BRAINCHILD
The Official Publication of HKCNDP

The Hong Kong Society of Child Neurology and Developmental Paediatrics
香港兒童腦科及體智發展學會

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SPECIAL ISSUE ON SPECIFIC LEARNING DISABILITIES

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The Hong Kong Society of Child Neurology & Developmental Paediatrics

BRAINCHILD – MAY 2009 ISSUE: Editor's Note

Mental Health Services for Children in Hong Kong

Chok-wan CHAN

The current issue of Brainchild is devoted to child neurology and consists of the keynote lecture on "Approach to Childhood-Onset Muscle Cramps, Exercise Intolerance and Recurrent Myoglobinuria" by Professor Ingrid Tein from Division of Neurology, Department of Pediatrics, The Hospital for Sick Children and Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada. She was the Course Director for the 2008 Annual Scientific Meeting of our Society. The paper outlines strategic approach to the clinical condition and provides clinician practical clues to tackle the problem systematically. The paper on "High prevalence rate of Wilson disease in Hong Kong: 1 in 5400" is a special feature article based on an excellent presentation at the May 2008 Bimonthly Meeting of our Society at the Kwong Wah Hospital by Professor Ching-Wan Lam of the University of Hong Kong which explores the epidemiology, clinical features and genetics of this metabolic diseases in the Chinese community in Hong Kong. The papers on "A practical approach for the diagnosis of prevalent fatty acid β-oxidation defects presenting with neuromuscular symptoms among southern Chinese". "Genetic Study of CNS Diseases" and "Seating Adaptation for Children with Neuromuscular Diseases" cover different aspects of childhood diseases of the nervous systems including genetics, clinical features and practical management collectively constituting a comprehensive account for the subspecialty of child neurology. I would like to take this opportunity to thank Dr. Catherine Lam. Dr. Eva Fung and Dr. Wu Shun Ping for editing these excellent papers for our readers.

While we are preparing this issue of Brainchild, we are facing outcry from the media and the public for better mental health services for our children in Hong Kong. It is unbelievable that in an affluent community like Hong Kong with GDP amongst one of the best in the world, our children with mental health problems have to wait for up to 36 months before receiving attention and care from the professionals. Just imagine the effect of an untreated child with ADHD on the family, school and community! I hope the government officials have the good heart to share this unpleasant experience with the child, the family and the caring professionals. This is not just a piece of statistics. It is really suffering of all those involved. I always admire the Hong Kong parents for their submissiveness and tolerance in accepting these totally unacceptable provisions. The United Nations Charter for the Rights of the Child 1989 urges all government to provide the best environment and living conditions for our children to grow and develop while the Equal Opportunity Commission of Hong Kong (EOC) promises our children to have best services for their health and not to be deprived of adequate, timely and effective management for their health conditions including mental health. The gist for all these problems are due to *insufficient professionals* (child psychiatrists, clinical psychologists and others), *inadequate resources support, poor manpower planning and lack of forward vision for Hong Kong children's needs*. This indeed is an example of poor government policy on child health and should be reviewed by the Hong Kong Ombudsman for adequacy of service provision by the SAR Government to our children with special needs. Under the pressure of the Hong Kong citizens, the SAR Government as always

starts to convene a group of people trying to study the mental health services including a section for children. Yet all members of the group were cherry-picked by the Government and there is very little involvement of professional bodies such as paediatricians for the child health sections. Paediatricians, being trained on child health and diseases, are the most ideal professionals to take care of our children. The under representation of paediatricians and the lack of knowledge about Hong Kong statistics on childhood mental health are limiting factors for the ultimate success of this group. We paediatricians deem it lamentable for such unwise and unbalanced arrangements by the HKSAR Government. In any case, paediatricians as responsible professionals would like to review our current state of conditions and to share our expertise, knowledge and experience with our Government.

The WHO definition of ***health*** evolves over the past five decades from "a state of free from diseases" to "a state of physical, mental, psychological, spiritual and social well being" and now "an ability to attain one's potential in life". This illustrates the concept of health consequent to the good control of infectious and genetic diseases, effective medical care of pregnancy and child delivery, excellent paediatric care in decreasing birth asphyxia and complication of prematurity as well as improvement of environmental health which we have just started to promote. We are now at a better stage of child survival and can afford to focus on the quality of life. Childhood mental health thus stands out as a subject of concern amongst professionals. The Hong Kong Joint Committee on Child Health formed by the Hong Kong Paedatiric Society, the Department of Health of the Hong Kong SAR Government and the Hospital Authority has as far back as 2001 created a Task Force on Mental Health Services for Children in Hong Kong headed by Dr. Ernest Luk (child psychiatrist) and Dr. William Wong (paediatrician) to study mental health problems of children and adolescents in this locality. Through the dedicated work of the Task Force, two notable products were achieved: "*The Survey on Mental Health Problems for Children in Hong Kong*" (which revealed major problems including Attention Deficit/Hyperactive Disorder (ADHD), Autistic Spectrum Disorders (ASD), Specific Learning Disabilities (SLD), Behavioural Disorders, and others amongst our children with clear statistics pertaining to each condition). The survey disclosed immense problems in the service for these conditions and their possible solutions. Based on these and as a compromise for the time being, the Task Force has recommended a "*Model for Child Mental Heath Services in Hong Kong*". The Model proposed to divide childhood mental health services into four levels (tiers) of care by different professionals (Level I by primary care health professionals, Level II by paediatricians trained in mental health of children including general paediatricians, child neurologists and developmental paediatricians, Level III by psychiatrists, and Level IV by child psychiatrists under hospital care). The model sets a good prototype for further study and alerts all professionals to gear up themselves so as to render the services effective, efficient, seamless and integrative. Active measures are being undertaken to update paediatricians to take up this challenging and yet important aspect in child health. Following this recommendation, the Task Force has trained in the year 2006 twenty paediatricians (from the Department of Health, Hospital Authority and the Private Sectors) via a 60-hour Course on common mental health problems and their basic management. The Course was well conducted with didactic lecture, clinical approach and case discussions. The results are outstanding and forecast promising. We plan to train more paediatricians on the subject in the coming years. This indeed is an innovative and practical approach to the problems. It is not ideal but is effective. It also allows time for the Government to train more local professionals and to consider employing more overseas specialists to fill up the deficiency as a long term plan.

HKCNDP has outstanding results for the advocacy work and management services for Specific Learning Disabilities (SLD)/Dyslexia via our Working Group on SLD. Now we are pleased to witness the universal acceptance of its existence in the Chinese language and primitive availability of primitive measures for accommodation, compensations and remediation as well as resources support at school, family and the community. We are extremely encouraged to witness the "Read and Write Project" generously sponsored by the Hong Kong Jockey Club Foundation which provides significant support to our pioneering work and above all exemplifies official recognition of the good work by all professionals on the subject in Hong Kong.

Encouraged by this success, *the HKCNDP Working Group on ADHD* was established by the Society Council in October 2005 with membership consisting of Professor Patrick Leung (CUHK), Professor Tatia Lee (HKU), Professor Shiu Ling Po (CUHK), Mr. Joseph Lau (Child Assessment Service) and Dr. Stephanie Liu (Child Assessment Service), Dr. Catherine Lam (HKCNDP) and Dr. Chok-wan Chan (HKCNDP). The Group was charged with the terms of reference to equip local professionals in child health with the most up-to-date information and knowledge on the subject so that their work and services can converge well with our child psychiatrists at the tertiary and quaternary levels (service system recommended by Dr. Ernest Luk, Convenor of the Task Force for Mental Health Service for Children in Hong Kong). The Group met three times to discuss practical approach, do mapping of local experience and literature, and set recommendations for management of this disorder in Hong Kong with the ultimate target to formulate a position paper to set directions for future services in our locality. In order to bring cutting-edge information to Hong Kong, the Society organized a series of meetings in Hong Kong. This started with the innovative lecture jointly hosted by our Society together with the reputable organization FOCUS (Focus On Children's Understanding in Schools) on "Advanced Assessment and Treatment of ADHD" by Dr. Thomas Brown Ph. D., Clinical Psychologist from Yale Clinic for Attention and Related Disorders and world authority on the subject, held on 4th October 2005 in Queen Elizabeth Hospital. This successful kick-off was followed by significant series of scientific activities working towards professional solidarity on the subject in Hong Kong.

The Hong Kong Society of Child Neurology and Developmental Paediatrics hosted our 2006 Annual Scientific Meeting (ASM) in November 2006 on ADHD. The Course Director was Professor *Drake Duane MD* of the Institute for Developmental Behaviour Neurology, Arizona State University, Scottsdale, Arizona, USA. Professor Duane is an experienced child neurologist cum developmental paediatrician currently ranked as top world expert in the area of childhood ADHD in private practice which is appropriate and relevant to upgrade local service standard for ADHD in the private sectors and at primary care levels. During the same period, we also hosted our *Joint Meeting on Developmental Paediatrics on ADHD* with invited experts from the Mainland of China (Beijing, Shanghai, Guangzhou, Chengdu and Chongqing), Hong Kong, Macau, Singapore and Taiwan to share experiences for our children with ADHD within the Chinese speaking community. The goal was to study the incidence, morphology, genetics and management of children within our region and to identify any special features in children with ADHD which might be different from our Caucasian counterparts. It is obvious that with all these efforts, we were able to provide optimal management to our children with ADHD in Hong Kong and within our Region which all child health professionals should strive to achieve!

The Monumental Milestone on the excellent work of all professionals in Hong Kong was our ability to successfully convince the Rehabilitation Advisory Council of Hong Kong to include SLD

and ADHD into the Rehabilitation Planning Programme (RPP). Henceforth, SLD, ADHD (and Autistic Spectrum Disorders ASD which is always within RPP) are officially taken as disabilities in Hong Kong. Such recognition enables individuals with ADHD to have access to accommodation, remediation, compensation, and resources provisions at health, medical, education, transport, housing, social (community) and other sectors heavily involved in the care of such individuals.

Encouraged by this success of our work at the RPP as well as urged by our promise to the Hong Kong professionals, we successfully published the Position Paper on SLD in 2006 and on ADHD in 2007. The battle is not completely won as yet because we need persistent and constant effort working with our parent groups, namely the Association for Specific Learning Disabilities (ASLD), the Hong Kong Association for ADHD (HKAA), and the Hong Kong Autism Parents Alliance (HKAPA), the non-governmental organizations (NGO), the legislators (parliamentarians), policy makers, media and the general public to tackle many of the challenges such as public awareness, professional readiness (medical, social and educational interventions), social justice, resources availability and government endorsement, may be given an opportunity for significant breakthrough. Given the multidisciplinary and multi-sectoral nature of management programmes, there is a need for all concerned parties to rapidly arrive at a consensus on the prevention, early identification, effective and timely intervention, and management of potential adverse social outcomes. Organized leadership is needed for developing roadmaps to achieve objectives of different stakeholders, and those of public offices responsible for much of the work and funding. Listing of SLD, ADHD and ASD in the official document will provide an immediate impact on public awareness on the subject, spanning numerous government departments, service providers and interest groups. Official resource support will doubtless provide the much needed incentive for cooperation among key-players.

This is thus a prime opportunity to plan for a coherent and comprehensive schedule for developments in SLD, ADHD and ASD and other mental health problems for children in Hong Kong as outlined in Hong Kong's rehabilitation policy with the view of maximizing life goals and participation of affected individuals. With SLD/ADHD/ASD individuals' innately adequate intelligence and frequent areas of strength as well as the availability of effective interventions, the spirit of Hong Kong's rehabilitation policy can hope to witness full rewards in this population.

Finally do accept our deepest appreciation for the good work of all responsible professionals and key players for child health services in Hong Kong in providing quality management and for striving best welfare and rights for our children with mental health problems. Together we should be able to achieve the noble goal of *Healthy Children for a Healthy World!*

I wish you all reading pleasure and best of health!

Dr. Chok-wan CHAN
Editor-in-Chief, *The Brainchild*
President, The HK Society of Child Neurology & Developmental Paediatrics
25th April 2009

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Approach to Childhood-Onset Muscle Cramps, Exercise Intolerance and Recurrent Myoglobinuria

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I. Introduction

Defects of energy metabolism may cause profound disturbances in the function of muscle and other highly energy-dependent tissues such as brain, nerve, heart, kidney, and bowel. The precise clinical phenotype of exercise intolerance for a specific group of defects can be predicted on the basis of the type, intensity and duration of exercise. In muscle, disorders of glycogen, lipid or mitochondrial metabolism may cause two main clinical syndromes, namely (1) progressive weakness (e.g. acid maltase, debrancher enzyme and brancher enzyme deficiencies among the glycogenoses, long and very-long-chain acyl-CoA dehydrogenase (LCAD, VLCAD) and trifunctional protein/ long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiencies among the fatty acid oxidation (FAO) defects and mitochondrial enzyme deficiencies) or (2) acute, recurrent, reversible muscle dysfunction with exercise intolerance and acute muscle breakdown or myoglobinuria (with or without cramps) (e.g. phosphorylase (PPL), phosphofructokinase (PFK), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGAM) and lactate dehydrogenase (LDH) among the glycogenoses and carnitine palmitoyltransferase II (CPT II) deficiency among the disorders of FAO) or (3) both (e.g. LCAD, VLCAD, short-chain L-3-hydroxyacyl-CoA dehydrogenase [SCHAD], and trifunctional protein/LCHAD deficiencies among the FAO defects as well as multiple mtDNA deletions) (Table 1).

Table 1. Heat, Fever and Myoglobinuria

	Exercise-Induced Myoglobinuria	Malignant Hyperthermia	Malignant Neuroleptic Syndrome	Heat Exhaustion/ Heat Stroke
Myoglobinuria	+	+	+	+
Provoking Factor	Exercise	Halothane	Neuroleptics	Exercise/ Exposure
Tachycardia	+	+	+	+
Acidosis	+	+	+	+
DIC	+	+	+	+
Muscle Rigidity	0	+	+	0
Onset Duration	Minutes	Minutes	Days	Minutes
Familial Attacks	Rare*	Rare	None	None

* Hereditable biochemical abnormality may be identified
• DIC, disseminated intravascular coagulation
Taken with permission from Rowland (1984): Can J Neurol Sci : 11 : 1-13

II. Definition of Clinical Syndrome of Myoglobinuria

As defined by Rowland (1984), myoglobinuria is a clinical syndrome, not just a biochemical state. If the patient is alert, there is myalgia or limb weakness. The colour of the urine is usually brownish, rather than red, and the urine gives positive chemical tests for both albumin and heme (at a concentration of at least 4 μg/ml), but there are few or no red blood cells. Myoglobin is identified by immunochemical methods. The sarcoplasmic enzymes, including serum creatine kinase (CK) is usually more than 100 times normal. Inconstant features include hyperuricemia, hyperphosphatemia and hypo- or hypercalcemia. If there is renal failure, serum levels of potasium and calcium may rise. If the patient is comatose, or if the presenting disorder is one of acute renal failure, there may be no muscle symptoms or signs. The diagnosis can be made if (a) the serum sarcoplasmic enzymes are 100 times normal; and (b) there is renal failure. The main hazards of an attack of myoglobinuria are potentially life-threatening respiratory failure, renal failure, and cardiac arrhythmias.

III. Identification of Myoglobin

In the past, myoglobin was identified by solubility characteristics, spectroscopy or electrophoresis which have now been replaced by immunochemical methods. Antibodies raised against purified myoglobin do not react with albumin, hemoglobin or muscle proteins. Visible pigmenturia occurs at a concentration of approximately 250 μg/ml of myoglobin. The sensitivity of assays for myoglobin in urine include radial immunodiffusion (5.0 μg/ml), peroxidase-sensitive chromogens (orthotolidine) (0.5 μg/ml), hemagglutination inhibition (0.3 μg/ml) and complement fixation (0.15 μg/ml); in serum these include hemagglutination inhibition (5 ng/ml), radioimmunoassay (5 ng/ml) and ELISA (5 ng/ml) (Penn 1986).

IV. Biochemical Classification and Pathogenesis

Etiologies of myoglobinuria can be divided into hereditary and sporadic forms. A comprehensive list of the diverse sporadic etiologies which may precipitate an attack in an otherwise normal individual is given by Rowland (1984). These can be divided into the following groups, namely those related to exertion, crush injury, ischemia, toxins and drugs, metabolic depression or distortion, abnormalities of body temperature, infections, progressive muscle disease and those which appear to be idiopathic. A comparison of myoglobinuria related to exertion, heat stroke, neuroleptic malignant syndrome and malignant hyperthermia is given in Table 1. We will consider only the heritable causes of myoglobinuria here which predispose to recurrent attacks of myoglobinuria under specific precipitating conditions or stressors. It should however be remembered that individual attacks may be quite complex in nature and that there may be more than one cause in each attack for both the hereditary and sporadic forms.

The hereditary forms (Table 2) are particularly important because they are recurrent and suggest underlying pathogenic mechanisms. This may have implications for future treatment strategies and preventative measures in the management of both the heritable and sporadic causes of myoglobinuria. These forms may be divided into three groups based upon whether the biochemical abnormality is (1) known (2) incompletely characterized or (3) unknown. In the first group, there are at least 22 recognized disorders, 7 affecting glycolysis or glycogenolysis, 6 affecting fatty acid oxidation, one involving the pentose phosphate pathway, one involving the purine nucleotide pathway, 6 involving the mitochondrial respiratory chain, and one involving triglyceride and membrane phospholipid biosynthesis. All are autosomal recessive in inheritance with the exception of PGK and G6PD deficiencies which are X-linked. The mitochondrial defects may be either autosomal recessive, dominant or inherited by maternal mitochondrial transmission.

Table 2. Heritable Causes of Exercise Intolerance and Recurrent Myoglobinuria

I. Biochemical Abnormality Known

1. Glycolysis/ Glycogenolysis
   (1) *Phosphorylase (McArdle, 1951)
   (2) Phosphofructokinase (Tarui et al., 1965; Layzer et al., 1967)
   (3) *Phosphoglycerate kinase (DiMauro et al., 1981a)
   (4) *Phosphoglycerate mutase (DiMauro et al., 1981b)
   (5) *Lactate dehydrogenase (Kanno et al., 1980)
   (6) Phosphorylase "b" kinase (Abarbanel et al., 1986)
   (7) Debrancher (Brown, 1986)

2. Fatty Acid Oxidation
   (1) *Carnitine palmitoyltransferase II (DiMauro and DiMauro, 1973)
   (2) Long-chain acyl-CoA dehydrogenase (Stein, 1987)
   (3) Very long-chain acyl-CoA dehydrogenase (Ogilvie et al., 1994)
   (4) Medium-chain acyl-CoA dehydrogenase (Rinaldo et al., 1995)
   (5) *Short-chain L-3-hydroxyacyl-CoA dehydrogenase (Tein et al., 1991)
   (6) *Trifunctional protein/ long-chain L-3-hydroxyacyl-CoA dehydrogenase (Spiekerkoetter et al., 1995)

3. Pentose Phosphate Pathway
   (1) *G6PDH (Bresolin et al., 1989)

4. Purine Nucleotide Cycle
   (1) Myoadenylate deaminase (Ilyser et al., 1989)

5. Respiratory Chain
   (1) *Complex II and aconitase (Haller et al., 1991)
   (2) Coenzyme Q deficiency (Ogasahara et al., 1989)
   (3) *Multiple mitochondrial DNA deletions (Ohno et al., 1991)
   (4) Complex I deficiency (de Lonlay-Debeney et al. 1999)
   (5) Complex III deficiency (cytochrome b) (Andreu et al. 1999)
   (6) Complex IV deficiency (Cytochrome oxidase deficiency) (Keightley et al. 1996)

6. Triglyceride and Membrane Phospholipid Biosynthesis
   (1) *LPIN1 - muscle specific phosphatidic acid phosphatase (Zeharia et al. 2008)

II. Biochemical Abnormality Incompletely Characterized
   (1) *Impaired long chain fatty acid oxidation (Engel et al., 1970)

   (2) *Impaired function of the sarcoplasmic reticulum (?)
   in familial malignant hyperthermia (predisposition
   in central core disease, Duchenne muscular dystrophy,
   Becker muscular dystrophy, myotonic dystrophy,
   myotonia congenita, Schwartz-Jampel syndrome,
   King-Denborough syndrome)

   (3) *Abnormal composition of the sarcolemma in Duchenne and
   Becker muscular dystrophy (Bonilla et al., 1989; Hoffman et al., 1989; Medori et al., 1989)

III. Biochemical Abnormality Unknown
   (1) *Familial recurrent myoglobinuria
   (2) *Repeated attacks in sporadic cases

*Etiologies that have been documented to cause recurrent myoglobinuria beginning in childhood

Modified from Tein I, DiMauro S, Rowland LP (1992) in Rowland LP, DiMauro S (eds): Handbook of Clinical Neurology, Vol. 18 (62): Myopathies pp 553-593 with permission of Elsevier Science Publishers, Amsterdam, The Netherlands.

It has been postulated that the mechanism of myoglobinuria in the glycolytic and fatty acid oxidation disorders may be the result of a fall in adenosine-5'-triphosphate (ATP) stores below some critical level needed to maintain the integrity of the muscle surface membrane (Rowland 1984). The fuels generated from these two biochemical pathways are used to replenish high-energy phosphate compounds, such as phosphocreatine and phosphoenolpyruvate, which in turn are used to regenerate ATP (Penn 1986). The dependence of skeletal muscle on different metabolic pathways depends upon the type of muscular activity. Resting muscle is heavily dependent on free fatty acids and fatty acid oxidation (Felig and Wahren 1975).

At rest, glucose utilization accounts for approximately 10-15 % of total oxygen consumption (Wahren 1977). Both slow and fast twitch fibres have similar levels of glycogen content at rest (Essen 1977). In working muscle, the relative utilization of triglycerides and stored glycogen versus free fatty acids and glucose depends upon the type, duration, and intensity of the exercise (Gollnick et al 1974, Essen 1978). In moderate exercise, ATP is first regenerated from high-energy phosphates. This is followed by muscle glycogen for the first 5 to 10 minutes which is indicated by the sharp rise in lactate. It then falls as muscle triglycerides and blood-borne fuels are utilized (Felig and Wahren 1975; Lithell et al 1979). The major fuels after 90 minutes are free fatty acids and glucose. Free fatty acid uptake increases by 70% in mild to moderate prolonged exercise between 1 to 4 hours, and after 4 hours, free fatty acids are used twice as much as carbohydrate sources.

In the glycolytic disorders, the muscle is therefore most vulnerable during the initial stages of intense exercise when carbohydrates constitute the major energy source. The development of a "second wind" phenomenon probably indicates a switch from carbohydrate to fatty acid utilization (Rowland et al 1986). A critical fall in ATP leading to both muscle contracture, failure of maintenance of muscle surface membranes and ultimately necrosis has not yet been proven. A fall in ATP in muscle, that had already entered a state of contracture, could not be demonstrated by biochemical studies of open muscle biopsies of patients with McArdle's disease (Rowland et al 1965) or by nuclear magnetic resonance (NMR) studies of PPL-deficient (Ross et al 1981) or PFK-deficient (Chance et al 1982) muscle. Supportive evidence was provided by experimental animal work (Brumback et al 1983). It was suggested by Rowland et al (1986) that the cramping may be a focal event involving a few fibres only, as supported by the work of Brandt et al (1977). This would make the measurement of total muscle ATP by biopsy or NMR inaccurate. Although a drop in ATP has not been documented, this would still appear to be the most logical mechanism.

Identified defects in fatty acid oxidation (FAO) include carnitine palmitoyltransferase II (CPT II) (DiMauro and DiMauro 1973), long-chain acyl-CoA dehydrogenase (LCAD), very long-chain acyl-CoA dehydrogenase (VLCAD) (Ogilvie et al 1994), short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD) (Tein et al 1991), medium-chain acyl-CoA dehydrogenase (MCAD) (Ruitenbeek et al 1995), and trifunctional protein/long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiencies (Dionisi-Vici et al 1991). In FAO disorders, attacks of myoglobinuria are precipitated after mild to moderate prolonged exercise when fatty acids are the key energy source in exercising muscle. These attacks may be further exacerbated by inadequate caloric intake as in fasting or infection with vomiting, which further limit blood glucose. In fasting, not only are muscle glycogen and blood glucose decreased, but there is defective ketogenesis due to impaired hepatic FAO. Therefore, a drop in ATP may occur resulting in myoglobinuria. Other possible mechanisms relate to the toxicity of elevated free fatty acids arising proximal to the block. Excessive long-chain fatty acids which may accumulate proximal to a block in long-chain fatty acid (LCFA) oxidation may have detergent properties on muscle and mitochondrial membranes leading to the potentiation of free-radical mediated lipid membrane peroxidative injury (Mak et al 1986). Excessive free fatty acids and their metabolites from compensatory omega-oxidation may also inhibit key metabolic pathways (e.g. glucogenesis, ß-oxidation, urea cycle) (Tonsgard and Getz 1985; Tonsgard 1986; Corkey et al 1988) and thus contribute to a further decrease in ATP. Other risk factors include infection, during which metabolic processes preferentially favour FAO, which persists despite glucose administration (Robin et al 1981), thereby increasing the dependence on FAO. Cold exposure may be detrimental as cold stimulates ketogenesis in normal individuals (Johnson et al 1961) and shivering depends upon involuntary muscle activity which is primarily dependent upon LCFA (Bell and Thompson 1979). Emotional tress has also

been a recognized precipitant which theoretically may relate to catecholamine-induced lipolysis increasing the stress on the FAO pathway.

In the respiratory chain disorders, a drop in ATP production could again be postulated as the mechanism. Haller et al (1991) identified a combined complex II-aconitase deficiency in muscle in a Swedish man who was strikingly similar to the large Swedish kindred described by Larsson et al (1964). This kindred had exercise intolerance, dyspnea, palpitations, excessive exertional rise in lactate and pyruvate and recurrent myoglobinuria. Multiple deletions of mitochondrial DNA have been described in two brothers with recurrent exertional myoglobinuria (Ohno et al 1991). Coenzyme Q10 deficiency was described in two sisters with encephalomyopathy and recurrent myoglobinuria (Ogasahara et al 1989).

Myoadenylate deaminase (Hyser et al 1989; Tonin et al 1990) and G6PD deficiencies (Bresolin et al 1989) have not been conclusively linked to a logical theory of causation because both enzymes may be absent in asymptomatic people.

Most recently, recurrent myoglobinuria was described in children who presented between two to seven years of age in the context of febrile illnesses. Deleterious mutations were documented in the LPIN1 gene which encodes the muscle-specific phosphatidic acid phosphatase, a key enzyme in triglyceride and membrane phospholipid biosynthesis (Zeharia et al 2008). Analysis of the phospholipid content disclosed accumulation of phosphatidic acid and lysophospholipids in muscle tissue of the more severe genotype which was speculated to result in myoglobinuria during stress. This disorder appears to be autosomal recessive in inheritance. As one individual who developed statin-induced myopathy was found to be a carrier for a pathogenic mutation in the LPIN1 gene, it was speculated that a carrier state may predispose to statin-induced myopathy.

In the second group, Engel et al (1970) described twin girls with recurrent myoglobinuria whose clinical and biochemical features suggested a defect in long-chain fatty acid oxidation in whom CPT deficiency was excluded (DiMauro and Papadimitriou 1986).

In familial malignant hyperthermia, there appear to be diverse underlying etiologies, however the final triggering event appears to be a sudden increase in sarcoplasmic calcium in response to anaesthetics or stress (Gronert 1980). Among these is an autosomal dominant disorder that maps to chromosome 19q12-13.2 which involves an abnormality of the calcium-release channel in the sarcoplasmic reticulum (SR) or the ryanodine receptor (MacLennan et al 1990). Among the genetic myopathies predisposing to MH, is central core disease which is also autosomal-dominant and found to be strongly linked to MH (Shuaib et al 1987). Further, the gene for central core disease has been mapped to the same position as MH (Kausch et al 1991).

In the Xp21-linked myopathies, potential membrane instability due to the missing dystrophin protein may be etiological in the mechanism for the myoglobinuria. Several patients with Becker's muscular dystrophy have been documented to have exertional myoglobinuria in adolescence (Bonilla et al 1989, Hoffman et al 1989, Medori et al 1989). In Duchenne dystrophy patients, a predisposition to myoglobinuria has been documented in association with anaesthetic agents. This reaction is distinctly different from the classic malignant hyperthermia reaction as in Duchenne patients the heart rate is usually decreased rather than increased and may proceed to cardiac arrest, the temperature may be normal, rigidity is uncommon and dantrolene does not appear to alter the course of the anaesthetic reaction (Karpati and Watters 1980). Given the severe weakness in Duchenne dystrophy, patients may be protected from exertional myoglobinuria by their limited capacity for exercise.

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V. Glycolytic/ Glycogenolytic Defects

Pathophysiology

The hydrolysis of adenosine triphosphate (ATP) provides the immediate source of energy for contraction and relaxation. The largest contribution of energy overall is derived from oxidative phosphorylation, whereas anaerobic glycolysis plays a relatively minor role, primarily limited to conditions of sustained isometric contraction when blood flow and oxygen delivery to exercising muscles are drastically reduced. Aerobic glycolysis is particularly important during the dynamic form of exercise, such as walking or running, therefore the pathophysiology of glycogenoses relates more to the impairment of aerobic than anaerobic glycolysis (Lewis and Haller 1986, Lewis et al 1991).

A useful test for the detection of enzymatic defects in the non-lysosomal glycogenolytic pathway and in glycolysis is the 'forearm ischemic exercise test' developed by McArdle (1951) which can be successfully performed in young cooperative children even down to 6 years of age. An indwelling needle is place in a superficial antecubital vein and a basal lactate and ammonia are obtained without stasis. A sphygmomanometer cuff is placed above the elbow and inflated above arterial pressure. The patient is asked to rhythmically squeeze another rolled-up cuff to well above 120 mm Hg for 1–2 minutes of exercise which requires constant encouragement from the observer. This can produce significant discomfort in normal individuals and should be truncated if the patient develops an acute cramp as myonecrosis may occur in an individual with a glycolytic disorder. After one minute of exercise, the cuff around the arm is deflated and blood samples are sequentially obtained at 1,3,5,7,10 and 15 minutes. In normal subjects, there is a 3- to 5-fold increase of lactate in the first 3 minutes and this declines to baseline values by 15 minutes. This is paralleled by a similar increase in ammonia. In individuals with a defect in glycolysis/glycogenolysis, there is an insufficient rise in lactate (less than 2-fold) and there will be a compensatory increase in ammonia, which also serves to indicate sufficient effort on the part of the individual. An insufficient lactate rise has been demonstrated in PPL, debrancher, PFK, PGK, PGAM and LDH deficiencies, but not in acid maltase or phosphorylase b kinase deficiency. A major limitation of the test is that the rise of venous lactate in patients not having a defect in this pathway depends on the patient's ability and willingness to exercise.

As previously mentioned, the energy substrates used by muscle for aerobic metabolism depends upon the type, intensity and duration of exercise as well as on physical conditioning and diet. During intense exercise (close to one's maximal oxygen uptake or V02max in dynamic exercise or maximal force generation in isometric exercise), energy is derived from anaerobic glycolysis, particularly when there is a 'burst' of activity with rapid acceleration to maximal exercise (DiMauro and Tsujino 1994). At low intensity exercise (below 50 % V02max), blood glucose and free fatty acids are the primary source of energy, whereas at higher intensities the proportion of energy derived from carbohydrate oxidation increases, and glycogen becomes an important fuel. At 70 to 80 % of V02max, aerobic metabolism of glycogen is the critical energy source, and fatigue occurs when glycogen stores are exhausted (DiMauro and Tsujino 1994). Therefore, the initial stages of intense exercise would be the time of greatest vulnerability for individuals with defective glycolysis/glycogenolysis. Patients note that they have to rest soon after the beginning of exercise due to muscle cramps, but if they continue to exercise at low intensity, they are then able to continue for a longer time which is known as the 'second-wind' phenomenon. This has been attributed to a metabolic switch from carbohydrate to fatty acid utilization (Felig and Wahren 1975) and by increased circulation (Haller et al 1985) which is an important clinical clue on history taking.

Phosphorylase deficiency

Clinical features

Myophosphorylase deficiency (type V glycogenosis or McArdle's disease) is a rare disease but an important cause of exercise intolerance and recurrent myoglobinuria. The key clinical manifestation is exercise intolerance. The two primary precipitants are brief isometric contraction (e.g. lifting heavy objects) and less intense but sustained dynamic exercise (e.g. climbing stairs). In approximately 50 % of these patients, there are episodes of muscle necrosis and myoglobinuria after exercise, 27 % of whom develop acute renal failure. Following the episode of myoglobinuria, there is usually complete functional recovery. However, fixed mild proximal greater than distal weakness is seen in about one-third of typical cases, more commonly in older patients (DiMauro & Bresolin 1986). Careful history taking shows that exercise intolerance generally starts in childhood but overt episodes of muscle cramping and myoglobinuria usually develop later with the diagnosis being made in the second or third decade of life. There may be marked variation in the severity of symptoms; in some, progressive weakness begins late in life (sixth decade) with no history of cramps or pigmenturia (DiMauro and Bresolin 1986, Pourmand et al 1983). In 4 reported childhood cases there was severe generalized muscle and respiratory weakness at or soon after birth and death in infancy (DiMauro and Hartlage 1978, Milstein et al 1989).

Laboratory data

Between episodes of myoglobinuria, the serum CK is variably increased in 93% of cases which contrasts with CPT II deficiency in which the resting CK is generally normal. EMG between episodes of myoglobinuria demonstrates fibrillations, myotonic discharges and positive waves in up to 50 % of patients, suggesting a mild myopathy (DiMauro & Tsujino 1994). ^{31}P-NMR spectroscopy demonstrates lack of cytoplasmic acidification during aerobic or ischemic exercise as well as a greater-than-normal drop of the PCr/Pi ratio (Argov and Bank 1991, Ross et al 1981).

Inheritance

There is a marked male predominance, despite the autosomal recessive pattern of inheritance. The gene has been localized to chromosome 11 (Lebo et al 1984). Two families with apparent autosomal dominant transmission (Chui and Munsat, 1976; Schimrigk et al., 1967) may be explained on the basis of subsequent generations of homozygotes and manifesting heterozygotes, in whom the residual activity is below a critical threshold (Papadimitriou et al., 1990; Schmidt et al., 1987). In a third family, Tsujino et al (1993a) demonstrated that the affected mother was a compound heterozygote carrying two different point mutations in the PPL gene, the unaffected father was heterozygous for a third point mutation and the three affected children were compound heterozygotes.

Muscle biopsy

On Periodic acid-Schiff stain, there may or may not be focal accumulations of glycogen in the subsarcolemmal regions and between myofibrils. The phosphorylase (PPL) stain (Takeuchi and Kuriaki, 1955) will show no staining in muscle fibres, though smooth muscle in the walls of intramuscular vessels will stain normally. DiMauro and Tsujino (1994) point out that a positive histochemical reaction may be seen in McArdle's under two conditions: (1) when there is residual enzyme activity; (2) when there are regenerating fibres. "False-positive" reaction in regenerating fibres is due to expression of a different

isoenzyme in immature muscle cells (DiMauro et al. 1978; Sato et al. 1977). Electron microscopy shows accumulation of normal-looking glycogen ß particles under the sarcolemma and between myofibrils and myofilaments (DiMauro and Tsujino 1994).

Biochemical considerations

Muscle PPL exists as an active phosphorylated alpha form and a less active, dephosphorylated beta form. PPL activity is undetectable in most or up to 10 % of normal residual activity in the muscle of affected patients. Glycogen accumulation is moderate (about two-fold) or may be normal and is normal in structure. The majority of patients have no immunologically detectable enzyme protein in muscle by SDS-PAGE, immunoblot and enzyme-linked immunosorbent assay (ELISA) (Servidei et al 1988b, McConchie et al 1991). Normal mature human muscle has a single phosphorylase isoenzyme whereas cardiac muscle and brain have three different isoenzymes. Lack of the muscle isoenzyme should cause partial defects in the heart and brain.

Molecular genetics

The three PPL isoenzyme genes have been cloned, sequenced and localized to different chromosomes. Tsujino et al (1993a) identified three point different point mutations: a C-to-G mutation in codon 49 of exon 1 (converting an arginine to a stop codon), a G-to- A in codon 204 of exon 5 (converting a glycine to a serine), and an A-to-C in codon 542 of exon 14 (converting a lysine to a threonine). In an analysis of 32 patients, 15 were homozygous for the first mutation and 12 were compound heterozygotes (Tsujino et al 1993).

Therapy

Most therapeutic trials have attempted to bypass the metabolic block by providing the muscle with glycolytic substrates (DiMauro and Tsujino 1994). Efforts to raise blood glucose by oral administration of glucose or fructose has had inconsistent results and caused weight gain. Glucagon injections were impractical and had inconsistent results. Raising the serum free fatty acid concentration through the use of fat emulsions, administration of norepinephrine or heparin and fasting, increased exercise tolerance. However more practical regimens such as a high fat-low carbohydrate diet were not effective (DiMauro and Bresolin 1986). Another strategy was to supply branched-chain amino acids which are taken up rather than released during exercise by McArdle muscle (Wahren et al 1973). However this resulted in an impairment rather than an improvement of bicycle exercise capacity in five of six patients, possibly due to a lowering of free fatty acids by the amino acids (MacLean et al. 1998). Slonim and Goans (1985) instituted a high-protein diet in a patient with weakness and demonstrated an improvement of muscle endurance and strength. Vitamin B6 is another potential therapeutic aid, as overall body stores of pyridoxal phosphate are depleted in McArdle's disease due to the lack of enzyme protein to which pyridoxal phosphate is bound (Haller et al. 1983). One patient has been shown to have a beneficial effect with vitamin B6 supplementation (Phoenix et al. 1998) but further studies need to be done. In addition, oral creatine monohydrate supplementation in a placebo-controlled cross-over trial involving nine patients was shown to alleviate symptoms and increase their capacity for ischemic, isometric forearm exercise (Vorgerd et al. 2000). Another strategy has been the ingestion of sucrose prior to exercise to increase the availability of glucose (Vissing and Haller, 2003). In a single-blind randomized, placebo-controlled crossover study, 12 patients were studied. Ingestion of sucrose prior to exercise improved exercise tolerance and sense of well-being. Finally, aerobic training of four McArdle's patients improved peak cycle exercise capacity, circulatory capacity and oxygen uptake (Haller et al. 1998b).

VI. Fatty Acid Oxidation Disorders

Defects in FAO are an important group of disorders because they are potentially rapidly fatal and a source of major morbidity encompassing a spectrum of clinical disorders including recurrent myoglobinuria, progressive lipid storage myopathy, neuropathy, progressive cardiomyopathy, recurrent hypoglycemic hypoketotic encephalopathy or Reye-like syndrome, seizures, and mental retardation. As all of the known conditions are inherited as autosomal recessive traits, there is frequently a family history of sudden unexpected death (SIDS) in siblings. Early recognition and prompt institution of therapy and appropriate preventative measures, and in certain cases specific therapy, may be life-saving and may significantly decrease long-term morbidity, particularly with respect to central nervous system sequelae. There are at least 21 recognized enzyme defects in FAO, most diagnosed in the last 15 years.

Common clinical and biochemical features of fatty acid oxidation disorders

Hale and Bennett (1992) suggest that there are at least four clinical and laboratory features that should lead the clinician to suspect a genetic defect in FAO. These common features include (1) acute metabolic decompensation in association with fasting; (2) chronic involvement of tissues highly dependent upon efficient FAO (e.g. heart, muscle, liver); (3) recurrent episodes of hypoketotic hypoglycemia; (4) alterations in the quantity of total carnitine or of the percentage of esterified carnitine in plasma and tissue.

(1) Metabolic decompensation in association with fasting.

Children with FAO defects are most prone to decompensation during conditions that place stress on the FAO pathway for fuel generation in the context of depleted glycogen and glucose reserves. These conditions include fasting, prolonged exercise (particularly after one hour of mild to moderate aerobic exercise), infection with vomiting and cold-induced shivering thermogenesis. In cold exposure, ketogenesis is stimulated in normal individuals (Johnson et al 1961) and shivering, which is an involuntary form of muscle activity, also depends on long-chain FAO (Bell and Thompson 1979). Children are most likely to be found comatose in the early-morning hours after an overnight fast. During infection such as a viral illness, there may be an added problem with vomiting and decreased oral intake. Children may also present with a Reye-like syndrome. Infants and younger children are at greater risk during fasting because of their limited fasting adaptation capabilities; prolonged fasting for an infant of less than 1 year of age would be 6 to 10 hours, versus 12 hours for a child between 1 and 4 years of age (Hale and Bennett 1992).

(2) Involvement of fatty-acid oxidation dependent tissues.

Tissues with high energy demands that are therefore dependent upon efficient FAO include skeletal muscle, heart and liver. With deficient hepatic ketogenesis, glucose becomes the only available fuel and therefore becomes rate-limiting under conditions of FAO stress when glycogen and glucose stores have been depleted. As a result, free fatty acids which are liberated during fasting and which cannot be metabolized due to the block in FAO, may be stored in the cytosol as triglycerides, producing a progressive lipid storage myopathy with weakness as well as a hypertrophic and/or dilatative cardiomyopathy and a fatty liver with microvesicular steatosis. Increased content of short- or medium-chain fatty acids and in particular, their dicarboyxlic acid metabolites, from compensatory omega oxidation, may cause secondary metabolic abnormalities including an impairment of glucogenesis, ß-oxidation and the citric acid cycle

(Tonsgard and Getz 1985, Tonsgard 1986, Corkey et al 1988) leading to a further decrease in cellular ATP production. In the long-chain FAO disorders which are frequently characterized by recurrent episodes of acute muscle breakdown or myoglobinuria (e.g. CPT II, LCAD, VLCAD, and trifunctional protein/LCHAD deficiencies), the accumulation of long-chain fatty acids and long-chain acylcarnitine proximal to the FAO block may have detergent-like actions on muscle membranes which likely contribute to the muscle breakdown. Excessive amounts of palmitoyl-CoA and palmitoylcarnitine have been shown to have detergent properties on isolated canine myocytic sarcolemmal membranes and to potentiate free radical induced lipid membrane peroxidative injury in ischemia (Mak et al 1986). Long-chain acylcarnitines also activate calcium channels in cardiac (Inoue and Pappano 1983, Spedding 1985) and in smooth muscle myocytes (Spedding 1985, Spedding and Mir 1987) and thus may potentiate the increase in cytosolic calcium associated with arrhythmogenesis as seen in ischemic myocardium (Lee et al 1987).

(3) Hypoketotic hypoglycemia.

The pattern of hypoketotic hypoglycemia reflects the accelerated rate of glucose utilization that occurs when fatty acids cannot be used as fuels and ketone bodies are not generated to spare glucose/glycogen stores. An increase in the ratio of serum free fatty acids to ketones from the normal ratio of 1:1 to > 2:1, would suggest a block in ß-oxidation.

(4) Alterations in plasma and tissue concentrations of carnitine.

In most cases of intramitochondrial ß-oxidation defects, the total carnitine concentration is decreased (<50 % of normal) and the acylcarnitine fraction is increased (> 50 % esterified; normal = 10-25 % in the fed state and 30-50 % in the fasted state) (Hale and Bennett 1992). In the intramitochondrial ß-oxidation disorders, the excessive acyl-CoA's which accumulate proximal to the block may be converted into acylcarnitines by chain-length specific carnitine acyltransferases (Bremer 1983). In the case of the plasmalemmal carnitine transporter defect, the total carnitine is markedly reduced (e.g. < 5 % of normal) and the esterified fraction is normal (Tein et al 1990a) as the transport defect in kidney leads to a decreased renal threshold for carnitine reabsorption.

(5) Additional laboratory findings.

During acute catabolic crises, a number of other biochemical abnormalities may be noted. During the Reye-like syndrome presentation, a modest hyperammonemia (100-200 µmol/L) may be documented accompanied by 3-5 fold elevations of liver transaminases (Hale and Bennett 1992). In acute myoglobinuria, there are marked increases in the sarcoplasmic enzymes including serum CK which may rise to > 100, 000 U/L (normal < 250 µmol/L). There may also be increased serum concentrations of amino acids (especially taurine), creatinine, potassium, phosphate and urate (Tein et al 1990b). These changes may have deleterious effects on the kidneys and heart, exaggerating the damage (Knochel 1976). Lactic acidosis may also be noted during the acute catabolic presentations which may reflect poor perfusion or inhibition of critical enzymes such as pyruvate carboxylase by accumulated metabolites (Corkey et al 1988). Urine organic acid screening may demonstrate unusual or excessive amounts which may be diagnostic of a specific block in ß-oxidation.

CPT II deficiency

Classic CPT II deficiency is characterized by adolescent onset recurrent episodes of acute myoglobinuria precipitated by prolonged exercise or fasting, in which power between episodes is normal, and in which lipid accumulation in muscle is noted only under conditions of fasting and prolonged exercise (DiMauro and Papadimitriou 1986). Fasting ketogenesis is generally normal in this condition, though it may be delayed, and there is no fixed cardiomyopathy, though arrhythmias may occur secondary to hyperkalemia and hypocalcemia during the acute myoglobinuric crises. Recently several cases of a severe infantile presentation of CPT II deficiency have been described which have included recurrent Reye-like syndrome, hepatomegaly with hypoketotic hypoglycemia and elevated liver aminotransferase, cardiomegaly with cardiac arrhythmias and elevated serum creatine kinase, as well as evidence of lipid storage in heart, skeletal muscle, liver and kidney (Demaugre et al 1991, Hug et al 1991, Elpeleg et al 1993). In these cases the activity of CPT II in cultured skin fibroblasts was < 10 % of control values in contrast to the 25 % residual activity documented in classic CPT II deficiency (Demaugre et al 1991).

Differentiating Laboratory Features

Fatty acid intermediates in the serum or urine of affected children may suggest the site of defect depending upon the chain-length specificity and the species type of the intermediates. These intermediates require specialized biomedical technology which may require referral to specialized metabolic laboratories. Particularly useful are the assessment of plasma total and free carnitine, serum acylcarnitines, urine acylcarnitines and urine acylglycines and organic acids. It should be emphasized that these intermediates may be absent at times when the child is metabolically stable and receiving adequate supplies of glucose, thereby eliminating the stress on the FAO pathway. They are best detected in the serum and urine of children during acute catabolic crises or during times of fasting.

(1) Carnitine.

FAO disorders are associated with a decrease in plasma total carnitine concentration (< 30 μmol/L, normal 40 to 60 μmol/L). The lowest concentration is found in the carnitine uptake defect where concentrations are usually < 5 % of control (Stanley et al 1991). In the intramitochondrial ß-oxidation defects, the plasma total carnitine concentrations vary between 10 % to 50 % of normal. In contrast, the total plasma carnitine concentration may be normal or increased in a child with CPT I deficiency in which the esterification of palmitate to carnitine is defective (Stanley et al 1992). In most fatty acid oxidation defects with the exception of the carnitine uptake and CPT I defects, there is an increase in the ratio of esterified carnitine to total carnitine reflecting the esterification to carnitine of the excessive acyl-CoA's that accumulate proximal to the block in ß-oxidation. Estimates of the amount of acylcarnitines is based on the difference between the free and total carnitine measurements. Under normal conditions, the esterified carnitine is 10 to 25 % of total in the fed state and 30 to 50 % of total in the fasted state (Hale and Bennett 1992). The carnitine esters can be further separated on the basis of the acid insolubility of long-chain acylcarnitine esters (Hale and Bennett 1992). Defects involving long-chain fatty acid oxidation, have increased amounts of long-chain acylcarnitines (Glasgow et al 1983). Further separation and identification of the individual acylcarnitine esters has been facilitated by fast atom bombardment-tandem mass spectrometry and isotopic exchange high-performance liquid chromatography (Millington et al 1992, Kerner and Bieber 1976). Specific serum acylcarnitines have been useful in the diagnosis of certain defects (e.g. octanoylcarnitine in MCAD deficiency) (Millington

1986). They are particularly helpful in the diagnosis of long-chain FAO disorders, because they overcome the problem of the renal threshold effect whereby long-chain acylcarnitines are selectively reabsorbed at the renal carnitine transporter site at the expense of free carnitine, and overcome the problem of poor solubility of long-chain fatty acids in urine.

(2) Dicarboxylic acids (DCA).

Dicarboxylic acids (adipic, suberic, sebacic acids) are found in many identified intramitochondrial ß-oxidation defects (Gregersen et al 1982a). Hale and Bennett (1992) point out that there are several limitations to the value of these compounds in the recognition of FAO defects. (i) These dicarboxylic acids may be seen in children receiving certain formulas containing medium-chain triglycerides or in children who are seriously ill (e.g. diabetic ketoacidosis) (Mortensen and Gregersen 1982) or who are receiving certain medications which interfere with FAO such as valproic acid (Mortensen 1981). It should be underlined that in each of these cases, the amount of ketones exceeds the amount of DCA whereas in the intramitochondrial FAO defects, the amount of DCA equals or exceeds the amount of ketones when the children are fasting.(ii) Secondly, these DCA are not present when children are not catabolic and are well and eating regularly or are receiving intravenous glucose at rates in excess of normal hepatic glucose production rates, thereby decreasing the dependence on FAO and the production of fatty acid metabolites. (3) Increased concentrations of DCA in the urine are generally not seen in the disorders involving the transport of fats into the mitochondria. Therefore, a FAO defect can be suspected in the presence of an excess of DCA relative to ketones, but the absence of DCA does not rule out a defect. The organic acid pattern may suggest the site of defect. For example, children with LCAD deficiency excrete primarily medium- and long-chain saturated DCA in contrast to children with trifunctional enzyme deficiency that excrete almost equimolar amounts of the saturated and the 3-hydroxydicarboxylic acids (Hale et al 1990 b). However, the presence of 3-hydroxy compounds also may be seen in toxic reactions with acetaminophen and with intrinsic liver disease (Pollitt 1990). Further advances in stable-isotope dilution mass spectrometry have improved the ability to quantitate metabolites in very small quantities (Gregersen et al 1986) in plasma or urine. Acylglycines that are consistently excreted in small quantities in the urine do not appear to have the same limitations of DCA. To this end useful glycine metabolites have been identified for several defects including MCAD, SCAD, ETF, and ETF-coenzyme Q oxidoreductase deficiencies.

Diagnostic Approaches and Screening Methods in FAO defects

(1) History and physical examination.

A careful history and clinical examination remain the key to investigation of these patients. The presentation may be either acute and recurrent or more chronic and slowly progressive. The acute presentation is more typical in which the child has a history of decreased oral intake during the preceding 24 to 36 hours followed by increasing lethargy and progressive obtundation or coma. The initial key investigations in a comatose child should include serum glucose and urine ketone measurements. Determination of urine ketones may be complicated because ill children are often dehydrated and will therefore have concentrated urine. If the blood glucose is above 3.3 mmol/L (60 mg/dl) and if it is accompanied by large amounts of urinary ketones, this tends to rule out a FAO disorder. However, if the blood glucose is < 3.3 mmol/L and the urine ketones are trace or small in amount, this would

suggest the possibility of a FAO disorder and warrants further investigation. Most importantly, samples from the acute presentation, particularly prior to intravenous glucose therapy, should be saved for the determination of serum carnitine total and free, serum acylcarnitines, serum free fatty acids and ketones and urine organic acids, acylglycines and acylcarnitines. Ordinarily, the serum free fatty acid to ketone ratio is 1:1. In the event of a block in FAO, this ratio increases to > 2:1 and is therefore a useful initial screen. Serum and urine specimens during the acute episode can also be used to assess integrity and hormonal regulation of the biochemical pathways involved in glucose homeostasis.

(2) Total carnitine measurement.

The differentiation between the carnitine uptake defect, CPT I and the intramitochondrial defects on the basis of total and free carnitine levels has been previously discussed.

(3) Urinary Organic acids.

It is critical to obtain urine specimens during the acute catabolic episode prior to intravenous glucose, as production of fatty acid metabolites ceases during normoglycemia.

(4) Fasting studies.

If the important samples have not been taken during an acute catabolic event, a fasting study may be considered to distinguish a FAO defect from other causes of hypoglycemia. However, it must be emphasized that if a fasting study is to be undertaken, it must be done under very carefully controlled hospital conditions with continuous monitoring and by physicians who are knowledgeable with respect to hypoglycemia, hypopituitarism, hyperinsulinism and FAO disorders. It is felt by some authorities that fasting studies should not be performed in children with FAO disorders, because diagnostic fasting may precipitate an acute metabolic crisis, leading to further morbidity or death. They suggest instead, that loading tests with carnitine or phenylpropionate can be used to aid in diagnosis. As suggested by Hale and Bennett (1992), there are pros and cons with each method. The reasons for a fasting study are: (1) the duration of fasting tolerance can be determined under carefully controlled conditions which may provide useful information regarding the long-term management of the affected patient and provide guidelines for prevention; (2) the full spectrum of abnormal fasting adaptation can be studied through assessment of a number of laboratory parameters including hormonal measurements; (3) the time to precipitation of acute clinical decompensation can be documented. As previously emphasized the cardinal risk is the precipitation of an acute catabolic crisis leading to morbidity and death. It would instead be preferable to collect appropriate samples, during an acute catabolic event suffered by the child.

The primary advantage of loading tests and the measurement of specific metabolites is their safety. The disadvantages are: (1) They are only useful in certain FAO defects e.g. MCAD deficiency, thus a negative test does not exclude all FAO defects; (2) They do not evaluate fasting adaptation.

The purpose of the fasting study is to identify the defective metabolic pathway through an analysis of temporal changes in substrates (glucose, free fatty acids, lactate, ketones), metabolites (carnitine, dicarboxylic acids), and relevant hormones (growth hormone, cortisol, insulin). Children must fast to the point at which they have significant symptoms or have a blood glucose of < 3.3 mmol/L (60 mg/dl). If at this point there is a deficient ketogenic response in the face of a significant dicarboxylic aciduria and a serum free fatty acid:ketone ratio of > 2:1, there is strong presumptive evidence for a defect in FAO.

(5) Other studies.

Once presumptive evidence for a defect in FAO has been established, the clinical picture in combination with an analysis of serum acylcarnitines, urinary organic acid profiles and urinary acylglycines may suggest a specific site of defect and the chain-length specificity of the defect (e.g. short-, medium- or long-chain). Another method for quantitative acylcarnitine profiling by electrospray ionization-tandem mass spectrometry (ESI-MS-MS) in human skin fibroblasts using unlabelled palmitic acid as substrate has been developed which has revealed pathognomonic acylcarnitine profiles in a variety of short-, medium- and long-chain FAO defects (Okun et al. 2002). Further investigations to identify the specific site of defect are as follows:

(i) Fatty acid oxidation studies.

A useful screening tool, provided the defect is expressed in cultured skin fibroblasts, is the measurement of the oxidation rates of [1-^{14}C] -labeled palmitate (C16), octanoate (C8), and butyrate (C4) in the fibroblasts to establish the chain-length specificity of the defect. (Rhead 1990).

(ii) Enzymatic assays.

Depending upon the suspected site of defect, a direct enzymatic assays may then be performed for the specific enzyme. These assays can be performed in cultured skin fibroblasts or in muscle biopsy specimens for CPT I, CPT II, SCAD, LCAD, SCHAD and trifunctional enzyme deficiencies. The carnitine acylcarnitine transferase deficiency (Pande et al 1993) and MCAD deficiency can be measured in cultured skin fibroblasts. Evidence for a defect in ETF or ETF-CoQ rely on demonstration of a combined deficiency in the activities of SCAD, MCAD and LCAD activities.

(iii) Uptake studies.

For the carnitine transporter (OCTN2) defect, diagnosis is confirmed by in vitro studies of carnitine uptake in cultured skin fibroblasts which indicate negligible uptake of carnitine in the homozygote patients, thereby precluding the calculation of Km and Vmax values (Treem et al 1988, Tein et al 1990a). This supports the concept that primary carnitine deficiency is due to a defect in the specific high-affinity, low-concentration, carrier-mediated carnitine transporter. Heterozygotes may demonstrate normal Km values but reduced Vmax values of 13 to 44 % of control (Tein et al 1990a, Stanley et al 1991) suggesting a decreased number of normally functioning transporters. This is the most sensitive study for detection of the carrier state as serum carnitine concentrations in the heterozygotes may be normal.

(iv) Molecular studies.

Molecular characterization of the specific defects include Western blotting to determine whether the defects are cross-reacting material positive suggesting a kinetic deficiency or whether they are cross-reacting material negative suggesting a decrease in the production of the affected enzyme. Western blotting has also been used in the determination of the amounts of the alpha and ß-subunits of ETF (Finocchiaro et al 1990). A number of the enzymes have now been cloned (e.g. CPT I, CPT II, LCAD SCAD, MCAD, trifunctional enzymes). This has led to the discovery of specific mutations resulting in the defective enzyme activity which has given rise to the development of specific molecular probes which can now be used for the precise and rapid detection of certain specific defects.

Treatment

Because of the small number of patients in any given institution, it is difficult to systematically evaluate any single treatment regimen. However, the mainstay of therapy is the avoidance of precipitating factors, particularly prolonged fasting. General treatment strategies are discussed next.

Avoidance of Precipitating Factors

Avoidance of precipitating factors, such as prolonged fasting, prolonged aerobic exercise (>30 minutes), and cold exposure resulting in shivering thermogenesis, is key. Prolonged fasting would be 6 to 10 hours for the infant younger than 1 year of age or 12 hours for the child between 1 and 4 years of age. In the event of progressive lethargy or obtundation or an inability to take oral feedings because of vomiting, the child should be taken immediately to the emergency room for intravenous glucose therapy. Intravenous glucose should be provided at rates sufficient to prevent fatty acid mobilization (8 to 10 mg/kg/min) (Hale and Bennett, 1992). This regimen should be continued until the catabolic cascade has been fully reversed and the child is able to take oral feedings again. It is wise to avoid prolonged exercise (i.e. >30 minutes) because during this time there is increased fat mobilization. A high-carbohydrate load prior to exercise is advisable with a rest period and repeat carbohydrate load at 15 minutes. Avoidance of cold exposure is essential.

High-Carbohydrate, Low-Fat Diet.

In general, it is advisable to institute a high-carbohydrate, low-fat diet with frequent feedings throughout the day, which would be commensurate with the special nutritional needs of the child given his or her age. This goal is best achieved with the aid of a metabolic dietitian, aiming toward approximately 70% to 75% of calories from carbohydrate sources, 15% from protein, and approximately 10% to 15% from fat. Monitoring of essential fatty acid levels is important to ensure that the child is receiving adequate essential fatty acids, as this may require supplementation in a fat-restricted diet. Augmentation of the diet with essential fatty acids (at 1-2% of total energy intake) is often used to reduce the risk of essential fatty acid deficiency (Gillingham et al, 1999; Uauy et al, 1989; Solis and Singh, 2002). Flaxseed, canola, walnut or safflower oils can be used for this purpose. An older child should have three regular meals per day with three equidistantly placed intermeal snacks, including a bedtime snack. In younger children, oral or nasogastric tube administration of an appropriate formula is indicated. In HMG-CoA lyase deficiency a high-carbohydrate, low-fat, low-protein diet with leucine restriction should be implemented (Gibson et al., 1988).

Uncooked Corn Starch.

To delay the onset of fasting overnight, the nightly institution of uncooked corn starch, in doses similar to those used in the treatment of glycogen storage disease (1 to 2 g/kg body weight/day as a single nighttime dose), will prolong the postabsorptive state and delay fasting (Dionisi-Vici et al., 1991). Cornstarch provides a sustained release source of glucose from the gastrointestinal tract, thereby preventing hypoglycemia and lipolysis (Fernandes and Smit, 2001). Cornstarch is usually initiated at 8 months of age when pancreatic enzymes are first able to function at full capacity for appropriate absorption (Hayde and Widhalm 1990). The initial recommended doses are generally 1.0 gm/kg/day which can be gradually increased to 1.5 to 2.0 gm/kg/day by age 2 years as needed (Fernandes and Smit, 2001). This may result in excessive weight gain.

Specific measures for individual FAO disorders include the following:

Medium-chain triglyceride (MCT) oil

MCT oil as a nutritional source could be useful in long-chain FAO disorders because the medium-chain fatty acids would circumvent the block in long-chain FAO and thereby facilitate ATP production from the remainder of the patent FAO pathway. The MCT oil could be started at a dose of 0.5 g/kg/day divided in three daily doses and could be increased up to 1 or 1.5 g/kg/day as tolerated. The major side effect is diarrhea. The usefulness of this approach, however, may be limited because excess MCT would ultimately be stored as long-chain fats in adipocytes. Furthermore, the success of MCT oil supplementation in LCHAD deficiency has been highly variable (Tein et al., 1995). When a high percentage of energy from fat is provided by MCT oil, patients are at risk for essential fatty acid deficiency and their diet should therefore be supplemented with EFA (1-2% of total energy intake) (Solis and Singh, 2002).

Riboflavin

Certain cases of the multiple acyl-CoA dehydrogenase deficiencies (e.g., ETF or ETF-CoQ-linked deficiencies) are responsive to riboflavin supplementation (Gregersen et al., 1982b). The dosage is approximately 50 mg 3 times a day for infants and young children and 100 mg 3 times a day for older children.

Carnitine

The essential indication for carnitine therapy is the carnitine transporter (OCTN2) defect, which is characterized by carnitine-responsive cardiomyopathy and very low plasma and tissue concentrations of carnitine (generally <5% of normal) (Stanley et al., 1991; Tein et al., 1990a). All 22 affected patients treated with high-dose oral carnitine supplementation demonstrated a dramatic improvement in their cardiomyopathy and myopathy within the first few weeks of therapy, as well as a reduction of heart size toward normal within a few months of therapy. In addition, three children with significant failure to thrive before therapy demonstrated a marked improvement in growth after therapy (Tein et al., 1990a). Of 19 patients treated with carnitine therapy for 5 to 20 years, 18 continued to be healthy (Stanley et al., 1991; Cederbaum et al., 2002). Thus, in the carnitine transporter defect, high-dose oral carnitine supplementation at 100 mg/kg/day in four divided daily doses is critical and life-saving, significantly reversing the pathology in this otherwise progressive and lethal disease. Furthermore, if a child is prospectively diagnosed from birth, early carnitine therapy from birth has been shown to prevent the development of the clinical phenotype (Lamhonwah et al., 2002).

In the intramitochondrial beta-oxidation defects with secondary carnitine deficiency, the results of carnitine therapy have been highly variable and insufficiently evaluated. Theoretically, carnitine has been given to limit the intracellular concentrations of potentially toxic acyl-CoA intermediates within the cell through transesterification and to thereby liberate CoA, which is a critical intracellular cofactor (Hale and Bennett, 1992). However, there has been no objective prospective study to prove that carnitine administration has had a beneficial effect. In one study of a patient with MCAD deficiency, Stanley et al. (1990) demonstrated that the associated carnitine deficiency was not the cause of the defect in FAO as shown by the lack of effect of carnitine replacement on fasting ketogenesis. After 3 months of oral carnitine therapy, this patient had no increase in plasma ketones and still became ill and hypoglycemic

after 14 hours of fasting. Furthermore, there is increasing evidence to suggest that carnitine administration may have deleterious effects in the long-chain FAO disorders. In these disorders there is an accumulation of long-chain acyl-CoAs proximal to the metabolic block, which on esterification become long-chain acylcarnitines. Excessive palmitoylcarnitine may have detergent effects on membranes and arrhythmogenic effects as previously discussed (Inoue and Pappano, 1983; Lee et al., 1987; Mak et al., 1986; Spedding, 1985; Spedding and Mir, 1987). This field warrants further investigation.

Specific therapies for LCHAD/TFP deficiency

Oral prednisone has been shown to lead to a dramatic reversal of the limb-girdle myopathy and marked reduction in the episodic myoglobinuria in one boy with the myoneuropathic form of LCHAD deficiency (Tein et al., 1995). Several children with LCHAD deficiency who had associated pigmentary retinopathy were shown to have a deficiency of the (n-3- polyunsaturated fatty acid, docosahexaenoic acid (DHA) and subsequent supplementation with DHA led to some improvement in visual function (Gillingham et al., 1997; Harding et al., 1999). Furthermore, the daily oral administration of a cod liver oil extract containing high amounts of DHA led to a marked clinical and electrophysiological recovery of the progressive peripheral sensorimotor axonopathy in one boy with the myoneuropathic form of LCHAD deficiency (Tein et al., 1999).

Triheptanoin

Use of the anaplerotic odd-chain triglyceride, triheptanoic acid, has been reported to be of value in the therapy of long chain fatty acid oxidation defects (Roe et al., 2002). In three patients with very long-chain acyl-CoA dehydrogenase deficiency fed controlled diets in which the fat component was switched from medium-even-chain triglycerides to triheptanoin, this treatment led rapidly to clinical improvement that included the resolution of chronic cardiomyopathy, myoglobinuria and muscle weakness for more than 2 years in one child and of myoglobinuria and weakness in the others. More studies need to be done to assess efficacy.

VII. Mitochondrial Encephalomyopathies

Historical Considerations: The concept of mitochondrial disease was first introduced in 1962 when Luft et al (1962) reported a young Swedish woman who suffered from severe hypermetabolism which was not due to thyroid dysfunction. In the decades of 1960 and 1970, systematic investigation of muscle biopsies led to the recognition of different patterns of mitochondrial changes (Shy and Gonatas 1964, Shy et al 1966). In 1963, Engel and Cunningham modified the Gomori trichrome stain which allowed identification of abnormal deposits of mitochondria as ragged-red fibres (RRF). Systematic biochemical investigation in the 1970's and 1980's led to a rational biochemical classification of mitochondrial diseases into five main groups. With advances in mitochondrial genetics, which contain their own DNA (mtDNA), a genetic classification has subsequently been proposed based on nuclear and/or mitochondrial DNA inheritance.

Morphologic Considerations

The finding of RRF or ultrastructural alterations of mitochondria in muscle biopsy specimens provides an important diagnostic clue, however as pointed out by DiMauro (1993, 2004) there are important limitations as follows: (1) RRF or ultrastructural mitochondrial abnormalities can be seen in disorders

of non-mitochondrial etiology such as muscular dystrophies, polymyositis and some glycogenoses, in which they likely represent secondary changes; (2) conversely, many primary mitochondrial diseases such as enzyme defects in metabolic pathways other than the respiratory chain e.g. PDHC, CPT, ß-oxidation and fumarase deficiencies do not have RRF. Furthermore, there are defects of the respiratory chain such as the form of Leigh syndrome associated with cytochrome c oxidase (COX) deficiency which tend not to have RRF. Though RRF are usually present in defects of mtDNA which affect the respiratory chain, there are also exceptions such as Leber's hereditary optic neuropathy (LHON) in which the mitochondrial changes are subtle, without RRF (Uemura et al 1987). Furthermore, RRF may depend on the threshold effect (the percentage of mutant mtDNA) and on the stage of the disease. Two other useful histochemical stains are succinate dehydrogenase (SDH) and cytochrome oxidase (COX) stains. RRF are often COX-negative, though not all COX-negative fibres are RRF (DiMauro 1993). COX-negative RRF are seen in patients with progressive external ophthalmoplegia and mtDNA deletions and in MEERF (myoclonus epilepsy with RRF) syndrome but not in MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes) syndrome which has COX-positive RRF. E/M may show increased numbers of mitochondria (pleoconial myopathy), increased size (megaconial myopathy), disoriented or rarefied cristae and osmophilic or paracrystalline inclusions (DiMauro 1993). The paracrystalline inclusions are deposits of mitochondrial creatine kinase (Stadhouders et al 1990). On muscle biopsy, there may also be lipid and glycogen storage signifying a defect of terminal oxidation (Jerusalem et al 1973).

Clinical considerations

Mitochondrial diseases are clinically heterogeneous. Pure myopathies may vary in age at onset, course, and distribution of weakness (DiMauro 1993). Patients may also have exercise intolerance and premature fatigue. In the clinical classification, there has been controversy between the 'lumpers' and the 'splitters'. A number of distinct clinical syndromes have been described e.g. Kearns-Sayre, MERRF and MELAS syndrome, each due to 3 distinct mutations in mtDNA. Common features to all three include short stature, dementia, sensorineural hearing loss, lactic acidosis and RRF. In children, given the earlier age of onset and generally more severe clinical phenotype, there may be a number of overlap syndromes e.g. MELAS/Kearns-Sayre syndrome.

Biochemical classification

Mitochondrial encephalomyopathies can be classified into five groups according to the area of mitochondrial metabolism specifically affected, namely: (1) defects of transport (2) defects of substrate utilization (3) defects of the Krebs cycle (4) defects of the respiratory chain, and (5) defects of oxidation/phosphorylation coupling (DiMauro 1993). Limitations of this classification scheme relate to the respiratory chain defects which can be due to genetic defects of nuclear DNA or to defects of mtDNA which are usually heteroplasmic in nature and deletions of mtDNA or point mutations in tRNA which affect mtDNA translation as a whole leading, to multiple respiratory chain defects.

Genetic classification

Mitochondria are the only subcellular organelles with their own DNA (mtDNA) (Nass et al 1963) which are capable of synthesizing a vital set of proteins. Human mtDNA is a small (16.5 kb), circular, double-stranded molecule that has been completely sequenced (Anderson et al 1981) and encodes 13 structural proteins, all of which are subunits of respiratory chain complexes, as well as two rRNAs and 22

tRNAs needed for translation. Unique features of mtDNA are as follows (DiMauro 1993): (1) Its genetic code differs from that of nuclear DNA (nDNA), (2) it is tightly packed with information as it contains no introns, (3) it is subject to spontaneous mutations at a higher rate than nDNA, (4) it has less efficient repair mechanisms than nDNA, (5) it is present in hundreds or thousands of copies per cell, (6) it is transmitted by maternal inheritance. In the formation of the zygote, mtDNA is contributed only by the oocyte (Giles et al 1980). If there is a mutation in some mtDNA in the ovum or zygote, this may be passed on randomly to subsequent generations of cells, some of which will receive few or no mutant genomes (normal or wild-type homoplasmy), others will receive primarily or exclusively mutant genomes (mutant homoplasmy) and others will receive a mixed population of mutant and wild-type mtDNAs (heteroplasmy). There are important implications from maternal inheritance and heteroplasmy, as follows (DiMauro 1993): (1) inheritance of disease is maternal as in X-linked traits, but both sexes are equally affected, (2) phenotypic expression of a mtDNA mutation will depend upon relative proportions of mutant and wild-type genomes with a minimum critical number of mutant genomes being necessary for expression (threshold effect), (3) at cell division, the proportion may shift in daughter cells (mitotic segregation) leading to a corresponding phenotypic change, (4) subsequent generations are affected at a higher rate than in autosomal dominant diseases. The critical number of mutant mtDNAs needed for the threshold effect may vary depending upon the vulnerability of the tissue to impairments of oxidative metabolism, as well as on the vulnerability of the same tissue over time which may increase with aging (Moraes et al 1991a, Wallace et al 1988a). Although the mtDNA-encoded peptides are functionally important, they represent a small proportion of total mitochondrial protein. The majority of mitochondrial proteins are encoded by nDNA, synthesized in the cytoplasm and then imported into mitochondria. This transport of proteins requires a complex series of postranslational events and translocation machinery involving synthesis of larger precursors in the cytosol, amino terminal leader peptides which function as address signals and recognize specific mitochondrial membrane receptors, translocation across the mitochondrial membrane and cleavage of the leader peptides with assembly of mature peptides at their final intramitochondrial location (Schatz 1991). Genetic classification of mitochondrial diseases must take into account defects of nDNA or mtDNA and defects of communication between the two genomes.

Defects of mtDNA can be divided into mutations in mitochondrial protein synthesis genes and mutations in mitochondrial protein coding genes. Protein synthesis gene defects may include duplication/deletion as seen in Kearns-Sayre and Pearson Marrow Pancreas syndrome, mutations in tRNA as seen in MELAS and MERRFS, and defects in rRNAs. Single deletions are usually sporadic and duplications ± single deletions are rare and usually maternal. Mutations in protein coding genes affect 13 of 82 structural proteins and may be multisystemic (e.g. LHON, NARP, MILS) or tissue specific. To date over 120 point mutations have been documented in mtDNA.

The majority of mitochondrial diseases are due to defects in nDNA and many are autosomal recessive in inheritance though some are autosomal dominant or X-linked. Nuclear gene defects are generally more severe, uniform and earlier in onset than mtDNA defects. Diseases due to mutations in nDNA include mutations in genes encoding subunits of the respiratory chain, mutations in genes encoding ancillary proteins needed for proper assembly of the respiratory chain (e.g. SURF1, SCO1, SCO2, COX10 and COX15 which are involved in the assembly of Complex IV), defects of intergenomic signaling (e.g. multiple mtDNA deletions, mtDNA depletion), defects of mitochondrial protein importation (e.g. TIMM8A gene mutations resulting in Mohr-Tranebjaerg syndrome), alterations of the lipid milieu

of the inner mitochondrial membrane (e.g. TAZ gene mutations resulting in Barth Syndrome) and alterations of mitochondrial motility or fission (e.g. mutations in OPA1 gene). Structural nuclear genes encode all subunits of Complex II and most subunits of Complexes I, III, IV and V as well as CoQ10 and cytochrome c. Multiple deletions of mtDNA may be due to defects of thymidine phosphorylase (TP), adenine nucleotide translocator 1 (ANT1), and polymerase gamma (POLG) genes. POLG defects are now thought to be among the most common of mitochondrial diseases, particularly in the European population. MtDNA depletion may be due to defects in the thymidine kinase 2 gene (TK2) or deoxyguanosine kinase gene (DGK). TK2 defects are later onset and muscle-specific and may present with a spinal muscular atrophy-like picture. DGK defects are early onset hepatocerebral presentation or multisystemic.

Physiologic Considerations

Alterations of oxidative metabolism can be detected by standard exercise physiology tests such as cycle ergometers or treadmills (Haller et al 1989, Lewis and Haller 1991a & b). The most useful indicator of a patient's capacity for oxidative metabolism is their maximal oxygen uptake (DiMauro 1993).Typical physiologic responses in patients with defects in oxidative metabolism are as follows (Haller et al 1989, 1991): (1) The increase of cardiac output during exercise is greater than normal relative to the rate of oxidative metabolism. (2) Oxygen extraction per unit of blood remains almost unchanged from rest to maximal exercise, leading to a gross mismatch between oxygen transport and utilization. In patients with heteroplasmic mtDNA mutations, there is an inverse relationship between the proportion of skeletal muscle mutant mtDNA and peak O_2 extraction during exercise (Taivassalo et al 2003). (3) Ventilation is normal at rest but increases excessively relative to oxygen uptake. (4) Venous lactate which is usually elevated at rest increases excessively relative to workload and oxygen uptake.

Mitochondrial function in muscle *in vivo* can be quantitatively evaluated using ^{31}P-NMR (Radda et al., 1995). The ratio of phosphocreatine (PCr) to inorganic phosphate (Pi) can be measured in muscle at rest, during exercise, and during recovery. In patients with mitochondrial dysfunction, PCr/Pi ratios are lower than normal at rest, decrease excessively during exercise, and return to baseline values more slowly than normal (Argov and Bank, 1991).

Selected Mitochondrial Diseases

Complex I Deficiency

NADH-CoQ reductase is the largest complex of the respiratory chain. There are three main clinical syndromes as follows: (1) A fatal infantile multisystem disorder characterized by severe congenital lactic acidosis, psychomotor delay, diffuse hypotonia and weakness, cardiomyopathy, and cardiorespiratory failure leading to death in the neonatal period (Moreadith et al 1984, Robinson et al 1986). The enzymatic defect is multisystemic. Therapeutic trials with thiamine, biotin, carnitine and the ketogenic diet have been unsuccessful (Moreadith et al 1984). Some subunits may be encoded by genes on the X-chromosome (Day et al 1982). (2) Myopathy with exercise intolerance followed by fixed weakness, which can start in childhood or adult life and which is usually accompanied by lactic acidosis at rest which can be exaggerated by exercise (Morgan-Hughes et al 1988). Therapy with riboflavin was of benefit to one patient with weakness (Arts et al 1983). (3) Mitochondrial encephalomyopathy (excluding MELAS), with onset in childhood or adult life which can have multiple combinations of ophthalmoplegia, seizures, dementia, ataxia, sensorineural hearing

loss, pigmentary retinopathy, sensory neuropathy and involuntary movements (Morgan-Hughes et al 1988). This heterogeneous group may include patients with nDNA and others with mtDNA defects, as many cases were reported before systematic analysis of mtDNA (DiMauro 1993).

Complex II Deficiency

Clinical presentations have included encephalomyopathy (Sengers et al 1983) and myopathy (Garavaglia et al 1990, Haller et al 1991), one with exercise intolerance and exertional myoglobinuria and a lack of SDH stain on muscle biopsy (Haller et al 1991). Another patient had associated aconitase deficiency (Haller et al 1991).

Coenzyme Q10 (CoQ10) Deficiency

Two sisters developed exercise intolerance and slowly progressive weakness of axial and proximal limb muscles with sparing of facial and extraocular muscles (Ogasahara et al 1989). Both had episodes of myoglobinuria in association with seizures or intercurrent infections. Family history suggested autosomal recessive inheritance. Muscle biopsies revealed excessive lipid and mitochondria in type I fibres. Biochemical analysis suggested a muscle (5 % normal) and probably brain specific isoenzyme defect of CoQ10 and CoQ10 replacement therapy improved both muscle and brain function. It is now apparent that primary CoQ10 deficiency can cause three major syndromes, a predominantly myopathic disorder with recurrent myoglobinuria, a predominantly encephalopathic disorder with ataxia and progressive cerebellar atrophy and a generalized form (Lamperti et al, 2003). The diagnosis is critical given the significant clinical response to the CoQ10 supplementation.

Complex III Deficiency

The clinical presentation is heterogeneous, but patients can be divided into two major groups, namely those with multisystem disease (encephalomyopathy) and those with tissue-specific defects such as myopathy or cardiomyopathy. Among the multisystem group, a fatal infantile form has been described in a child with severe lactic acidosis and hypotonia with complex III deficiency in muscle, heart, liver and kidney and a decrease of cytochrome b in muscle. Later onset encephalomyopathies (childhood to adult life) have presented with combinations of weakness, short stature, dementia, ataxia, sensorineural deafness, pigmentary retinopathy, sensory neuropathy and pyramidal signs (Morgan-Hughes et al 1985, Kennaway et al 1988). The myopathic presentation is characterized by exercise intolerance with premature fatigue and hyperpnea which is often followed by fixed weakness (Morgan-Hughes et al 1985, Kennaway 1988). The existence of tissue-specific isoforms is suggested by the presence of normal complex III activity in fibroblasts and lymphoid cells of a patient with pure myopathy (Darley-Usmar et al 1986). Treatment strategies have included the administration of menadione (Vitamin K3) and ascorbate (Vitamin C) (Eleff et al 1984). In one young woman with myopathy (Darley-Usmar et al 1983), this resulted in prompt clinical improvement (Eleff et al 1984, Argov et al 1986). This treatment proved ineffective in two other patients including an infant with encephalomyopathy (Przyrembel 1987) and an adult with myopathy (Reichmann et al 1986). Complex III deficiency has also been described in one patient with an unusual form of facioscapulohumeral muscular dystrophy (Slipetz et al 1991). The cardiomyopathy presentation due to complex III and reducible cytochrome b deficiency in the myocardium was documented in one patient with a rare fatal histiocytoid cardiomyopathy of infancy (Papadimitriou et al 1984) which proved to be tissue-specific to heart.

mtDNA Depletion

mtDNA depletion, which results from defects of intergenomic signaling, is usually characterized clinically by congenital or childhood forms of autosomal recessively inherited myopathy or hepatopathy (Moraes et al 1991a; Vu et al., 1998). Although skeletal muscle and liver seem to be the main target tissues, other tissues are often affected in both conditions, including kidney (renal tubular acidosis) and the central nervous system (CNS). mtDNA depletion should be considered in children with the spinal muscular atropy (SMA) phenotype but without mutations in the SMN gene (Mancuso et al., 2002; 2003). The decrease in mtDNA is documented by densitometry of Southern blot analysis and confirmed by immunocytochemistry with anti-DNA antibodies and by in situ hybridization (Andreeta et al., 1991; Moraes et al, 1991a; Tritschler et al, 1992). In severe cases, depletion of muscle mtDNA varies between 83% and 98%. Mutations in two genes, both of which are involved in mitochondrial nucleotide homeostasis, have been associated with mtDNA depletion syndromes, even though they do not account for all cases. Mutations in the gene encoding thymidine kinase 2 (TK2) are frequently documented in patients with the myopathic mtDNA depletion syndrome (Saada et al., 2001; Mancuso et al., 2002). Mutations in the gene encoding deoxyguanosine kinase (dGK) predominate in patients with hepatic or multisystemic mtDNA depletion syndromes (Mandel et al 2001, Salviati et al., 2002) and may have an Alper's hepatocerebral phenotype. The Alper's-Huttenlocher phenotype with childhood encephalopathy and liver failure may also be due to mutations in the POLG gene which may be associated with mtDNA depletion or multiple secondary mtDNA deletions in varying degrees (Chinnery and Zeviani 2008). Recessive mutations in the POLG gene tend to cause mtDNA depletion and present in childhood and dominant POLG mutations tend to cause adult-onset disease with multiple secondary deletions of mtDNA.

VIII. Approach to Investigation of Recurrent Myoglobinuria

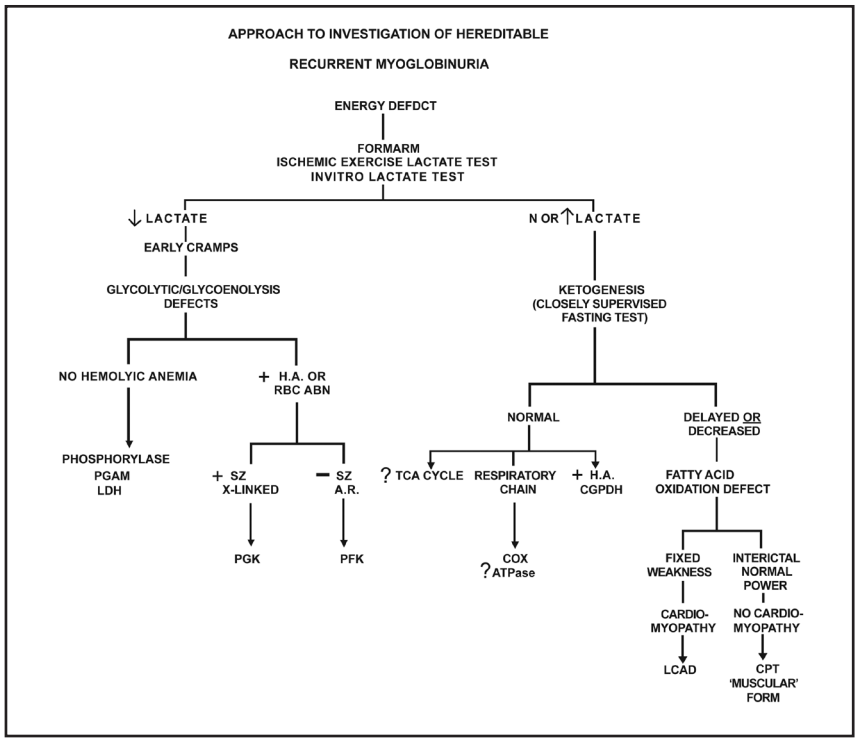
Based upon the clinical features and screening biochemical tests, a practical approach can be derived for the prioritized investigation of recurrent myoglobinuria (Fig. 1). If there is a history of true muscle cramps within the first minutes of high-intensity exercise or of a "second wind" phenomenon, this would suggest a glycolytic or glycogenolytic disorder. If there is a history of muscle stiffness after mild to moderate prolonged exercise (e.g.>1 hour) or of myoglobinuria precipitated by fasting or cold exposure, this would suggest a defect in FAO. The first clinical test would be an in vivo forearm ischemic lactate test. In the young child in whom this is not possible, an in vitro lactate test may be performed on the muscle biopsy to test the integrity of the glycolytic pathway. If lactate production is insufficient (less than a 3-4 fold rise) this would suggest a block in the glycogen pathway. The defects in glycolysis/glycogenolysis can be divided into two groups, those in which there is an associated hemolytic anemia such as PGK and PFK deficiency and those without hemolytic anemia e.g. PPL, PGAM and LDH deficiency. PGK can be further distinguished from PFK, as PGK is X-linked and may have associated seizures whereas PFK is autosomal recessive. The forearm ischemic lactate test can also be used to assess whether there is an appropriate rise (3-4 fold) in ammonia. If ammonia production is insufficient, this would suggest a defect in the purine nucleotide cycle such as myoadenylate deaminase deficiency.

If there is adequate lactate production, the next important question relates to whether there is any evidence for deficient ketogenesis. If ketogenesis appears normal, the considerations include a defect in the pentose phosphate pathway (G6PD deficiency) which may be distinguished by the presence of hemolytic anemia. Other considerations would include the mitochondrial encephalomyopathies secondary

to defects in the respiratory chain or mitochondrial DNA, which may be suspected if there are marked elevations of serum lactate (> 2-fold) and certain characteristic clinical features such as failure to thrive, short stature, and sensorineural hearing loss or perhaps a maternal pattern of inheritance. Another biochemical cycle in which defects could theoretically result in deficient energy production resulting in myoglobinuria, would be the tricarboxylic acid cycle, though no such underlying defects have been identified to date.

If there is evidence of delayed or deficient ketogenesis, a defect in FAO should be suspected. Of the possibilities, the most common defect is that of the classic "adult" myopathic form of CPT II deficiency in which there is typically adolescent-onset recurrent myoglobinuria, with normal power between episodes, and no associated cardiomyopathy or overt liver disease. This contrasts with the fixed lipid storage myopathy and cardiomyopathy seen in LCAD/VLCAD, LCHAD/TFP and SCHAD deficiency. The common "adult" myopathic form of CPT II deficiency contrasts with the rare "infantile" hepatic form of CPT I deficiency which presents with recurrent hypoglycemic hypoketotic encephalopathy and seizures, precipitated by fasting or infection, and in which there are no muscular manifestations (Demaugre et al 1988; Tein et al 1989). However, Demaugre et al (1991) described a unique "infantile form" of CPT II deficiency ("overlap" case) in a 3-month-old boy with fasting hypoketotic hypoglycemic encephalopathy, elevated serum CK, cardiac arrhythmias and cardiomegaly. The residual CPT II activity was decreased to 10 % of normal in the cultured skin fibroblasts of this severe infantile CPT II deficiency, in contrast to the 25% residual activity documented in 2 patients with the more "classic" adult form of CPT II deficiency (Demaugre et al 1991). Thus, the careful consideration of the history, and physical exam, followed by a series of selected, prioritized screening investigations, should therefore allow the clinician to reach a presumptive diagnosis, to be confirmed by more specific and intensive investigations.

Figure 1. Approach to Investigation of Recurrent Myoglobinuria



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High Prevalence Rate of Wilson Disease in Hong Kong: 1 in 5400

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Wilson disease (WD) (MIM # 277900) is an autosomal recessive disorder of copper transport. Clinical manifestations of Wilson disease vary widely. The age of onset ranges from three to more than 50 years of age. The initial onset of symptoms can be hepatic, neurological, psychiatric or as an acute hemolytic crisis. The prevalence of WD has been estimated to be approximately 1 in 30,000 in the Caucasian population.

The gene responsible for WD was identified, and the gene product was predicted to be a copper binding P-type adenosine triphosphatase. The *ATP7B* gene consists of 21 exons which span a genomic region of about 80 kb and encode a protein of 1465 amino acids. ATP7B is expressed primarily in the liver and kidney. The protein plays a dual function role in the hepatocyte. One role is biosynthetic, delivering copper to apoceruloplasmin within the Golgi network. The other role of ATP7B is to transport excess copper out of the cell and into the bile canaliculus for subsequent excretion from the body via the bile. ATP7B is localized in the trans-Golgi network of hepatocytes under low copper conditions, redistributes to cytoplasmic vesicles when cells are exposed to elevated copper levels, and then recycles back to the trans-Golgi network when copper is removed. Therefore, an *ATP7B* mutant will result in a reduction in the rate of incorporation of copper into apoceruloplasmin or a reduction in biliary excretion of copper, or both. For example, a Wilson disease variant protein, Arg778Leu, has recently been shown to be extensively mislocalized, presumably to the endoplasmic reticulum. Defective biliary excretion leads to accumulation of copper in the liver with progressive liver damage and subsequent overflow to the brain, causing loss of coordination and involuntary movements. Deposition in the cornea produces Kayser-Fleischer rings, and accumulation in other sites causes renal tubular damage, osteoporosis, arthropathy, cardiomyopathy, and hypoparathyroidism.

The prognosis for patients is excellent with early treatment with D-penicillamine, trientine, or zinc salts, but early detection, monitoring, and treatment of presymptomatic patients is critical to prevent irreversible liver damage requiring transplant. As described earlier, biochemical and symptomatic signs are not specific enough for effective diagnosis of all affected individuals. In addition, the clinical and laboratory parameters are not sufficient to exclude the diagnosis of Wilson disease in patients with liver disease of unknown origin. In these two groups of patients, the exclusion of a diagnosis of Wilson disease by clinical and biochemical parameters is very challenging. Direct detection of the mutations causing Wilson disease will eliminate these problems. Direct mutation detection in diagnosis of presymptomatic sibs is particularly important because of the difficulty in distinguishing presymptomatic patients from heterozygotes.

The most frequent *ATP7B* mutation in Caucasian patients is His1069Gln, which is found in 28-38% of all alleles, and the next most frequent is Gly1267Lys, which is found in 10%. No such mutations have so far been detected in Asian Wilson disease patients. This finding reveals that the mutation spectrum of the *ATP7B* gene shows a population-dependent distribution. No studies have yet been undertaken to elucidate the molecular basis of Wilson disease in Hong Kong Chinese. In one study performed in Northern Chinese using single strand conformational polymorphism analysis, however, less than 50% of Wilson disease patients had ATP7B mutations. As a second locus for Wilson disease has not been reported, the result probably reflects the insensitivity of the screening method. In addition, the mutational spectrum of a disease-causing gene might be different between Southern and Northern Chinese, e.g. phenylketonuria. Therefore, the data cannot be used directly in our population. A study has been performed on Southern Chinese Wilson disease. However, the investigators only screened 4 out of 22 exons of the *ATP7B* gene for mutations, and mutations were detected in less than 15% of Wilson disease patients. Neither of these studies has provided enough information to establish an easy and effective protocol for DNA-based diagnosis for Wilson disease in the Hong Kong Chinese population.

Toward this end, we have delineated the spectrum of mutations in the Wilson disease gene in patients in Hong Kong. This was the first local study to elucidate the molecular basis and establish an effective DNA-based diagnostic protocol. The ATP7B genes of 65 patients were amplified by polymerase chain reaction (PCR) and sequenced. Haplotype analysis was performed using D13S301, D13S314, and D13S316. The p.L770L/p.R778L status in 660 subjects was determined to estimate WD prevalence. Allele age of p.R778L was determined by the smallest homozygosity region between D13S301 and D13S270. We identified 42 different mutations with 17 being novel. p.R778L (17.3%) was the most prevalent. Exons 2, 8, 12, 13, and 16 harbored 70% mutations. Thirty-two haplotypes were associated with WD chromosomes. The estimated prevalence rate was 1 in 5,400. Three out of 660 normal subjects had p.L770L/p.R778L. In the remaining 657 individuals, neither p.L770L nor p.R778L was found. We characterized a Hong Kong Chinese-specific ATP7B mutation spectrum with great genetic diversity. Exons 2, 8, 12, 13, and 16 should be screened first. The perfect linkage disequilibrium suggested that p.R778L and its private polymorphism p.L770L originated from a single ancestor. This East-Asian-specific mutation p.R778L/p.L770L is aged at least 5,500 years.

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A Practical Approach for the Diagnosis of Prevalent Fatty Acid -oxidation Defects Presenting with Neuromuscular Symptoms among Southern Chinese

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Abstract

Fatty acid oxidation defects are a group of inherited metabolic diseases affecting the β oxidation pathway of fatty acids. It is one of the many groups of inherited metabolic diseases causing neurometabolic disorder. Early diagnosis and appropriate treatment reduce morbidity and mortality. Screening of blood acylcarnitines by tandem mass spectrometry can detect most of the fatty acid oxidation defect patients. However, a definitive diagnosis needs to be established for these patients. With recent advances in functional assays of the pathway, it is now possible to determine directly both the overall rate of β-oxidation and profiling of acylcarnitine intermediates for detection blockage in the pathway. First, the methodology is reviewed here together with a brief introduction of a new combined approach to perform both assays simultaneously. Second, the spectrum of fatty acid oxidation disorders among southern Chinese is also reviewed here. The pattern is different from Caucasians in that no definitive case of median chain acyl CoA dehydrogenase deficiency has been found so far. In contrast, it is the most common inherited metabolic diseases in the West. On the other hand, we diagnosed patients with carnitine uptake defect, multiple acyl CoA dehydrogenase deficiency, very long chain acyl CoA dehydrogenase deficiency and carnitine translocase defect. Our research has identified a common founder mutation in carnitine transporter gene (*OCTN2*) in southern Chinese suggesting carnitine uptake defect might be one of the prevalent inherited metabolic diseases in our locality. A diagnostic algorithm is proposed for definitive diagnosis of fatty acid oxidation disorders.

Introduction

Inherited neurometabolic disorders are caused by inborn errors of metabolism e.g. organic aciduria, aminoacidopathies, urea cycle disorders, mitochondrial fatty acid oxidation disorders (FAOD) etc., leading to progressive destruction of motor, mental and perceptual functions of the brain. The presenting symptoms are usually non-specific. They can affect single organ system but more often multiple organ systems are involved. They may manifest as acute life-threatening event or a sub-acute progressive degenerative disorder. On the other hand, due to the multiplicity of potential defects in metabolism and large number of various inborn errors in the biochemical pathways, different metabolic diseases would share similar clinical features.

Therefore, their differentiation and specific diagnosis of inherited metabolic diseases (IMD) requires good laboratory support, which needs to provide various assays of the biochemical pathways. Diagnosis of any metabolic diseases follows a stepwise approach, starting with clinical suspicion.

Step 1. Clinical features. Patients with metabolic diseases will present to pediatricians with atypical clinical features which are suggestive of an underlying metabolic disease.

Step 2. Delineating a category for the underlying metabolic defect. The pediatricians will request some first line metabolic disease laboratory screening tests or histopathology investigations. These first line screening tests are useful in indicating that (1) whether if the patients are likely suffering from a metabolic disease and (2) if so, which part of the metabolic pathways is likely to be defective, for example defects in the fatty acid oxidation pathway entails a list of more than 20 individual enzyme defects. Laboratory tests belonging to this group include urine organic acids, plasma amino acid and plasma carnitine analysis.

Step 3. Arriving at a definitive diagnosis. After confining the possible metabolic defect to a part in the biochemical pathway, the specific enzymes in the pinpointed pathway will need to be assayed in order to arrive at the definitive diagnosis i.e. which enzyme or specific action in the pathway is the cause of disease. These enzyme assays are performed either on peripheral blood samples or cultured fibroblasts derived from a skin biopsy. We have been providing fibroblast culture service for local hospitals free of charge since 1997. However, we are lack of personnel support in developing enzymes assays. Therefore, in many occasions, we have to rely on overseas laboratory for this service which may sometimes be quite expensive. Some patients cannot afford the expenses required for sending sample overseas.

Step 4. Arriving at a genetic diagnosis. This step may be complementary to enzyme assays in step 3. As genes causing many metabolic diseases are now known, genetic analysis may in some cases replace the need of enzyme assays/ biochemical assays in arriving at a definitive diagnosis.

In past years, we reported some preliminary data on the common IMD in a southern Chinese population in Hong Kong (1, 2). The results show that the pattern of these diseases observed in southern Chinese might be different from that of Caucasian and northern Chinese. For aminoacidemia, classical phenylketonuria is very rare in southern Chinese. However, similar incidences are reported for northern Chinese and Caucasians (3). Likewise, methylmalonic, propionic and isovaleric acidaemia are the three most frequent organic acidurias found in northern Chinese but not in southern Chinese (4).

There are many interesting studies revealed evidences supporting genetic differences between southern and northern Chinese. For example, a difference in genetic profiles using 30 microsatellites was observed in 28 population sampled in the southern and northern China (5). Other approaches including studies of frequencies of Gm markers (6) and Y-chromosomes (7), polymorphisms of blood groups, enzymes and HLA (8, 9), have come up with similar conclusions. As compared to other IMD, FAOD are less well studied in Chinese. The primary

reason might be due to a low incidence of the diseases, for example medium chain acyl-CoA dehydrogenase deficiency (MCADD) is virtually not found in the southern Chinese population. However, as reviewed here, certain kinds of FAOD are prevalent among southern Chinese. Secondly, insufficient awareness of local profile of FAOD may result in inadequate development of laboratory services to diagnose this group of diseases. Although screening methods using mass spectrometry for FAOD are available in many laboratories in China including Hong Kong, other functional tests e.g. in-vitro fatty acid oxidation (FAO) rate and probe assays are not in the common test repertoires in the locality. Therefore, a definitive diagnosis by a functional assay or DNA analysis has not been established in many patients. In order to fill this gap, we review here the functional laboratory assays for the diagnosis of FAOD and our recently developed novel in-vitro probe assay combining the overall FAO rate and acylcarnitine profiling using isotope ratio mass spectrometry (IRMS) and electro-spray tandem mass spectrometry (ESI-MSMS) to study FAOD (10).

In this paper, the laboratory techniques for investigation of FAOD and the latest data on the profile of the common FAOD among southern Chinese are discussed. A diagnostic algorithm is also proposed for guiding pediatricians in making a definitive diagnosis for patients suspected to have FAOD.

Mitochondrial fatty acid β-oxidation disorders

Mitochondrial fatty acid β-oxidation is essential for production of energy during acute stress e.g. infection and fasting and at the time of increased muscle activities (11). Moreover, FAO also provides over 80% of basal energy supply to the heart and liver at all times (12). There has been great advance in the understanding of inherited disorders of FAO (13-15). Up-to-date, over 20 disorders in fatty acid transport and mitochondrial β-oxidation have been reported, all are inherited as autosomal recessive diseases. The usual clinical presenting features of inherited FAOD may include neonatal onset with severe metabolic acidosis, hypoketotic hypoglycaemia, hyperammonia, cardiomyopathy, liver dysfunction, and sudden unexpected death, or infantile onset with intermittent myopathy, neuropathy, and retinopathy. However, the spectrum of presentation also includes adult onset with myopathy, rhabdomyolysis and cardiomyopathy (16). Therefore, other than those that are diagnosed by newborn screening of blood acylcarnitines, a high level of clinical awareness is required to diagnose patients with FAOD.

First-line laboratory investigations of suspected FAOD rely on analysis of carnitine and acylcarnitines in blood samples and urine organic acid profiling. There are occasional pitfalls in these basic routine metabolic tests for the screening of FAOD. For example, urine organic acid patterns in patients with FAOD usually show non-specific increases in dicarboxylic acids and cannot pinpoint to the specific disorders. Measurements of blood or serum acylcarnitines by ESI-MSMS are more robust and give rapid and accurate diagnosis of FAOD. However, this technique may still have the difficulties in making a definitive diagnosis in certain cases especially during stable phase of the disease and there is a co-incidental deficiency of carnitine (17).

After a preliminary diagnosis of a FAOD is made by these routine metabolic tests, it should be followed up by a definitive diagnosis of the specific defect with a functional assay of the FAO

pathway. There are two main types of in vitro functional assays including (I) determination of overall fatty acid oxidation rate and (II) acylcarnitine profiling. The principle and latest developments of these functional assays will be discussed here.

Overall fatty acid oxidation rate assays

Long chain fatty acids e.g. palmitic acid undergo β-oxidation in the mitochondria by repetitive cycles of cleavage of 2-carbon fragments in the form of acetyl-CoA and reduction of NAD and FAD to their reduced forms. Acetyl-CoA enters the tricarboxylic acid cycle to be metabolized to CO_2, and additional molecules of reduced NAD and FAD are produced. The latter pass their reducing equivalents to the respiratory chain pathway with the help of electron transfer flavoprotein (ETF) and ETF dehydrogenase (ETFDH), energy in the form of ATP and H_2O are formed as the end products by the oxidative phosphorylation process. Therefore, the rate of production CO_2 and H_2O is proportional to the β-oxidation rate of the fatty acids.

For analysis of overall FAO rate, one approach is to measure the rate of production of radioactive CO_2, either as $^{14}CO_2$ and/or the radio-active acid soluble fatty acid metabolites after loading cells with ^{14}C labeled substrates, such as oleate or octanoate (18). Table 1 summarizes the in vitro overall FAO rate assays published in the past two decades. In general, these assays can give a satisfactory result on confirming defective overall FAO oxidation rates. But at times it might be difficult in result interpretations due to the wide normal range and poor inter-assay variability. Furthermore, during early phase of FAO only approximate 20% of acetyl moiety is converted to carbon dioxide which has limited the sensitivity of the method. On the other hand, the determination of acid soluble products is only feasible for medium- and long chain-fatty acids.

Table 1. Summary of assays of fatty acid β-oxidation rate in different biological samples.

AUTHOR (Reference)	Year of publication	Cell/Specimen	Probes, μmol/L	Duration of incubation	Measurement techniques
Saudubray el al (18)	1982	Fibroblasts	Oleate C-14, 250	6 hours	$^{14}CO2$ release
Moon et al (19)	1987	Fibroblasts	[9,10-^{3}H]-Palmitate, 22	4 hours	^3H_2O release
Manning et al (20)	1990	Fibroblasts	[9,10-^{3}H]-Palmitate/ myristic, 100	4 hours	^3H_2O release
Brivet et al (21)	1995	Lymphocytes	[9,10-^{3}H]-Palmitate/ myristic, 100	2 hours	^3H_2O release
Olpin et al (22)	1997	Fibroblasts	[9,10-^{3}H]-Palmitate/ myristic/oleic acids,100	4 hours	^3H_2O release
Seargeant et al (23) Narayan et al (24)	1999 2005	Whole blood Fibroblasts	[9,10-^{3}H]-Palmitate, [9,10-^{3}H]-Palmitate, 110	2 hours 2-3 hours	^3H_2O release ^3H_2O release

Oleate C-14=^{14}Carbon labelled oleate; ^{3}H=tritium labelled substrates

In view of these limitations in assays using 14C labeled substrates, tritium-labeled substrates based assays were developed. These assays are based on generation of 3H2O from cultured cells fed with various tritium-labeled fatty acids e.g. [9,10-3H]-palmitic acid, [9,10-3H]-myristic acid

and [9,10-3H]-oleic acid (19-24). There are obvious advantages of using tritium-labeled over 14carbon-labeled substrates include their higher specific activities and lower cost. Moreover, over 75% of the label is recovered as 3H2O in the FAD- and NAD-linked dehydrogenation steps of the β-oxidation spiral, and subsequent release during oxidation of acetyl-CoA in the Kreb's cycle allowing a better assay sensitivity than using radio-active labeled carbon substrates.

The oxidation rate assay provides an assessment of the overall measurement of flux rate through the FAO spiral. A defect in FAO will lead to a reduced rate of FAO but the oxidation rate result does not locate where the enzyme blockage occurs. Therefore, a different kind of assay was developed to locate the blockage in the FAO pathway.

Profiling of accumulated acyl-CoA and acylcarnitine intermediates due to an enzyme blockage

In order to detect a specific defect in the FAO pathway, in vitro methods have been developed to determine the acyl-CoA and/or acylcarnitine profiles by use of radio-HPLC and later by tandem MS. Any blockage in the pathway should result in accumulation of intermediate metabolites preceding the blockage.

Table 2 summarizes these profiling methods published since 1991. Kler et al was the first to develop a profiling method using isolated mitochondria or permeabilized cells fed with radio-labelled palmitoyl-CoA (25). The radio-active acyl-CoA and acylcarnitine esters formed were separated by HPLC and the radio-activities were measured by radiation counter (radio-HPLC). This method proved to be able to differentiate patients with electron transfer flavoprotein (ETF), ETF-dehydrogenase (ETFDH) and medium chain acyl-CoA dehydrogenase deficiency from normal control subjects. However, wider application of this method is limited by use of radio-active substrate and labor-intensive separation by radio-HPLC. A few years later, a novel method was developed by Nada et al, in which intact fibroblasts or lymphoblastoid cells were incubated for 96 hours with an in-house synthesized stable isotope labeled substrate ([17,17,18,18-$^{2}H_{4}$]linoleic acid) and followed by analysis of the acylcarnitines generated using the ESI-MSMS (27). The profiles obtained were always characteristic and specific to most individual enzyme defects in the FAO pathway. Although this deuterated linoleic acid was not commercially available, it has been shown that other substrates such as [16-$^{2}H_{3}$]palmitic acid, [U-^{13}C]palmitic acid and many others, can also be used (see references in Table 2). Nowadays, these acylcarnitine profiling methods have gained wide acceptance in many diagnostic laboratories as the first line assay to diagnose FAOD.

Combined assay for overall FAO rate and acylcarnitine profiling

Narayan et al have pointed out that the main advantage of the overall FAO rate assay is that it remains as the only true kinetic (rate) measurement of flux through the FAO pathway, whereas the acylcarnitine profiling method using ESI-MSMS can accurately delineate the exact site of an enzyme defect, making the two approaches complementary (24). In fact, both assays are complimentary to each other. However, two separate incubations are needed if both data of kinetic rate and profiling are required. Because of this, we recently developed a novel

functional assay for total FAO rate assay by measurement of deuterated water enrichment using isotope ratio mass spectrometry (IRMS) which also simultaneously performs the conventional acylcarnitine profiling by ESI-MSMS using a single tracer incubation experiment (10). The method has a few unique features which are distinct from previously methods. Firstly, a universal deuterium-labeled palmitic acid as substrate is used which is oxidized to form deuterated water and deuterium-labeled acylcarnitine intermediates in the FAO spiral and Kreb's cycle. Secondly, we employed a glucose free medium for cell culture that simulates fasting and will probably increase the sensitivity of the assay to detect defects in FAO. In this study, we demonstrated that using the combined assay, most FAO defects including carnitine-palmitoylcarnitine transferase I, carnitine-palmitoylcarnitine transferase II , carnitine-acylcarnitine translocase, very-long-chain acyl-CoA dehydrogenase, medium-chain acyl-CoA dehydrogenase, short-chain acyl-CoA dehydrogenase, long-chain 3-hydroxyl-acyl-CoA dehydrogenase and mitochondrial trifunctional protein deficiencies, could be differentiated from the control cells by their decrease in the delta ppm of $^{2}H_{2}O$ enrichment measured by IRMS and concomitant accumulation of characteristics acylcarnitines resulting from the blockage of specific enzymes.

Table 2. Comparsion of acylcarnitine profiling assays for detection of blockage in the fatty acid β-oxidation pathyway

Author (Ref.)	Year of publication)	Cells	Palmitate probes, μmol/L	L-Carnitine mmol/L	Incubation time	Measurement techniques
Kler et al (25)	1991	Fibroblasts	C-14, 0.12	1	30 minutes	radio-HPLC
Pourfarzam et al (26)	1994	Fibroblasts	C-14, 0.073	1	60 minutes	radio-HPLC
Nada et al (27)	1995	Fibroblasts	H-2 , 400	0.4	72 hours	ESI-MSMS
Schaffer et al (28)	1995	Leukocytes	C-14, 50	2.5	4 hours	radio-HPLC
Ventura et al (29)	1999	Fibroblasts	UC-13, 200	0.4	96 hours	GC-CI-MS,FABMS
Shen et al (30)	2000	Fibroblasts	Unlabelled, 200	0.4	96 hours	ESI-MSMS
Jones et al (31)	2001	Fibroblasts	Unlabelled, 100	not mentioned	24 hours	GCMS
Tyni et al (32)	2002	Fibroblasts	UC-13, 300	1.8	90 minutes	ESI-MSMS
		Myoblasts	UC-13, 440	1.72	90 minutes	ESI-MSMS
Okun et al (33)	2002	Fibroblasts	Unlabelled, 200	0.4	96 hours	ESI-MSMS
Sim et al (34)	2002	Fibroblasts	H-2 , 110	0.4	72 hours	ESI-MSMS
Young et al (35)	2003	Fibroblasts	UC-13,200; Unlabelled, 200	0.4	120;72 hours	ESI-MSMS
Schulze-Bergkamen et al (36)	2005	Peripheral blood mononuclear cells	Unlabelled palmitic,100	0.4	120 hours	ESI-MSMS
Roe et al (37)	2006	Fibroblasts	H-2 , 200	0.4	72 hours	ESI-MSMS
Law et al (10)	2007	Fibroblasts	UH-2, 200	0.4	96 hours	ESI-MSMS/IRMS

C-14=^{14}Carbon labelled palmitate; H-2=deuterium labelled palmitate; UC-13=Universal ^{13}Carbon labelled palmitate; radio-HPLC=radioactive tracer separation high performance liquid chromatography; ESI-MSMS=electrospray ionization tandem mass spectrometry; GC-CI-MS=chemical ionization gas chromatograph mass spectrometry; FABMS=fast-atom bombardment mass spectrometry; GCMS=gas chromatograph mass spectrometry; IRMS=isotope-ratio mass spectrometry

BRAINCHILD
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Fatty acid β-oxidation defects in Hong Kong Chinese

The Joint Metabolic Clinic at PWH/CUHK is the first multi-disciplinary Metabolic Clinic in Hong Kong which has been operating for almost 10 years. The clinic takes care of patients suffered from inherited metabolic diseases. The clinic receives input from multiple disciplines; include Pediatrics, Chemical Pathology, Anatomical and Cellular Pathology, Obstetrics and Gynecology, and bone marrow transplantation team. The JMC-laboratory, led by Prof. Nelson Tang, supports first-line diagnostic investigations, like plasma and urine metabolites (organic acids, and carnitine profiling) and advanced investigations like skin fibroblast cultures, enzyme assays and mutation analysis which are essential for making a definitive diagnosis. Samples for first-line investigations came from hospitals of the NTE cluster and other clusters, like UCH, KWH and private hospitals.

The 10 years' experience of treating more than 100 families with various IMD provided us a unique opportunity to reveal the spectrum of common IMD in the southern Chinese population, which had little information in the past. Here, I briefly review the spectrum of fatty acid *β*-oxidation defects found in our population (Fig. 1). FAOD commonly causes acute metabolic crisis during infancy and early childhood and is also a common metabolic cause of neuromuscular symptoms during infancy and early childhood. Most FAOD share a common prototype of clinical presentation: an acute metabolic presentation in a previously well infant/child, clinically with metabolic decompensation, hypoglycemia, liver derangement. The acute attacks are commonly precipitated by other trivial events like mild infection and fever. The characteristic hypoketotic hypoglycaemia is the well-recognized diagnostic indicator for FOAD.

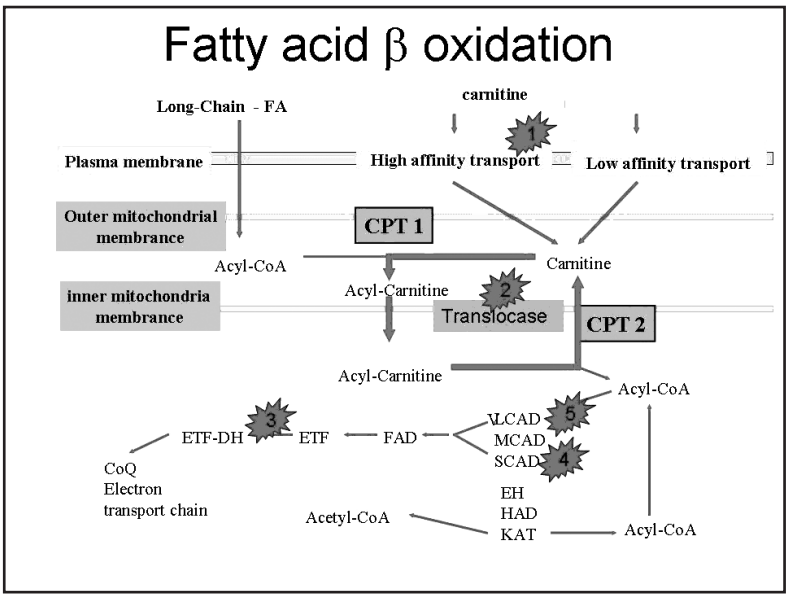

Fig. 1. Pathway of β-oxidation of fatty acid. Various defects found in Chinese are marked by numbers. 1. Carnitine transporter defect, 2. Carnitine-acylcarnitine translocase defect, 3. multiple acyl-CoA dehydrogenase deficiency, 4, short chain acyl-CoA dehydrogenase deficiency and 5. very long chain acyl-CoA dehydrogenase deficiency.

While medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is very common in Caucasians, no definitive case has been diagnosed in Chinese. Instead, several other FAOD are common in southern Chinese: namely primary carnitine deficiency, carnitine-acylcarnitine translocase defect, multiple acyl-CoA dehydrogenase deficiency and short-chain acyl-CoA dehydrogenase deficiency. These disorders will be reviewed here with a particular emphasis on the occurrences in southern Chinese..

Carnitine uptake defect (CUD)

Long chain fatty acids are transported across mitochondrial membranes by an acylcarnitine transport system where intra-mitochondrial β-oxidation is taking place. Thus, a sufficient intracellular level of carnitine is essential for fatty acid oxidation to proceed. Carnitine is taken up into the cytoplasm by an active transporter at the plasma membrane. Loss of function of this plasmalemmal transporter due to mutations will lead to deficiency of carnitine and impaired fatty acid oxidation (38). CUD (OMIM:212140) was also found responsible for significant proportion of sudden infant death cases in a prospective study (39). CUD is one of the IMD which is very responsive to treatment. Carnitine supplement corrected tissue carnitine deficiency, eliminated cardiomyopathy and little complication had been observed in the original case treated for over twenty years (40).

We diagnosed the first case of CUD in a family with sudden death of 2 infants in Hong Kong. The defect is caused by a mutated plasma membrane carnitine transporter, which actively pumps carnitine against a concentration gradient into the cell. The defect leads to an intracellular deficiency of carnitine. In-vivo investigation of the parents also demonstrated a renal wastage of carnitine. Subsequent genetic research in families described by various research teams led to the discovery of the disease gene, *OCTN2*, also known as SLC22A5 (41, 42, 43). Now this disorder has been diagnosed in patients from Macau, Mainland China and Taiwan. Studies of these samples revealed a common founder mutation (R254X) of *OCTN2* among southern Chinese (44). As most Chinese patients carried this mutation, it could be used as a screening marker in high-risk patients. Furthermore, the mutation is likely descended from a single ancestral founder, its frequency would be high in the population and we determined that carrier frequency was as high as 1% in southern China. Similarly, in a Japanese population in Akita, heterozygote frequency of another *OCTN2* mutation was also as prevalent as 1% in the population. The estimated incidence of live births affected was 1 in 40,000 (45). It suggests that CUD may be prevalent in Oriental populations.

Another common FAOD in Chinese is multiple acyl-CoA dehydrogenase deficiency. It can be caused by a genetic defect of either one of the subunits of electron transfer flavoprotein (ETF) or electron transfer flavoprotein dehydrogenases (ETF-DH). ETF and ETF-DH are responsible for the final steps of β-oxidation where electrons generated from different acyl-CoA dehydrogenases and branched-chain amino acid metabolism are transferred to the respiratory chain. Defects in either ETF or ETF-DH result in blockage of acyl-CoA dehydrogenase activity and characteristic organic acid pattern including elevation of 2-hydroxyglutaric acid, short to medium chain ayclglycines as well as dicarboxylic acids of different lengths.

Our patient registry had three unrelated southern Chinese families of MADD. All probands in these families presented with typical features of fatty acid oxidation defects. Mutation analysis on the genomic DNA confirmed that mutations of ETF-DH are involved in two families and ETF-A in one family (unpublished data). Furthermore, one additional case had been report from a previous series of our laboratory (2).

Other fatty acid oxidation defects

Carnitine-acylcarnitine translocase deficiency frequently presents with severe illness and causes death in neonatal period. In older child, it causes severe cardiomyopathy and has a high mortality. Notably, this defect was first discovered in a patient born to an American-Chinese parent in 1992 (46). Furthermore, the same splicing mutation (IVS2-10T/G) was found in another British-Chinese family and a local case of sudden death (47, 48). We have also performed pre-natal molecular diagnosis for a couple carrying the same mutation.

Short-chain acyl-CoA dehydrogenase deficiency is a defect among the last steps of the chain shortening reactions of FAO, breaking down short-chain fatty acid which has already been oxidized down to 6-carbon in length. Therefore, these patients could have some degree of emergency energy supply from FAO and are expected to present with mild disease. While most patients have mild phenotypes, however, some patients had more severe disease and died for yet unknown reason. The severe phenotype might be related to secondary blockage of other mitochondrial reactions due to accumulation of toxic metabolites. Furthermore, we diagnosed a local case of very long-chain acyl-CoA dehydrogenase deficiency in an adult patients presented with muscle weakness (49).

A diagnostic flowchart with criteria for differential diagnosis of FAOD using the new combined FAO flux and acylcarnitine profiling assay as second level investigation is proposed (Fig. 2). Similar protocol was proposed by Sim *et al* using two separate *in-vitro* assays, namely the conventional tritiated water release assay for FAO flux measurement and acylcarnitine profiling (50). We have incorporated a new parameter namely total even-chain acylcarnitines (TC) which can reliably differentiate CUD from other FAOD and normal controls, into this modified protocol. When the first line investigation is inconclusive e.g. when the serum acylcarnitine profile is abnormal but non-specific, or the results of confirmatory tissue enzyme assays are equivocal, the new *in-vitro* combined assay can be used for further investigation of the possible diagnosis. The findings of the combined assay would provide clues on one of the four possibilities: (i) a FAOD is unlikely; (ii) if there is an abnormal acylcarnitine profile and ^2H_2O enrichment is less than 55 ppm/mg protein/96h, the probable defect will be at the carnitine cycle or the FAO spiral; (iii) if the acylcarnitine profile is normal, ^2H_2O enrichment is less than 109 ppm/mg protein/96h and TC is less than 9.3 nmol/mg protein/96h, the defect is likely to be CUD; (iv) if the acylcarnitine profile is normal, ^2H_2O enrichment is less than 55 ppm/mg protein/96h and TC is less greater than 9.3 nmol/mg protein/96h, a probable defect in mitochondrial respiratory chain or non-FAO disorder is likely. This information will help the selection of confirmatory tissue enzyme or mutational analyses.

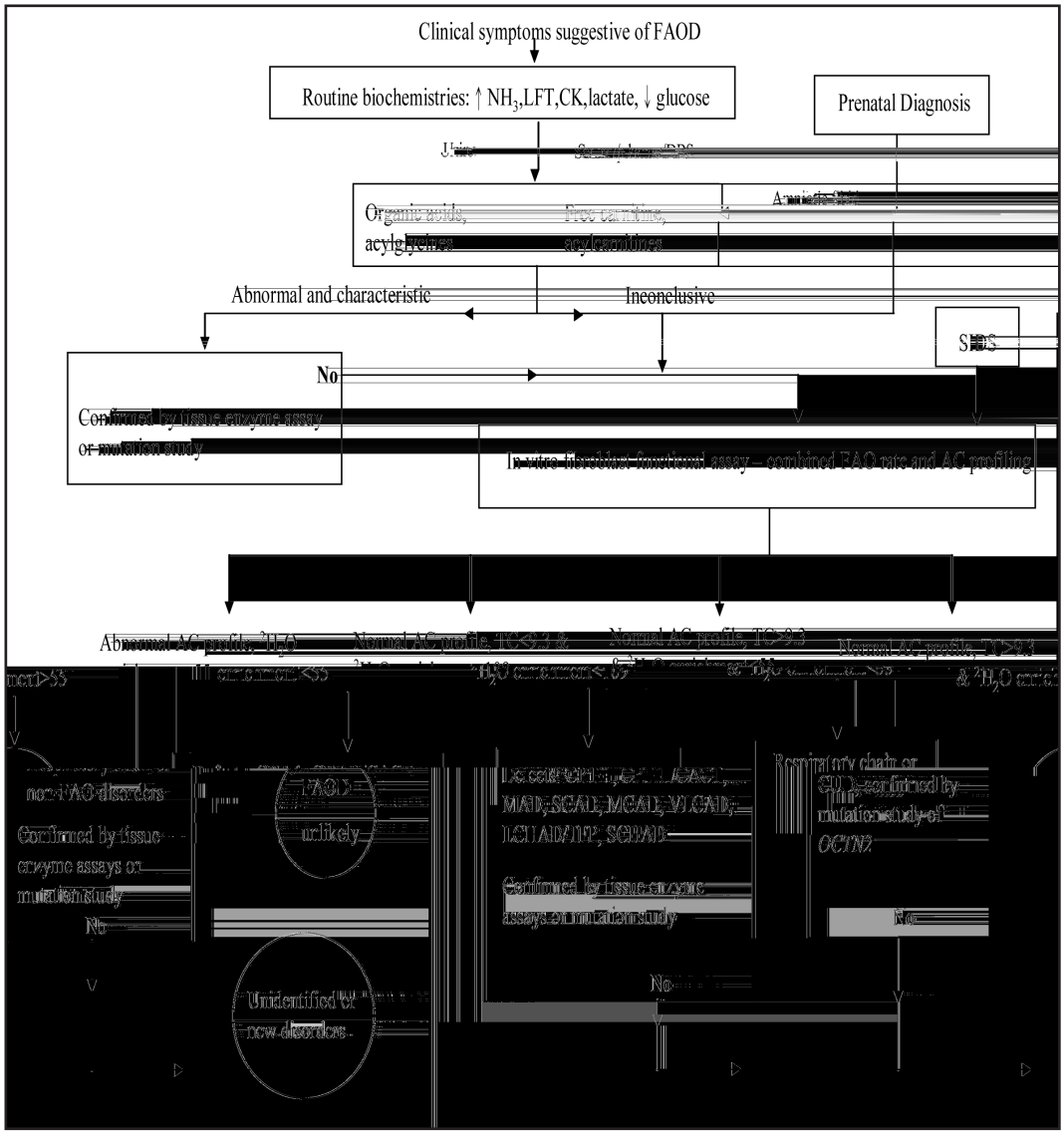

Fig. 2. Investigation flowchart for patients with suspected fatty acid oxidation disorders (FAOD). Criteria for differential diagnosis by combined overall FAO flux and acylcarnitine profiling assay are shown. Abbreviations: NH3: ammonia, LFT: liver function tests, CK: creatine kinase, SIDS: sudden infant death syndrome, FAO: fatty acid oxidation, AC: acylcarnitine, TC: total even chain acylcarnitines, CPT-1: hepatic carnitine palmitoyltransferase I, CPT-II: carnitine palmitoyltransferase II, CACT: carnitine acylcarnitine translocase, MAD: multiple acyl-CoA dehydrogenases, SCAD: short-chain acyl-CoA dehydrogenase, MCAD: medium-chain acyl-CoA dehydrogenase, VLCAD: very long-chain acyl-CoA dehydrogenase, LCHAD: long-chain L-3-hydroxyacyl-CoA dehydrogenase, TFP: trifunctional protein, SCHAD: short-chain L-3-hydroxyacyl-CoA dehydrogenase, CUD: carnitine uptake defect, OCTN2: sodium dependent organic cation/carnitine transporter.

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Genetic Study of CNS Diseases

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Introduction

Clinical Genetic Service (CGS) is the major service provider of genetic testing in Hong Kong. The development of various genetic investigations serves several purposes. First, genetic disorders can be confirmed, which has implications on prognosis and subsequent clinical management. Second, risk assessment during genetic counselling can be done more accurately, allowing patients or parents to make informed decisions on reproductive options. Third, the genetic defect can serve as a marker with which one can perform cascade screening in the family and prevent recurrence of the disease by means of prenatal diagnosis or even pre-implantation genetic diagnosis.

Chromosomal disorders

A lot of patients are referred to CGS for CNS diseases. Particularly, children with developmental delay, autism, and mental retardation constitute a major category of referrals. Karyotyping has been an important investigation in this group of patients, because a small but significant percentage of these patients are caused by various chromosomal abnormalities. From 1991 to 2007, 184 (6.6%) chromosomal abnormalities were detected in ~2791 patients referred for these reasons, not counting Down syndrome and other microdeletion syndromes (22q11.2 deletion syndrome, Williams syndrome, Smith-Magenis syndrome, etc.) that require fluorescence in-situ hybridization (FISH) for confirmation. Over the last 10 years or so, it became more and more recognized that cryptic subtelomeric chromosomal aberrations account for 5-10% of idiopathic mental retardation. It is important to identify these cases, because recurrence is not uncommon in these families. However, being "cryptic" means that these subtelomeric aberrations are difficult to detect with traditional cytogenetic techniques. In CGS, a combined molecular and FISH approach is adopted. A small study was done and detected 3 (15%) subtelomeric deletions among 20 unrelated MR patients.[1]

Fragile X syndrome

Fragile X syndrome is regarded as the commonest hereditary MR syndrome with an incidence of 1/4,000 – 1/6,000 males and accounts for 1-2% of MR patients. However, it is not so easily recognizable clinically. It is so named because in these patients the X chromosome may bear a fragile site at Xq27.3 region and the detection of this fragile site by cytogenetic technique was mainstay of diagnosis till early 90's. It is the first genetic disease found to be caused by trinucleotide repeat expansion. There is a polymorphic stretch of CGG trinucleotide repeats in the 5'UTR of exon 1 of the *FMR1* gene. In normal people, the number of CGG repeats is in the range of 6-54. Individuals with 55-200 CGG repeats are said to have a premutation; meiotic instability may occur, resulting in contraction or further expansion of the repeats in the offspring.

Individuals with >200 CGG repeats are said to have a full mutation. Full mutations are almost invariably associated with abnormal methylation of the gene and transcriptional silencing, with the result of a Fragile X syndrome phenotype. All males with full mutation manifest variable degree of mental retardation, whereas about half of the female full mutation carriers manifest. Therefore, Fragile X syndrome is said to be inherited in an X-linked semidominant fashion. In our genetic laboratory, Fragile X is detected by an initial screening with polymerase chain reaction (PCR) followed by confirmation with Southern blotting. A total of 34 index cases were identified since 1987. There was a positive family history of MR in about half of the patients. Mothers who had been tested were confirmed to be either premutation or full mutation carriers.

Rett syndrome

Rett syndrome (RS) is an interesting neurodevelopmental disorder. It predominantly affects females. The typical presentation is apparently normal development in the first 18 months, followed by developmental regression with loss of learned hand use and language skills, seizures, gait apraxia, deceleration of head growth, and midline stereotypic hand movements. The gene implicated in RS has been elusive until 1999, when Amir et al. (1999) found mutations in the *MECP2* gene in some patients with RS.[2] As a result of collaboration with the research group from Stanford University, a RS patient from Hong Kong was among the first patients who had the causative *MECP2* mutations identified.[3] Soon Lam et al. (2000) reported 6 MECP2 mutations in 13 local patients with classical RS.[4] CGS has been providing *MECP2* gene analysis for patients with suspected Rett syndrome since 2001. Up to now, 14 different mutations were identified in 18 unrelated patients. The mutations are heterogeneous, including *MECP2* gene deletion, single exon deletion, missense mutations, nonsense mutations, frameshift mutations and in-frame deletion. All these confirmed cases were sporadic and were females. None of the mothers who underwent testing carried the mutation. It is now known that *MECP2* mutations not only cause a classical RS phenotype in female, it also causes a severe encephalopathic phenotype in male. On the other hand, atypical or milder cases of RS were also found to be due to *MECP2* mutations, which aroused the debate whether routine *MECP2* testing should be offered to children with non-specific mental retardation or autism. So far evidence showed that *MECP2* mutations probably account for no more than 1% of these cases.

Prader-Willi and Angelman syndromes

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are clinically distinct disorders that are the result of similar genetic defects. These genetic defects involve the chromosome region 15q11-13. Owing to the presence of low copy repeats, this region is prone to deletion or duplication. To complicate it further, this region contains imprinted genes. Genomic imprinting is a term that describes the phenomenon of differential gene expression dependent upon the parent of origin of the alleles. Some genes express only when they are inherited from the father, while some others express only when they are inherited from the mother. About 70% of both PWS and AS patients are caused by microdeletion of this region. A PWS phenotype occurs when the microdeletion happens on the paternally inherited chromosome 15; an AS phenotype results when the microdeletion happens on the maternally inherited chromosome 15. The rest of the

PWS or AS patients are caused by other genetic or epigenetic defects like uniparental disomy 15 (UPD15) and imprinting defects. For AS, about 10% of patients are caused by mutations of a gene known as UBE3A. CGS has been using FISH to detect 15q11-13 microdeletion in cases of suspected PWS/AS since mid-90's. With the subsequent advances in molecular techniques, we are now able to diagnose over 98% of the PWS and about 90% of AS cases. At present, we have confirmed the diagnosis of 56 PWS and 38 AS patients. The genetic defects in the PWS patients are microdeletion (59%), matUPD15 (39%), and imprinting defect (2%). The genetic defects in the AS patients are microdeletion (66%), patUPD15 (11%), imprinting defect (5%), *UBE3A* mutation (13%) and undefined (5%).

Hereditary neurodegenerative diseases

This is a group of neurological disorders that usually present in adulthood and show anticipation, a phenomenon in which disease severity is increased and disease onset is pushed to an earlier age when the disorder is transmitted from one generation to the next. Quite a number of them are caused by polyglutamine tract expansion as a result of CAG trinucleotide repeat expansion in the respective genes. There are at least 10 disorders due to this kind of mutation. In our laboratory, we are testing for Huntington disease, spinal cerebellar ataxias (SCA type 1, 2, 3, 6, 7, 8, 12), dentatorubropallidoluysian atrophy (DRPLA), and Kennedy disease. With the exception of the X-linked recessive Kennedy disease, these disorders are of autosomal dominant inheritance. To date, we have confirmed the molecular defect of over 78 local families of SCA. SCA3 is the commonest type, with over 56 positive families. Among these neurodegenerative disorders, DRPLA may be of particular interest to paediatric neurologists, because in all three DRPLA families that we have confirmed, there are affected individuals who presented before 18 years of age.[5] Other neurodegenerative disorders which we have seen disease onset before 18 years are SCA3 (3/54 families) and Huntington disease (1/22 family).

Congenital central hypoventilation syndrome

Congenital central hypoventilation syndrome (CCHS) is a rare autosomal dominant disorder of the autonomic nervous system (ANS) characterized by an abnormal autonomic ventilatory response to progressive hypercarbia and sustained hypoxemia. Patients typically present in the newborn period with hypoventilation or apnea asleep, awake, or both, without any associated cardiac, pulmonary, neuromuscular or brainstem lesions. Rarely, some patients may present at a later age and are diagnosed to have late onset central hypoventilation syndrome (LOCHS). The central hypoventilation can occur as an isolated feature or in association with anatomic defects of the ANS. Notably Hirschsprung's disease (Haddad syndrome) was also present in 16-50% of patients; and tumours of the sympathetic nervous system such as neuroblastoma, ganglioneuroblastoma, and ganglioneuroma, were found in 5-10% of patients. *PHOX2B* gene defects cause >90% of cases, and more than 90% of the mutations result in polyalanine tract expansion. We reported three local male patients with CCHS ascertained because of persistent hypoventilation in the absence of primary pulmonary, cardiac, neuromuscular, brainstem and metabolic causes. Two of them also had Hirschsprung's disease. *PHOX2B* gene analysis detected a single nucleotide insertion and two other mutations that led to polyalanine tract expansion.[6] Two more patients, a boy and a girl, were diagnosed subsequent to that report.

Pelizaeus-Merzbacher disease

Pelizaeus-Merzbacher disease (PMD) is an X-linked disorder caused by *PLP1* gene defects. *PLP1* encodes the myelin proteolipid protein, an important component of CNS myelin formation. Patients, usually males, present with severe hypotonia of neonatal or infantile onset, nystagmus, and cognitive impairment, with progression to spasticity and ataxia at the later stage. MRI scan of the brain typically shows reduced white matter and myelination. The most common *PLP1* mutation is gene duplication. At CGS, 7 local PMD patients were confirmed, with 4 *PLP1* duplications and 3 point mutations.

Mitochondrial disorders

Suspected mitochondrial disorder is a fairly common reason of referral to CGS. This group of disorders are not easy to diagnose and probably are very much under-diagnosed currently for a number of reasons. There is a wide spectrum of clinical phenotypes. Some patients present with the well defined phenotypes of MELAS, MERRF, NARP, LHON, KSS and CPEO; while many others have non-specific phenotypes. Clinical severity also varies with the level of heteroplasmy and the distribution of the mutant in the body. Biochemical and even histological evidence of mitochondrial dysfunction are sometimes just secondary changes. Apart from those that cause the aforementioned, well defined phenotypes, mutations are generally heterogeneous. Furthermore, mitochondrial disorders are not necessarily caused by mtDNA mutations; mutations of nuclear encoded genes, notably those encoding subunits of the respiratory chain complexes, also cause mitochondrial dysfunction. So far, 10 families with MELAS, 2 families with NARP, 1 family with MERRF and 1 with LHON have been confirmed by CGS.

Conclusion

During the last 15 years, genetic study of CNS diseases has moved from the cytogenetic era into the molecular era. This is not to say that cytogenetics is dispensable; it remains an essential tool for the diagnosis of chromosomal disorders. With the completion of the Human Genome Project, knowledge of disease causing genes is growing with unprecedented pace. Together with advances in molecular technologies, genetic diagnosis for single gene disorders is more readily available. The demand for genetic study is ever increasing; CGS is prepared to meet the demand from the public.

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Seating Adaptation for Children with Neuromuscular Diseases

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Abstract

Children with severe neuromuscular diseases require different seating systems to reduce the formation of skeletal deformities. Customized seating system of suitable dimensions and various adaptive parts will maintain the best body alignment to reduce muscular effort in relation to gravitational force. Different considerations for children with various degree of impairment are discussed. Wheelchair with tilt-in-space features is a common prescription for children with poor head and trunk control by making use of the biomechanical advantage of the gravitational force. Children with progressive weakness may need different types of wheelchair at different stages of their disease progression. Key control for proximal stability for those with spasticity or dystonia can enable them to carry out higher cognitive functions, whereas those with poor cognitive function and severe physical impairment may have better basic functions and ease carers' burden in the daily care processes.

Keywords

Seating, Mobility, Neuromuscular Diseases, Wheelchair, Posture, Service

Introduction

Children with severe neuromuscular diseases who cannot ambulate or even maintain the sitting position are provided with a proper seating system with suitable mobility base as they have higher risk of developing deformities because of their abnormal muscle tone, poor head and trunk control, muscular weakness and skeletal asymmetry. Early provision of correct seating system together with appropriate orthoses could result in significant reduction in the formation of severe skeletal deformities such as scoliosis, kyphosis, hip dislocation and multiple joint contractures[1-3]. A proper seating system and mobility base can enhance the daily functions such as feeding, learning and ambulation. It can also facilitate development of self esteem and satisfies the inborn curiosity for children to explore the environment.

Seating Management and Considerations

The objectives of seating management are: (1) to provide sufficient external support to restore normal sitting posture and (2) to maximize upper body function and (3) to attain an even pressure distribution over the entire torso and extremities for preventing pressure sores.

1. Postural Management

For young children with severe neuromuscular diseases, a seating system with suitable dimensions to maintain the body alignment can prevent formation of deformities. Adaptive parts like head or neck supports, lateral trunk supports, pelvic stabilizers, thigh abduction devices and adjustable footrests could be applied.

For children with postural deformities which are still flexible, every effort is made to correct such deformities. When structural deformities have been developed over pelvis and spine, such as unilateral dislocated hip causing tilted pelvis with obliquity which can potentially lead to development of scoliosis, it is recommended to respect and accommodate the deformities to enhance comfort, to reduce pain by even out the pressure distribution and to facilitate ease of care. Children with good upper trunk control but poor sitting balance and poor lower limb control, such as Spina Bifida with high lesion, can be benefited from molded seat cushion to control pelvis position and increase stability so as to free the hands for wheelchair manipulation and other functional activities.

Children are prone to develop spinal deformities if foldable wheelchairs with sling back and seat upholstery have been used as the long-term seating devices. The sling seat could encourage hammock effect in which the thighs are kept in adducted and internally rotated positions. Children usually find it difficult to maintain balance in a wide chair, and hence the trunk leans to the sides especially when skeletal asymmetry has already developed. Besides, they have a tendency to slide out of the chair because upright sitting posture cannot be maintained by weakness of head and trunk control, increased muscle tone and tightness of hamstrings in relation to the position of the footrest.

Wheelchair with tilt-in-space features is a common prescription for children with poor head and trunk control by making use of the biomechanical advantage of the gravitational force (Figure 1). The feature provides a continuous range of tilt angle selection from -5° to 60°. The children can remain in the backward tilted position for taking rest, relieving pressure, and staying in the more upright position without altering the seat-to-back angle for active functions. However, the wheelchair dimensions increase gradually as the child grows. Such changes will result in great difficulties in relation to public transports and small household living area. The latest design makes the wheelchair more compact in size with increased stability and flexibility.

Fig. 1: Tilt-in-space wheelchair with wide range tilt angle selection

Wheelchair with back recliner can alleviate the muscular effort in maintaining the sitting posture but there is a strong tendency for the pelvis, together with the trunk, to slide forward (Figure 2). Reclined chair is prescribed for those with limited hip flexion range and those who cannot tolerate a 90° seat angle. It is also used to manage fracture or post-operative patients with long leg casts. In addition, the back reclination feature is useful during bladder catheterization for children with Spina Bifida.

Fig. 2: Wheelchair with back reclining system

Children with Spinal Muscular Atrophy presented with poor head and trunk control have difficulty in positioning the head with a simple head or neck support. Custom molded head support or modular supporting system could help in maintaining the head position.

Children might have a strong tendency to flex head forward involuntarily. The more backward tilting of the chair will enhance a more kyphotic posture. It is a difficult dilemma to balance the situation. A semi-rigid neck collar could be prescribed for controlling the head position in certain activities such as learning.

2. Seated Mobility

The requirements in ambulation are quite different for children with various neuromuscular diseases. Simple self-propelled manual wheelchair is prescribed for indoor mobility or outdoor transportation. For those children with progressive weakness in managing wheelchairs, they begin with a light-weight manual wheelchair and gradually move to power-assisted wheelchair. Power mobility is recommended eventually.

For Children with poor cognitive function, severe physical impairment and poor potential for self ambulation, the major concern will be the ease of carers in assisting ambulation. In view of the environmental limitations in Hong Kong, postural positioning and wheelchair accessibility have to be compromised. Carers are advised to have a simple light-weight foldable transit chair for outdoor transportation and to have another rigid type wheelchair adapted with specialized seating devices to provide optimal postural support for prolonged sitting during the daytime in school or at home.

3. Support for Functions

Limiting uncontrolled movements and maintaining head, trunk and pelvis alignment by appropriate supports, restraint systems and seating configuration definitely enhance proximal stability for children with spasticity or dystonia. With the key control of head, trunk and pelvis, postural stability and security can enable the child to carry out precise activities requiring high cognitive functions such as computer use, integrated environmental control and power mobility. Through this type of proper positioning, feeding, swallowing, chest function, visual perceptual abilities and communication can be improved. The children can have better appearance and thus self esteem is enhanced.

Biomechanics in Therapeutic Seating

1. Centre of Gravity and Sitting Posture

In normal sitting posture, the centre of gravity (CG) of head is located anterior to the thoracic spine. The spine experiences highest loading during sitting compared to standing and lying. Muscular force is required to maintain the upright position, and hence, muscular fatigue will develop. This results in kyphotic or scoliotic postures due to gravitational forces. A backward tilted chair will allow the head and upper trunk to be more posteriorly orientated in relation to the thoracic and lumbar spine so that the CG will be directed to the back (Figure 3). The relative change of orientation will reduce the muscular force and further minimize the axial loading applied to the spine.

Fig. 3: Tilt-in-space wheelchair with wide range tilt angle selection

2. Four-Point Support in Seating

Scoliosis could be corrected by applying forces at the end of rib at the apex of curve and counterbalanced by forces at the opposite side. The correction of scoliotic curvature can be achieved by making use of lateral supports arranged in 4-point manner (Figure 4). If the correction requires a very strong corrective force, lateral supports attached on the wheelchair are insufficient to control the curvature. In this situation, a spinal brace could be prescribed. Children with neuromuscular diseases usually have poor cardiopulmonary function and poor tolerance towards rigid spinal bracing, the semi-rigid spinal brace, which is made from soft lining reinforced by rigid thermoplastic material, can exert correctional forces to prevent deterioration of spinal deformities. With the spinal brace, children can be easily positioned in the wheelchair with optimal body alignment. Children with very rigid severe kypho-scoliosis and pelvis obliquity could be managed by molded back and seat cushion. Corrective acting and counteracting forces are applied in the supporting region of the molded cushion.

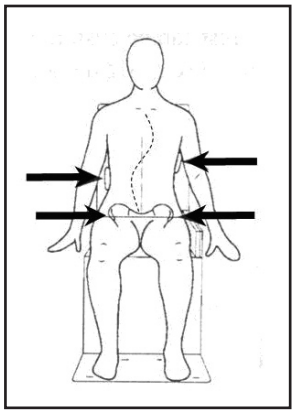

Fig. 4: 4-point support design to correct scoliotic curve

3. Seat Pressure Management

Decubitus ulcers are common in patients with poor nutrition (paucity of subcutaneous fat), poor sensation and poor ability in relieving pressure. Ulcers are also found in those who like to rock their trunk back and forth or slide against the seat surface resulting in a shearing force between contact areas. Even pressure distribution will minimize the risk of ulcer formation. Different materials can be used to relieve seat interface pressure. The main idea is to maximize the contact area and thus minimize local pressure by forming a contoured seat interface.

Air cushion is prescribed for prevention of sore formation in clients with poor lower limb sensation. However, it is unstable for those with fair sitting balance. Gel cushion has the same function in seat pressure redistribution but it is quite heavy. Gel and foam composite cushion is characterized by satisfactory cushioning performance and their weights are within manageable limits. As the gel material could be displaced from the pressure area after prolonged sitting, the gel content has to be reorganized regularly to ensure the desired effect is maintained. If ulcer has already developed, pressure mapping can be used to access the pressure area and to find out the causes by simulating different arms and trunk movements as well as different sitting postures. If the causes cannot be rectified, attempts to change cushioning materials will not solve the problem at all. Foam cushion with cut-out area under the pressure area could be used until the ulcer heals.

Recent Advances in Seating

There is significant reduction in the price of power wheelchair, thus high technology design is more affordable for wheelchair users. Manual wheelchairs with power assist design can allow children with progressive muscular weakness, such as Duchenne Muscular Dystrophy, to prolong their manual wheelchair stage and have a gradual change over to power mobility. The power

add-on design adapted to manual foldable wheelchair provides flexibility to switch over from power to manual chair for different occasions and to serve as backup while the motor unit is not functioning.

Features like manual or electrical tilting and standing can minimize repositioning of the child for certain activities. Standing exercise can be continued at home. Power wheelchair with function of seat elevation provides the clients with greater freedom to gain access to higher workspaces and achieves independence in daily activities.

Integrated Seating Clinic

In view of the increasing demand for specialized seating and wheelchair services, the first comprehensive multi-disciplinary Seating Clinic was established in 1994. The aim is to provide early intervention with respect to seating for children with neuromuscular diseases in Hong Kong. The service was initiated by the Department of Orthopaedics and Traumatology of the Chinese University of Hong Kong, in collaboration with the Rehabilitation Engineering Centre of the Hong Kong Polytechnic University. The clinic was established in Prince of Wales Hospital. The seating clinic is run by the seating team which composes of paediatric orthopaedic surgeons, rehabilitation engineers, physiotherapists, orthotists and occupational therapists. The seating team assesses the skeletal and neuromuscular conditions of the children and makes planning on the adaptive seating system. Custom-made seating devices are designed and fabricated to suit special demands. In order to ease the financial burdens on families in meeting the changing needs with growth of children who are still skeletally immature, the "Cathay Pacific Wheelchair Bank" was established with a charitable donation from the Cathay Pacific Airways in 1996. The wheelchair bank carries stocks of various types and sizes of wheelchairs with different replacement parts and a speedy service is available to the clients on a loan and recycle basis.

Conclusion

Proper seating for children with severe neuromuscular diseases can be a challenging task for both the children and the caregivers in that many issues including clinical, functional, technical and environmental factors have to be dealt with. The quality of service also depends on the professional knowledge of the seating team and the resources available to them. However, when one considers that the final beneficiary is the unfortunate child suffering from disabling neuromuscular diseases, provision of seating service is always an extremely worthwhile undertaking to accomplish.

Acknowledgement

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