Laboratory investigations of inherited metabolic diseases

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More than 3500 inherited metabolic diseases are known to exist and their rates of morbidity and mortality vary enormously. Identification of these patients, usually neonates or young children, is important for appropriate treatment, prognosis, and genetic counselling. Patients suspected of having an inherited metabolic disease should be investigated for abnormal elevation of metabolites in the urine and blood. Laboratory techniques for the determination of endogenous substances in body fluids are remarkably diversified, ranging from simple chemical tests to sophisticated chromatographic analysis coupled with information-rich detectors. Definite diagnosis of most disorders requires identification of the deficient enzyme or aberrant transport proteins. Analysis may be carried out directly on urine, plasma, or blood cells, or in cultured fibroblasts or lymphocytes requiring cell culture facilities. For many inherited metabolic diseases, recombinant DNA technology has been proven reliable in the detection of affected patients and disease carriers. DNA analysis has also elucidated the molecular genetics and pattern of inheritance involved.

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Introduction

Inherited metabolic diseases (IMDs) are a group of genetically determined disorders involving abnormalities in trans-membrane transport of specific metabolites or in metabolic enzymes of carbohydrate, amino acid, nucleic acid, or lipid metabolism.[1] More than 3500 IMDs have now been identified and they vary considerably in morbidity and mortality.[2] Some are relatively harmless, such as cystinuria and pentosuria, and patients can lead a virtually normal life. Others, including glucose-6-phosphate dehydrogenase deficiency (G6PD), require avoidance of some extrinsic agents. With some IMDs, severe handicap and premature death can be avoided if the diagnosis is made early and the patient treated appropriately. Examples are phenylketonuria (PKU) and maple syrup urine disease (MSUD). In more severe diseases, including most lysosomal enzyme disorders, permanent neurometabolic damage or early fatality cannot be prevented.[2]

Identification of an IMD is based on a wide spectrum of information. Usually, family history and clinical presentation at disease onset give useful indications.[3] Identification of accumulated or missing metabolites may provide the diagnosis, although direct analysis of enzymes and proteins in blood, fresh tissue specimens, or cultured cells is needed to confirm the exact biochemical problem.[1] Since IMDs are predominantly monogenic disorders, recombinant DNA techniques have been used to detect gene defects and obtain information on the molecular genetics and inheritance patterns.[4]

An investigation protocol devised by Chalmers and Lawson in 1982 was able detect up to two-thirds of the then known IMDs involving an enzyme defect, including amino acidopathies, organic acidurias, and hyperammonaemias.[5] This involves a progressive and systematic approach in the investigation of infants with unexplained acute illness. First-line tests include radiology, heaematology, microbiology, and CSF

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examination.These are aimed at excluding trauma, infection, and acquired metabolic derangements. First-line biochemistry tests including urinary sugars and ketones, blood pH, blood gases and glucose, plasma electrolytes, and renal and liver function tests. These are used to establish the presence of hypoglycaemia, ketosis, or metabolic acidosis. Second-line biochemistry tests include determination of blood ammonia and lactate, and the qualitative analysis of plasma and urinary amino acids and sugars. The third-line biochemistry tests are quantitative plasma amino acid analysis and urine organic acid analysis. The final biochemical procedures are confirmatory tests such as specific loading tests and enzymology. This protocol can be adopted fully or in part, according to the availability of laboratory personnel and facilities. It also provides a guideline for the acquisition of appropriate techniques and equipment. This approach to laboratory investigations is now standard procedure for IMD investigations.[6]

In the past two decades, developments in automated biochemistry analysers, chromatographic equipment, and recombinant DNA technology have provided invaluable tools for the investigation of IMD. In this paper, essential laboratory tests and recent advances in laboratory technology for the investigaton of IMDs will be described. A recommended investigation protocol is shown in Figure 1. It should commence with documentation of clinical features and family history, followed by general biochemistry tests to detect metabolic disorders, metabolic screening tests to establish metabolic anomalies, detection of abnormal elevation of metabolites in body fluids, enzymology to confirm deficiencies in metabolic enzymes, and DNA analysis for gene defects.

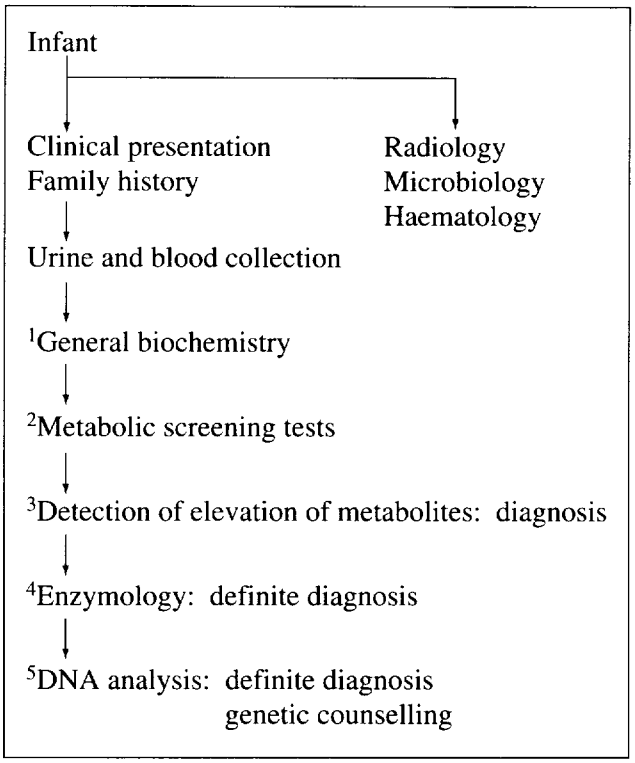

Fig 1. Laboratory investigations of infants suspected of having an inherited metabolic disease. Tests 1 and 2 can be carried out in most biochemistry laboratories while 3 to 5 require more sophisticated equipment, facilities, and expertise.

Clinical presentation

Most neonates with an IMD are born near- or full-term with no specific abnormal features.[7] Symptoms usually develop during the first week of life, although some mild forms of IMD and progressive neuromuscular dystrophies become evident as the child grows. At disease onset, clinical features indicative of IMD include: failure to thrive, seizures, convulsions, hypotonia, persistent vomiting, drowsiness, lethargy, peculiar odour, fragile connective tissues, muscle weakness, hepatosplenomegaly, and adverse reaction to feeding.[3,7] Patients with one or more of these symptoms should have appropiate radiology, haematology, and microbiology tests carried out to identify possible non-metabolic causes of the symptoms such as trauma, infection, and major developmental anomalies. Patients with IMD may be prone to infections and sepsis is not uncommon in neonates with a metabolic defect. Any symptom that is associated with feeding should be recognised, such as the effect of fruit juice in infants with hereditary fructose intolerance.

For further investigations, a blood specimen and a spot urine specimen should be collected as soon as possible for immediate biochemical analysis. Ideally, these specimens should be collected during the acute phase of the illness.

General biochemistry

Blood gases, pH, and glucose should be measured immediately. Plasma sodium, potassium, chloride, bicarbonate, calcium, urea, creatinine, total protein, albumin, bilirubin, and alkaline phosphatase should be determined. In metabolic acidosis, the anion gap should be calculated; a gap greater than 20 mmol/L suggests an organic aciduria while a normal gap (range, 6-14 mmol/L),[8] indicates renal tubular acidosis or bicarbonate loss from the gastrointestinal tract. Unexplained hyponatraemia in an infant, particularly one with ambiguous genitalia, is an indication of congenital adre-

Table 1. Metabolic screening tests for the investigation of inherited metabolic diseases

Specimen	Test	Test result	Commonly used methodology
Urine	Creatinine	Quantitative	Jaffe reaction on a chemistry analyser
	Reducing substances	Qualitative	Benedict's reagent in tablets,manual
	Acetoacetate,acetate	Qualitative	Nitroprusside in tablets, manual
	Glycos-aminoglycans	Quantitative	Carbazole sulphuric acid reaction on a chemistry analyser
	Reducing sugars	Qualitative	TLC*
	Amino acids	Qualitative	TLC
Plasma	Amino acids	Qualitative	TLC
	Ammonia	Quantitative	Glutamate dehydrogenase-catalysed amination of 2-oxoglutamate on a chemistry analyser
	Lactate	Quantitative	Lactate dehydrogenase reaction on a chemistry analyser
	Uric acid	Quantitative	Uricase reaction on a chemistry analyser

* TLC thin layer chromatography

nal hyperplasia. Young children with urea cycle disorders may have neurological dysfunction with respiratory alkalosis, those with organic acidurias have hyperventilation with metabolic acidosis. Persistent hypoglycaemia infers a carbohydrate storage disorder or amino acidopathy. Prolonged jaundice is commonly found in infants with α_1-antitrypsin deficiency, galactosaemia, tyrosinaemia type I, Zellweger's syndrome, and cystic fibrosis.[3]

These tests can all be performed rapidly and reliably on automated analysers for blood gas measurement and general biochemistry.

Metablic screening tests

Metabolic screening tests that should be available in all clinical laboratories are listed in Table 1. They require no special equipment or technology. Dry reagents for reducing substances, glucose, and ketones provide a rapid screening method for these substances in urine. The remaining tests use either simple chemical procedures readily adaptable on automated chemistry analysers or thin layer chromatography (TLC). With TLC, no expensive instrumentation is required and TLC protocols can be established for the semi-quantitative analysis of a wide range of substances by optimising the chromatographic parameters and the compositions of the mobile phase and the staining reagent. However, technical competence is required and the interpretation of TLC results needs experience.

Urinary tests

Measurement of urinary creatinine is mainly for the adjustment of urine volume for amino acid or organic acid analysis. The presence of reducing substances in the urine is detected by the Benedict's copper reduction reaction using reagent tablets. If positive, the specimen should be further analysed for reducing sugars. Urinary acetone and aceto-acetate but not β-hydroxybutyrate can be detected by nitroprusside reaction tablets. The simple urinary reaction with 2,4-dinitrophenylhydrazine detects keto-acids on the formation of the chromogenic phenylhydrazones. When ketouria is detected, the urine should be further analysed for its organic acid profile.

Reducing sugars

One-dimensional TLC differentiates at least five reducing sugar residues simultaneously in urine: lactose, galactose, glucose, fructose, and xylose.[9] For glucose, there is the convenient paper test that uses glucose oxidase in a chromogenic reaction.

Qualitative amino acids

Two-dimensional TLC can differentiate about 18 amino acids in both urine and plasma, especially

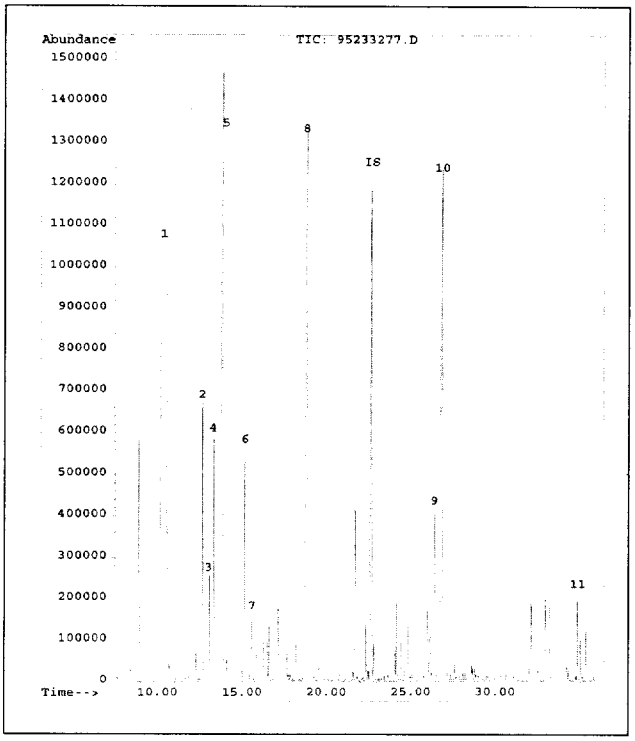

Fig 2. A total ion chromatogram of organic acid analysis of a urine specimen from a patient with multiple carboxylase deficiency. The trimethylsilyl esters of the organic acids were separated on an Ultra-1 column, 25 m, 0.22 mm internal diameter, 100 μ film thickness (Hewlett Packard) and ion masses obtained by negative electronic ionisation in a Hewlett Packard 5890A-5972A GCMS system (Hewlett Packard, Palo Alto, Ca, US). The peaks are: (1) lactic acid, (2) 2-hydroxybutyric acid, (3) 3-hydroxypropionic acid, (4) pyruvic acid, (5) 3-hydroxybutyric acid, (6) acetoacetate, (7) 3-hydroxyisovaleric acid, (8) succinic acid, (9) tiglyglycine, (10) 3-methylcrotonylglycine, (11) 2S,3S-methylcitric acid. The internal standard was 3-phenyl-n-butyric acid. (GCMS conditions and ionic spectra are available on request)

the aromatic and branched-chain amino acids. [10] It is a reliable screening test for phenylketonuria. However, amino acid patterns on TLC plates can be inconclusive and sometimes need follow up by quantitative amino acid analysis.

Urinary glycosaminoglycans

There is some evidence that the frequency of mucopolysaccharidosis (MPS) in Chinese may be higher than it is in Caucasians.[11] This can be screened for by determining total urinary glycosaminoglycans (GAG) using a chromogenic reaction on chemistry analysers. Urine with high total GAG should be analysed for the specific GAG by electrophoresis.

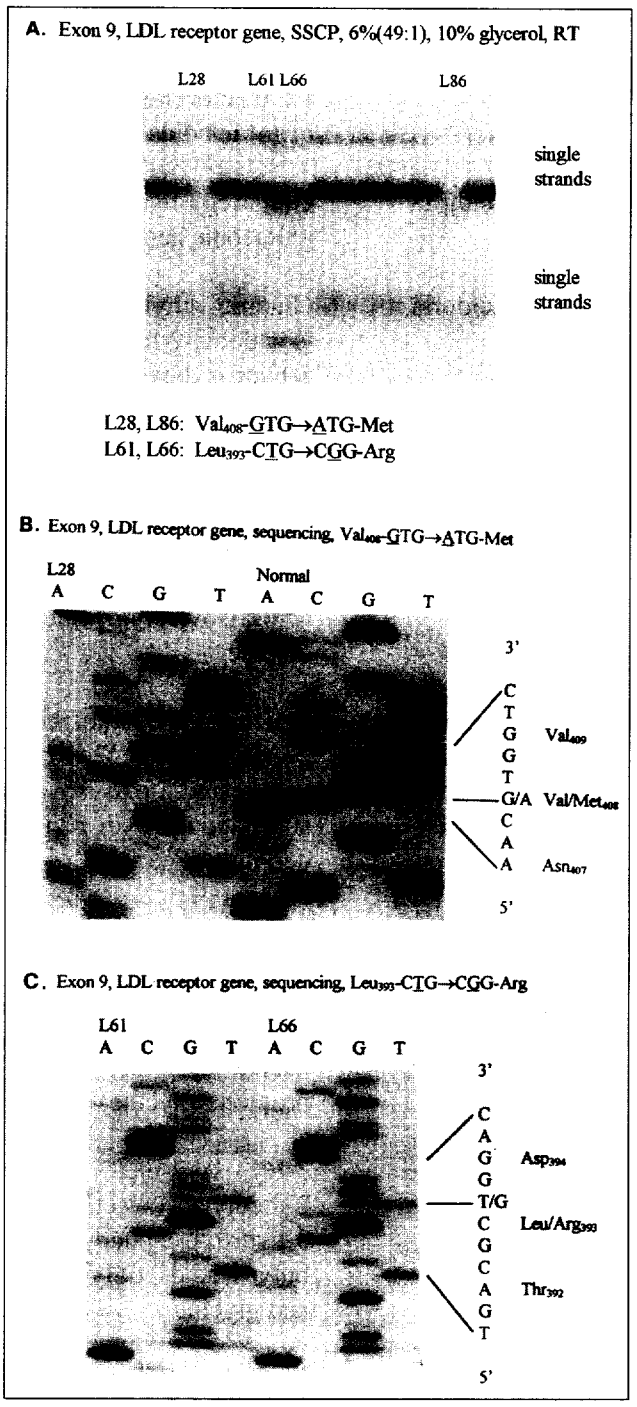

Fig 3. Direct mutation analysis of the low-density-lipoprotein receptor gene in Chinese patients with familial hypercholesterolaemia. Two mutations were identified in exon 9. (A) Detection of base changes in PCR products by single strain conformation polymorphism analysis. (B) Sequence analysis of the G to A change in codon 408 of patients L28 and L86. (C) Sequence analysis of the T to G change in codon 393 of patients L61 and L66. (Details of the PCR, SSCP, and sequencing conditions are available on request)

Table 2. Examples of hereditary organic acidurias that may lead to acute illness in young infants.

Disorder	Characteristeric urinary organic acids or metabolites	Enzyme defect	Reference
Glutaric aciduria type I	Glutaric, 3-hydroxyglutaric and glutaconic acids	Glutaryl-CoA dehydrogenase	51
Glutaric aciduria type II	Glutaric, ethylmalonic, 3-hydroxy-isovaleric, 2-hydroxyglutaric, 5-hydroxyhexanoic, adipic, suberic, sebacic and lactic acids, isovalerylglycine and isobutyrylglycine	Electron transfer flavoprotein (ETF) and ETF-ubiquinone oxidoreductase	52
Isovaleric acidaemia	Isobutyric, 2-methylbutyric, isovaleric and 3-hydroxyisovaleric acids, isovaleryglycine disease	Isovaleryl-CoA dehydrogenase	53
Maple syrup urine	Leucine, isoleucine, valine, 2-oxoisocaproic, 2-oxo-3-methyl-valeric and 2-oxo-isovaleric acids and the hydroxyl acid derivatives	Branched-chain keto acid decarboxylase	54
Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	Adipic, suberic, sebacic, lactic, 3-hydroxybutyrate, acetatacetate, 5-hydroxyhexanoic and dehydrosebacic acids, hexanoylglycine, phenyl-propionylglycine	Medium-chain acyl-CoA dehydrogenase	55
2-Methylaceto acetyl CoA thiolase deficiency	3-Hyroxy-2-methylbutyric, 2-methylacetoacetic and 3-hydroxy-2-phenyl-butyric acids, tiglyglycine and glycine	2-Methylaceto-acetyl CoA thiolase	56
Methylmalonic acidaemia type I	Methylmalonic acid, methylcitric and 3-hydroxypropionic acids	Methylmalonyl-CoA apomutase	57
Multicarboxylase deficiency	Lactic, propionic, 3-hydroxy-propionic, 3-hydroxyisovaleric, 3-hydroxy-n-valeric and methylcitric acids and 3-methylcrotonylglycine	Holocarboxylase synthetase	58
Propionic acidemia	Propionic, 3-hydroxypropionic, methyl citric, 3-hydroxy-n-valeric acids and propionylglycine	Propionyl-CoA carboxylase	59

Plasma ammonia

Hyperammonaemia in infants or young children is often found in Reye's syndrome, liver disease, heart disease, and sepsis.[12] It is detected in all forms of urea cycle disorders and some organic acidurias such as propionic acidaemia, methylmalonic acidaemia, and multiple carboxylase deficiency. Blood ammonia should be determined in infants with acute illness, particularly in those with unexplained neurological deterioration, lethargy, and vomiting. Regardless of cause, hyperammonaemia is a serious condition. Acute hyperammonaemia may result in vomiting and seizures leading to coma and death, while chronic hyperammonaemia is associated with brain damage.[7] For ammonia measurement, the heparinised blood specimen must be transported in ice to the laboratory soon after collection.[13]

Plasma lactate

Lactic acidosis is a common form of metabolic acidosis in infants. It may be caused by tissue hypoxia or hypoperfusion, intoxication, IMD, and systemic diseases such as diabetes mellitus, liver failure, renal failure, or a tumour.[14] Hereditary defects in glycogen metabolism, gluconeogenesis, pyruvate metabolism, the Krebs cycle, and the mitochondrial respiratory chain lead to primary hyperlacticacidaemia. Secondary hyperlacticacidaemia is often associated with certain organic acidurias, fatty acid oxidation defects, and urea cycle disorders.[7] For lactate measurement, the plasma must be obtained as quickly as possible from blood specimens in a heparin tube containing chilled protein precipitants such as perchloric acid.[14]

Plasma uric acid

Three inherited enzyme defects are associated with gross hyperuricaemia during early childhood or adolescence. In glucose-6-phosphatase deficiency (glycogen storage disease type I), hyperuricaemia is due to the excessive production and impaired excretion of uric acid.[15] Over-production of purine nucleotides and uric acid occurs with a severe deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT), which leads to Lesch-Nyhan syndrome, and in phosphoribosyl pyrophosphate synthetase (PPRS) superactivity.[16,17] High plasma uric acid and hypoxanthine levels and high urinary output of uric acid in childhood are diagnostic for these two inherited purine disorders, although the two have to be differentiated by clinical features and enzyme assays.

Detection of elevation of metabolites

The identification and quantitation of metabolites of intermediary metabolic pathways give important diagnostic clues and sometimes confirmation of an IMD. The methods of determination, however, often require sophisticated equipment and technical expertise that are available in only some clinical laboratories in Hong Kong.

Amino acid analysis

An amino acid disorder should be identified as soon as possible to avoid permanent damage, especially neurological impairment. Most amino acidopathies can be detected by the quantitative analysis of free amino acid residues in plasma or urine.[18] Examples include phenylalanine for phenylketonuria, tyrosine for tyrosinaemia, branch-chain amino acids (valine, leucine, and isoleucine) for maple syrup urine disease, and homocysteine for homocysteinaemia.

Reversed-phase high performance liquid chromatography (RP-HPLC) is commonly used for the quantatitive analysis of amino acid profiles in clinical laboratories.[19] When equipped with an autosampler-injector and a high-capacity computer, HPLC can be rapidly manipulated for complex solvent programming and chromatographic data analysis. For consistent performance and to save time, it should be programmed for automated derivatisation of the amino acid residues, which is possible for the two commonly used reagents of o-pthalaldehyde and 9-fluorenylmethyl chloroformate. High-quality HPLC columns of a homogenous monolayer coating of octadecyl-hydrocarbon on fully porous and spherical silica or polyvinyl resin less than 5 μ in size ensure high efficiency and reproducibility of separation.[20] To detect amino acid disorders in mentally retarded patients, we use a binary solvent system on a complex gradient elution programme to analyse 28 free plasma amino acids simultaneously, using an automated procedure for o-pthalaldehyde/β-mercaptoethanol derivatisation of the amino acid residues.[21]

However, HPLC requires skilled technical personnel for its operation and maintenance. If the number of specimens for amino acid analysis is large, for instance more than 15 per day, then using a dedicated automated amino acid analyser saves manpower and ensures consistent performance.

Organic acid analysis

The accumulation of organic acids in physiological fluids, especially blood and urine, is associated with many disorders in the metabolism of amino acids, carbohydrates, and lipids (Table 2). There are hundreds of such organic acids, which can be aliphatic, aromatic, dicarboxyclic, oxo-, keto-, poly-hydroxyl, and their acylglycines.

Table 3. Direct DNA diagnosis of some inherited metabolic diseases

Disorder	Defect	Methodology
α_1-Antitrypsin deficiency	α_1-Antitrypsin, ZZ alleles	PCR,* restriction analysis
Cystic fibrosis	Cystic fibrosis transmembrane conductance regulator	PCR, ASO†
Duchenne's muscular dystrophy	Dystrophin	Multiplex PCR, Southern blotting
Familial hypercholesterolaemia	Low-density lipoprotein receptor	PCR, gene sequencing
Fragile X syndrome	FMR-1 gene	PCR, Southern blotting
Gaucher's disease	Glucose-6-phosphatase	PCR, ASO
Huntington's disease	HD gene	PCR, Southern blotting
Lesch-Nyhan syndrome	Hypoxanthine-guanine phosphoribosyl transferase	PCR, gene sequencing
Phenylketonuria	Phenylalanine hydroxylase	PCR, ASO
Tay-Sachs disease	β-D-N-acetylhexosaminidase A	PCR, ASO

* PCR polymerase chain reaction
† ASO allele specific oligonucleotide

Gas chromatography-mass fragmentography (GCMS) is now the technique-of-choice for the identification and quantitation of organic acids.[6,22] Open, tubular, fused-silica capillary columns longer than 10 m and less than 0.3 mm internal diameter are used, giving more than 10 000 theoretical plates with helium as the carrier gas. The stationary phase can be 100% methyl silicon or have up to 50% phenyl silicon.[23,24] Electronic ionisation is reliable and reproducible in modern bench-top quadruple mass spectrometers for the collection of ion mass spectra. Analytes are identified by mass spectra, instead of being recognised only by retention time, as in conventional gas chromatography (GC). Advanced computerisation also enables effective control of autoinjection and chromatographic parameters, and efficient collection of ionisation data for mass analysis. Figure 2 shows the GCMS analysis of a patient with multicarboxylase deficiency.[25]

Gas chromatography-mass fragmentography can also carry out quantitative analysis of individual organic acid residues, although the protocol requires more detailed optimisation.

Information-rich detection systems

Improvements in computer and interface systems have led to the development of information-rich detector systems for use in clinical laboratories, especially in mass spectrometry.[26] Tandem mass spectrometry (MS-MS) provides a powerful methodology for the separation and identification of long-chain carboxylic acids in physiological fluids and tissue homogenates, including all types of saturated, unsaturated, branched-chain, hydroxy, epoxy, and postanoid compounds; and complex esters such as glycerolipids, glycerophospholipids, ceramides, and sphingomyelins.[27]

High performance liquid chromatography can be coupled to mass spectometry (LCMS) by different interface systems to identify analytes not detectable by conventional HPLC or GC systems. Atmospheric pressure chemical ionisation mass spectrometry (LC-APCI-MS) has been used to analyse N-acetylasparagine, N-acetylglutamine, and their acids.[28] Thirteen bile acids in serum and bile can be quantified simultaneously by HPLC-electrospray mass spectrometry analysis,[29] while conventional HPLC can detect up to 10 conjugated bile acids in bile, but with much lower sensitivity.[30] HPLC-thermospray mass spectrometry is capable of analysing w-carboxyleukotriene B4 and w-hydroxyleukotriene B4 in colon homogenate.[31] Nuclear magnetic resonance

(NMR) spectroscopy is a powerful tool for urine analysis.[32] The more recently developed capillary electrophoresis-mass spectrometry (CEMS) system has immense potential for the investigation of abnormal metabolites.[33]

Such sensitive equipment is understandably expensive, costing millions of Hong Kong dollars and requiring an isolated laboratory with high standards of ventilation, temperature control, and organisation; operation and maintenance costs are also expensive. There is also the need for specially trained technical and scientific personnel, which can take years of intensive work to develop the necessary expertise. Most metabolites known to have clinical relevance can be analysed by conventional GC or GCMS techniques, with the exception of substances at extremely low concentrations. Gas chromatography-tandem MS is required for the analysis of 3-hydoxyisovalerylcarnitine[34] and tandem MS provides rapid measurement of acylcarnitine metabolites.[35] Most urinary acylcarnitines, however, can be analysed by standard GC[36] and GCMS systems.[37]

Deficiency in metabolic enzymes

A confirmatory diagnosis of most IMDs requires identification of the defective enzyme.[1] For the detection of galactosaemia, there is a commercially available assay kit that asesses galactose-6-phosphate uridyl transferase activity in erthrocytes.[38] The assay protocol to detect deficiency of arylsulphatase A activity in metachromatic leukodystrophy is relatively easy, with p-nitrocatecol sulphate as the enzyme substrate.[39] Recently a spectrophotometric assay has been established for porphobilinogen deaminase to detect acute intermittent porphria.[40] For most of the other thousands of enzymes associated with IMD, establishment of an assay protocol requires laborious optimisation, even though some enzyme assays are facilited by HPLC.[41] Very often, substrates and standards are not commercially available. Strong personnel commitment and financial backing are required, so only a small number of selected enzyme assays can be established in a local clinical laboratory in Hong Kong. In our recent analysis of mucopolysaccharidosis, we sent blood cells to reference laboratories abroad for assays of the leukocyte and lymphocyte enzymes.[12] This arrangement will probably continue in the future.

Direct DNA analysis

The advent of polymerase chain reaction (PCR) and cyclic DNA sequencing techniques has led to the establishment of rapid and reliable recombinant DNA protocols for direct DNA analysis.[42] Some IMDs for which such protocols have been established are listed in Table 3. One prerequisite of a DNA diagnostic method is that there is a predominant segregation of a detectable mutation. For example, the 3-base pair deletion leading to a loss in phenylalanine in codon 508 in cystic fibrosis (CF) has a frequency as high as 75% among Caucasian CF patients.[43] Short deletions of the dystrophin gene account for about 60% of cases of Duchenne's muscular dystrophy and they can be detected by multiplex PCR.[44] Another example is fragile X syndrome, which is caused by the expansion of CGG trinucleotide repeats in the FMR-gene.[45]

None of the diseases in Table 3 are known to be of high frequency in Hong Kong. Fragile X syndrome, the most common inherited cause of mental retardation in the West, with an incidence among males of approximately 1:1250 and females 1:2500, has appeared in fewer than 20 patients in Hong Kong since 1986, based on cytogenetic analysis (ST Lam, personal communication). Although direct DNA analysis, which can detect both affected patients and carriers, is well-established, it does not seem to be in high demand in Hong Kong. Familial hypercholesterolaemia (FH) is the most common genetic disease in lipid disorders, with a carrier rate of 1:500. More than 150 mutations have been detected in the low-density lipoprotein receptor (LDLR) gene that causes FH and they occur in the promoter and all the 18 exons.[46] Our study of the LDLR gene revealed three possible common mutations in local Chinese FH patients.[47] Still, together they only account for a mere 16.7% of all the known LDLR gene mutations. In clinical laboratories, DNA diagnostic tests for genetic diseases having heterogeneous and scattering mutations such as FH require a reliable screening methodology. Single strand conformation polymorphism (SSCP) is commonly used for this purpose [48]

Although DNA analysis requires special laboratories and personnel and is therefore costly, the detection of gene mutations does not merely serve diagnostic functions. It also provides a means to detect disease carriers. Genotypic features are essential to understanding the pattern of inheritance and are thus vital for genetic counselling. In Hong Kong, genotypic features of most IMDs in the local population are still largely unknown and possible common mutations in individual diseases remain to be established. There is an urgent need to establish recombinant DNA techniques in more clinical laboratories for the investigation of IMDs.

Conclusion

Investigation of IMD demands extensive laboratory support, because of the great variety of enzymes and metabolites involved. The flow-chart in Figure 1 illustrates the approach for laboratory investigation. It also shows the importance of basic biochemistry and clinical skills. Simple chemistry and chromatographic tests plus an accurate and logical interpretation of biochemical and clinical data often prove to be adequate for making a diagnosis. The investigation of some inherited disorders remains difficult, even with advanced technology. For example, disorders of mitochondrial long-chain fatty acid oxidation are difficult to diagnose correctly[49] while a complex and lengthy process is required for the diagnosis of peroxisomal disorders.[50] Research on the methods of investigation of IMD continues.

The techniques, facilities, instrumentation, and personnel required for all the tests mentioned in Figure 1 are available in Hong Kong. Most clinical laboratories should be able to carry out the metabolic screening tests described in Table 1. A few reference laboratories should provide quantitative analysis of metabolites in urine and blood. Perhaps one or two laboratories should undertake the enzymological and DNA techniques.

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