0.27% of female neonates.[20] Congenital hypothyroidism has also been found to contribute significantly towards mental retardation, so screening for this condition was performed simultaneously with the G-6-PD deficiency. Since 1984, more than 150 babies have been diagnosed and treated, giving an incidence of 1/3100 live births.[21]

Prenatal diagnosis

Despite advances in the diagnosis and treatment of IMDs, and improvements in the technologies employed in screening for probands and carriers, many patients do not respond to therapy, hence the need for prenatal diagnosis (PND) for IMDs. The following discussion focuses on the rationale for PND, those who should be offered these procedures, the methodologies employed, and the current development of such services.

Rationale

Since treatment may not be available, parents of children born with an IMD are left with limited options. Based on estimates of their risk of having further children with the same disease, parents can opt for having no further children, do nothing to prevent further pregnancies, request artificial insemination by donor, or embark on PND with the intention of aborting any affected foetus.[22] Such options should be offered during genetic couselling sessions, before future pregnancies are planned. Every effort should be made to help these couples make informed decisions. To the parents and families, this is one way of ameliorating further suffering. For the population in general, this is a proactive approach to public health.

Who should be offered prenatal diagnosis?

Prenatal diagnosis should be offered to those families who are known to be at risk for an IMD. These include families with previously affected children, or known carriers of the mutant gene such as some mothers of children with an X-linked condition, or parents of those suffering from AR inherited diseases. For X-linked diseases that defy biochemical or genetic diagnosis, one could still perform foetal sexing with the intention of aborting a male foetus. Autosomal dominant conditions may give rise to difficulties in counselling, because of the variability in clinical expression. In these instances, family members should be exhaustively examined to rule out their carrier status.

Methodologies

More than 100 IMDs can now be diagnosed during the prenatal period, using one or more of the following methods: amniocentesis, chorionic villous sampling, foetal blood sampling, foetal tissue biopsy, and embryo studies.[23] Samples from these procedures can be studied using techniques in biochemistry, molecular biology, or histopathology.

a) Amniocentesis

For the past two decades, mid-trimester amniocentesis has been the mainstay of prenatal diagnosis for IMDs. Amniotic fluid samples can be examined for accumulated metabolites such as 17α–hydroxyprogesterone in congenital adrenal hyperplasia, methylmalonic acid in methylmalonic aciduria, and more recently, 4-hydroxybutyric acid in succinic semialdehyde dehydrogenase deficiency (4-hydroxybutyric aciduria).[24] However, most diagnoses are made on cultured amniotic cells, if the enzymes express in this medium. Nearly 100 enzymatic defects can now be diagnosed by this means. Problems with this technol-

ogy include failure to establish a culture and the contamination of cultures by maternal cells. Special precautions must be taken to overcome these problems.[25] Molecular and morphological studies can also be conducted in this tissue.

b) Chorionic villous sampling
Compared with amniocentesis, chorionic villous sampling is associated with a higher miscarriage rate. This procedure, however, offers the advantage of diagnosis at an earlier stage of pregnancy, at around 8 to 11 weeks of gestation. This means much less stress for the parents, and more time for the laboratory to confirm the diagnosis.

c) Foetal blood sampling
For failed diagnosis by amniocentesis or delayed referrals, foetal blood sampling is a final approach whereby biochemical and molecular studies can be performed for some IMDs. Usually conducted after 18 weeks of gestation, this procedure requires expertise and patience. It also has a higher miscarriage rate than does amniocentesis.

d) Foetal biopsies
For enzymes that do not normally express in the aforementioned samples, organ biopsies may have to be resorted to for diagnosis. For example, in Type 1a glycogen storage disease, or Von Gierke's disease, deficiency of glucose-6-phosphatase has to be demonstrated in foetal liver samples. Similarly, demonstration of enzyme deficiency in foetal liver has been employed for the diagnosis of ornithine transcarbamylase deficiency. Since this procedure carries a much higher miscarriage rate than do other methods, an alternate diagnostic approach, such as molecular studies on other tissues, should be considered.

Gene therapy

When PND is not feasible, due to the unavailability of a test, or parental objection due to social or religious reasons, the option of carrying on with the pregnancy is a valid one. When a neonate has a congenital disease, the only option left is to manage it as well as possible. Besides conventional therapy, as mentioned in a related article in this seminar (Low), gene therapy will soon become a possibility. In general, most people agree that for ethical reasons, gene therapy should be considered for somatic cells only and not for gonadal cells. This approach fits well with our scenario of treatment for newborns with an IMD.

There are basically three approaches to the correction of genetic defects: replacement of the defective gene by a normal one; correction of the specific genetic defect (site–directed mutagenesis); or gene augmentation, which refers to the introduction of a normal gene without the removal of the defective one. While all these methods are applicable to recessively inherited disorders, gene augmentation cannot be used in dominantly inherited diseases, since the aberrant proteins produced by the remaining defective gene can still cause pathology. In terms of technology, three methods of gene therapy are available: the introduction of genes by physical methods; via viral vectors such as a retrovirus or adenovirus; or via non-viral vectors.[26]

Theoretically, metabolic disorders are good candidates for gene therapy, since they will probably not require strict gene regulation.[27] For example, one only needs to have 10% to 20% of normal factor IX to function without manifestations of haemophilia B, which can theoretically be treated by introducing the normal gene into an ectopic tissue. However, for other IMDs, gene therapy may be more complicated. For example, in phenylketonuria, hepatic-specific cofactors are required for phenyalanine hydroxylase activity. Hence, for gene therapy of any IMD, one has to consider the basic genetic defect, whether there is any need for cofactors, and the nature of the target tissue.

Lysosomal storage disorders represent a special category of IMD for which much progress has been made in their treatment. Allogenic bone marrow transplantation and enzyme infusion have been offered for treatment of diseases such as mucopolysaccharidosis type I, II, VI, Gaucher's type I disease, Krabbe's disease, Niemann-Pick A disease, metachromatic leukodystrophy, and Fabry's disease. For gene therapy, extensive experimentation is in progress for Gaucher's disease and Sly's syndrome.[28]

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