Early diagnosis of maple syrup urine disease using polymerase chain reaction-based mutation detection

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Abstract

Background: Maple syrup urine disease (MSUD) is an autosomal recessive disorder caused by defective activity of the branched-chain α-ketoacid dehydrogenase enzyme complex. Early diagnosis and management of MSUD are imperative for preventing permanent neurological impairments. In the Philippines, a 4.7 kb deletion in the dihydrolipoamide branched-chain transacylase E2 (DBT) gene has been commonly identified in MSUD patients. Polymerase chain reaction (PCR) amplification of a junction fragment between intron 10 and exon 11 has been used to detect this deletion. The purpose of the present paper was to use PCR-based mutation detection of the deletion mutation to diagnose MSUD in neonates in order to provide proper diagnosis and effective treatment.

Methods: A region encompassing exon 11 and the junction fragment of the E2 (DBT) gene was PCR amplified from genomic DNA prepared from two neonates at risk for MSUD.

Results: PCR amplification of both exon 11 and the junction fragment from one of the neonates demonstrated that this case was a heterozygous carrier of the deletion. Thus, normal feeding was started. For the other neonate, PCR amplification of the junction fragment was successful, whereas the region encompassing exon 11 was not amplified. This neonate was genotyped as homozygous for the deletion, and treatment for MSUD was provided immediately.

Conclusion: Examination of the deletion mutation in the E2 (DBT) gene facilitated early MSUD diagnosis and was beneficial for the determination of the proper course of treatment.

Key words deletion mutation, diagnosis, maple syrup urine disease, PCR.

Maple syrup urine disease (MSUD) is an autosomal recessive metabolic disorder caused by defective activity of the branched-chain α-ketoacid dehydrogenase (BCKAD) enzyme complex, which is composed of the dihydrolipoamide branched-chain transacylase E2, the α and β subunits of the BCKAD decarboxylase E1, and the dihydrolipoamide dehydrogenase E3. The resulting metabolic block causes accumulation of the branched-chain amino acids (BCAA) leucine, isoleucine, and valine. Elevated levels of these BCAA and the corresponding branched-chain α-ketoacids (BCKA) induce ketoacidosis, neurological impairment, developmental disturbances, and coma and can be fatal if the patients are not treated. 

In classic MSUD, the most common form of the disorder, affected newborns appear normal at birth but symptoms appear within 3–5 days, when BCAA levels surpass critical concentrations. Early metabolic insult due to postnatal metabolic decompensation underlies the frequently encountered mental impairment in MSUD patients. Presymptomatic diagnosis and subsequent metabolic control cannot be overemphasized and are crucial determinants of the outcome of afflicted individuals.

Therefore, early diagnosis with a sensitive, reliable, and rapid method is imperative. Every neonate in developed countries is usually screened for MSUD during neonatal screening. In the Philippines, however, MSUD is not yet included in the newborn screening panel, but because the urine of MSUD patients has a characteristic maple syrup or burnt sugar smell, many cases of classical MSUD have been identified in the Philippines, leading to the supposition that there is a genetic predisposition for MSUD among Filipinos.

In our previous study, a 4.7 kb deletion from intron 10 to exon 11 of the E2 (DBT) gene was found in a group of Filipino MSUD patients. Remarkably, the identified deletion was found to be common, affecting more than 70% of the E2 alleles in the MSUD patients. Furthermore, simple polymerase chain reaction (PCR) amplification of the junction fragment from genomic DNA was shown to be a powerful method to identify the mutation. Here, we report the benefits of the molecular diagnosis of MSUD in two neonates using PCR-based mutation screening for this common mutation. This method allows genetic diagnosis, within 6–7 h, of neonates suspected of having MSUD and is therefore beneficial for early intervention.
Methods

Case ascertainment

Two unrelated neonates suspected of having MSUD were included in the study. Neither of these neonates was a product of a consanguineous marriage. Informed consent was given by the family of each patient, allowing the use of the patients’ blood samples for biochemical and genetic analysis. All samples were collected in accordance with local institutional ethics review board guidelines.

Analysis of the 4.7 kb deletion

Peripheral blood samples were obtained from the patients, a normal control subject, and a previously identified MSUD patient homozygous for the 4.7 kb deletion in the E2 (DBT) gene. Genomic DNA was extracted from the blood samples using standard methods. To detect the deletion mutation, a junction fragment connecting intron 10 to exon 11 of the E2 (DBT) gene was PCR amplified. In addition, we attempted to amplify a terminal region of exon 11 that is absent in the allele encoding the deletion as described before. Genetic diagnosis was established within 6–7 h after blood sampling.

Results

Case 1

Patient 1, a boy, was born term as the first child of healthy unrelated Filipino parents. The family history of the parents was negative for symptoms related to MSUD. The patient was asymptomatic at birth. After 3 days of age, however, he presented with lethargy, vomiting, poor suck, and reduced activity. Seizures and deterioration of sensorium suggestive of the classic type of MSUD were then observed. Finally, the characteristic maple syrup smell of the patient’s urine prompted the attending doctor to consider MSUD. At 14 days of age the patient was referred to the Philippine General Hospital.

To confirm the clinical diagnosis of MSUD, laboratory examinations were carried out, including thin-layer chromatography to determine the plasma leucine level, quantitative plasma amino-acid analysis using automated cation-exchange chromatography, and molecular analysis. First, genetic analysis was performed. PCR amplification of the junction fragment connecting intron 10 to exon 11 of the E2 (DBT) gene produced a 775 bp band, whereas the fragment encompassing exon 11 was not PCR amplified (Fig. 1). These results showed that this patient had a homozygous deletion in the E2 (DBT) gene, which confirmed the diagnosis of MSUD. As soon as we confirmed the diagnosis and 6–7 h after obtaining the blood sample, this patient was placed on a formula specifically designed for MSUD patients. After 10–12 h both thin-layer chromatography and quantitative plasma amino-acid analysis produced results characteristic of MSUD. Quantitative cation-exchange chromatography demonstrated that the patient had markedly high leucine levels (>2300 μmol/L; ref. range: <200 μmol/L), which correlated with the results obtained with thin-layer chromatography (leucine level: 2000 μmol/L; normal value: <300 μmol/L).

Case 2

Patient 2 was the third child of a healthy Filipino couple. His eldest brother was clinically diagnosed with MSUD at the Philippine General Hospital but died due to metabolic crisis at 1 year of age. Molecular analysis of the first brother’s DNA sample showed that he was homozygous for the 4.7 kb deletion in the E2 (DBT) gene (data not shown). His second brother was born normal. Mutational analysis of both parents demonstrated that they were both carriers of the 4.7 kb deletion (data not shown).

Armed with the knowledge that patient 2 was at risk for MSUD, the parents readily consented to have their baby’s blood extracted for DNA and biochemical analysis immediately after birth. Under the guidance of the Genetic Metabolic Unit, the patient was treated for MSUD until this disorder could be ruled out.

The junction fragment was obtained as a PCR-amplified product from genomic DNA. In contrast to the eldest brother, the region encompassing exon 11 was also successfully PCR amplified (Fig. 2). Patient 2 was found to be a heterozygous carrier of the common deletion and was phenotypically cleared of having MSUD. Thus, this patient was immediately started on regular milk formula. The plasma leucine levels of patient 2 were also found to be within the normal range.

Discussion

Because MSUD is rare in most populations, there have been very few reports published on prospective diagnostic methods and
Fig. 2 Amplification of the 775 bp junction fragment connecting intron 10 and exon 11 of the E2 gene. Polymerase chain reaction (PCR)-amplified products are shown. A clear band corresponding to the 775 bp junction fragment was obtained from patient 1 (lane 1). A product of the same size was obtained from patient 2 (lane 2). No amplified product, however, was obtained from the normal control patient (lane 3). An amplified 775 bp product was obtained from a patient known to be homozygous for the 4.7 kb deletion in the E2 gene (lane 4). No DNA sample was included in the PCR amplification reaction (lane –). The marker lane (M) was loaded with HaeIII-digested ΦX174 phage DNA.

therapies for this disorder. A novel 4.7 kb deletion in the E2 (DBT) gene caused by non-homologous recombination between an L1 repeat in intron 10 and an Alu repeat in exon 11 was previously identified in Filipino individuals affected by MSUD. It has been noted that the molecular identification of this relatively common deletion in affected Filipinos can be a valuable diagnostic tool, providing early identification of the disease or the carrier state of tested individuals. This approach can be done immediately following birth and requires only 6–7 h to obtain results. Therefore, preventive measures can be started earlier to avoid the extraneous detoxification needed to clinically diagnose at-risk newborns and prevent permanent neurologic insults caused by a delay in diagnosis.

The direct examination of the E2 (DBT) gene provided definitive diagnosis for patient 1 and readily determined the carrier status of patient 2. Patient 1 was found to have the deletion mutation in both of his alleles and was put on the special milk formula within 1 day of admission. Patient 2 clearly benefited from the molecular analysis, which immediately provided a carrier diagnosis and avoided unnecessary detoxification. This molecular approach for diagnosing MSUD has proved to be a simple, sensitive, definitive, and rapid test, thus avoiding the need for expensive measures requiring unnecessary special milk formula, laboratory tests, intensive care, and prolonged hospitalization.

Furthermore, identification of patient 1 as homozygous for the E2 (DBT) gene deletion will be useful if future children are born to his parents. In lieu of newborn screening in the Philippines, this procedure has proved to be very effective in diagnosing suspected MSUD cases; therefore, it represents an essential advance in the diagnostic arm of molecular genetics by providing an early and appropriate intervention strategy, thereby decreasing treatment costs.

The discovery of the E2 (DBT) gene deletion has allowed its use as a tool for DNA-based diagnosis of MSUD. As is the case for the Ashkenazi Jewish population, effective carrier detection may decrease the morbidity associated with delayed diagnosis of this disorder among future Filipino MSUD patients.

Although molecular analysis potentially facilitates prenatal diagnosis of affected families, this diagnostic method is not practiced in this predominantly Roman Catholic country, in which Philippine laws deem abortion illegal and not acceptable. Prenatal diagnosis for the purpose of early intervention, however, may be unnecessary, because, as we have shown here, the diagnosis can be made using molecular analysis within the first 10 h of life.

Because we have found that many other Filipino MSUD patients are homozygous for the E2 (DBT) gene deletion, we conclude that this approach for MSUD management is practical for use in the Philippines. Considering that this mutation has also been found in an Austronesian tribe in Taiwan, it is suggested that the present diagnostic approach for MSUD can be used in other populations.

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