RESEARCH BRIEF

Diagnostic Strategy for Mucolipidosis II/III

J SHETH, M MISTRI, M KAMATE*, S VAJA# AND FJ SHETH

*From the FRIGE's Institute of Human Genetics, FRIGE House, Jodhpur Gam Road, Satellite, Ahmedabad. India; *KLES, Prabhakar Kore Hospital, Belgaun, Karnataka, India; and #Enzyme Laboratory, Guy's Hospital, London.*

Correspondence to:
Dr Jayesh J Sheth,
FRIGE's Institute of Human Genetics,
FRIGE House, Jodhpur Gam Road,
Satellite, Ahmedabad-380015.
jshethad1@gmail.com
Received: March 27, 2012;
Initial review: April 23, 2012;
Accepted: May 04, 2012.

Overlapping clinical phenotypes are a diagnostic challenge to the clinician, especially in the cases of mucolipidosis (ML) and mucopolysaccharide disorders (MPS), due to overlapping phenotypes. Present study was carried out in 147 children suspected to have ML or MPS and 100 controls. They were screened for ML II/III by colorimetric method using substrate pNCS. Six children were found screen positive for ML II/III and further confirmatory study showed significantly raised activity in plasma confirming high specificity of the ML screening test. Forty-two (28.5%) children out of remaining 141 children that were screen negative, were found to have various MPS disorders, while rest 99 had normal enzyme activity in plasma and leucocytes. Present study demonstrates prompt and specific chemical method that can be used as a tool for estimating ML II/III, with high specificity.

Key-words: *Mucolipidosis (ML), Screening test*.

Published online: June 10, 2012. PII: 097475991200279-2

Mucolipidosis II/III [ML II/III] are rare autosomal recessive lysosomal storage disorders with a joint incidence of 1 in 325,000 live births [1]. It is a progressive disorder involving multiple organ and tissues with a wider clinical spectrum and fatal outcome. It has overlapping clinical phenotypes with mucopolysaccharidosis (MPS) disorders. ML II/III collectively results from a deficiency of the enzyme N-acetylglucosamine-1-phosphotransferase, which phosphorylates target carbohydrate residues on N-linked glycoproteins [2]. In absence of this phosphorylation, the glycoproteins are not destined for lysosomes, and escape outside the cell resulting in an extracellular accumulation of the hydrolases producing variable phenotypes with multi-organ involvement [2-5].

Various strategies for early detection such as lysosomal protein markers LAMP-1 and Sapocin-C [6], tandom mass spectroscopy (TMS) [7], and more recently reported, high throughput multiplex assay [8] are available; however, underutilized [8]. In the present study, a simple chemical screening method using plasma sample is used, which is followed by confirmative enzyme study.

METHODS

Present study includes 147 children in the age range of 3 months to 16 years having dysmorphic features such as coarse facial features, enlargement of liver and spleen, skeletal abnormality, respiratory complications and neuroregression as the presenting features. Simultaneously, 100 age-match normal healthy controls were also included for the screening test. Institutional ethical committee approval, and an informed written consent were obtained from each enrolled subjects prior to the study initiation.

Plasma was separated from the heparinised blood by centrifugation and stored at -20°C till further analysis. All the subjects were initially studied by chemical screening method where 30µL of plasma was mixed with the enzyme p-nitrocatechol sulphate (pNCS) or (2-hydroxy-5-nitrophenyl sulphate - pNCS) prepared in sodium acetate buffer (pH: 5.0) and incubated at 37ºC for 30 minutes. The reaction was terminated using 1N NaOH. Parallel standard p nitrocatechol (pNC) and blank (distilled water) were also processed with each set of experiments. Development of brown color was suggestive of screen positive for ML II/III, similar to pNC standard. Yellow or no change in color was suggestive of normal for ML II/III, similar to blank.

All test subjects including screen positive and screen negative were further processed for lysosomal enzyme - Hexosaminidase-T, Arylsulfatase-A, β-galactosidase, α-fucosidase and β-glucuronidase in plasma using techniques described earlier [9-11].

RESULTS

Of the 147 test subjects investigated, six (4.08%) were found to be screen positive for ML II/III. Further confirmative study carried out in plasma showed significantly raised activity of all five enzymes (hexosaminidase-T, arylsulphatase-A, β-galactosidase, β-glucuronidase and α-fucosidase) ***Table* I**. Remaining 141 children had normal enzyme activity for the above mentioned leukocyte enzymes. Of these, 42 (28.5%) were confirmed to have various types of MPS. All 100 age match controls were screen negative.

DISCUSSION

At present, very few screening tests are available for lysosomal storage disorders, such as urine GAG study for MPS, Chitotrosidase for Gaucher and Niemann Pick type-A/B but none exists for ML II/III that can be rapidly performed with high specificity and sensitivity, and is cost effective. In this study of 147 clinically suspected children and 100 normal controls evaluated using a two-tier process, there were no false negatives or false positives for ML II/III, as confirmed by plasma lysosomal enzymes.

The foremost suspicion of ML II/III is the clinical presentation followed by decreased lysosomal enzyme activities in cultured cells and increased enzyme activities in plasma/serum samples to indicate the presence of the disease [10,12,13]. However, it should be noted that not all lysosomal enzymes are affected with the same degree; sulphatase enzymes are nearly 10-100 fold in ML II/III, which forms the basis of the present screening strategy. Nonetheless, the definitive diagnosis of ML II/III disease involves the identification of a functional deficiency of N-acetylglucosamine 1-phosphotransferase enzyme activity and molecular genetic lesions associated with either one of the two subunits of N-acetylglucosamine 1-phosphotransferase. This is the difficult exercise mainly due to the disease complexity and limited availability of N-acetylglucosamine 1-phosphotransferase enzyme study. Currently, the only screening test available for ML is multiplex assay where deficient protein is used as marker for LSD [8]. Multiplex assay requires high technical sophistication and its use as a screening strategy for low prevalence diseases like ML II/III, is inappropriate, and led to the search for another alternative. Present study has demonstrated the simple chemical test where synthetic substrate pNCS gets hydrolysed in presence of Arylsulfatase-A (ASA) when excessively present in the plasma and catalyzes to form excess pNC, and gives dark brown colour in alkaline solution which is visible to the naked eye [14,15]. This test assay can be processed by any laboratory for screening suspected cases for ML II/III, followed by further confirmatory study from plasma enzymes at a specialized centre. The sensitivity and specificity of the test was found to be 100 percent with high positive and negative predictive values. This study clearly demonstrates its usefulness in the children with aforementioned clinical phenotypes as a first tier screening test followed by enzymatic activity from the plasma as a confirmative tool.

TABLE I ENZYME CONFIRMATION IN 147 CASES SUSPECTED OF HAVING ML II/III

Confirmed diagnosis	*Enzyme activity*
Screen positive (*n*=6)	↑ Arylsulfatase-A;
Mucolipidosis II/III	↑ α-hexosaminidase T; ↑ α-galactosidase;
Screen negative (*n*=42)	↑ α-fucosidase;
	↑ β-glucuronidase
13, MPS I	↓ α-Iduronidase
8, MPS II	↓ α-Iduronate sulphate
5, MPS IIIA	↓ Heparan sulphamidase
1, MPS IIIB	↓ N-Ac-β-D-glucosaminidase
8, MPS IVA	↓ β-galactosidase-6-sulphate sulphatase
1, MPS IVB	↓ β-galactosidase
5, MPS VI	↓ Arylsulfatase-B
1, MPS VII	↓ β-glucuronidase*

*↑ increased enzyme activity; ↓decreased enzyme activity; *Absent enzyme activity. 99 individuals had normal enzyme activity for all the above assays.*

Acknowledgment: Dr Chaitanya Datar, Dr. Sureshkumar EK, Dr. Raju C. Shah, Dr. Nidhish Nanavaty, Dr. Harshuti Shah and Dr. Sanjiv Mehta for their referrals.
Contributors: JJS: designed the study and written the manuscript. MM: carried out the technical work. MK: provided clinical details and SV: helped in standardizing the assay. FS has critically evaluated the manuscript.
Funding: ICMR Grant No.54/1/2005-BMS;
Competing interests: None stated.

REFERENCES

1. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. JAMA. 1999;281:249-54.
2. Mariko K, Michael B, Canfield W. Mucolipidosis II (I-Cell Disease) and mucolipidosis IIIA (Classical Pseudo-Hurler Polydystrophy) are caused by mutations in the GlcNAc-phosphotransferase á/â–subunits precursor. Am J Hum Genet. 2006;78:451-63.
3. Raas-Rothschild A, Cormier-Daire V, Bao M, Genin E, Salomon R, Brewer K, *et al.* Molecular basis of variant pseudo-Hurler polydystrophy (mucolipidosis IIIC). J Clin

WHAT THIS STUDY ADDS?

- A simple screening strategy demonstrating a prompt and reliable chemical method that can be used as a tool for identifying ML II/III, and distinguishing it from MPS disorder.

Invest. 2000;105:673-81.
4. Staretz-Chacham O, Lang TC, LaMarca ME, Krasnewich D, Sidransky E. Lysosomal storage disorders in the newborn. Pediatrics. 2009;123:1191-1207.
5. Aracena M, Mabe P, Mena M, Andreani S, Daza C. Mucolipidoses type II. Rev Med Child. 2003;131:314-9.
6. Chang MHY, Bindloss CA, Grabowski GA, Qi X, Winchester B, Hopwood JJ, *et al.* Saposins A, B, C and D in plasma patients with lysosomal storage disorders. Clinic Chem. 2000;46:167-74.
7. Meikle P, Ranieri E, Simonsen H, Rozaklis T, Ramsay SL, Whitfield PD, *et al.* Newborn screening for lysosomal storage disorders: clinical evaluation of a two-tier strategy. Pediatrics. 2004;114:909-16.
8. Fuller M, Tucker JN, Lang DL, Dean CJ, Fietz MJ, Meikle PJ, *et al.* Screening patients referred to a metabolic clinic for lysosomal storage disorders. J Med Genet. 2011;48: 422-5.
9. Raine DN, Crossley JE, Kennedy JF. Lysosomal'acid' hydrolases in normal individuals controls and patients with inborn errors of metabolism. British Polymer Journal. 1983;15:139-48.
10. Wiesmann U, Vasella F, Herschkowitz N. I-cell disease: leakage of lysosomal enzymes in to extracellular fluid. N Engl J Med. 1971;285:1090.
11. Sheth JJ, Oza N, Mistri M, Naik P, Kumar S, Sheth F. Mucolipidosis II (I-cell) in two children with skeletal abnormality, Dysmorphism and Hepatosplenomegaly. Pediatric On call [series online] 2009; [cited 2009 May 1];Vol 6, Art # 24. Available from: http://www.pediatriconcall.com/fordoctor/casereports/mucolipidosis.asp
12. Hwu WL, Chuang SC, Wang WC, Wang TR. Diagnosis of I cell disease. Zhonzhua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi. 1994;35:508.
13. Natowicz MR, Wang Y. Plasma hyaluronidase activity in mucolipidoses II and III: Marked differences from other lysosomal enzymes. AJMG. 1996;65:209-12.
14. Lee-Vaupel M, Conzelmann EA. Simple chromogenic assay for arylsulphatase A. Clin Chim Acta.1987;164:171-80.
15. Clark AG, Jowett DA, Smith JN. A continuous spectrophotometric assay for arylsulfatase activity dependent on the formation of complex between cupric ions and notrocatechols. Analytical Biochemistry. 1981;118:231-9.