HK J Paediatr (new series) 2006;**11**:337-340

Diagnostic Difficulties in Sanfilippo Disease

DKH ***CHAN,*** CW ***LAM,*** STS ***LAM***

Abstract We reported on a 21 months old girl, diagnosed as Sanfilippo disease, MPSIIIB. Her mother was at 21 weeks of pregnancy when she first presented to us. The diagnostic pitfalls in the clinical, biochemical and genetic aspects were highlighted. The limited time frame for urgent molecular diagnosis, prenatal diagnostic tests and counselling issues were discussed.

Key words Alpha-N-acetylglucosaminidase; Diagnostic pitfalls; MPSIIIB; Sanfilippo disease type B

Introduction

Sanfilippo disease, abbreviated as MPSIII, is a rare lysosomal storage disease (LSD) involving specific enzymatic defects in the degradation pathway of heparan sulphate. The prevalence of MPSIII subtypes varies from 1 in 114,000 to 1 in a million. To the best of our knowledge, there is no available Chinese data at present. The hallmark of this disease is disproportionate and progressive neurological deterioration without striking somatic involvement. The subtypes MPSIII A/B/C/D are caused by the deficiencies of heparan N-sulfatase (SGSH), alpha-N-acetylglucosaminidase (NAGLU), acetyl CoA: alpha-glucosaminide N-acetyltransferase and N-acetylglucosamine 6-sulfatase (GNS) respectively.[1]

Here we reported on a girl who presented early with only mild somatic features of mucopolysaccharidosis (MPS) and absence of significant mental deterioration. DNA-based analysis identified known compound heterozygous disease-causing mutations for both R482W and R565W of the NAGLU gene.[2-4] The reliability and limitations in terms of clinical and laboratory diagnoses were reviewed.

Clinical Genetic Service, Department of Health, Hong Kong, China

DKH **CHAN** （陳錦雄） *MBChB(CUHK), MRCP(UK)*
STS **LAM** （林德深） *FHKAM(Paed), FRCP*

Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, N.T., Hong Kong, China

CW **LAM**（林青雲） *FHKAM(Pathology)*

Correspondence to: Dr STS **LAM**

Received July 11, 2006

Case Report

This 21 months girl was referred to the Clinical Genetic Service (CGS), HKSAR, for assessment of suspected MPS. Antenatal history was uneventful except that her mother was found to have iron deficiency anemia and was treated with iron supplements. She was born at full term by normal delivery, weighing 3.3 kg. Macrocephaly and coarse facial features was noted since 6 months of age. Her neurodevelopment was up to her age. She had no known history of hyperactivity and sleep disorders. Her parents and elder sister all enjoyed good health.

Investigations showed normal liver function test, skeletal survey and CT scan (Figure1). First urinary screening by toluidine blue test for glycosaminoglycan (GAG) was "negative".

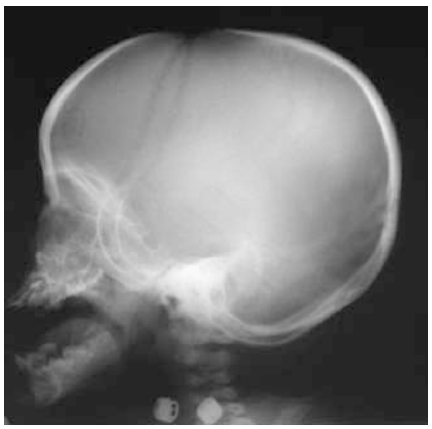
When the proband attended CGS at 21 months, her mother was noted to be pregnant at 20 5/7 weeks of gestation, posing an urgent concern for a possible genetic problem in the family. The assessment of the girl revealed macrocephaly and coarse facial features including hirsuitic forehead, short nose and thick lips. There was neither abnormal behaviour

nor other somatic features of MPS like hernia, skeletal problems and hepatosplenomegaly (Figure 2).

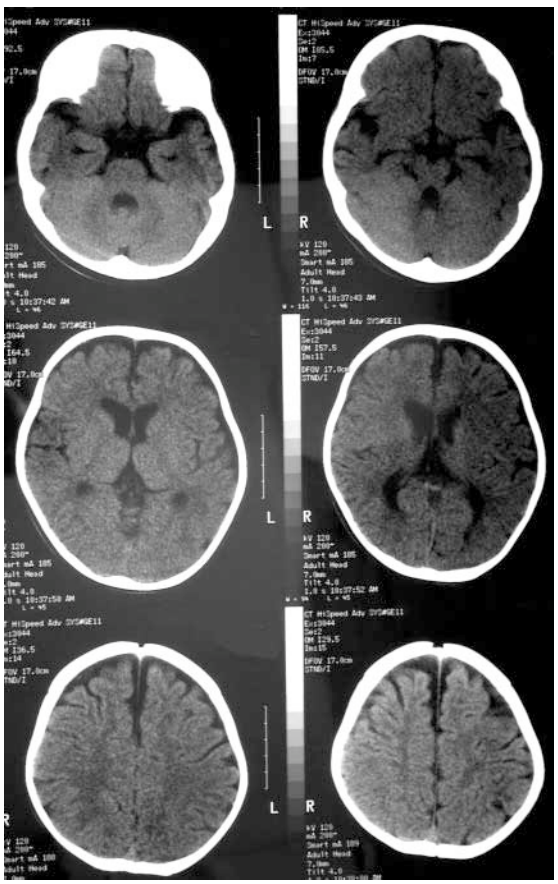
Urgent blood for karyotyping and molecular testing for Fragile X syndrome yielded normal results. Even though a second urine specimen for GAG screening by toluidine blue test was again "negative", the diagnosis of MPS was still highly suspected because of the coarse facial features. Further examination of urine by two-dimensional electrophoresis (2D-EP) identified abnormal hyper-excretion of heparan sulphate. The finding was diagnostic of Sanfilippo disease (MPSIII). Urine for oligosaccharide did not identify any abnormality by thin layer chromatography.

The family returned at 23 weeks of gestation for counseling of MPSIII. Clinical details about the natural history, prognosis and availability of treatment of MPSIII were discussed. Genetic counselling had been offered regarding this autosomal recessive condition, with recurrence risks up to 25% for every pregnancy. The parents were informed that this is a progressive neurodegenerative disorder with no available curative treatment. Prenatal diagnostic counselling was arranged in view of the urgency of enzymatic and molecular confirmation for the fetus at this critical moment. Peripheral blood was taken from all family members and urgent amniocentesis was performed with consent for testing of alpha-N-acetylglucosaminidase activity in an oversea center. The cultured amniocytes of the fetus showed that there was marked deficiency in the level of alpha-N-acetylglucosaminidase activity (Table 1).

Their genomic DNA was extracted for mutational analysis for both the Sanfilippo A and B genes. The proband and the fetus both inherited a compound heterozygous for R482W and R565W mutations of Sanfilippo B (NAGLU) gene (Figure 3). These were known disease-causing mutations. Her father and mother were found to be



(a)



(b)

Figure 1 Normal findings of imaging investigations for the propositus including: (a) radiograph of the skull and (b) computer tomographic (CT) film of the brain.

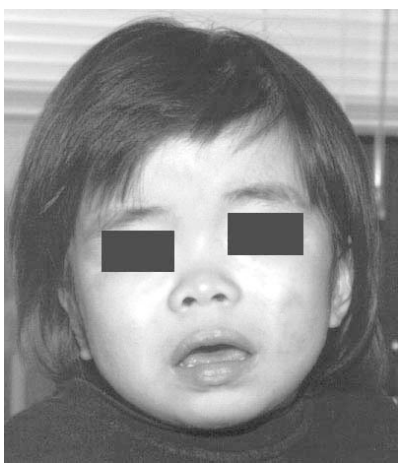


Figure 2 Frontal view of the propositus at age 21 months. Coarse facial features were noted including hirsuitic forehead, short nose and thick lips.

heterozygous R565W and R482W carrier respectively. Her elder sister was not affected.

The parents finally decided to terminate the pregnancy just before 24 weeks of gestation, based on the earliest return of molecular confirmation. The proband was referred for developmental assessment to detect early complications of this disease. Informed consent for publication of medical information including clinical details, imaging and clinical photos had been obtained.

Discussion

Mucopolysaccharidosis is a form of lysosomal storage disease (LSD) due to enzymatic defects in the degradation pathways of the GAGs. Clinical manifestations include a combination of significant coarse facial features and various multi-systemic involvements, including hepatosplenomegaly, dysostosis multiplex and developmental delay. Sanfilippo disease, MPSIII, is distinctive in its disproportionate involvement in the neurological system with minimal somatic manifestation. Early diagnostic difficulties in a few aspects will be highlighted.

Firstly, the clinical diagnosis of MPSIII usually only becomes evident when neurological symptoms became apparent; including intractable aggression, hyperactivity and sleep disturbance. In this case, the proband only exhibited somatic signs of macrocephaly and coarse facial features on examinations, which were non-specific indicators to any subgroups of MPS. The characteristic features of progressive neurodegeneration and behavioural problems might take a few more years to become clinically evident.

Secondly, difficulties in employing simple biochemical screening for GAGs are clearly exemplified by this case. It had been well reported that false negative results were commonly seen in all urine screening assays, including the CTAB test and the toluidine blue test.[5-7] MPSIII and IV are the more commonly encountered cases in clinical practice which will be "missed". In this case, toluidine blue test was employed. This test is mainly used to detect chondroitin sulphate, but is less sensitive towards heparan sulphate. 2D-electrophorsis is more reliable for quantifying the amount of specific GAGs. It is the recommended test for accurate diagnosis of MPS, when clinical suspicion remained unresolved.

Table 1 Alpha-N-acetylglucosaminidase activity of the cultured amniocytes

	Cultured amniocytes Alpha-N-acetylglucosaminidase (pmol/min/mgprotein)
Fetus	0.1
Intrabatch controls	12.4
Reference range	1.6-28

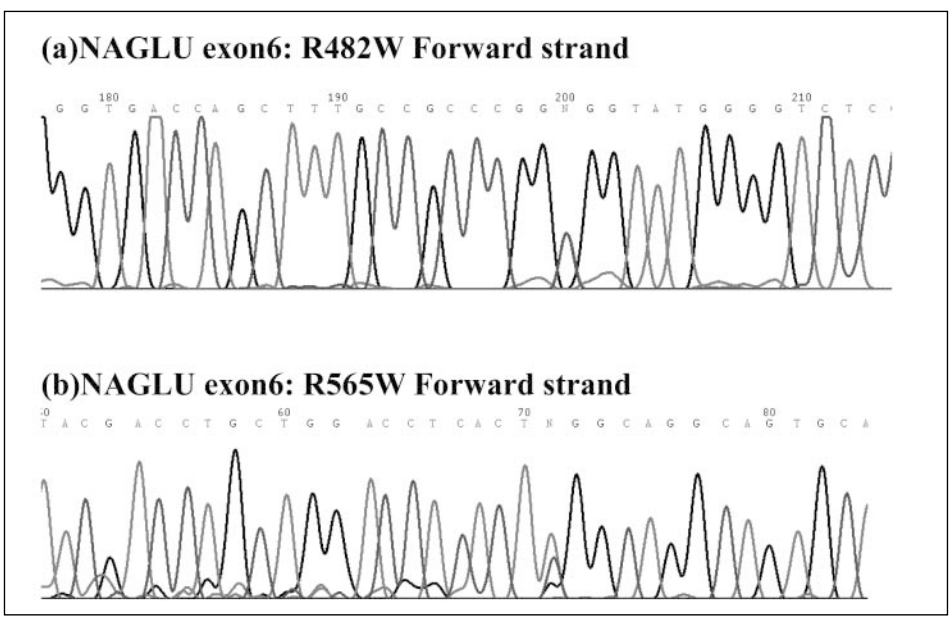


Figure 3 Mutational analysis of NAGLU gene (The propositus and fetus).

Thirdly, enzymatic assay was not available in our locality. The use of this alternative diagnostic procedure was precluded by the time constraint required for urgent prenatal diagnosis in this family. Molecular diagnosis of MPSIII subtypes was thereby the most reliable confirmatory test in this situation. However, technical difficulties always existed.[8-10] Since the presentations of various forms of MPSIII were quite indistinguishable clinically, it was logical but also time consuming to perform mutational analysis of four specific genes. To our knowledge, there was no reported information regarding the epidemiology of Sanfilippo disease and mutational hotspots of these four specific genes in Chinese patients for reference. Obviously, the diagnostic complexity and urgency was well illustrated.

All these aspects of diagnostic difficulties do not only frustrate the physician, but also cause tremendous psychological impact to this family. Quite often we do encounter families who desperately want another healthy child, even before the medical diagnosis and genetic counselling of a previous affected child could be confirmed.

Based on the experience from this case, we do advocate a high index of suspicion among clinicians for earlier diagnosis of MPSIII, even though no curative treatment is available to these subjects. Sanfilippo disease is unique among MPS because of technical difficulties in delivering the deficient enzyme to the brain efficiently across the blood-brain barrier (BBB). However, anticipatory care is still beneficial to the family, especially regarding possible behavioural problems and sleep disturbances. On the other hand, this case best illustrates the importance of early genetic diagnosis, carrier detection and possibly prenatal diagnostic test for timely counselling and intervention.

Acknowledgements

We express our in-depth thank to Staff of Department of Chemical Pathology, Princess Margaret Hospital and Dr. M Tang, Prenatal Diagnostic Clinic, Tsan Yuk Hospital of Hong Kong University for their invaluable help in urgent urinalysis of MPS and amniocytes collection respectively.

We also thank Dr. Lo Ling, Paediatrics Department, United Christian Hospital and Dr. Lau Wai Lam, O&G Department, Kwong Wah Hospital for referring this family to us for assessment.

References

1. Yogalingam G, Hopwood JJ. Molecular genetics of mucopolysaccharidosis type IIIA and IIIB: Diagnostic, clinical, and biological implications. Hum Mutat 2001;18:264-81.
2. Zhao HG, Li HH, Bach G, Schmidtchen A, Neufeld EF. The molecular basis of Sanfilippo syndrome type B. Proc Natl Acad Sci U S A 1996;93:6101-5.
3. Weber B, Guo XH, Kleijer WJ, van de Kamp JJ, Poorthuis BJ, Hopwood JJ. Sanfilippo type B syndrome (mucopolysaccharidosis III B): allelic heterogeneity corresponds to the wide spectrum of clinical phenotypes. Eur J Hum Genet 1999;7:34-44.
4. Tanaka A, Kimura M, Lan HT, Takaura N, Yamano T. Molecular analysis of the alpha-N-acetylglucosaminidase gene in seven Japanese patients from six unrelated families with mucopolysaccharidosis IIIB (Sanfilippo type B), including two novel mutations. J Hum Genet 2002;47:484-7.
5. de Jong JG, Wevers RA, Laarakkers C, Poorthuis BJ. Dimethylmethylene blue-based spectrophotometry of glycosaminoglycans in untreated urine: a rapid screening procedure for mucopolysaccharidoses. Clin Chem 1989;35:1472-7.
6. Leistner Sandra and Giugliani Roberto. A useful routine for biochemical detection and diagnosis of mucopolysaccharidoses. Genet. Mol. Bio. Vol 21 n.1 Sao Paulo Mar.1998
7. Chih-Kuang C, Shuan-Pei L, Shyue-Jye L, Tuen-Jen W. MPS screening methods, the Berry spot and acid turbidity tests, cause a high incidence of false-negative results in sanfilippo and morquio syndromes. J Clin Lab Anal 2002;16:253-8.
8. Beesley CE, Young EP, Vellodi A, Winchester BG. Identification of 12 novel mutations in the alpha-N-acetylglucosaminidase gene in 14 patients with Sanfilippo syndrome type B (mucopolysaccharidosis type IIIB). J Med Genet 1998;35: 910-4.
9. Durkovic J. Diagnostics of mucopolysaccharidoses presented through the case of Sanfilippo syndrome. Srp Arh Celok Lek 2004;132(5-6):174-8
10. Zhao HG, Aronovich EL, Whitley CB. Genotype-phenotype correspondence in Sanfilippo syndrome type B. Am J Hum Genet 1998;62:53-63.