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 Combined Issue on Inborn Error of Metabolism and Neurogenetics

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The Hong Kong Society of Child Neurology & Developmental Paediatrics

EDITOR'S NOTES for the August 2017 Issue

Inborn Error of Metabolism and Neurogenetics

Dr. Kwing Wan TSUI

This issue of BrainChild brings together two important areas in Developmental Paediatrics and Paediatric Neurology, inborn error of metabolism (IEM) and genetics. In Hong Kong, universal newborn metabolic screening has been supported by the Government and will be rolled out to each public hospital in stages. Dr. Josephine Chong highlighted in her article the importance of newborn metabolic screening and early detection and intervention can improve cognitive outcomes. Genetic testing is also recommended as the first line investigation in children with intellectual disability. Dr. Ivan Lo explained from chromosomal abnormalities to single gene defects and the less well studied epigenetic disorders as underlying etiology of intellectual disability. He would foresee that whole exome or whole genome sequencing would probably be the ultimate answer to some of the undiagnosed cases. Neuroimaging is another important modality that neurologists often use as part of workup for children with neurological problems. Dr. Jane Tsang provided a systemic approach to interpretation of MRI brain of IEM in children, which would be very helpful to the clinicians in their daily practices.

There are rare diseases which are always puzzling the clinicians. High index of suspicion and judicial use of investigational modalities could help diagnosing uncommon clinical conditions. Good clinical judgment comes with knowledge and experience. Dr. Eric Yau, Dr. Hencher Lee, Dr. Carol Siu and Dr. Wai Wai Cheng shared with us their cases and approach in some for the rare conditions, namely biotinidase deficiency, recurrent rhabdomyolysis, monoamine neurotransmitter diseases and vitamin B12 deficiency. I am sure these will be very interesting and educational.

Advocacy and community support is one of the objectives of HKCNDP. There are minorities with rare diseases and their needs are often overlooked, especially in a medical system with limited resources and overwhelming demands. An article in this issue, "A Message from Rare Disease Patients" from Hong Kong Mucopolysaccharidosis & Rare Genetic Diseases Mutual Aid Group pointed out various needs of this small patient group. A holistic approach should be adopted by clinician who is managing these patients despite the limitation in social resources.

Last but not the least, I would like to thank all the aforementioned authors, our guest editors, Prof. Marc Patterson, course director of our Annual Scientific Meeting 2013 on "Paediatric Neurometabolic Disorders" and Dr. Chloe Mak, Consultant Pathologist of Princess Margaret Hospital; and members of the editorial board, namely Dr. Florence Lee, Dr. Catherine Lam, Dr. Eric Yau, Dr. Josephine Chong, Dr. Jasper Chow and Dr. Wing Cheong Lee, who are very hardworking and make publication of this issue successful.

Dr. Kwing Wan TSUI

President
The Hong Kong Society of Child Neurology and Developmental Paediatrics

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Preface

Neurogenetic disoders are a group of clinically and genetically heterogeneous disorders affecting the differentiation and function of the neurological system. With the recent advances in genetics and genomics, there are expanded understanding into the disease pathogenesis, classification, investigations, diagnosis and management. This issue focuses on the discussion on clinical features, neuroradiologic studies, biochemical and genetic diagnostic approach of neurogenetic disorders. Three articles written by local experts are included: "The Genetics of Intellectual Disability" by Dr. Ivan Lo, "An Approach to Recurrent Rhabdomyolysis" by Dr. Hencher Lee, and "MRI brain of Inborn Errors of Metabolism in Children" by Dr. Jane Tsang.

2017

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Inborn Errors of Metabolism disorder causing Intellectual disability: What is the role of newborn metabolic screening?

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Introduction

Intellectual disability (ID) is one of the largest health care, economic, and social burdens in the modern health care system and society. It affects around 2-3% of children and adults worldwide. ID is a lifelong and debilitating condition; it is defined as a disorder with onset during the developmental period that includes both intellectual and adaptive functioning deficits in conceptual, social, and practical domains, and it is associated with a wide range of neurological disabilities, epilepsy, and behavioural symptoms with autism, hyperactivity, self-injurious behavior, and aggressive behaviour[1]. Of the diverse aetiologies of ID, more than 50% of them are genetic. Other aetiologies of ID may include antenatal, perinatal, and postnatal factors; it may result from neurological disease, infection, prematurity, intoxication, trauma, teratogens, and radiation. Children below 5 years of age, typically present with significant deficits (below 2 standard deviations) in two or more developmental domains, which is defined as global developmental delay. Genetic anomalies associated with ID include chromosomal abnormalities, copy number variation, methylation disorders, and single gene mutations. Disease-modifying therapy is not available for most ID patients with rare genetic conditions. Those with inborn errors of metabolism (IEM) represent a small subgroup, for some of which disease-modifying therapies are available. The outcome is expected be significantly improved if IEM patients are identified and treated before neurological injury occurs. Literature review showed that IEM disorders could be identified in 2.8% of ID patient with second line biochemical metabolic testing. Among inborn errors of metabolism, 81 disorders are identified as amendable to treatment[2,3]. The benefits of early intervention for treatable inborn errors of metabolism justify population-based screening, despite the low incidence of these disorders. This article highlights the history and principles of newborn metabolic screening, in addition the supplementary biochemical testing will be discussed as investigations for patient with intellectual disability.

Inborn Errors of Metabolism and history of newborn metabolic screening

"Inborn errors of metabolism" (IEM) describes a class of genetic disorders, which are mostly due to single gene defects resulting in defective function of gene products, mainly enzymes, that catalyze the conversion of substrates into products. These disorders are associated with the accumulation of toxic intermediary metabolites or reduced production of essential downstream metabolites. Although the incidence of individual IEM is low, the collective incidence of IEM is estimated to be around 1 in 4,122 to 5,000 live births in

Hong Kong in local case series[4,5,6]. Based on screening of over 17,000,000 newborns, the prevalence of IEM in China[7,8] was estimated to be 1 in 5,800.

Expanded newborn metabolic screening (NBS) of inborn errors of metabolism (IEM) is a comprehensive program for early detection of pre-symptomatic IEM patients. The goal of such programs is to reduce disease related mortality and morbidity by offering affected individuals early treatment and intervention. 50 years ago, Robert Guthrie demonstrated the feasibility of using dried blood spot (DBS) filter paper cards to perform a bacterial inhibition assay to detect abnormal level of phenylalanine metabolites in patients with phenylketonuria (PKU) presenting with intellectual disability[9]. PKU is an inborn error of metabolism, usually caused by a deficiency of phenylalanine hydroxylase, which leads to intellectual disability and neurobehavioral abnormalities if not treated early. This was the first IEM disorder included in the mandatory newborn screening panel in many countries. Through early diagnosis and treatment with a phenylalanine-restricted diet, long-term intellectual disability can be prevented in PKU patients.

As diagnostic technology has evolved, gas chromatographic (GC) analysis or high-pressure liquid chromatography (HPLC), coupled with mass spectrometry (MS), were later used to detect abnormal metabolites for the diagnosis of IEM[10,11]. In the last two decades, a major revolution took place in the field of expanded newborn metabolic screening when tandem mass spectrometry (MS/MS) technology was employed to characterize diagnostic acylcarnitine and later amino acid profiles. Electrospray ionization (ESI) coupled with MS/MS[12,13] can now offer a high throughput screening measuring multiple analytes of amino acids and acylcarnitines simultaneously. By incorporating this technique, the newborn screening panel could be "expanded" to simultaneously detect numerous IEM. There are still vast differences in the practice of NBS in different countries[14-18] depending on individual country's public health care system burden, public awareness, professional education, and available expertise in the field.

Example of IEM disorders presenting with intellectual disability

A recent literature review 2 identified a total of 81 IEM disorders presenting with intellectual disability as a major feature, including disorders of amino acids, cholesterol and bile acids, creatine, fatty aldehydes; glucose homeostasis and transport; hyperhomocysteinemia; lysosomes, metals, mitochondria, neurotransmission; organic acids, peroxisomes, pyrimidines, urea cycle, and vitamins/co-factors. (Table.1)

Table 1. List of 81 IEM disorders leading to intellectual disability

Biochemical category	Disease name	
Amino acids	HHH syndrome (hyperornithinemia, hyperammonemia, homocitrullinemia)	PHGDH deficiency (Serine deficiency)
	Non-ketotic hyperglycinemia	PSAT deficiency (Serine deficiency)
	Phenylketonuria	PSPH deficiency (Serine deficiency)
		Tyrosinemia type II
Cholesterol & bile acids	Cerebrotendinous xanthomatosis	
	Smith–Lemli–Opitz Syndrome	

Biochemical category	Disease name	
Creatine	AGAT deficiency	
	Creatine transporter Defect	
	GAMT deficiency	
Fatty aldehydes	Sjögren–Larsson syndrome	
Glucose transport & regulation	GLUT1 deficiency syndrome	
	Hyperinsulinism hyperammonemia syndrome	
Hyperhomocysteinemia	Cobalamin C deficiency	Cobalamin G deficiency
	Cobalamin D deficiency	Homocystinuria
	Cobalamin E deficiency	MTHFR deficiency
	Cobalamin F deficiency	
Lysosomes	α-Mannosidosis	Niemann–Pick disease type C
	Aspartylglucosaminuria	Sanfilippo syndrome A (MPS IIIa)
	Gaucher disease type III	Sanfilippo syndrome B (MPS IIIb)
	Hunter syndrome (MPS II)	Sanfilippo syndrome C (MPS IIIc)
	Hurler syndrome (MPS I)	Sanfilippo syndrome D (MPS IIId)
	Metachromatic leukodystrophy	Sly syndrome (MPS VII)
Metals	Aceruloplasminemia	
	Menkes disease/Occipital horn syndrome	
	Wilson disease	
Mitochondria	Co enzyme Q10 deficiency	
	MELAS	
	PDH complex deficiency	
Neurotransmission	DHPR deficiency (biopterin deficiency)	SPR deficiency (biopterin deficiency)
	GTPCH1 deficiency (biopterin deficiency)	SSADH deficiency
	PCD deficiency (biopterin deficiency)	Tyrosine Hydroxylase Deficiency
	PTPS deficiency (biopterin deficiency)	
Organic acids	3-Methylcrotonyl glycinuria	HMG-CoA lyase deficiency
	3-Methylglutaconic aciduria type I	l.o. Isovaleric academia
	β-Ketothiolase deficiency	Maple syrup urine disease (variant)
	Cobalamin A deficiency	Methylmalonic academia
	Cobalamin B deficiency	MHBD deficiency
	Ethylmalonic encephalopathy	mHMG-CoA synthase deficiency
	Glutaric acidemia I	Propionic academia
	Glutaric acidemia II	SCOT deficiency
Peroxisomes	X-linked adrenoleukodystrophy	
Pyrimidines	Pyrimidine 5-nucleotidase superactivity	
Urea cycle	Argininemia	
	Argininosuccinic aciduria	CPS deficiency
	Citrullinemia	NAGS deficiency
	Citrullinemia type II	OTC Deficiency
Vitamins/co-factors	Biotinidase deficiency	Imerslund Gräsbeck syndrome
	Biotin responsive basal ganglia disease	Molybdenum co-factor deficiency type A
	Cerebral folate receptor-α deficiency	Pyridoxine dependent epilepsy
	Congenital intrinsic factor deficiency	Thiamine responsive encephalopathy
	Holocarboxylase synthetase deficiency	

Adapted from Van Karnebeek, C. D., & Stockler, S. (2012). Molecular genetics and metabolism, 105(3), 368-381.

More than half of all IEM can be identified by metabolic screening tests in blood (plasma amino acids, homocysteine) and urine (creatine metabolites, glycosaminoglycans, oligosaccharides, organic acids, pyrimidines). Newborn metabolic screening can screen out subgroup of the listed disorders, including amino acid disorders, organic acid disorders, and fatty acid oxidation disorders (Table 2). In Hong Kong, the current mandatory newborn screening program[19] uses cord blood to screen for Glucose-6-phophate dehydrogenase deficiency (G6PDD) and congenital hypothyroidism only. The Center of Inborn Errors of

Metabolism (CIEM) of the Chinese University of Hong Kong (CUHK) implemented a voluntary participation fee-for-service expanded NBS screening program[20] in July 2013. The program offers screening for 30 IEM using dried blood spot filter paper cards and tandem mass spectrometry (MS/MS) technology. This expanded newborn metabolic screening programme identifies some of the treatable IEM disorders, which, when untreated, lead to intellectual disability. In the latest Government policy address[21], it states that the Pilot Study on Newborn Screening for Inborn Errors of Metabolism implemented in two public hospitals since October 2015 has proven effective, the Department of Health (DH) and the Hospital Authority (HA) plan to extend the screening service to all public hospitals with maternity wards in phases from the second half of 2017-18.

Table 2: Examples of IEM disorders detectable with tandem MSMS

Inborn Errors of Metabolism Categories	Example of target IEM disorders
Amino acid disorders	Phenylketonuria, Maple syrup urine disease, Citrullinaemia type 1, Argininosuccinic aciduria, Homocystinuria, Tyrosinaemia type 1, Arginase deficiency, Defects of biopterin cofactor biosynthesis and regeneration, Citrullinaemia type 2, Hypermethioninaemia,
Organic acid disorders	Propionic acidaemia, Isovaleric acidaemia, Glutaric acidemia type 1 , Methylmalonic aciduria , Beta-ketothiolase deficiency, Multiple carboxylase deficiency
Fatty acid oxidation disorders	Carnitine uptake defect, Medium-chain acyl-coA dehydrogenase deficiency, Very long-chain acyl-coA dehydrogenase deficiency, Carnitine palmitoyltransferase I /II deficiency, Multiple acyl-CoA dehydrogenase deficiency

Adapted from J.S.C. Chong (2014), The Hong Kong Medical Diary, 19 (12), 5-8

IEM causing intellectual disability: Role and limitations of expanded newborn metabolic screening

The composition of the panel of each newborn metabolic screening programme determines the efficiency and limitations of that screening test in detecting IEM. It is essential to be aware of the biochemical analytes being screened in the local screening programme. Most of the expanded newborn metabolic screening panels include amino acid and acylcarnitine analytes. Those IEM disorders that will present with intoxication or acute metabolic decompensation, can be detected by newborn metabolic screening. The intoxication or decompensation may lead to permanent neurological damage with or without hyperammonaemia, and thus intellectual disability. The goal of all screening programmes is timely diagnosis, which result would be available within 1-2 days and offer the opportunity for treatment before symptom develop.

It must be acknowledged that not all IEM disorders are detected by the most of the current newborn metabolic screening programmes. In assessing patients presenting with intellectual disability, secondary biochemical testing should be considered if the newborn metabolic screening is unremarkable. The clinical history of the presenting condition and associated symptoms of the patient should be analyzed for planning proper biochemical investigations.

Table 3. List of biochemical testing in investigation of the treatable IEM disorders

Test	IEM disorders	
Urine organic acids	Beta ketothiolase Deficiency Cobalamin A deficiency Cobalamin B deficiency Cobalamin C deficiency Cobalamin D deficiency Cobalamin F deficiency Ethylmalonc encephalopathy Glutaric acidemia type I & type II HMG-CoA lyase deficiency Holocarboxylase synthetase deficiency	Homocystinuria Isovaleric acidemia -methylcrotonyl glycinuria 3-methylglutaconic aciduria Methylmalonic acidemia MHBD deficiency HMG-CoA synthetase deficiency Proprionic acidemia SCOT deficiency SSADH deficiency Tyrosinaemia type II
Urine glycosaminoglycans	MPS I, MPS II, MPS III, MPS VI	
Urine creatine metabolites	AGAT deficiency, GAMT deficiency, Creatine transporter deficiency	
Urine oligosaccharides	Mannosidosis Aspartylglucosaminuria	
Urine purines & pyrimidines	Purine 5' nucleotide superactivity Molybdenum Cofactor type A deficiency	
Plasma amino acids	Arginosuccinic aciduria Citrullinemia Citrullinemia type II CPS deficiency Argininemia HHH syndrome Maple syrup urine disease	MTHFR deficiency NAGS deficiency OTC deficiency Phenylketonuria PDH complex deficiency Tyrosinemia type II
Plasma total homocysteine	Homocystinuria MTHFR deficiency Cobalamin C deficiency, Cobalamin D deficiency, Cobalamin E deficiency, Cobalamin F deficiency, Cobalamin G deficiency	

Another concern is the availability of treatment, availability of special medications and medical foods are a concern in the medical system. It is an important element for preparation of metabolic screening, as the treatment should be commenced without delay for patient identified from screening programme.

Conclusion

Intellectual disability (ID) affects around 2-3% children and adults worldwide. It is one of the largest health care, economic, and social burdens in the modern health care system and society. Newborn screening (NBS) of inborn errors of metabolism (IEM) by tandem mass spectrometry (MS/MS) is a world recognized cost effective public health program to minimize morbidity and mortality associated with various IEM conditions. It helps to identify that subgroup of ID patients who have treatable IEM. The newborn metabolic screening programme plays a major role in identifying IEM patients in the neonatal period, and offering them early intervention, including dietary treatment and medical treatment before they experience metabolic decompensation. The prognosis for this group of patients is significantly improved with early identification and early intervention. In view of a territory-wide mandatory expanded newborn metabolic screening program will be implemented in Hong Kong. It is essential for physician to understand the scope and limitation of newborn metabolic screening testing. There are some IEM patients who will present with intellectual disability beyond neonatal period, who may have had negative newborn metabolic screening. The key of newborn screening programme depends on the coverage and design of the screening panel. Secondary biochemical testings are as essential as part of the initial workup,

so as to identify this group of patients for early treatment and intervention, and aiming at a better outcome. Genetic testing including array comparative genomic hybridization and exome sequencing should also be considered as part of investigations for patient with intellectual disability in complementary to negative metabolic biochemical testing to delineate the underlying aetiology of intellectual disability.

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MRI brain of Inborn Errors of Metabolism in Children

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Introduction

The diagnosis of the inborn errors of metabolism (IEM) is challenging. Their presenting symptoms and signs are often non-specific and with variable chronicity. The imaging appearance of many IEM overlaps and, frequently, varies with the stage and the variant of the disorder. Neuroradiologists largely rely on the magnetic resonance imaging (MRI) pattern recognition coupled with clinical information and laboratory results to narrow down the differential diagnosis to a certain type of disorder – metabolic/toxic or demyelinating – but rarely is diagnostic.[1] Almost 60% of inherited white matter diseases remain without a specific diagnosis.[2,3]

Furthermore, many acquired diseases, such as periventricular leukoencephalopathy, inflammatory and infectious processes, acquired metabolic disorders with nutritional deficiencies, and toxic injuries, can present with radiological appearance that mimic those changes similar to those associated with IEM.[4]

A systematic approach based on the pattern of brain involvement can be useful in the analysis of neurometabolic disorders by imaging.[1,3,5] In this article, a brief overview of MRI brain pattern based approach to the investigations of IEM will be presented; a complete discussion of IEM is well beyond the scope of this article. Interested readers are referred to textbooks on the subject, such as those by Backovich1, van der Knaap and Valk[5,6] or Scriver et al.[7,8]

In addition, it is of paramount importance to beware of the sensitivity and specificity of individual radiological signs might be hard to determine among rare disease such as IEM, thus the absence of certain radiological signs cannot perfectly "rule out" a specific disease.

Normal myelination

Before analyzing the pathologies, it is important to be familiar with the imaging appearance of the normal myelination progression. The myelination process starts during intrauterine life and continues after birth in an orderly timely manner, commencing in the brainstem with progression to the cerebellum and the cerebrum.[4,9] A simplified general rule of myelination progression is that it proceeds from caudal to rostral, from central to peripheral, and from dorsal to ventral. Myelination is usually complete on T1-weighted images by age 1 year (between 8 and 12 months) and on T2-weighted images at age 2 years (between 18 and 24 months).[9,10] T1-weighted images are very useful to assess myelin injury and delayed or hypomyelination during the first year of life. T2-weighted images are usually much more useful than T1-weighted images in the assessment of myelination and white

matter injury after the first birthday; however, T1-weighted images are still useful to look for discrepancies between the T1 and T2 appearances, which might give a clue to diagnosis in hypomyelinating disorders (such as in 18q deletion syndrome). T2 FLAIR images are superb for the detection of supratentorial white matter changes in children beyond the age of 1 to 2 years (after myelination is largely completed).[1]

Overview of Simple Pattern Approach to the MRI Brain of Inborn Errors of Metabolism in Children

Gray Matter vs White Matter

In the global analysis of MRI images of brain, the first consideration is to determine which structure is predominantly involved: gray matter, white matter, or both. In general, disorders that primarily affect cortical gray matter will cause thinning of the cortex and, thus, have prominent cortical sulci. In the acute phase (during metabolic decompensation), most disorders primarily affecting deep gray matter will show swelling, reduced diffusivity, and either low attenuation on CT or T1 hypointensity and T2/FLAIR hyperintensity on MRI in the involved structures; chronically, the structures are shrunken and have increased diffusion. The cerebral white matter will often have an abnormal appearance in disorders of gray matter, as Wallerian degeneration of axons causes diminished white matter volume and decreased white matter attenuation on CT or mildly to moderately T2W hyperintense on MRI. This white matter appearance can often be differentiated from that of primary white matter disorders if the imaging study is performed early in the course of the disease, as the affected white matter will often be edematous and , therefore, brighter and more voluminous (causing compressed, smaller sulci) than the white matter that has undergone Wallerian degeneration.[11] Those disorders primarily affecting white matter (other than hypomyelinating disorders) cause marked hypodense lesion on CT or T2W hyperintense lesion on MRI before any volume loss is apparent.[1] Many white matter diseases can result in devastation of the involved areas, with necrosis and cavitation of the affected region and subsequent ex-vacuo dilation of the ventricles, whereas the abnormal white matter in gray matter disorders appears less severely damages. Finally, the clinical presentation of patients with cortical gray matter disorders (seizures, dementia in early stages) differs from that of deep gray matter disorders (chorea, athetosis, dystonia) and both differ from the presentation of white matter disorders (spasticity, hyperreflexia, ataxia); clinical information in very useful to get started on the right track.

1. Predominant Gray Matter Disorders

Once identified as being primarily of gray matter, the next step is to determine whether the cerebral cortex or the deep gray matter nuclei are primarily involved. This would be determined by assessing the deep gray nuclei for abnormal signal intensity on T2W or FLAIR imaging.[11] To determine cortical involve, a specific scrutiny for sulcal effacement, cortical swelling and reduced diffusion in acute phase or cortical thinning with sulcal enlargement in chronic phase, and abnormal signal intensity of the cortex may be helpful.[1]

1.1 If only deep gray matter is involved, then the specific structure that are affected and their signal intensity should be identified.

1.1.1 *Striatum (caudate and putamen)*

Involvement of the striatum (caudate and putamen) is observed in Leigh syndrome (a.k.a.subacute necrotizing encephalomyelopathy)[12], Mitochondrial myopathy, encephalopathy with lactic acidosis, and stroke-like episodes (MELAS)[13], propionic academia14, Wilson disease[15], Hypermanganesemia with Dystonia, Polycythemia and Cirrhosis (HMDPC) due to mutation in the *SLC30A10* gene[16], juvenile Huntington's disease[17], molybdenum cofactor deficiency, isolated sulfite oxidase deficiency[18], asphyxia, and hypoglycemia (Table 1). Many of these diseases often have associated white matter or cortical injury.[11]

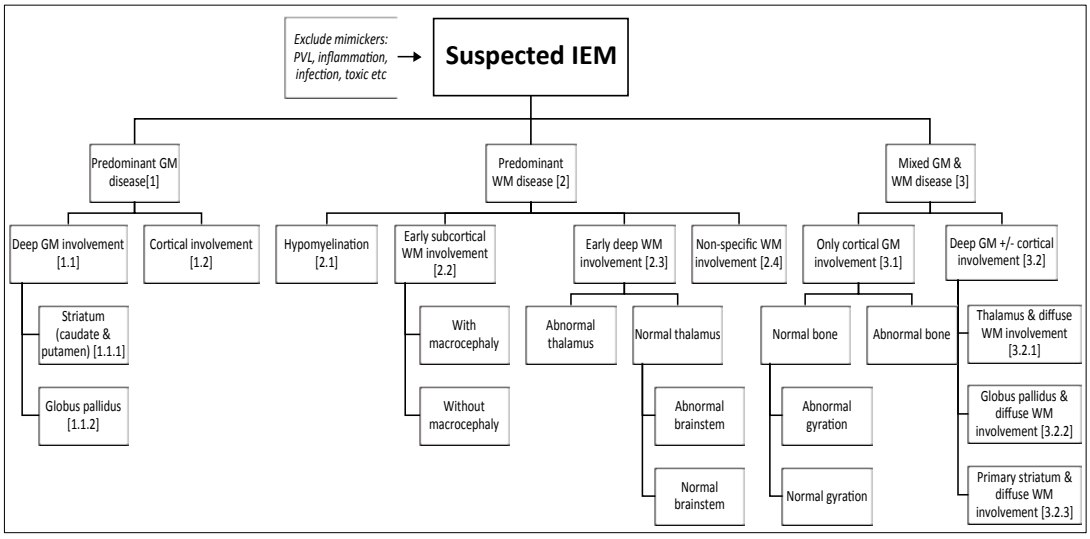


Figure 1. Algorithm of the pattern approach of neuroimaging in the analysis of inborn errors of metabolism (IEM). GM=Gray matter, WM=White matter. Please refer to text for the disease entities. [ ] indicates the related paragraph.

Table 1. Pediatric metabolic disorders with T2W or FLAIR hyperintensity of striatum
Leigh syndrome (usually with white matter involvement)
Propionic acidemia (usually with white matter involvement)
Wilson disease (often with white matter involvement, periaqueductal)
Juvenile Huntington disease
Molybdenum co-factor deficiency, isolated sulfite oxidase deficiency

Leigh syndrome follows a similar pattern of bilateral, symmetrical basal ganglia or brainstem changes. Lesions in Leigh syndrome evolve over time and a lack of visible lesions does not exclude the diagnosis. Reversibility of lesions is seen in some patients, making the continued search for treatment and prevention a priority for clinicians and researchers.[19] Lesions of the lower brain stem were always present when patients had near fatal respiratory failure. However, upper brain stem lesions were transient and were found in parallel to reversible respiratory disorder. Fatal respiratory failure was unpredictable from clinical or neuroradiologic findings.

Brain stem lesions are associated with the loss of respiratory control in patients with Leigh syndrome, but the time at which fatal respiratory failure will occur is unpredictable.[20]

1.1.2 *Globus pallidus*

If only globus pallidus is involved, showing T2W hypointensity or T2W hypointensity with central T2W hyperintensity, the diagnosis of pantothenate kinase associated neuropathy (formerly called Hallervorden-Spatz disease)[21,22] or oculodentodigital dysplasia[23] can be suggested; these are easily differentiated on clinical examination[1].

If isolated globus pallidus involvement shows T2W hyperintensity, methylmalonic acidemia[24], guanidinoacetate methyltransferase (GAMT) deficiency (being one of the creatine deficiencies, which shows low creatine level on MR spectroscopy[25,26], isovaleric academia[27], pyruvate dehydrogenase E2 deficiency (due to mutation of the dihydrolipoamide acetyltransferase (E2) component)[28], carbon monoxide poisoning, or the chronic phase of kernicterus should be considered.[1,6] The neuroimaging findings of succinic semialdehyde dehydrogenase deficiency may show T2W hyperintensities in multiple regions, most commonly in the globus pallidus, or may reveal delayed hypomyelination or may demonstrate no specific abnormality.[29]

If T2 or FLAIR hyperintensity of the globus pallidus is seen in association with subcortical white matter demyelination and involvement of the cerebellar dentate nuclei, L-2-hydroxyglutaric aciduria[30,31] and Kearns–Sayre syndrome[32] should be considered. If T2W or FLAIR hyperintensity of the globus pallidus is associated atrophy of the dorsal brain stem and cerebellar dentate nuclei is seen, consider dentatorubropallidoluysian atrophy.[33-35] T2W hyperintensities in basal ganglia associated with T2W hyperintensity of the peri-insular cortex with or without perirolandic cortex involvement would suggest a diagnosis of urea cycle disorders, such as ornithine transcarbamylase deficiency and citrullinemia type 1.[36-38] However, in urea cycle disorders, cerebral abnormalities change depending on the stage of disease.[39]

If T1W hyperintensity of the globus pallidus is seen associated with normal T2W signal, consider acute kernicterus, while T2W hyperintense change of the globus pallidus is observed in chronic stage kernicterus.[40]

1.2 If the imaging pattern indicates that it is primarily one of cortical involvement (cortical thinning with enlarged cortical sulci), consideration should be given to such disorders as the neuronal ceroid lipofuscinoses (which might associated with T2W hypointense change at thalami)[41], Rett syndrome (in girls)[42], Alpers disease (though MRI and pathological changes in white matter have been reported in Alpers syndrome)[43], glycogen storage diseases[44,45] or type 2 GM1 gangliosidosis[1,46].

2. Predominant White Matter Disorders

In many cases, symmetry is a striking feature of inherited white matter disorders and toxic encephalopathies, although not without exceptions, whereas asymmetry is most often seen in acquired white matter disorders, particularly inflammatory disorders and infections. The appearance of the lesions is important: isolated, or confluent, or both. In most inherited white matter disorders, in toxic encephalopathies, and in diffuse white matter injury after irradiation and chemotherapy, the lesions are confluent; as a rule no isolated lesions are seen. Multifocal and isolated lesions are more commonly seen in acquired conditions.[5]

White matter disorders in IEM can be segregated based on three factors: whether white matter never myelinate completely (hypomyelination) or whether myelin forms and is subsequently destroyed (demyelination); whether myelin destruction is in the periventricular, deep or subcortical white matter; and what part of the white matter (specific gyrus or lobe) is affected.[11]

2.1 The pattern of a lack of myelination, or hypomyelination, is seen in very few disorders. It is most commonly found in Pelizaeus–Merzbacher disease, a disorder that affects the PLP1 gene that codes for the production of proteolipid protein, one of the major structural proteins of myelin.[47,48] The appearance of the brain in this disorder is that of a normal, much more immature brain. For example, the MRI of a 5-year-old child with this disorder might be mistaken for that of a 5-month-old infant. Similar appearances can be seen in disorders of trichothiodystrophy with photosensitivity[49,50], in patients with the 18q-syndrome (deletion of a large portion of the long arm of chromosome 18)[51,52], and in patients with Sala disease (a disorder of sialic acid transport)[53,54]. If there is a question about the diagnosis of Pelizaeus–Merzbacher disease, proton MRS may help, as it shows an elevated NAA peak.[55] The reduction of Cho on MRS might be a common marker for hypomyelinating disorders.[56]

If myelin develops but is subsequently damaged, the brain should be analyzed to determine whether the region primarily affected is the deep white matter or the subcortical white matter.

2.2 *Leukodystrophies with early involvement of subcortical white matter*

If the subcortical white matter is involved, it should be carefully analyzed to see if the subcortical U fibers are affected. If so, an attempt should be made to find out whether the patient has macrocephaly. Bilateral, symmetrical, frontal white matter involvement involving the U fibers in a macrocephalic patient is quite specific for Alexander disease, particularly if it extends posteriorly to involve the caudate heads.[57] Bilateral, diffuse and symmetric, peripheral white matter involvement without macrocephaly should raise suspicion for organic acidurias[14] or early Kearns–Sayre syndrome.[1,58] Diffuse white matter abnormality involving the subcortical U fibers and associated with subcortical cysts suggests megalencephalic leukoencephalopathy with subcortical cysts (MLC)[59-61].

2.3 *Leukodystrophies with early involvement of deep white matter and sparing of subcortical U-fiber*

If early myelin injury is restricted to primarily deep white matter, the thalami should be specifically analyzed. High attenuation on CT or T1W hyperintensity or T2W hypointensity on MR bilaterally in the thalami strongly suggests globoid cell leukodystrophy (Krabbe disease)[62] or GM2 gangliosidosis.[1,63,64] If the thalami are normal, the brain stem should be evaluated for involvement of specific tracts, particularly the corticospinal tracts. If specific tracts (the corticospinal tracts, in particular) are involved, peroxisomal disorders such as cerebral form of X-linked adrenoleukodystrophy should be strongly considered.[65,66] If not, consideration should be given to metachromatic leukodystrophy (typically, a pattern of radiating stripes with a signal intensity closer to normal is seen within the abnormal cerebral white matter)[67], cerebellar ataxia with cerebral hypomyelination (vanishing white matter disease)[68], phenylketonuria (earliest and most frequently in the parieto-occipital periventricular white matter)[69], Lowe syndrome (oculocerebrorenal syndrome)[70], mucolipidosis type IV, merosin deficient congenital muscular dystrophies, and, in the proper clinical setting, damage from radiation or chemotherapy.[1,6] Among these, vanishing white matter disease should be considered if the clinical history is one of periodic acute worsening after trauma or infection and if areas of cystic degeneration (seen as hypointense on FLAIR images) develop in the hemispheric white matter.[71,72] Lowe syndrome should be suspected in many small cysts are seen in the affected white matter, particularly in the periventricular region.[70,73,74]

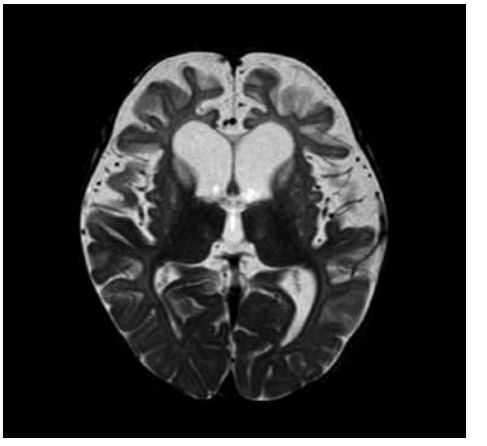


Figure 2. Leigh syndrome in a 6-month-old infant. Axial T2 weighed image shows symmetrical hyperintense signal at bilateral caudate and putamen.

Table 2. Pediatric metabolic disorders with T2W or FLAIR hyperintensity of globus pallidus
Methylmalonic acidemia
Guanidinoacetate methyltransferase (GAMT) deficiency
Isovaleric acidemia
Succinic semialdehyde dehydrogenase deficiency
Urea cycle disorders
Pyruvate dehydrogenase (E2) deficiency
Bilirubin toxicity
Cyanide and carbon monoxide intoxication

If cerebellar white matter, dorsal brain stem, cerebral peduncles and internal capsules are affected in a *newborn*, maple syrup urine disease should be considered, which the diagnosis can be confirmed by the presence of restricted diffusion on diffusion weighted images at the affected area and a broad peak from the branched-chain keto acids at 0.9ppm on proton MR spectroscopy. The images of patients with classical maple syrup urine disease during the neonatal period are diagnostic. All areas which are normally myelinated at that age have

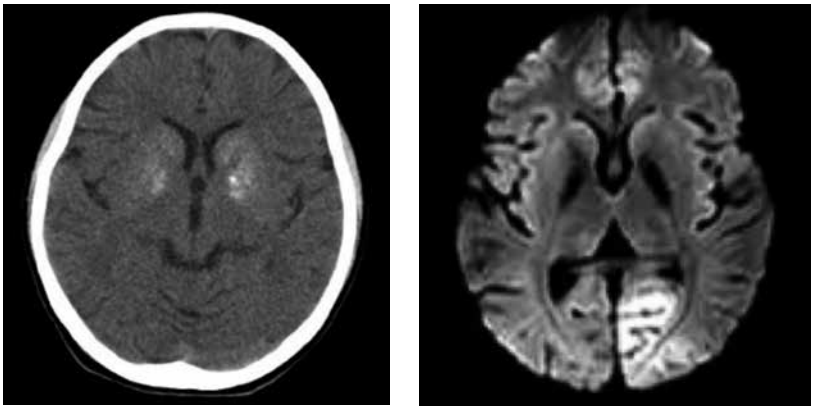
an abnormal signal intensity and are swollen. This pattern is exclusively seen in vacuolating myelinopathies of neonatal onset.[75]

2.4 End stage white matter disease of any cause results in diffuse (superficial and deep), bilateral white matter damage that is completely nonspecific.[11]

3. Disorders Affecting Gray and White Matter

Exclusive damage of either gray matter or white matter structures is rather rare in neurometabolic disorders. Most commonly both are involved, but in variable proportions. Disorders with significant damage to both gray and white matter can be divided into those involving only the cerebral cortex and those involving deep gray nuclei (with or without cortical involvement).[1]

3.1 Those disorders involving only cortical gray matter can be subdivided depending on whether the patient has normal long bones and spinal column. *If the bones are normal*, the cortex should be analyzed for areas of abnormal gyration pattern. If abnormal gyration pattern is present in addition to a lack of myelination, the differential diagnosis includes the generalized peroxisomal disorders[76], congenital cytomegalovirus disease[77-79], and congenital muscular dystrophies with cerebral involvement (these disorders will typically have pontine hypoplasia and cerebellar dysplasia, as well.[80] If no abnormal gyration pattern is demonstrated, differential considerations include Alpers disease and Menkes disease (also called kinky hair disease), both of which cause considerable brain destruction.[58] One of the most striking findings on MRI in patients with classical Menkes disease is excessive tortuosity of the cerebral arteries. MRA confirms tortuosity, dilatation, and kinking of the arteries.[81] If the bones are abnormal, the differential includes primarily storage diseases, such as the mucopolysaccharidoses (multiple small spotlike lesions dispersed in the white matter, with a predilection for the parietal and occipital white matter)[82-86] and lipid storage disorders.[11]



A B

Figure 3 Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like Episodes (MELAS) in 13-year-old patient. A. CT reveals bilateral caudate and putaminal calcifications. B. Diffusion weighed image demonstrates restricted diffusion at left occipital lobe, suggestive of acute infarction.

Table 3. Leukodystrophies with early involvement of subcortical white matter
Alexander disease
Kearns-Sayre syndrome
Megalencephalic leukoencephalopathy with subcortical cysts
Galactosemia

3.2 If deep gray matter is involved, differential diagnosis is dependent upon which nuclei are primarily involved.

3.2.1 *Leukodystrophies with thalamus involvement*

If the thalami are involved, associated with diffuse white matter disease, differential considerations include Krabbe disease[62], the GM1 gangliosidoses[46] and GM2 gangliosidoses[63] and Fabry disease[87]. Krabbe disease is distinguished from the others by the presence of abnormal T2W hyperintensity along the corticospinal tracts. Another consideration is autosomal dominant acute necrotizing encephalitis[88,89], particularly if T2W hyperintensity is also seen in the dorsal brain stem.

GM1 and GM2 gangliosidoses (GM2 gangliosidoses aka Tay-Saches disease) have high attenuation on CT and hyperintense on T1 weighted images and hypointense on T2 weighted images on MR, and neonatal profound asphyxia, which typically involves the ventrolateral thalamus along with posterior putamina and perirolandic cortex.[46,63,90-92] The neuronal ceroid lipofuscinoses shows T2W hypointense thalami and cerebral atrophy.[93]

Fabry disease (FD) shows T1W hyperintensity or calcification at the pulvinar of the thalami.[87] Fabry disease is inherited in an X-linked recessive manner and is related to a deficiency of a-galactosidase, which leads to accumulation of globotriaosylceramide-3 in lysosomes. The prevalence is estimated to be approximately 1 in 50,000 persons. The vast majority (nearly 60% of men and 75% of women) are diagnosed after 20 years of age, whereas the mean age at onset of the first cerebrovascular event is 38 years for men and 43 years for women. The disease has several phenotypes, for which the presentations can be varied. The deposition of globotriaosylceramide-3 in the endothelium and smooth muscles leads to involvement of multiple organ systems, including the blood vessels, heart, and kidneys. Vascular complications are believed to be responsible for the central nervous system (CNS) manifestations. Stroke (ischemic or hemorrhagic) is the most common macrovascular complication. Microvascular manifestations in the brain include white matter as well as gray matter disease. Notably, among younger patients with cryptogenic stroke, FD has been diagnosed in 0.7%-4.9% of patients.[94] Ischemic stroke may result from cardiac embolism, large and small vessel disease, while hemorrhagic stroke is usually attributed to hypertension.[95] Young adults with FD show a high frequency of preexisting and clinically silent infarcts and a relative preference for acute ischemia in the posterior circulation.[96] Gradual deterioration of renal function to end-stage renal disease (ESRD) usually occurs in the third to fifth decade in untreated patient.[97] The high prevalence of parapelvic cyst in classically affected FD males and females, although to date parapelvic cyst cannot be considered a pathognomonic sign of FD, its presence should alert both nephrologists and

radiologists to consider the diagnosis of FD, especially in subjects with an unclear family history of renal disease and when other stigmata of the disease are evident.[98] The common cardiac involvement and clinical characteristics in FD including: left ventricular hypertrophy, conduction abnormalities and arrhythmias, coronary artery disease and valvular infiltrative myopathy.[99] Early treatment of enzyme replacement therapy with agalsidase Beta is related to a better outcome regarding stability and regression of signs and symptoms in Fabry disease.[100]

Thalami may also be affected in mitochondrial disorders, Wilson disease[15], and Canavan disease[101]; typically other deep gray matter nuclei will be affected as well (putamina in mitochondrial disorders and Wilson disease, globi pallidi in Canavan disease).

3.2.2 *Leukodystrophies with globus pallidus involvement*

Globus pallidus involvement in association with diffuse white matter disease including the subcortical, deep, and periventricular regions suggests a diagnosis of Canavan disease. Bilateral involvement of the globus pallidus is seen and involvement of the thalamus often present. The resulting image with sparing of putamen and caudate nucleus is very typical of Canavan disease. Brain stem tracts are often involved and the cerebellar white matter may also be affected.[101,102] Symmetrical globus pallidus and thalamus involvement, association with subcortical white matter and sparing of periventricular white matter suggests Kearns–Sayre syndrome, especially later phase (Often extensive brain stem abnormalities are seen, however, the pattern develops over time and in the earlier stages only limited abnormalities in some of the structures may be seen)[32] or L-2-hydroxyglutaric aciduria; the latter will often show involvement of the cerebellar dentate nuclei and cerebellar vermis atrophy and sparing the brain stem.[30,103]

Globus pallidus and diffuse white matter involvement sparing the subcortical white matter during the early stages of the disease might suggest methylmalonic academia[24,104], maple syrup urine disease, carbon monoxide toxicity, or cyanide toxicity. Maple syrup urine disease typically has involvement of the globus pallidus, corticospinal tracts in the centrum semiovale, internal capsules, cerebral peduncles, dorsal pons, and cerebellar white matter reduced diffusivity in the affected regions, and a characteristic peak at 0.9 ppm on proton MR spectroscopy.[75,105-107] The images of patients with milder variants of maple syrup urine disease during the second year of life are very similar to those of Canavan disease. However, clinical history and laboratory findings differentiate between the two.[75] Carbon monoxide and cyanide toxicity typically involve the cerebral cortex and striatum and, sometimes, the cerebellum.[108-111]

3.2.3 *Leukodystrophies with striatal (caudate and putaminal) involvement*

Primary striatal (putamen and caudate) involvement suggests Leigh syndrome[12], MELAS[112], propionic academia[14], glutaric aciduria type 1 (glutaryl CoA dehydrogenase deficiency)[113], molybdenum cofactor deficiency, isolated sulfite oxidase deficiency[18], Cockayne syndrome[114], hypomyelination with atrophy of the basal ganglia and cerebellum,

toxic exposure, later infantile or childhood profound hypoxic–ischemic injury, or childhood hypoglycemia. Regions of involvement in Leigh syndrome vary with the underlying molecular cause of the disorder, although no consistently reproducible genotype–phenotype associations have been identified. In MELAS, cortical lesions are seen more commonly than basal ganglia lesions, and are usually present when the basal ganglia are involved. Two important features that differentiate the cortical lesions from those of ischemic infarcts are the locations (they do not correspond to vascular territories) and the common association of premature senescent calcifications in the globi pallidi. The cortex is often more severely involved than the underlying white matter and that the periventricular white matter is most often preserved in MELAS.[112] Glutaric aciduria type 1 is typically associated with enlarged subarachnoid spaces, particularly in the anterior sylvian fissures, and central white matter T2 hyperintensity.[113] Isolated sulfite oxidase deficiency is rapidly progressive and causes multicystic encephalomalacia of the cerebral white matter.[18] Cockayne syndrome shows calcification of the striatum, as well as characteristic facies and other aspects of the syndrome.[114,115]

Spread of Disease

Adding the pattern of spread to the analysis further helps to distinguish certain entities. In infants with Canavan disease, diffuse cerebral white matter changes are seen. In older patients with Canavan disease, MRI shows that the most severe abnormalities are present in the subcortical white matter of the cerebrum and cerebellum. Central white matter structures, such as the periventricular rim of white matter, internal capsule, and corpus callosum are better preserved. As the disease progresses, the central white matter also becomes involved. Thus, the spread of the white matter changes is centripetal.[101] The cerebral form of X-linked adrenoleukodystrophy usually spreads in a dorsoventral direction; the direction of spread of Alexander disease is ventrodorsal, that of Canavan disease centripetal, and that of metachromatic leukodystrophy centrifugal.[5]

Table 4. Leukodystrophies with early involvement of deep white matter
Krabbe disease
GM2 gangliosidoses
Cerebral form of X-linked adrenoleukodystrophy
Metachromatic leukodystrophy
Childhood ataxia with CNS hypomyelination (vanishing white matter disease)
Phenylketonuria
Lowe syndrome
Mucolipidosis type IV
Merosin deficient congenital muscular dystrophy
Damage from radiation or chemotherapy

Confounding Factors and Remarks

The approach afore-described would hopefully limit the differential diagnosis to a list of disorders of reasonable length. However, the approach is not perfect and will not always allow a diagnosis to be made. The approach is complicated by several confounding factors. The first of these is the fact that many metabolic disorders have a different appearance on

imaging studies when imaged at different stages of the disease (therefore, this approach is most useful in the early stages of the disease). Another potential source of error is that some disorders are diagnosed biochemically while others are diagnosed genetically. Atypical imaging patterns can be seen in disorders diagnosed biochemically because many diseases that manifest the same serum/urine biochemistry actually have different underlying genetic and neurochemical defects. Disorders diagnosed genetically may appear heterogeneously because different mutations of the gene may result in abnormalities of different pathways (different parts of proteins may participate in different chemical pathways). Nonetheless, this systematic approach will allow the user to get close to the diagnosis much of the time.[11] Moreover, many acquired diseases, such as periventricular leukoencephalopathy, inflammatory and infectious processes, acquired metabolic disorders with nutritional deficiencies, and toxic injuries, which are not included in the simple pattern approach afore-described, can present with radiological appearance that mimic those changes similar to those associated with IEM.[4]

Table 5. Leukodystrophies with globus pallidus involvement
Canavan disease
Kearns-Sayre syndrome
L-2-Hydroxyglutaric aciduria
Methylmalonic acidemia
Maple syrup urine disease
Carbon monoxide poisoning
Cyanide toxicity

Table 6. Leukodystrophies with striatal involvement
Leigh syndrome
MELAS
Propionic acidemia
Glutaric acidemia type I
Molybdenum cofactor deficiency, Isolated sulfite oxidase deficiency
Cockayne syndrome
Hypomyelination with atrophy of the basal ganglia and cerebellum
Late infantile/childhood profound hypoxic-ischemic injury
Childhood hypoglycemia

Conclusion

The diagnosis of IEM is challenging. "Piecing together the puzzle", said Dr. Elaine Kan in Hospital Authority Convention 2017, Hong Kong. Coupled with clinical information and laboratory results, the use of the systematic MR pattern recognition may hopefully narrow down the differential diagnosis, looking forward for a timely and appropriate diagnosis for the patients under the conjunct effort from the multidisciplinary team. The number of potentially treatable neurometabolic disease in increasing.[116-122] "**HOPE** would be the future for our patients", said Dr. Fung Cheuk Wing in Hospital Authority Convention 2017, Hong Kong. Thinking further out of the box, treatable IEM in adults might fill some of the potential gaps between pediatric and adult medicine for the better future of our patients.[121]

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BRAINCHILD
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The Genetics of Intellectual Disability

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Introduction

A high level of intelligence is what makes human beings so unique and superior to the rest of the mammalian species. Intelligence is probably the most complex of the complex traits, partly because intelligence has a lot of different elements. For instance, when we make comments on the intelligence of a person, what are we referring to exactly? Do we mean the ability of that person to follow logic, to comprehend a piece of information, to analyze a situation, to solve a problem, to communicate with other people, or to stay focused on a task? That is why there are various elaborate tests to measure our intelligence, or intelligence quotient (IQ), and the same is true about the assessment of the lack of intelligence.

Being a complex trait, intelligence has a normal or Gaussian distribution in the population, and intellectual disability (ID) is arbitrarily defined as an IQ of 70 or lower. Like all other complex disorders, there are many genetic and environmental factors underlying ID, and etiologic heterogeneity is the rule. In some cases there may be a stronger genetic component, while in others a stronger environmental component. When we discuss about the genetics of complex traits we often say there are "common variants with small effects" and "rare variants with large effects". Every person has a unique genetic makeup, except for monozygotic twins. This is because our genome allows for a certain degree of variation. After the completion of the Human Genome Project, we learnt that if we compare the genome of a normal person with that of another normal person, there is on average 1 sequence variant per thousand bases (0.1%). These variants are called single nucleotide polymorphisms (SNP). The percentage looks small, but given the size of our genome which consists of about 3 billion bases, 0.1% would mean 3 million SNPs. Soon later, scientists were also aware of the existence of copy number variations or structural variations. Together these make up at least 0.2% difference between the genomes of any two persons.[1,2] A lot of these variants are "common variants with small effects", and their additive or multiplicative effects, together with other environmental factors, contribute to the normal variation in intelligence. If a person carries a single "rare variant with large effect", it may suffice to cause a dramatic shift in IQ to either side of the normal curve, resulting in ID or intellectual giftedness. One might say that environmental factors probably have a small role now, especially in an affluent society like Hong Kong where antenatal and perinatal care of high standard is readily available both in the public and private sectors. Yet the Zika virus story also reminds us that these environmental factors can be secretly at work for a long period of time without our notice.

Working as a clinical geneticist for over 25 years, I have had the chance to see a lot of patients with ID. My job is to try to identify the genetic defects in these patients that may

account for their ID. Having a genetic diagnosis can inform prognosis and guide long term management. It can also reduce unnecessary investigations. For the family, more accurate risk assessment can be provided through genetic counselling and, if necessary, genetic testing for the family members, and advice on reproductive options can be given. The genetic defects that we try to find are the "rare variants with large effect". We can classify them into gross chromosomal abnormalities, submicroscopic chromosomal abnormalities, single gene defects, and epigenetic defects. I will discuss these accordingly.

Chromosomal abnormalities

When I entered this field in the early 1990's, karyotyping was almost the only genetic testing available across the territory; at least this was true in the public service. When people wanted to order a genetic study, they simply meant karyotyping. Practicing clinical genetics was straight forward; we performed karyotyping for almost every referred case. As for cases of ID or autism, a combo of karyotyping, or G-banding, and Fragile X study was the rule. Fragile X study was really a cytogenetic study in those years. Using folate deficient culture medium, the fragile site on the X chromosome could be induced and then observed under the microscope. I shall come back to Fragile X syndrome later as it is not a chromosomal disorder per se. Chromosomal abnormalities detected in patients with ID or autism were very heterogeneous, including aneuploidies and all possible structural abnormalities.

Down syndrome

Notably, Down syndrome, or trisomy 21, was still a significant cause of intellectual disability in those days, with an incidence of about 1 in 700. With the subsequent introduction of Down syndrome screening and prenatal diagnosis in Hong Kong, we saw a significant reduction of Down syndrome in live births. Although not all cases of Down syndrome were confirmed by our centre, we did observe an obvious decreasing trend of the number of Down syndrome cases confirmed by our cytogenetics laboratory (79 cases in 1992 vs. 15 in 2016). With prenatal screening technology using cell free fetal DNA gaining popularity in recent years, we will probably see a further reduction of Down syndrome in live-born babies.

Sex chromosomal aneuploidies

The other chromosomal aneuploidies that are more prevalent in ID cases include XXY or Klinefelter syndrome, triple X syndrome, and a supernumerary inv dic(15) chromosome. The effect of an extra X chromosome on intelligence is generally mild but definite. Studies show that an extra X would cause a left shift of the IQ distribution curve by 5-15 points. That means a higher percentage of these patients would have an IQ within the ID range compared to the general population. Genetic counselling to pregnant women whose fetuses are found to have sex chromosomal aneuploidies is not an easy task largely because of the uncertainty with neurodevelopmental outcome. Nevertheless, most patients with one extra X still have an IQ within normal range, despite greater likelihood of learning difficulties. Rare cases with 2 or 3 extra X chromosomes are also seen, and these patients invariably have significant ID.

Inverted dicentric chromosome 15

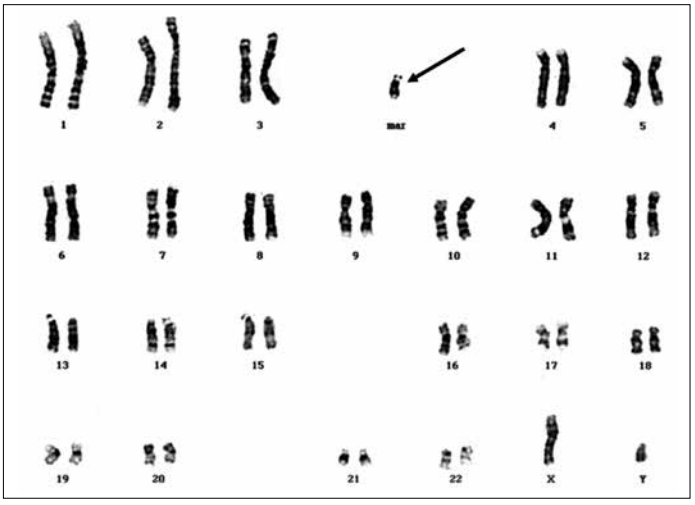

Fig. 1: Karyotype with a supernumerary bi-satellited marker chromosome.

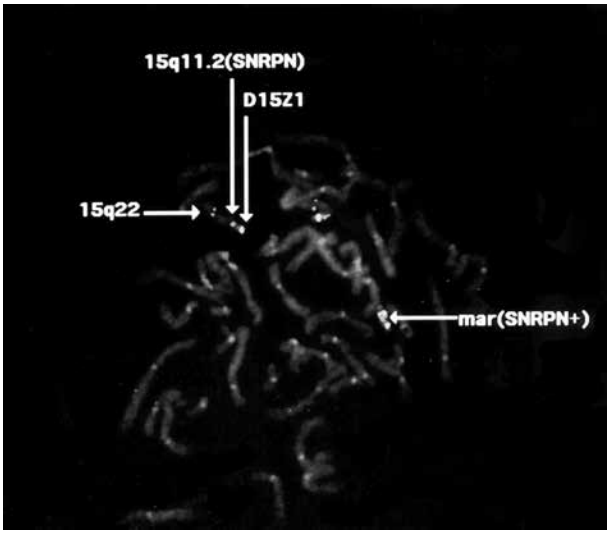

Fig. 2: FISH study showed that the marker chromosome is derived from chromosome 15 and involved the SNRPN locus.

Inverted dicentric chromosome 15 [inv dic (15)], is a recurrent chromosomal abnormality found in patients with ID/autism. It is usually first recognized in chromosomal analysis as a supernumerary, bi-satellited, marker chromosome, which means an extra piece of chromosomal material of unknown origin (Fig. 1). Upon further studies by Fluorescence In Situ Hybridization (FISH) (Fig. 2), multiplex ligation dependent probe amplification (MLPA), or chromosomal microarray (aCGH), the chromosome 15 origin can be confirmed. Interestingly, the size and parent-of-origin of the inv dic(15) have great bearing on whether it would cause ID. For an inv dic(15) to cause ID, it must be of a size large enough to encompass the Angelman syndrome/Prader-Willi syndrome (AS/PWS) critical region at 15q12, and be of maternal origin. The parent-of-origin specific nature is due to the fact that the 15q12 region is imprinted, containing genes whose expression is dependent on the parent of origin. Therefore, patients carrying an inv dic(15) have two extra copies of the AS/PWS critical regions. Less commonly, patients carrying extra copies of the AS/PWS critical

regions without an inv dic(15) are also known; these patients have tandem duplication of the 15q12 region on one of the chromosomes 15 instead. The clinical phenotype is similar to that caused by inv dic(15), and is collectively known as 15q duplication/triplication syndrome, characterized by variable developmental delay, autism, epilepsy, and subtle dysmorphism. Refractory epilepsy or Lennox-Gastaut syndrome is a concern in some patients. Inv dic(15) is usually sporadic, but tandem duplication/triplication can be inherited from a phenotypically normal mother, in which case genetic counselling is important for the prevention of recurrence in the family.

Structural chromosomal abnormalities

This is an even more heterogeneous group compared to the aneuploidies, and includes cytogenetically detectable deletions, duplications, insertions, inversions, ring chromosomes, and translocations. The phenotypic consequences of these abnormalities are dependent upon the chromosome region involved, and the size of the gain or loss of chromosomal material that is resulted. A lot of these chromosomal abnormalities are private to the individual families and therefore not easy to find another case for reference in the literature. Nevertheless, there are recurrent chromosomal abnormalities associated with well documented phenotypes or syndromes, e.g. terminal deletion of 4p and 5p associated with Wolf-Hirschhorn syndrome and Cri du chat syndrome, respectively; 9p deletion syndrome, and 18q deletion syndrome.

Submicroscopic chromosomal abnormalities

In the mid-1990's, a molecular cytogenetics technique was introduced to our Service. It was known as FISH. Using commercially available genomic probes, we were able to interrogate different chromosomal regions known to have recurrent microdeletions. These microdeletions are so small that they escape detection by conventional karyotyping, which has a resolution of 5-10 Mb. At the time, the better known microdeletion syndromes were Williams-Beuren syndrome (7q11.23 deletion), DiGeorge/Velocardiofacial syndrome (22q11.2 deletion; now collectively known as 22q11.2 deletion syndrome), Angelman syndrome and Prader-Willi syndrome (15q11-13 deletion), Smith-Magenis syndrome (17p11.2 deletion), and Miller-Dieker syndrome (17p13.3 deletion).

The most notable microdeletion syndrome in my opinion is the 22q11.2 deletion syndrome (Fig. 3), mainly because, with an incidence of approximately 1 in 3,000, it outnumbers other microdeletion syndromes. Patients with 22q11.2 deletion syndrome have a variable combination of clinical features including congenital heart defects, cleft palate, hypocalcaemia, immunodeficiency, and neurodevelopmental delay, so they can be ascertained for quite different reasons. These patients invariably have ID, but the degree of ID is quite variable. ADHD is also quite common in children (24-37%), and schizophrenia is common in adults (25-30%).[3,4] The great majority of the cases are sporadic. Among the handful of familial cases, the mother is usually the transmitting parent. In early years, full blown cases of "DiGeorge" syndrome were occasionally seen, often with complex congenital heart defects and immunodeficiency, and hence poor survival. Nowadays, with the wide availability of fetal anomaly scan and invasive or noninvasive prenatal diagnosis, such full-blown cases are rarely seen. Instead, 22q11.2 deletion syndrome is more often diagnosed in patients with ID but without other major manifestations.

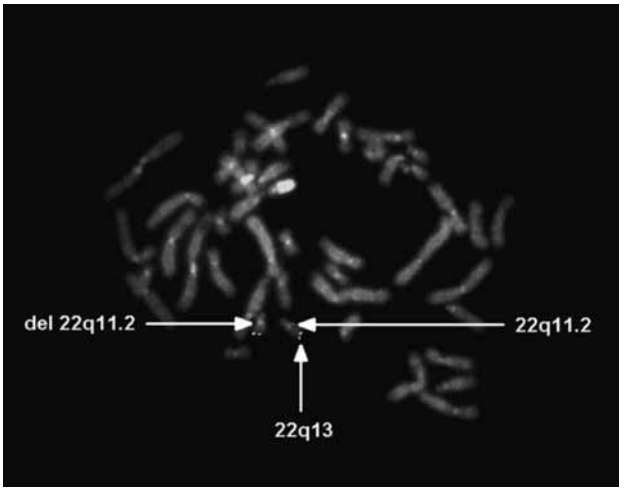

Fig 3: FISH confirmed absence of signal from 22q11.2 region on one of the chromosomes 22.

Angelman syndrome (AS) and Prader-Willi syndrome (PWS) are among the most well-known microdeletion syndromes, and also are the first examples of imprinting disorders. Genomic imprinting refers to differential gene expression dependent on the parent of origin. In the imprinted region on chromosome 15q11-13 region, there are genes with monoallelic expression. Some only turn on the paternal allele while the others only turn on the maternal allele. This phenomenon explains why the same microdeletion of the region results in two clinically distinct phenotypes. Deletion of the paternally derived chromosome 15 causes PWS, while deletion of the maternally derived chromosome 15 causes AS. Yet, it is also known that FISH cannot diagnose all cases of AS/PWS; only 60-70% of these cases are caused by FISH-detectable microdeletion. The other cases are caused by other genetic mechanisms like uniparental disomy, imprinting defect, and gene mutation (e.g. *UBE3A* in AS) that were not detectable until the subsequent development of molecular diagnostics in our laboratory. One can refer to Luk et al.[5] and Lo et al.[6] for local experience in AS and PWS, respectively.

In the late 1990's, a lot of attention was drawn to cryptic abnormalities, either deletion or duplication, that occurred at the subtelomeric regions, i.e. the very tips at either end of the chromosomes that are known to be gene rich regions. These subtelomeric deletions and duplication are not detectable by conventional karyotyping and require the application of FISH or MLPA technique to diagnose. The detection rate is variable, ranging from 2% to 29%, dependent on the stringency of inclusion and the sample size. A study with compiled data from 11,688 cases from three different centres in the United States showed an approximately 2.5% detection rate, with about half of the clinically significant abnormalities being terminal deletions either de novo or due to unbalanced translocation. The most frequently affected chromosomes were 1p, 22q, 4p, 9q, 8p, 2q, and 20p.[7]

The arrival of chromosomal microarray (aCGH) technology in 2012 has significantly reduced the use of FISH and MLPA for the detection of submicroscopic chromosomal abnormalities (Fig. 4). Detection rate in local cohorts of ID patients was 11% to 19%.[8,9] A

normal aCGH study pretty much excludes unbalanced chromosomal abnormalities, gross or cryptic. The wider application of aCGH in patients with ID also reveals some "novel" recurrent microdeletions and microduplications, e.g. 2q23.1 microdeletion, 16p11.2 microdeletion/microduplication, 16p13.11 microdeletion/ microduplication, 17p11.2 duplication (Potocki-Lupski syndrome), and 22q13.3 deletion (Phelan-McDermid syndrome), etc. Interestingly, it is realized that some of these microdeletions or microduplications are not fully penetrant. In other words, these copy number variants can be found in normal population, but prevalence in ID patients is higher. Genetic counselling is challenging especially when these variants are found via prenatal diagnosis.

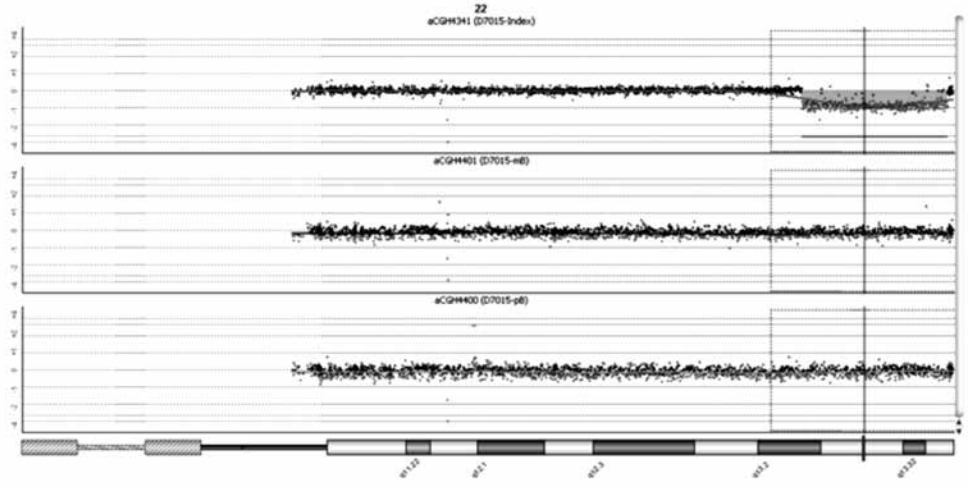

Fig. 4: A patient with de novo 22q13.2-q13.33 deletion (top tracing: patient; lower two tracings: parents), detected by aCGH, confirming Phelan-McDermid syndrome.

Single gene defects

There are hundreds of monogenic disorders with ID as the only or one of the manifestations. In clinical practice, it is easier to differentiate between syndromic causes and non-syndromic ones. Syndromic causes can be identified with a clinical approach, judging from the dysmorphic features, pattern of organ malformations, and inheritance. Sometimes, one is able to make a clinical diagnosis with certainty even without confirmatory genetic testing. On the other hand, non-syndromic causes are much more difficult to diagnose. In the following discussion I will focus on syndromic causes only.

Fragile X syndrome

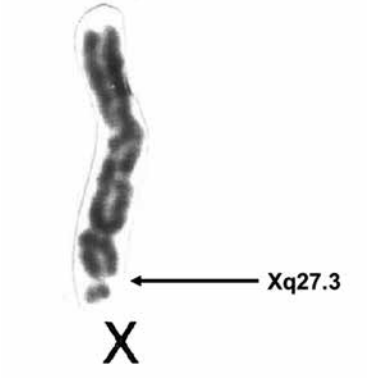

Fig. 5: An X chromosome with fragile site at Xq27.3 region.

Fragile X syndrome is the most well-known X-linked ID. Although it is a "syndrome", the clinical characteristics are not usually distinct enough for clinical inclusion or exclusion with confidence. For instance, the macro-orchidism (large testes) is not present before puberty. Therefore, diagnosing Fragile X syndrome requires a screening approach in patients with ID and autism, unless the patient has features clearly indicative of an alternative cause. I have mentioned above that we used to diagnose Fragile X syndrome by cytogenetics technique using folate deficient culture medium (Fig. 5). It was an insensitive method. The molecular basis of Fragile X syndrome was discovered in 1991.[10] There is a stretch of polymorphic CGG trinucleotide repeat sequence in the 5' untranslated region of exon 1 of the FMR1 gene at Xq27.3 region. The number of CGG repeats is variable in normal people and is up to 54. Patients with Fragile X syndrome, however, carry more than 200 CGG repeats in their *FMR1* gene. This is called full mutation, which results in gene silencing through abnormal methylation of the gene promoter. The neurodevelopmental phenotype of Fragile X syndrome is largely due to deficiency of the gene product, FMRP. Carriers with *FMR1* gene containing 55-200 CGG repeats are said to have a premutation. Fragile X syndrome is the first example of human disease caused by trinucleotide repeat expansion, a novel kind of mutation. To address the low sensitivity of cytogenetic technique in detecting Fragile X syndrome, FMR1 gene analysis was the first molecular testing developed after the introduction of molecular techniques in our laboratory. In Western literature, Fragile X syndrome is said to be the commonest inherited cause of ID, with a prevalence of 1 in 4,000 males and 1 in 6,000 females. After 20 years of Fragile X testing in patients with ID/autism, we observed that it was present in about 1% of patients with ID. In a local study of Fragile X carrier screening in pregnant women, among 2,650 Chinese pregnant women screened, only 3 Fragile X carriers were detected, 2 with premutation and one full mutation, giving an overall prevalence of Fragile X carriers 1/883 females, which was several folds lower than the frequently quoted 1/200-300 females in Western literature.[11]

Rett syndrome

Rett syndrome is a peculiar neurodevelopmental disorder that mainly affects females. These girls typically present with severe global delay and autism with or without a noticeable developmental regression. They are non-dysmorphic, though usually have microcephaly of postnatal onset. Early-onset epilepsy is almost the rule. Other tell-tale signs include mid-line hand stereotypies, gait apraxia, bruxism, and episodic hyperventilation. The strong sex bias had once led to the postulation of an X-linked dominant inheritance with male lethality. However, excessive male lethality was not really evident in the affected families. The answer was finally revealed by the identification of *MECP2* gene mutations in these patients.[12] MeCP2, the gene product, was then known to be a transcriptional regulator through chromatin modulation. In other words, it mediates epigenetic control of the expression of other genes. Through collaboration with a US centre, one of our Rett patients was among the first to have the diagnosis confirmed.[13] It was then understood that while Rett syndrome is truly X-linked dominant, no excessive male lethality is observed because the great majority of mutations arise de novo on the paternal allele. In fact, rare cases of males with *MECP2* mutations were reported and these usually have a more severe phenotype as neonatal encephalopathy. More common in males, however, is a neurodevelopmental disorder caused

by *MECP2* duplication, characterized by severe ID, hypotonia progressing to spasticity, epilepsy, mild dysmorphism, and occasionally midline stereotypic hand movements. In contrast to Rett syndrome, *MECP2* duplication syndrome is X-linked recessive, and in most cases the mother is a carrier, so genetic counselling and prevention are especially important.

Mowat-Wilson syndrome

Mowat-Wilson syndrome (MWS) is a relatively new syndrome, first described by Mowat et al. in 1998 in six patients who shared features of Hirschsprung's disease, microcephaly, mental retardation, and dysmorphic facial features.[14] Actually quite a few of our local patients were seen well before that and the diagnosis remained unknown until several years after the first publication. In 2001, loss-of-function mutations of the ZFHX1B gene were found to be the cause of this syndrome.[15,16] The gene product, Smad interacting protein-1, is a transcription factor that belongs to two-handed zinc finger/homeodomain family, and plays an important role in the development of the neural crest. There were about 10 local cases confirmed. They shared characteristic facial dysmorphism, marked developmental delay and mental retardation, epilepsy of early onset, and ataxia. However, only two patients had Hirschsprung's disease. They showed a happy disposition and friendliness to strangers, which, together with the ataxia, had led to the misdiagnosis of Angelman syndrome in a few patients.

Kabuki syndrome

Previously known as Kabuki Make-up syndrome, Kabuki syndrome (KS) is also a relatively new multiple congenital anomalies/mental retardation (MCA/MR) syndrome, first described in Japanese patients in 1981.[17] It was later found to be pan ethnic. The craniofacial dysmorphic features, the high arching eyebrows and eversion of the lateral portion of the lower eyelids in particular, are reminiscent of the make-up of Japanese Kabuki artist, hence the previous name of the syndrome. Almost all KS patients have ID of variable degrees. Short stature is also common. Other organ anomalies that are more common and contribute significantly to morbidity include congenital heart defects and renal malformation/dysplasia. The genes underlying KS were elusive until 2010 with the advent of next generation sequencing technology.[18] It was also found to be genetically heterogeneous, with the KMT2D gene being the major culprit and the X-linked KDM6A gene accounting for a small percentage of cases. So far the great majority of KS cases were sporadic. I am not aware of a single familial case in our locality. Lastly, there is some phenotypic overlap between KS and Turner syndrome, so karyotyping is recommended before the much more costly molecular testing, especially in female patients.

Nicolaides-Baraitser syndrome

Nicolaides-Baraitser syndrome (NBS) is an interesting neurodevelopmental disorder that attracted more and more attention over the past 5 years. These patients present variable developmental delay, epilepsy, hirsutism, coarse facial features, and prominent interphalangeal joints. When the patient has significant failure to thrive, sparse hair and hypoplastic fifth fingers, the diagnosis is probably Coffin-Siris syndrome instead. Like Mowat-Wilson syndrome, these patients have been seen for decades and a common

phenotype was recognized, but the diagnosis remained uncertain until more related publications in recent years. With the advent of NGS, the genetics underlying NBS and CSS are becoming clear. They are both disorders of the SWI/SNF complex, which plays a role in chromatin modulation and thus regulation of gene transcription. NBS is caused by dominant mutations of the *SMARCA2* gene, while CSS is more heterogeneous and can be caused by mutations of the *ARID1A, ARID1B, SMARCA4, SMARCB1,* or *SMARCE1* gene. These are all genes encoding the subunits of the SWI/SNF complex. So far all patients reported were sporadic.

Rasopathies

Noonan syndrome (NS), cardiofaciocutaneous syndrome (CFCS) and Costello syndrome (CS) together constitute the major rasopathies. Noonan syndrome is one of the commonest dysmorphic syndrome, with an incidence of 1/1,000-2,500. Although only 1/3 to 1/4 of patients have significant ID, a lot more patients have learning difficulties and attention deficit. CFCS and CS are much rarer, but ID is almost universal. The *PTPN11* gene is the first gene identified to cause Noonan syndrome, but its mutations only account for about 40% of NS cases. Subsequently about 10 other genes were identified one by one, and the roles of these genes in the Ras/MAPK signaling pathway became better understood. The Ras/MAPK signaling pathway is important to the regulation of cell growth, proliferation, differentiation and cell death in response to extracellular signals. It is noteworthy that while germline mutations of these genes cause one of these rasopathies, somatic mutations in these genes are commonly found in malignancies.

Overgrowth syndromes

Sotos syndrome (SS), also known as cerebral gigantism, is the most classic example of overgrowth disorder associated with ID. The overgrowth is of prenatal onset and continues postnatally. These patients invariably have ID, hypotonia, and a characteristic facial gestalt that includes macrocephaly, frontal bossing, broad forehead, frontoparietal balding, hypertelorism, downward slanting palpebral fissures, and pointed chin. Epilepsy is also common, affecting about 25% of patients. The gene implicated in Sotos syndrome is *NSD1*. Another overgrowth syndrome that shows phenotypic overlap with Sotos syndrome is Weaver syndrome (WS), the causative gene of which is *EZH2*. Weaver syndrome is less common than Sotos syndrome and has less severe ID. The products of both *NSD1* and *EZH2* genes are SET-domain containing proteins that affect gene expression by chromatin modification. They are also proto-oncogenes with somatic mutations reported in malignancies.

Simpson-Golabi-Behmel syndrome (SGBS) is an X-linked recessive disorder characterized by ID, overgrowth, coarse facial features, postaxial polydactyly, and other dysmorphism. The causative gene is *GP3*, which encodes an extracellular proteoglycan. The mother is often an asymptomatic carrier; molecular confirmation and genetic counselling are thus especially important for the prevention of recurrence in the family.

In a few patients referred to our Service for ID and autism, we detected mutations of the *PTEN* gene, a tumour suppressor gene. The diagnosis is PTEN hamartoma tumour syndrome.

Probably not a common cause of ID, but it is noteworthy that these families have different phenotypes among the family members; some have ID/autism, macrocephaly and slight overgrowth, while other family members may have normal intelligence but history of benign or malignant tumours. Papillomatous skin lesions, goiter/thyroid cancer and breast cancer are the relatively common abnormal growths in these patients. *PTEN* is a well-known cause of familial breast cancer after *BRCA1* and *BRCA2*, and is usually included in NGS panels designed for hereditary breast cancer.

Neurocutaneous syndromes

Neurofibromatosis type 1 (NF1) is a relatively common genetic disorder, with an incidence of about 1/3,000. The majority of patients with NF1 have normal intelligence and indeed I have seen patients who perform very well at school. However, overall speaking, many patients have mild developmental delay in early childhood and learning difficulties in school. A small percentage of patients have frank ID, especially those with heterozygous deletion of the whole gene. NF1 is also related to the Ras signaling pathway; the protein product, neurofibromin, is a GTPase activating protein that negatively regulates the Ras activity.

Tuberous sclerosis complex (TSC) is several folds rarer but cause more severe morbidities than NF1. The percentage of patients with ID/autism is about 50%. Epilepsy, subependymal giant cell astrocytoma, renal angiomyolipomas and pulmonary lymphangioleiomyomatosis are the other major morbidities, the treatment of which is often challenging, and regular monitoring is required. TSC can be caused by dominant mutations of either the TSC1 or TSC2 gene, encoding hamartin and tuberin, respectively. These proteins form a complex which is a negative regulator of the mTOR (mechanistic target of rapamycin) signaling pathway. The knowledge of the role of hamartin and tuberin in negatively regulating the mTOR signaling pathway has led to application of mTOR inhibitors in treating some of the manifestations of TSC, e.g. refractory seizures.

Metabolic disorders

This is a highly heterogeneous group of disorders. Of the nearly 20,000 protein coding genes in our genome, about 3,000 metabolic genes are operating in at least one tissue type, involving over 6,000 reactions. Among these genes, over 2,500 are operating in the brain.[19] In other words, the number of genes that directly or indirectly affect brain function through metabolism is huge. Yet, it is important to identify these metabolic causes of ID because they are potentially treatable. A systematic review has identified a total of 81 treatable inborn errors of metabolism (IEM) with ID as a major manifestation. Among these, 62% can be identified by metabolic testing on blood and urine specimens, while others require a molecular approach.[20] The widely adopted newborn IEM screening with tandem mass spectrometry technology can only cover a fraction of these treatable IEM. In the genomics era, whole exome (WES) or whole genome sequencing (WGS) is probably the ultimate test. In a study of 41 patients with ID associated with a metabolic phenotype, the use of WES identified the diagnosis in 28 (68%). More importantly, over half of those with a definitive diagnosis can benefit from more targeted treatment.[21]

Epigenetics

This is probably a less well studied area. To define "primary" epigenetic disorders, these are not caused by abnormalities at the sequence level, but are instead due to abnormal chromatin modification like methylation and structure (open and accessible vs. compact and inaccessible). Not many diseases are caused by primary epigenetic defects. For instance, some AS and PWS cases are caused by patUPD and matUPD, respectively, and methylation defects. Methylation defects are occasionally reported in patients born with AS or Beckwith-Wiedemann syndrome (BWS) who were conceived by assisted reproductive technology. In contrast, abnormal epigenetic control of gene(s), in cis or in trans, can be secondary to a primary genetic defect at the sequence level. These have been referred to as 'Mendelian disorders of the epigenetic machinery".[22] A few of these have already been mentioned above, including Fragile X syndrome, Rett syndrome, Kabuki syndrome, Nicolaides-Baraitser/ Coffin-Siris syndrome, Sotos syndrome, and Weaver syndrome. Some disorders which I have not discussed but also fall into this category are Rubinstein-Taybi syndrome, Cornelia de Lange syndrome, Alpha-Thalassaemia mental retardation X-linked syndrome, and Borjeson-Forssman-Lehmann syndrome.

Conclusion

This is a brief overview of the genetic causes of ID based on personal experience. Given my profession as a clinical geneticist, I believe I have seen many more genetic causes of ID than other doctors, but even then it is just the tip of an iceberg. Our capability in diagnosing genetic causes of ID has ever been tightly linked with technological advancement. In the discussion above, I have left out the non-syndromic causes, not that they are unimportant, but only that we have not had the right weapon to tackle them until recently. These are a highly heterogeneous group; in Online Mendelian Inheritance in Man (OMIM), there are over 40 genetic loci for autosomal dominant mental retardation, 59 loci for autosomal recessive mental retardation, and more than 100 loci for X-linked mental retardations. Whole exome or whole genome sequencing is probably the ultimate answer. In particular, whole genome sequencing will be the "one" test that detects all genetic defects from gross chromosomal rearrangements to single nucleotide variations.

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BRAINCHILD
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Biotinidase Deficiency – a biotin-responsive inherited metabolic disorder

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Biotinidase deficiency is a rare treatable inherited metabolic disorder with a variable combination of neurological manifestations (hypotonia, seizures, ataxia, developmental delay, hearing and visual impairment, etc.), cutaneous lesions (dermatitis and alopecia), respiratory problems and immunological disorders. Early identification of this disorder is important and treatment with biotin can prevent long-term irreversible neurological dysfunction. Pre-symptomatic treatment is feasible, as newborn screening of biotinidase deficiency can be routinely performed in dried blood spots by colorimetric enzyme assay.

Case Report

The proband was the product of non-consanguineous marriage. She was born full-term by vaginal delivery with body weight of 2.59 kg. Perinatal history was unremarkable. She was referred to our unit at the age of 2 months for inspiratory stridor, suspected infantile spasms, eczema and developmental delay.

Inspiratory stridor was noted at 2-3 weeks of life but then subsided spontaneously. One week before admission, stridor recurred. No other symptom of respiratory tract infection was evident. A few days before admission, the patient was noted to have brief episodes of sudden staring and extension of four limbs associated with cessation of her cry. Each attack lasted for few seconds.

Physical examination on admission revealed eczema over the face and scalp. Inspiratory stridor was noted. There were no dysmorphic features. The head circumference was normal (75^{th} percentile). There was no visual fixation and she had no social smile. Complete head lag was noted. Both of her hands fisted. There was generalized hypotonia but her muscle stretch reflexes were brisk.

Seizures were noted after admission. They usually began with gurgling sounds, followed by tonic extension of the lower or upper limbs with impaired consciousness occasionally and mild oxygen desaturation at times. Most episodes lasted for few seconds, but some attacks lasted up to 30 seconds. Seizures commonly occurred when the patient was about to sleep and could occur more than 10 times per day. Phenobarbital was given initially, and phenytoin and levetiracetam were added subsequently because of unsatisfactory seizure control.

Preliminary blood tests, including a complete blood count, electrolytes, glucose and ammonia were normal. Alanine transaminase (ALT) and alkaline phosphatase (ALP) were

mildly elevated. Blood gas revealed metabolic acidosis with respiratory compensation (pH 7.28; HCO3 14.6; base excess -12.1).

Computed tomography (CT) scan of the brain at 2 months was normal. An electroencephalogram (EEG) revealed episodic sharply contoured slow waves over the right frontal and bilateral posterior regions. Magnetic resonance imaging (MRI) of brain revealed early cerebral atrophic changes and diffuse T2 hyperintensity involving bilateral cerebral white matter and peripheral cerebellar white matter.

Urine analysis showed markedly elevated excretion of 3-OH-isovaleric and 2-OH-glutaric acids and moderate elevations of 3-OH-propionic acid, 2-ketoglutaric acid, lactate, 3-methylcrotonylglycine and 2-ethyl-3-OH-propionic acid and mild increase in methylcitrate.

Developmental milestones were at a standstill until she began treatment with multivitamins (including biotin) at 3-months. The metabolic acidosis resolved and the acylcarnitine profile showed no abnormality by LC-MS/MS. Urine organic acids were normal. Her skin lesions improved within weeks of treatment.

Serum biotinidase activity was 0.4 (at 37^{O}C, nmol pABA/min/ml; healthy subjects: >4.4; biotinidase deficient: <0.7). DNA sequencing revealed homozygous BTD gene mutations at c.476G>A and confirmed the diagnosis of biotinidase deficiency.

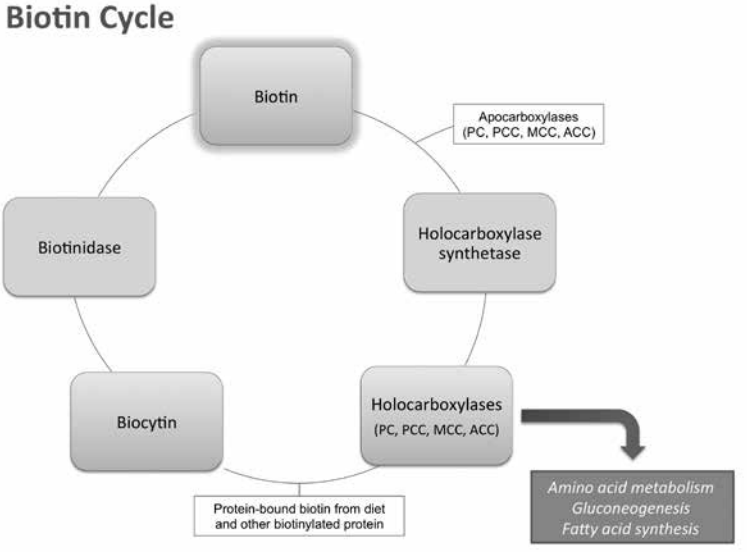
The seizures stopped initially after biotin supplementation began, but recurred later again in form of epileptic spasms attacks that occurred in clusters at 9-months. This time, the EEG showed modified hypsarrhythmia. Seizure control was achieved subsequently with vigabatrin and topiramate. She has been seizure-free since 13-months and all anti-epileptic drugs were discontinued at the 3 years.

Her head circumference grew along 10th percentile after 15 months of age. MRI of the brain performed at 3-years was normal. Brainstem evoked responses were within normal limit, but pure tone audiometry showed bilateral mild to moderate impairment of mainly high tone range, and visual reinforcement audiometry revealed mild low tone sensorineural hearing loss. Ophthalmological assessment found mild hyperopia, but no other visual abnormality. Despite biotin treatment, she still has developmental delay and is attending school with support for children with intellectual disability.

Discussion

Biotinidase releases free biotin from biocytin and biotinylated peptides. It is essential in biotin homeostasis. Biotin is a water-soluble B-complex vitamin (also known as vitamin B7 or vitamin H) that serves as the coenzymes for pyruvate carboxylase, propionyl-CoA carboxylase, methylcrotonyl-CoA carboxylase (all resided in mitochondria) and acetyl-CoA carboxylase (located in cytosol). These carboxylases are involved in gluconeogenesis,

amino acid metabolism (leucine, valine, isoleucine, methionine, threonine) and fatty acid biosynthesis. (Fig. 1) Deficiency in biotinidase will therefore impair these carboxylases' function and lead to secondary organic acidaemia, ketoacidosis and hyperammonaemia.



Biotinidase deficiency is a rare (approximately 1:60,000) autosomal recessive disorder of biotin recycling. Profound biotinidase deficiency is defined as having less than 10% of mean normal enzyme activity in serum; partial deficiency is defined as 10 – 30% of normal biotinidase activity. The ratio of profound to partial biotinidase deficiency cases is approximately 1:1.

Biotinidase deficiency is also known as "late-onset" multiple carboxylase deficiency because the first symptoms usually start between the 3rd and 6th month of life, as compared with the "early-onset" multiple carboxylase deficiency associated with holocarboxylase synthetase deficiency, which often becomes symptomatic in neonatal period. However, neonatal onset or late presentation in older children or adolescents may also be seen in some cases of biotinidase deficiency.

Patients with biotinidase deficiency frequently present with neurological symptoms such as hypotonia, developmental delay and seizures (predominantly myoclonic and some with epileptic spasms). Respiratory problems such as hyperventilation, laryngeal stridor or apnea may be the early symptoms in some biotinidase-deficient patients. Other common symptoms include conjunctivitis, intermittent ataxia, optic atrophy and sensorineural deafness. Acute metabolic decompensation with lactic acidosis, ketosis, hyperammonaemia, lethargy or coma may be the presenting symptoms in untreated cases. However, older children or adolescents may present with limb weakness, spastic paresis or visual problems such as scotoma and visual loss. Some patients also have immunodeficiency and are susceptible to severe fungal and bacterial infections. There may be abnormal findings in magnetic resonance imaging of brain, usually involving the white matter and such changes tend to improve after biotin treatment.

Cutaneous lesions and organic aciduria are important clues to the diagnosis. Skin manifestations including eczematous dermatitis and alopecia are common. Urine organic acid analysis may show 3-OH-propionic acid, 3-OH-isovaleric acid, lactic acid and 3-methylcrotonylglycine and the plasma acylcarnitine profile shows 3-OH-isovalerylcarnitine (C5-OH). However, both cutaneous lesions and biochemical changes may be intermittent and not present in the initial phase or sometimes absent, even in symptomatic cases. Therefore, the diagnosis of biotinidase deficiency and a therapeutic trial of biotin should be considered in children with progressive neurological symptoms of unexplained origin, particularly in infants with severe seizures and significant developmental delay even in the absence of organic aciduria and cutaneous lesions.

The diagnosis of biotinidase deficiency can be made by serum enzyme assay and can be further confirmed with mutational analysis of the BTD gene. Newborn screening is possible using the enzyme assay on dried blood spots.

Oral biotin at 5 - 20mg per day usually leads to clinical improvement in all symptomatic cases and early treatment in pre-symptomatic children may prevent the development of symptoms. Nevertheless, developmental delay and visual / hearing impairment, having once appeared, are usually irreversible, despite biotin supplement.

Conclusion

Early recognition of biotinidase deficiency is extremely important, particularly in localities without newborn screening for this disorder, as most cases will improve dramatically with biotin, and timely treatment in asymptomatic children may prevent metabolic decompensation in the newborn period and avoid long-term irreversible neurological deficits. Diagnosis may be difficult in some cases because of variable neurological symptoms and biochemical changes. With the availability of screening by colorimetic assay in dried blood spots and availability of effective treatment with biotin, screening for biotinidase deficiency in universal newborn screening programs warrants serious consideration and further studies.

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BRAINCHILD
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An approach to recurrent rhabdomyolysis

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Introduction

Rhabdomyolysis, caused by rapid significant dissolution of skeletal muscle fibres, is generally associated with a marked elevation of creatine kinase (CK) concentration in plasma, and/or detectable myoglobin in urine. Patients classically complain of muscle cramp or pain, muscle weakness, and/or pigmented urine. The release of large amounts of myoglobin into the circulation, when exceeding the capacity of binding proteins such as haptoglobin, could cause acute tubular necrosis and subsequently acute kidney injury, which is potentially life-threatening.

Case 1

The girl was born full-term with history of perinatal depression requiring resuscitation. At 5 months she was noted to have poor weight gain and developmental delay, with spasticity in limbs. Plasma lactate was elevated up to 6.6 mmol/L, with an elevated L/P ratio of 39; cerebro-spinal fluid lactate was 1.4 mmol/L. Dried blood spots tests and urine organic acids profiling were unrevealing. Serum TSH and prolactin were normal, and there was no hypoglycaemia documented. MRI brain findings suggested previous hypoxic brain injury as well as delayed myelination. Plasma CK had been mildly elevated to just above the upper reference limit.

At age 2 she presented with fever and vomiting, with cough and sputum. On admission hypernatraemia and pre-renal failure were noted, with plasma sodium 163 mmol/L, potassium 6.3 mmol/L, urea 33 mmol/L and creatinine 133 μmol/L. Serum and urine osmolality were 351 mOsm/kg and 939 mOsm/kg respectively. Plasma CK was once elevated up to above 62,000 U/L, which subsequently returned to previous levels with correction of dehydration and electrolyte disturbances.

Case 2

The child had his first episode of rhabdomyolysis at age 2 and subsequently another one at age 7, with plasma CK elevated to above 27,000 U/L, as well as detectable myoglobinuria. Both episodes were precipitated by fever. Dried blood spots tests showed normal patterns of amino acids and acylcarnitines, while urine organic acids profile showed no pathological pattern. The family history was unremarkable. Genetic testing for common known mutations in *RYR1* was negative, yet two heterozygous novel variants were subsequently detected in *LPIN1*. Parental studies confirmed compound heterozygosity of the genetic variants.

Case 3

Patient 3 enjoyed good past health with normal development. At age 7 he had his first episode of rhabdomyolysis, which was triggered by fever and flu-like illness. He had suffered from five non-exertional attacks by the age of 22. Plasma CK concentrations were elevated to more than 110,000 U/L, which returned to normal in between attacks. Renal function test was normal and autoimmune markers were negative. Fasting plasma lactate was 1.9 mmol/L. Dried blood spots tests showed normal patterns of plasma amino acids and acylcarnitines, and plasma free carnitine concentrations in between attacks were normal. Urine organic acids profiling was also unrevealing. Muscle biopsy showed query ragged red fibres shown by trichrome staining and accumulation of mitochondria in the subsarcolemmal regions by ultra-structural studies, but overall findings were inconclusive. The family history was unremarkable and none of his tested family members had elevated CK concentrations. Genetic tests for *CPT2* and *LPIN1* were negative.

Rhabdomyolysis is defined as the rapid breakdown of striated skeletal muscle fibres. Patients with rhabdomyolysis can present with myalgia, muscle weakness, muscle swelling and/or coloured urine,[1] which can progress to develop fever, malaise, tachycardia, vomiting, acute kidney injury, and ultimately disseminated intravascular coagulation and multi-organ failure.[2]) Regardless of the cause, dissolution of the muscle fibres cause systemic release of intracellular content, including myoglobin, CK, lactate dehydrogenase, as well as hyperkalaemia, hyperphosphataemia and hyperuricaemia.[3] The diagnosis of rhabdomyolysis is usually established with a plasma CK concentration that increased to greater than 5-fold,[3,4,5] or 10-fold,[6,7] of the upper reference limit, and subsequently returned to normal values. Myoglobin is believed to cause acute kidney injury by precipitation in the glomerulus and other mechanisms, and determination of myoglobin in urine or serum confirms the diagnosis of rhabdomyolysis.[4] The sensitivity of myoglobinuria to detect rhabdomyolysis is however limited, due to the relatively short half-life of myoglobin (2–4 hours) compared with that of CK (1–2 days), and myoglobin can rapidly decrease to normal levels within 24 hours.[8] Common causes of rhabdomyolysis are summarized in **Table 1** with elaborations below.

CAUSES

Acquired Causes. Rhabdomyolysis can be caused by trauma to muscles, as in crush injury, compartment syndrome or after prolonged surgical operations, or induced by significant muscle contractions as in strenuous exercises, convulsions or tetany.[4] Statins are known to induce muscle toxicity and rarely rhabdomyolysis in susceptible individuals.[7] Although rarely seen in younger children, rhabdomyolysis has been attributed to use of alcohol and substances of abuse such as heroin, cocaine and methamphetamine.[9] Recently emerging drugs of abuse has also been described to cause rhabdomyolysis in local cases.[10] In addition, antipsychotics and antidepressants have been associated with rhabdomyolysis, with or without neuroleptic malignant syndrome.[5,11] Therefore a carefully taken history and comprehensive toxicology screening may aid with the diagnostic evaluation. On the other hand, severe electrolyte disturbances especially hypokalaemia, hypophosphataemia, hypo-/hypernatraemia, which may be related to underlying endocrine disorders or abuse of

diuretics/laxatives, can lead to rhabdomyolysis. Other acquired causes of rhabdomyolysis include exposure to various toxins, venoms and poisons, as well as infections, heatstroke and hypothermia.[3,5] As in ***Case 1***, the acute episode of rhabdomyolysis was likely triggered by the severe hypernatremia secondary to dehydration and possibly the infection, although an underlying inherited susceptibility to rhabdomyolysis such as mitochondrial myopathy could not be excluded.

Inherited Causes. The possibility of an inherited cause should be considered when rhabdomyolysis is recurrent and an acquired cause is not apparent, especially in the paediatric group.

Conditions that are known to predispose rhabdomyolysis include metabolic myopathies such as disorders of glycogenolysis and glycolysis, and fatty acid oxidation defects; mitochondrial disorders; muscular dystrophies; and channelopathies.[5]

Clinical features are of utmost importance in delineating the underlying cause and a detailed history should be taken, including any muscle weakness, exercise intolerance, precipitating factors of attacks, extramuscular complaints, and family history of neuromuscular disorders. In patients with metabolic myopathies, rhabdomyolysis is often triggered by metabolic derangements, such as excessive exercise, prolonged fasting and infection. The knowledge of any consanguinity in the family may also help with the evaluation when many of the genetic causes are inherited in an autosomal recessive manner.

Disorders of glycogenolysis and glycolysis

Glycogen storage diseases (GSD) are known to be associated with rhabdomyolysis, which typically occurs after strenuous exercise. Of note, the classical example is McArdle disease (GSD type V), in which muscle glycogen phosphorylase is deficient due to mutations in PYGM. The second-wind phenomenon, which describes a sudden improvement of tolerance and attenuation of the early fatigue after exercising for about 10 minutes, is a pathognomonic feature for McArdle disease.[12] A modified forearm ischaemic test may help to establish the diagnosis, in which with adequate exercise plasma lactate fails to rise.[13] Other GSD known to cause rhabdomyolysis include muscle phosphofructokinase deficiency (GSD type VII), muscle phosphorylase kinase alpha-1 subunit deficiency (GSD type IXd), phosphoglycerate mutase 2 deficiency (GSD type X), lactate dehydrogenase A subunit deciency (GSD type XI) and fructose-1,6-bisphosphate aldolase A deficiency (GSD type XII). An increase in glycogen content might be seen in muscle biopsy for GSD. Rhabdomyolysis associated with phosphoglucomutase deficiency (congenital disorder of glycosylation type It) and phosphoglycerate kinase-1 deficiency are also reported.[5]

Fatty acid oxidation defects

Rhabdomyolysis related to fatty acid oxidation defects classically occurs after prolonged exercise when glycogen stores have been depleted. Recurrent myoglobinuria is a classical

presentation of carnitine palmitoyltransferase II (CPTII) deficiency caused by mutations in *CPT2*. Rhabdomyolysis is also known to be associated with very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency with mutations in *ACADVL*, and mitochondrial trifunctional protein deficiency with mutations in *HADHA* or *HADHB*. Less commonly cases are also reported in carnitine-acylcarnitine translocase deficiency with mutations in *SLC25A20*, and myopathic form of glutaric acidaemia type II mostly caused by mutations in *ETFDH*. Rhabdomyolysis has been previously reported in local cases of CPTII deficiency and VLCAD deficiency.[14,15] Characteristic abnormalities for individual disorders could be evident on plasma acylcarnitine profiling or dried blood spots metabolic screening, although patients might be biochemically normal in between attacks. Lipid content is uncommonly increased in muscle fibres on muscle biopsy.[16]

Mitochondrial myopathies

The presence of multi-systemic involvement suggests mitochondrial myopathies, which can be caused by mutations in mitochondrial DNA or nuclear genes. Ptosis or chronic progressive external ophthalmoplegia may also be apparent. Rhabdomyolysis has been attributed to coenzyme Q10 deficiency; defects in cytochrome b, cytochrome c oxidase and mitochondrial transfer RNA due to mutations in mitochondrial DNA (including m.3243A>G which also causes mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) can also present with rhabdomyolysis.[17]

Plasma lactate is often elevated, with an increased lactate-to-pyruvate ratio. Muscle biopsy can show ragged red fibres with Gomori trichrome stain as well as COX-negative fibres with oxidative stains; subsarcolemmal mitochondrial aggregates and pleomorphic mitochondria, with or without paracrystalline inclusions, can also be evident on ultra-structural studies, although these features are not specific and may be seen in other muscular disorders. Analysis of the electron transport chain complexes may also be considered when common mitochondrial mutations are not detected.

Muscular Dystrophies

When most patients with muscular dystrophies present with muscle weakness, these patients are prone to have rhabdomyolysis upon exertion due to sarcolemmal instability. Increased risks of rhabdomyolysis have been described in dystrophinopathies and autosomal recessive limb girdle muscular dystrophies including types 2B (dysferlinopathy), 2E (beta-sarcoglycanopathy), 2I (muscular dystrophy-dystroglycanopathy type C5) and 2L (anoctaminopathy).[5,17] An elevated baseline plasma CK is usually observed in muscular dystrophies, and immunohistochemical stains on muscle biopsy could aid in diagnosis.

Channelopathies

The skeletal muscle ryanodine receptor, encoded by *RYR1*, is a calcium release channel of the sarcoplasmic reticulum. Mutations in *RYR1* have been associated with susceptibility to malignant hyperthermia, central core disease and minicore myopathy.[18] Defective ryanodine

receptor in skeletal muscle has been an important cause of recurrent rhabdomyolysis.[19] Areas of central cores or minicores in muscle fibres that represent mitochondria depletion and reduced oxidative activity may be revealed on muscle biopsy. Mutations in *SCN4A*, which encodes a voltage-gated sodium channel in skeletal muscle, are also reported to cause rhabdomyolysis, in addition to paramyotonia congenita and periodic paralysis.[20]

Myoadenylate deaminase deficiency

Myoadenylate deaminase, encoded by *AMPD1*, catalyses the deamination of adenosine monophosphate in skeletal muscle. Myoadenylate deaminase deficiency may manifest as exertional myalgia or susceptibility to rhabdomyolysis. Patients may also be asymptomatic, probably due to certain compensatory mechanisms, and the clinical significance of this condition is often questioned.[21] A suboptimal increase in ammonia in modified forearm ischaemic test is a hallmark of myoadenylate deaminase deficiency, although the specificity is limited.[13]

Lipin-1-related recurrent rhabdomyolysis

Mutations in *LPIN1* are recently identified to be one major cause of autosomal recessive childhood-onset recurrent rhabdomyolysis.[22] The gene encodes lipin-1, a magnesium-dependent phosphohydrolase that catalyses the dephosphorylation of phosphatidic acid during synthesis of membrane phospholipids. Patients typical have their first episode of rhabdomyolysis between one and seven years old, and as in ***Case 2*** the attacks are most often precipitated by febrile illnesses.[23] It is now suggested that the diagnosis of lipin-1 deficiency should be considered prior to muscle biopsy in paediatric patients presenting with recurrent rhabdomyolysis associated with fever.

Exertional rhabdomyolysis

Benign exertional rhabdomyolysis has been an entity to describe otherwise normal individuals in whom clinical rhabdomyolysis is triggered by strenuous exercises. The diagnosis is often established by exclusion when investigations have been unrevealing.[24,25] It is now believed that certain yet unidentified genetic causes could underlie the susceptibility to rhabdomyolysis in these individuals.[5] Molecular analysis for most of the aforementioned disorders are available. Cases with high clinical suspicion of an underlying inherited cause may warrant target gene panel testing by next-generation sequencing.

Management

Regardless of the underlying cause of rhabdomyolysis, serial monitoring of renal function and coagulation profile is warranted for prompt detection of acute kidney injury and disseminated intravascular coagulation. On the other hand, plasma CK and urine myoglobin might not be adequate to predict the severity of rhabdomyolysis and risk of acute kidney injury.[26] Forced hydration by administration of saline or glucose fluid is often indicated, while the evidences for using bicarbonate for urine alkalinisation and mannitol for diuresis are considered controversial.[3,4] Renal replacement therapies such as continuous veno-venous

haemoperfusion and haemodialysis can be considered in severe cases.[27] Disease-specific treatments are available for individual groups of metabolic myopathies, while awareness and avoidance of the triggers such as fasting, strenuous exercises or high temperatures is the mainstay to prevent further attacks in all groups of inherited susceptibilities to rhabdomyolysis.

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Table 1. Summary of Major Causes of Recurrent Rhabdomyolysis.

Aetiologies	Potentially useful workup
Acquired Causes	
• Trauma, injury, compartment syndrome, strenuous exercises	History
• Heatstroke, hypothermia	History
• Infections	Acute phase reactants; microbiology and serology tests
• Drugs e.g. statins, alcohol and substances of abuses, antipsychotics and antidepressants	History; toxicology screening
• Toxins, venoms, poisons	History
• Electrolyte disturbances	Renal function test and bone profile
Inherited Causes	
• Disorders of glycogenolysis and glycolysis: *glycogen storage diseases e.g. type V or McArdle disease, types VII, IXd, X, XI and XII; congenital disorder of glycosylation e.g. type It; phosphoglycerate kinase-1 deficiency*	Modified forearm ischaemic test; muscle biopsy
• Fatty acid oxidation defects: *e.g. carnitine palmitoyltransferase II deficiency, very long-chain acyl-CoA dehydrogenase deficiency, mitochondrial trifunctional protein deficiency, carnitine-acylcarnitine translocase deficiency, glutaric acidaemia type II myopathic form*	Acylcarnitine profiling or dried blood spots tests; muscle biopsy
• Mitochondrial myopathies: *e.g. coenzyme Q10 deficiency, defects in cytochrome b, cytochrome c oxidase and mitochondrial transfer RNA*	Lactate and lactate-to-pyruvate ratio; muscle biopsy; electron transport chain complexes analysis
• Muscular Dystrophies: *e.g. dystrophinopathies; limb girdle muscular dystrophies types 2B, 2E, 2I and 2L*	Creatine kinase; muscle biopsy
• Channelopathies: *e.g. defects in RYR1 and SCN4A*	Muscle biopsy
• Miscellaneous: *myoadenylate deaminase deficiency; lipin-1-related recurrent rhabdomyolysis; benign exertional rhabdomyolysis*	

Laboratory investigations for monoamine neurotransmitter diseases

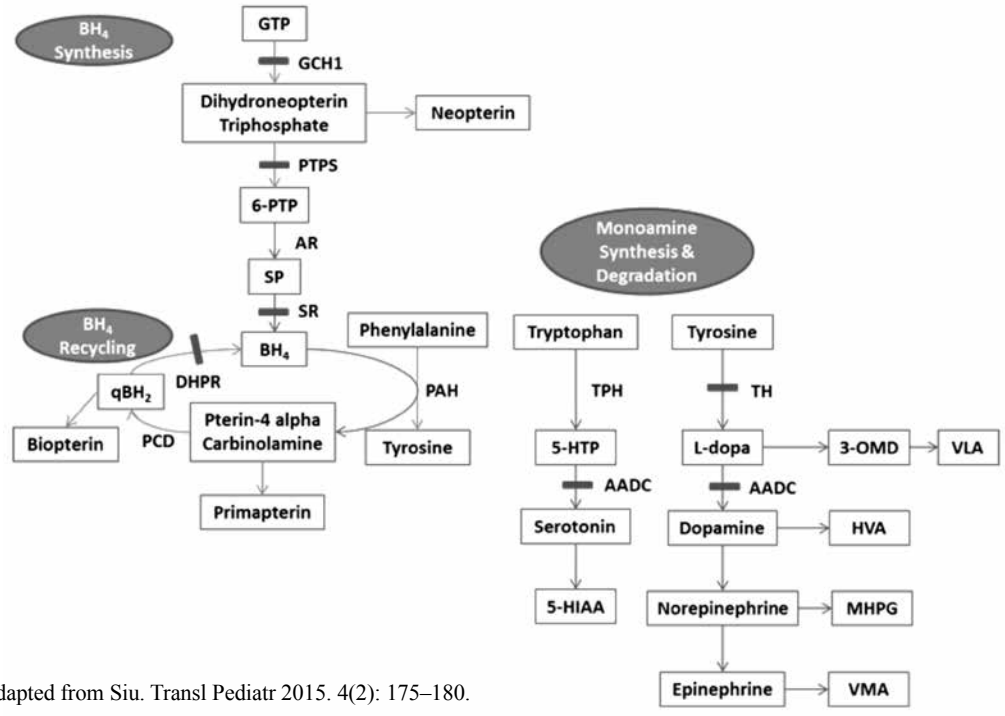
Dr. Carol SIU

Introduction

Defects in the synthesis and degradation of monoamine neurotransmitters represent a group of inherited diseases that are increasing recognised the paediatric population. Dopamine, epinephrine, norepinephrine and serotonin are the monoamine neurotransmitters with pivotal role in the functions of movement, mood and behaviour.[1] The synthesis and degradation pathways of monoamine neurotransmitters is shown in ***Figure 1***. Regarding the disease presentations, the age of disease onset is usually in infancy and childhood but can be variable. Neurological symptoms are the predominant features which include dystonia, dyskinesia, spastic paraparesis, choreoathetosis, oculogyric crises, seizures and autonomic dysfunctions. The clinical phenotypes overlap substantially among this group of disorders as well as mimicking many other neurological diseases. Accurate diagnosis is crucial for

Figure 1. Metabolic pathway of monoamine neurotransmitter synthesis and degradation.
BH_4 is an obligate co-factor for PAH, TPH and TH. The enzyme defects causing neurotransmitter diseases are depicted by solid bar.

AADC=aromatic L-amino acid decarboxylase. AR=aldose reductase. DHPR=dihydropteridine reductase. GTP=guanosine triphosphate. GCH1=guanosine triphosphate cyclohydrolase 1. 5-HIAA=5-hydroxyindoleacetic acid. 5-HTP=5-hydroxytrytophan. HVA=homovanillic acid. MHPG=3-methoxy-4-hydroxyphenylglycol. 3-OMD=3-O-methyldopa. PAH=phenylalanine hydroxylase. PCD=pterin-4-alpha-carbinolamine dehydratase. 6-PTP=6-pyruvoyl-tetrahydropterin. PTPS=6-pyruvoyl-tetrahydropterin synthase. qBH2=q-dihydrobiopterin. SP=sepiapterin. SR=sepiapterin reductase. TPH=tryptophan hydroxylase. TH=tyrosine hydroxylase. VLA=vanillactic acid. VMA=vanilmandelic acid.



Adapted from Siu. Transl Pediatr 2015. 4(2): 175–180.

Table 1. List of investigations for monoamine neurotransmitter diseases

Investigations for monoamine neurotransmitter diseases
- CSF neurotransmitter metabolites - Dried blood spot/ plasma amino acids - Serum prolactin - Urine metabolic profiling by GC-MS - Urine pterins - Mutation analysis for specific genes

prompt intervention as many of them are amendable to treatments and some conditions can have complete resolution of symptoms. Laboratory investigations are indispensable to delineate the exact diagnosis which is the key for therapeutic interventions, defining prognosis, and assessing recurrence risk.[2,3] In this article, the laboratory investigations for monoamine neurotransmitter diseases are reviewed. A list of useful tests is provided in ***Table 1***. The diseases covered in this article include autosomal dominant and autosomal recessive GTP cyclohydrolase 1 (GCH1) deficiency, deficiencies in 6-pyruvoyl-tetrahydropterin synthase (PTPS), tyrosine hydroxylase (TH), aromatic L-amino acid decarboxylase (AADC), sepiapterin reductase (SR) and dihydropteridine reductase (DHPR).

Cerebrospinal fluid analysis for neurotransmitters

For the diagnosis of monoamine neurotransmitter diseases, the metabolites related to the synthesis of degradation of monoamine neurotransmitters are measured in the cerebrospinal fluid (CSF). The analytes include 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), 5-hydroxytrytophan (5-HTP), neopterin, biopterin, sepiapterin, 3-O-methyldopa (3-OMD) and 5-methyltetrahydrofolate (5-MTHF). 5-HIAA and HVA are the metabolites of serotonin and dopamine respectively. 5-HTP is the precursor of serotonin.3-OMD is derived from L-dopa. The pathways also involve the synthesis and regeneration of tetrahydrobiopterin (BH_4) where neopterin, biopterin and sepiapterin are the metabolites in the BH_4 cycle. Each particular disorder gives a specific pattern in the CSF analysis (Table 2).

Table 2. Biochemical abnormalities in monoamine neurotransmitter diseases

	Enzyme Deficiencies						
Biochemical Markers	**GCH1(AD)**	**GCH1(AR)**	**PTPS**	**TH**	**AADC**	**SR**	**DHPR**
CSF 5-HIAA	N or ↓	↓	↓	N	↓	↓	↓
CSF HVA	↓	↓	↓	↓	↓	↓	↓
CSF/Urine Neopterin	↓	↓	↑	N	N	N	N
CSF/Urine Biopterin	↓	↓	↓	N	N	↑/N*	↑
CSF/Urine 3-OMD	N	N	N	N	↑	N	N
CSF Sepiapterin	N	N	N	N	N	↑	N
CSF 5-MTHF	N	N	N	N	N	N	↓
Serum prolactin	N	N/↑	N/↑	N/↑	N/↑	N/↑	N/↑
Blood Phenylalanine	N	↑	↑	N	N	N	↑

AADC=aromatic L-amino acid decarboxylase. AD=autosomal dominant. AR=autosomal recessive. CSF=cerebral spinal fluid. DHPR= dihydropteridine reductase. GCH1=guanosine triphosphate cyclohydrolase 1. 5-HIAA=5-hydroxyindoleacetic acid. HVA=homovanillic acid. 5-MTHF=5-methyltetrahydrofolate. 3-OMD=3-O-methyldopa. PTPS=6-pyruvoyl-tetrahydropterin synthase. SR=sepiapterin reductase. TH= tyrosine hydroxylase.

* Urine pterins are generally normal in SR deficiency.

Properly collected CSF samples are critical for reliable results. Traumatic taps should be avoided in lumber puncture since neurotransmitter metabolites are susceptible to rapid oxidation when exposed to red cells. Blood-stained CSF must be immediately centrifuged to obtain the clear supernatant. To eliminate the chance of having red cells in the CSF samples, the initial 0.5 mL CSF would not be put forward for analysis. Furthermore, there is rostrocaudal gradient for both 5-HIAA and HVA which the levels double in every 5-10 mL of CSF collected. Thus, the collection should follow standardized fractions labeled on the tubes and appropriate fraction is selected for analysis, on which the reference intervals are based on. Pterins are extremely sensitive to light and temperature. Immediately after collection, the CSF should be sent to the laboratory on ice and light protected. The method of analysis is high performance liquid chromatography with electrochemical detector and fluorimeter. The reference intervals are age-adjusted since the concentrations of dopamine and serotonin metabolites are at high level at birth and drop in the first few months of life then more gradually in adulthood.

Investigations in blood and urine samples

Various blood and urine tests are the integral part of the diagnostic work-up for neurotransmitter diseases. Notably, pterin analysis can be performed in urine samples, which are the choice of non-invasive investigations. The urine also gives specific abnormal pattern for a few defects including GCH1, PTPS and DHPR deficiencies. Moreover, detection of increased 3-OMD and vanillactic acid with reduced vanilmandelic acid in urine is useful as screening for AADC deficiency.[4]

Another important marker for neurotransmitter diseases is serum prolactin. The release of prolactin from pituitary gland is under tonic inhibition by dopamine. Hence, inherited conditions leading to dopamine deficiency will increase the prolactin level. Elevated serum prolactin has been demonstrated in certain neurotransmitter diseases. Galactorrhoea with increased serum prolactin has been reported in TH deficiency.[5] Elevation has also been reported in AADC and SR deficiencies.[6,7] The serum prolactin level has been considered to be a useful tool for monitoring the treatment in PTPS and DHPR deficiencies.[8,9] In addition, blood phenylalanine concentrations are increased in PTPS, DHPR and autosomal recessive GCH1 deficiencies. Plasma or dried blood spot amino acid analysis should be part of the investigations.

Genetic testing

The role of genetic testing is on diagnostic confirmation. Specific genes of the neurotransmitter diseases are analyzed to detect the presence of mutations. The conventional methods are polymerase chain reaction and Sanger DNA sequencing. Precise diagnosis by genetic testing is crucial as the therapies are disease-specific and mechanism-based. It is noteworthy that the genetic results are pivotal for proper genetic counselling and prognosis prediction. The risk of recurrence in future pregnancy can be determined and prenatal diagnosis is also feasible.

Biochemical and molecular defects of monoamine neurotransmitter diseases

Autosomal dominant GTP cyclohydrolase 1 (GCH1) deficiency

GCH1 is the enzyme that catalyses the rate-limiting step of guanosine triphosphate (GTP) conversion to dihydroneopterin triphosphate in BH4 synthesis.[2] *GCH1* gene, located at chromosome 14q22.2, is the disease-causing gene. Heterozygous GCH1 mutations cause autosomal dominant GCH1 deficiency which account for 90% of cases. The mechanism leading to partial BH4 deficiency is the dominant negative effects exerted by these mutants on the normal alleles.[10] Having higher infinity with BH4, tyrosine hydroxylase has more significant decrease in activity compared to tryptophan hydroxylase. Thus, the symptoms are mainly driven by dopamine deficiency. Biochemically, biopterin, neopterin and HVA are reduced in CSF. The low neopterin and biopterin are also shown in urine. The phenylalanine levels in blood are normal. The mutation detection rates for sequencing analysis and deletion/duplication analysis are 60% and 5-10% respectively.[11,14]

Autosomal recessive GTP cyclohydrolase 1 (GCH1) deficiency

Homozygous or compound heterozygous mutations of *GCH1* gene result in autosomal recessive GCH1 deficiency. The GCH1 enzyme activity is markedly reduced to less than 10% of control leading to deficiency of both serotonin and dopamine.[10] Hence, the clinical symptoms are usually severe with early disease onset. In CSF and urine, 5HIAA, HVA, biopterin and neopterin are all at very low levels. Hyperphenylalaninaemia is also often present.

6-Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency

PTPS is the key enzyme for BH_4 synthesis converting dihydroneopterin triphosphate to 6-pyruvoyl-tetrahydropterin.[2] The production of serotonin and dopamine are severely impaired since BH4 synthesis is remarkably reduced in the absence of PTPS.[15] It is the most common disorder in BH4 metabolic defects. Autosomal recessive mutations in the *PTS* gene at chromosome 11q23.1 are the culprit of the disease and there is reasonably good genotype-phenotype correlation. The CSF profile shows increased neopterin with reduced 5-HIAA, HVA and biopterin. Elevated neopterin and decreased biopterin are also present in urine. There is hyperphenylalaninaemia and raised serum prolactin has also been reported.

Tyrosine hydroxylase (TH)

TH catalyses the rate-limiting reaction of tyrosine conversion to L-dopa in catecholamine production. The clinical features of TH reflect cerebral catecholamine deficiency.[16] Characteristic CSF findings include a decrease in HVA with normal 5-HIAA which gives rise to an abnormally low HVA to 5-HIAA ratio. The biochemical abnormalities are usually not present in the urine. Raised serum prolactin is another biochemical feature and galactorrhoea could be one of the clinical presentations.[5] TH deficiency is an autosomal recessive condition caused by mutations in in TH gene on chromosome 11p15.5. This disease in relatively more prevalent in Chinese population and the mutation spectrum is heterogeneous.[17]

Aromatic L-amino acid decarboxylase (AADC) deficiency

AADC is the enzyme involved in the decarboxylation of 5-HTP and L-dopa into serotonin and dopamine synthesis using cofactor pyridoxal 5'-phosphate. Severe deficiency in dopamine and serotonin are the basis of the clinical presentations for AADC deficiency.[7] In CSF, the concentrations of 5-HIAA and HVA are decreased and the 3-OMD and 5-HTP are raised. High levels of 3-OMD with decreased vanilmandelic acid concentration are also detected in the urine.[4] Serum prolactin is elevated in several reported cases. The disease is caused by autosomal recessive mutations in DDC gene at chromosome 7p12.2 and a hotspot mutation c.714+4A>T (IVS6+4A>T) has been reported in Chinese.[18]

Sepiapterin reductase (SR) deficiency

SR is the enzyme responsible for the final step of BH4 metabolism. This autosomal recessive disease is due to mutations in SPR gene at chromosome 2p13.2.[6,10] The synthesis of serotonin and dopamine are both impaired. Elevated biopterin and sepiapterin, reduced 5-HIAA and HVA and normal neopterin are detected in the CSF.[2,19] The urine pterin profile usually does not have any abnormality. The blood phenylalanine at baseline is often normal owing to sufficient phenylalanine hydroxylase activity in peripheral tissue with the presence of dihydrofolate reductase, aldose and carbonyl reductase to maintain adequate BH_4 level. Raised serum prolactin might be observed.

Dihydropteridine reductase (DHPR) deficiency

DHPR is involved in the pathway of BH_4 regeneration. Mutations in QDPR at chromosome 4p15.3 cause DHPR deficiency and the inheritance is autosomal recessive. Enzyme defect of DHPR results in BH4 deficiency and accumulation of the metabolite q-dihydrobiopterin which in turn exerts inhibitory effects on downstream enzymes including PAH, TPH, TH and AADC. Consequently, there is hyperphenylalaninaemia as well as impaired production of serotonin and dopamine.[20] In addition, DHPR is vital for maintaining folate in its active form so the deficiency will lead to folate depletion in the central nervous system.[21] The clinical symptoms of DHPR deficiency are generally more profound than other monoamine neurotransmitter diseases. In CSF, the levels of 5HIAA, HVA and 5-MTHF are low while the biopterin is elevated. Hyperphenylalaninaemia and hyperprolactinaemia are both present.

Conclusion

It is imperative to come to the realisation that monoamine neurotransmitter diseases is a heterogeneous group of inherited disorders which can have non-specific presentations and significant clinical overlap with other neurological conditions. The role of laboratory investigations to identify the underlying defects is immense as the specific patterns of biochemical abnormalities and the mutation detection in relevant genes confirm the diagnosis. Accurate and prompt diagnosis is of paramount importance guiding for specific treatments which often leads to improved clinical outcomes in patients.

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A Girl with an Abnormal Gait

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This was a sixteen-year-old girl who was referred from orthopaedic surgeon to paediatrician because of abnormal gait. The girl is from Pakistan origin and was born in Pakistan. The whole family immigrated to Hong Kong in June 2014. The girl has poor vision since birth and the poor vision remains static. She can best differentiate the number of fingers. The abnormal gait has been there for a long time. She is otherwise healthy. She has no history of convulsion or other neurological problems. Family history revealed that both biological parents are from consanguineous marriage. Father has poor vision since birth. Father has two daughters (one of them is the patient) and two sons from the first wife and another daughter from the second wife. All the children except the patient are normal. Functionally, due to poor vision, the patient's activities of daily living are all dependent on her sisters. She received no formal education in Pakistan.

On physical examination, the patient had no dysmorphism. The patient could walk unaided. The gait was non-specific which was a bit wide-based with very short strides. She could not walk on heels or on tip-toes. The neurological examination revealed normal tone, normal reflexed on upper limbs but absent reflexes on lower limbs. There were no definite cerebellar signs.

The differential diagnosis at this juncture was: gait anomaly due to visual impairment, peripheral neuropathy, myopathy, syndromal or chromosomal cause. Baseline blood investigations including muscle enzymes, metabolic studies, and blood investigations for peripheral neuropathy including glucose, immune markers, vitamin B12 were done. Magnetic Resonance Imaging (MRI) of brain and orbit was also ordered. Nerve conduction study was also arranged.

A batch of nerve conduction studies was done in April 2015. The following abnormalities were shown: (1) Motor study on right tibial nerve showed a very small amplitude of compound motor action potential (CMAP) of 0.5mV at a stimulation current of 100mA. (2) The CMAP at stimulation of right peroneal and left peroneal nerves were at the low normal range. (3) Sensory study on both sural nerves showed no reproducible sensory nerve action potential (SNAP). At the same time, serum vitamin B12 level was found to be 68 pmol/L (normal reference: 133-675 pmol/L). Vitamin D2 was also shown to be at a severe deficiency level. With the above nerve conduction study results and serum vitamin B12 level, the patient was diagnosed to have peripheral neuropathy secondary to vitamin B12 deficiency. Vitamin B12 supplement was started on 2nd May 2015, initially 1mg daily for one week, followed by 1mg weekly for four weeks and then 1mg monthly. It was given by intramuscular route. Just before vitamin B12 treatment, serum vitamin B12 level was repeated and was found to be

163pmol/L. Two months after vitamin B12 treatment, the level was repeated and was shown to be 250 pmol/L.

A batch of nerve conduction studies was repeated in August 2015. The following significant findings were shown: (1) Motor study on right tibial nerve showed the amplitude of CMAP of 0.8mV at a stimulation current of 100mA. (2) An increase in amplitude of CMAP was seen on stimulation of right peroneal, left tibial and left peroneal nerves. The increase was 50% to 100%. (3) Normal SNAP was seen on stimulation of bilateral sural nerves. Clinically the patient could walk better with a faster stride velocity. The girl could walk on heels which she could not do so before.

Nerve conduction study was done again in February 2016 with the following significant findings: (1) The amplitude of CMAP on stimulation of right tibial nerve was 8.3mV at a stimulating current of 100mA. (2) There was further improvement in amplitude of CMAP on stimulation of right peroneal nerve. The increase was about 30%. Clinically the patient could walk even better and could walk on tip-toes. The stride distance also improved.

Subsequently, MRI was done on this patient showing the following abnormalities: dilated fourth ventricle, elongated superior cerebellar peduncles, hypoplastic cerebellar vermis simulating the molar tooth sign and suggestive of Joubert syndrome-related disorder. This syndrome is characterized by hypotonia, abnormal ocular movements, intellectual disability of variable severity. Very often, ataxia with broad based gait was found. Later, eye assessment on our patient revealed retinitis pigmentosa. Despite the likely fact that the girl got the second diagnosis of Joubert syndrome, literature review showed no correlation between vitamin B12 deficiency and Joubert syndrome. The patient was placed in Ebenezer School (special school for visually blind children) for further education. The patient came to hospital for monthly vitamin B12 injection. So far, she enjoyed good result from vitamin B12 injection as seen clinically and electrophysiologically. She experienced no side effects from treatment.

Discussion

Acquired peripheral neuropathy accounts for <30% of all cases of peripheral neuropathy in children. (Common causes are actually hereditary causes like Charcot-Marie Tooth disease.) ***Table 1*** lists out the causes of acquired peripheral neuropathy in children. Among them, vitamin B12 deficiency, although rare, is a treatable condition of peripheral neuropathy.

The recommended dietary intake of vitamin B12 is 2.4 μg/day. (Usual western diet contains 5-7μg/day.) Major dietary sources are meat, eggs, milk, shellfish. The release of vitamin B12 from food for absorption into the body is complex and requires intact function of stomach, pancreas and ileum. The etiology of vitamin B12 deficiency could be dietary like in chronic alcoholism, in long-term vegetarian diet, or in infants who are exclusively breast-fed.[1] Autoimmune gastritis resulting from destruction of gastric parietal cells and the

associated lack of intrinsic factor to bind ingested vitamin B12 is another common etiology. (This abnormal autoimmune disorder very often is associated with thyroid disease, type I diabetes mellitus, or vitiligo.) Other gastrointestinal etiologies includes gastrectomy, chronic pancreatitis, ileum resection, Crohn's disease, short-gut syndrome, intestinal bacterial overgrowth, Helicobacter pylori infection of stomach, prolonged antacid therapy, etc.

Table 1. Acquired causes of peripheral neuropathy

Inflammatory	Acute inflammatory demyelinating polyneuropathy (Guillain Barre syndrome)
	Chronic inflammatory demyelinating polyneuropathy
Infection	Lyme disease
	Chagas disease (American trypanosomiasis)
	Diphtheria
	Leprosy
	Rabies
Rheumatic Diseases	Churg-Strauss syndrome (allergic granulomatosis and angiitis)
	Henoch Schonlein purpura
	Inflammatory bowel disease
	Juvenile idiopathic arthritis
	Systemic lupus erythematosus
Organ Failure	Renal failure
	Hepatic failure
	Critical illness polyneuropathy
Others	Diabetes Mellitus
	Porphyria
	Hypothyroidism
	Coeliac disease
	Malignancy
	Drugs like isoniazid, vincristine
	Toxins like lead, arsenic, mercury
	Vitamin deficiency like vitamin B12 (cobalamin) deficiency, vitamin B6 (pyridoxine) deficiency

Lastly, certain hereditary enzymatic defects and mutations in genes encoding endocytic receptors involved in ileal absorption and cellular uptake, such as mutations in the gene encoding for the gastric intrinsic factor, can also cause as vitamin B12 deficiency. Looking back to our case, the likely cause in this child is dietary given the fact that the patient was also found to have severe vitamin D deficiency.

Neurologic manifestations may be the earliest and often the only manifestation of vitamin B12 deficiency.[2] Hematologic manifestation, namely macrocytic anemia is another manifestation. The severity of the hematologic and neurologic manifestations may be inversely related in a particular patient. In our patient, she had no anemia with normal MCV, normal iron profile and normal folate level.

Neurologic presentation, when present, consists of the classic picture of subacute combined degeneration of the dorsal (posterior) and lateral spinal columns. This lesion, specific for vitamin B12 deficiency, is due to a defect in myelin formation of unknown mechanisms. The lateral column consists of the corticospinal tract and the dorsal column is responsible for proprioception and vibration. The overall effect of subacute combined degeneration will be paresthesia of limbs, impaired vibration and position sense, sensory ataxia, then progress to spastic paraparesis, clonus, extensor planter response. Another neurological manifestation of vitamin B12 deficiency is peripheral neuropathy which is typically sensorimotor, symmetrical axonopathy and affects the legs more than the arms. Other neurologic manifestations are optic neuropathy and neuropsychiatric symptoms like impaired memory, personality change, cognitive decline, dementia in elderly.[3] Our patient demonstrated absent reflexes and a non-specific abnormal gait. The differential diagnosis covered a range of neurological conditions including myopathy and neuropathy. Hence, the initial investigation was quite extensive and included blood tests, imaging and electrophysiological studies. It is fortunate that we did the nerve conduction study meticulously and discovered abnormalities of peripheral neuropathy. Among the many causes of acquired peripheral neuropathy, we found vitamin B12 deficiency in this patient, and vitamin B12 therapy was started. Subsequently, the patient showed continuous clinical and electrophysiological improvement after vitamin B12 treatment and this also gave strong evidence that vitamin B12 deficiency was the culprit.

Tests to measure serum vitamin B12 levels in the body are readily available and inexpensive. The cut-off level to define deficiency level is < 150 pmol/L. The level of 150-250 pmol/L is defined as a possible vitamin B12 deficiency because neurological symptoms can occur at this range. A level > 250 pmol/L is accepted as the normal level. Levels of serum methylmalonic acid and homocysteine are ancillary diagnostic tests of vitamin B12 deficiency but they too have their limitations. (***Figure 1*** shows the metabolism of vitamin B12.)[4] In our patient, homocysteine was actually checked but this was not elevated. Therefore, in the end, we rely on the serum vitamin B12 level, clinical assessment and nerve conduction study as monitoring. We also did the assay of anti-intrinsic factor and anti-parietal cell antibodies as part of the workup for the underlying etiology of vitamin B12 deficiency but they were found to be negative.

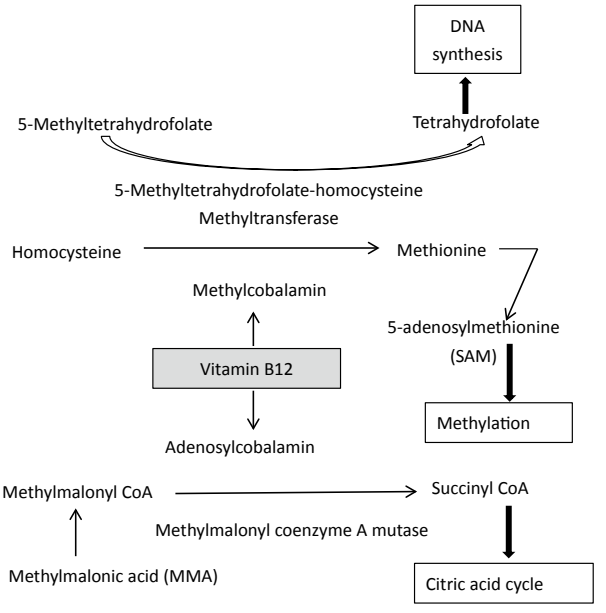

Figure. 1 Metabolism of Vitamin B12

Treatment of vitamin B12 deficiency is by vitamin B12 replacement - vitamin B12 1mg intramuscular route weekly for one week, followed by weekly for four weeks. If the etiology is not possibly eliminated, vitamin B12 1mg in intramuscular route given monthly should be continued for the rest of patient's life. The alternative is to give oral vitamin B 1-2mg daily as maintenance when the serum vitamin B12 has reached normal level. Neurologic abnormalities, if present, improve over the ensuing three months, with the maximum improvement attained at six to twelve months. The degree of improvement is inversely related to the extent and duration of disease. The ability to reverse symptoms like cognitive impairment may not be promising. Nevertheless, continuous replacement therapy may still help to prevent symptoms from deteriorating. Treatment is started soon after diagnosis of vitamin B12 deficiency. Response is promising and the girl showed improvement in gait. We are still not sure how much the peripheral neuropathy contributes to this girl's overall functional disability initially given the fact that the child also suffers from Joubert syndrome with retinitis pigmentosa and intellectual problem. Nevertheless, we believe that vitamin B12 therapy does help significantly as reported by the whole family.

In conclusion, in face of unexplained neurological signs and symptoms like paresthesia, limb weakness, ataxia, dementia, extensive investigations are warranted. Very often electrophysiological studies like nerve conduction studies are unexpectedly helpful. Vitamin B12 is one of treatable cause of peripheral neuropathy. Treatment is straight forward and rewarding.

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A Message from Rare Disease Patients

Hong Kong Mucopolysaccharidoses & Rare Genetic Diseases Mutual Aid Group

Our Introduction

It all started after a casual meeting of five families, each with a member diagnosed with a rare disease. This fellowship gradually grew into The Hong Kong Mucopolysaccharidoses & Rare Genetic Diseases Mutual Aid Group, which became a registered charitable organisation in 2005 and now has some 50 members, all of whom are rare disease patients. About half of our members are patients diagnosed with Mucopolysaccharidoses, while the rest are patients diagnosed with other rare diseases, including Gaucher Disease, Pompe Disease and Huntington Disease. (details in the Table 1 below)

Table 1. Members of Hong Kong Mucopolysaccharidoses & Rare Genetic Diseases Mutual Aid Group

Type	No. of Members
MPS1	3
MPS2	4
MPS3	8
MPS4	7
MPS6	3
MPS7	0
MPS9	0
Sub total	**25**
Atypical Hemolytic Uremic Syndrome	2
Fabry disease	2
Gaucher's disease	1
Glutaric aciduria Type I	1
Glycogen storage disease	1
Hereditary epidermolysis bullosa	1
Huntington's Disease	1
Interstitial deletion of chromosome 3q23 to 3q25	1
Kabuki Syndrome	1
Leigh Disease	1
Mucolipidosis	1
Multiple sclerosis	1
Osteogenesis Imperfecta	1
Phenylketouria (PKU)	2
Pompe disease	5
Prader-Willi Syndrome	1
Sotos Syndrome	0
Spinocerebellar Atrophy Type 35 and Spondyloepiphyseal Dysplasia	1
Urea cycle disorder	1
WAGR syndrome	1
Williams Syndrome	1
Unknown	1
Sub total	**28**
Total	**53**

(Updated on 12 May 2017)

Our Objectives

In addition to supporting and encouraging one another, our members and their families often share news about the latest medical developments and promote rare disease awareness to the public. As patient advocates we also appeal to the government for support, with good success. For example, in 2010, the Hospital Authority finally responded to our pleas to include Enzyme Replacement Treatment in the Drug Formulary. This meant that patients who had long been agonised by and suffered from rare diseases could finally receive affordable treatment, and have a hope for the future.

Our Aspirations

Recently, Secretary for Food and Health Dr. Ko Wing-man announced that the Food & Health Bureau would expand the Newborn Screening System to cover over 20 rare diseases. This is great news to us, as early detection means early treatment, and both the patients and their families can better plan for the treatment regimen, daily medical care and the life to come. But screening alone is still far from enough, we hope that patients whose diseases are treatable could receive treatment as early as possible, so as to catch the "Golden Treatment Period" and achieve maximum medical efficacy. We also hope that the government could formulate a Rare Disease Policy and provide on-going medical sponsorship to all rare disease patients, to allow them to live with dignity and hope, and to know that society cares.

Our sincere gratitude and appreciation goes to all medical personnel who have cared for us; your professionalism has allowed our patients to receive the best care available. Going forward, we hope that the following suggestions can also be implemented, so that the "body, mind and soul" of the rare disease patients and their families can be holistically attended to:

1. ***Psychological counselling:***
   a. Post-diagnosis psychological counselling
      Early psychological counselling is particularly crucial, as the emotional turmoil experienced by rare disease patients and their families—from the initial agony, self-blame, anxiousness to final acceptance—takes time to settle. To prevent the "traumatised" patients and their families from falling into a downward spiral of negative thinking and causing harm to themselves, early professional intervention and treatment are imperative.

2. ***Community support:***
   a. Community care
      With the large amount of patients requiring medical attention, relying solely on the counselling offered by public hospitals is not enough. On-going support requires the participation from the community, including psychologists from community groups and social workers to provide proactive, effective and continual emotional support.

b. Referral to patient groups (if there is one), as mutual support among patients is equally important.

c. Financial support
Social workers should assist patients and families with limited financial means to identify suitable sources of financial support and work out financial solutions to reduce the burden incurred by medical treatment.

d. Community rehabilitation service
Rare disease patients often require long-term rehabilitation therapy, and yet the waiting period in hospitals is usually too long to meet the demand. As such, the establishment of more community rehabilitation centres can help to provide simple but effective physiotherapy and occupational therapy to the patients and slow down the physical decline caused by rare diseases.

3. ***Specialist care:***

a. Establish a specialty for rare diseases to provide specialist care to rare disease patients
Hong Kong's hospitals do not currently have a rare disease specialty; patients are usually assigned to other specialties (such as paediatric, geriatric, internal medicine or metabolic, etc.) by age or medical symptoms. Without a clear clinical focus, rare disease patients are unable to receive the best, targeted treatment and medical professionals cannot accumulate the experience required to provide better care. We therefore suggest the Hospital Authority to establish a rare disease specialty in one large-scale hospital in each cluster to facilitate both treatment and medical research.

If we can all work together towards the above goals, we can significantly allay the mental and physical strain suffered by the patients and their families.

Hong Kong Mucopolysaccharidoses & Rare Genetic Diseases Mutual Aid Group
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