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■ Case Report ■

Transient neonatal diabetes mellitus with macroglossia diagnosed by methylation specific PCR (MS-PCR)

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= Abstract =

Transient neonatal diabetes mellitus (TNDM) has been associated with paternal uniparental isodisomy of chromosome 6, paternally inherited duplication of 6q24, or a methylation defect at a CpG island of the *ZAC* or *HYMAI* gene. We experienced a case of TNDM in which the patient presented with hyperglycemia, macroglossia, and intrauterine growth retardation, caused by a paternally derived *HYMAI*. An 18-day-old female infant was admitted to the hospital because of macroglossia and recurrent hyperglycemia. In addition to the macroglossia, she also presented with large fontanelles, micrognathia, and prominent eyes. Serum glucose levels were 200-300 mg/dL and they improved spontaneously 2 days after admission. To identify the presence of a maternal methylated allele, bisulfite-treated genomic DNA from peripheral blood was prepared and digested with BssHII after polymerase chain reaction (PCR) amplification with methylation-specific *HYMAI* primers. PCR and restriction fragment length polymorphism analysis showed that the patient had only the paternal origin of the HYMA1 gene. TNDM is associated with a methylation defect in chromosome 6, suggesting that an imprinted gene on chromosome 6 is responsible for this phenotype. (Korean J Pediatr 2010;53:432-436)

Key Words : Transient neonatal diabetes mellitus, Macroglossia, Methylation defect

Introduction

Neonatal diabetes mellitus (NDM), defined as hyperglycemia that presents within the first month of life and that requires insulin therapy, is a rare disorder, with an estimated incidence of 1 in 400,000 live births[1-3]. Clinically, NDM is classified into two main categories, transient neonatal diabetes mellitus (TNDM) and permanent neonatal diabetes mellitus (PNDM), which differ primarily in their duration of insulin dependence. In TNDM, insulin secretion is spontaneously recovered at a median age of 4 months[3]. However, PNDM requires lifelong insulin treatment[4].

Three genetic mechanisms have been shown to cause TNDM, including paternal uniparental disomy (UPD) of chromosome 6[5], paternally inherited duplication of chromosome 6q24[6], and a methylation defect at a CpG island (CGI) overlapping exon 1 of the *ZAC* or *HYMAI* gene[7]. These defects result in overexpression of a paternally expressed allele at chromosome 6q24, which alters pancreatic β-cell maturation and insulin secretion[7, 8].

So far, only a few cases of TNDM have been reported in Korea[9-12]. We experienced a case of TNDM who presented with hyperglycemia, macroglossia, and intrauterine growth retardation, apparently caused by a paternally-derived *HYMAI*.

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Case report

An 18-day-old girl was referred from the primary clinic due to facial dysmorphism, poor weight gain, and recurrent hyperglycemia detected at 16 days of life. She was born at 40 weeks of gestation by spontaneous vaginal delivery with a birth weight of 2,200 g (below 3rd percentile). No one in the family was reported to have diabetes mellitus

and the mother did not have gestational diabetes. The patient's length was 50 cm (50-75th percentile), her weight 2,690 g (10-25th percentile), and her head circumference 35.8 cm (75-90th percentile). She had extreme macroglossia, a prominent occiput, large fontanelles, micrognathia, and prominent eyes. Overall, subcutaneous tissue was decreased. Laboratory findings showed a blood glucose level of 280 mg/dL, serum C-peptide level of 0.79 ng/mL (normal range, 0.4-2.2 ng/mL), and 24hr urine C-peptide level of 1.0 μg/day (normal range, 2-260 μg/day). Glycosuria (3+) without ketonuria and acidosis was found and the serum insulin level was below 0.1 μIU/mL. Pancreatic computed tomography was negative. Chromosome analysis was performed and revealed a normal 46,XX female karyotype. Insulin therapy was initiated with regular insulin and insulin doses of 0.1 unit/kg were injected intermittently when blood glucose levels were above 200 mg/dL. Hyperglycemia improved spontaneously two days after admission and insulin therapy was discontinued thereafter (Fig. 1). She was discharged at 32 days of life. At 2 months of age, she weighed 4.7 kg (10-25th percentile), and macroglossia was in an improving state.

To test for methylation defect of chromosome 6, genomic DNA was extracted from peripheral blood leukocytes and was analyzed for methylation status of the *HYMAI* gene at the conserved region of the differentially methylated region (DMR) by methylation specific polymerase chain reaction (MS-PCR) and restriction fragment length polymorphism (RFLP). After PCR amplification of sodium bisulfite-treated DNA, PCR products were digested with restriction enzymes (BssHI) to distinguish between unmethylated (undigested) bands and methylated (digested) bands. The enzymes were chosen for their recognition sequences, which contain a CpG dinucleotide. In this assay, a methylated cytosine will maintain the enzyme recognition site and consequently will be cut, whereas an unmethylated cytosine will be converted to uracyl, which will destroy the recognition site and therefore the site will remain undigested. MS-PCR analysis identified the paternal origin of the *HYMAI* gene, which is DMR; such that the paternal CGI were unmethylated and the maternal CGI were methylated. No maternal part of chromosome 6 was detected in the patient (Fig. 2).

Discussion

We have found a patient with TNDM with macroglossia, who shows a lack of methylation of *HYMAI* gene in chromosome 6. UPD of chromosome 6 suggests that two copies of the paternal allele are necessary for the development of TNDM; therefore, it is likely that an imprinted gene on chromosome 6 is responsible for the diabetic phenotype[7]. Differential DNA methylation patterns between patients with UPD of chromosome 6 and normal controls have been demonstrated[7]. TNDM associated with partial duplication of the long arm of a paternal chromosome 6 also have also been reported[8, 13]. More recently, submicroscopic duplication and methylation mutations of the 6q24 region have been found in patients with TNDM, implying that more subtle changes of chromosome 6 may be involved[7]. In 2000, Gardner et al[7] showed that, in a number of TNDM cases, the TNDM CGI was completely unmethylated on both alleles, in the absence of UPD. They termed this a "methylation defect". Temple et al[14] performed genotype/phenotype study in TNDM patients confirmed by genetic analysis.

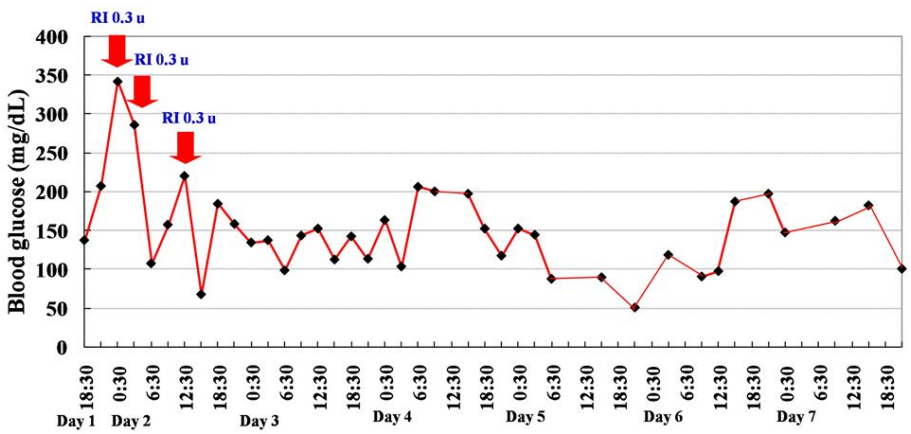

Fig. 1. Serial blood glucose changes in the patient.

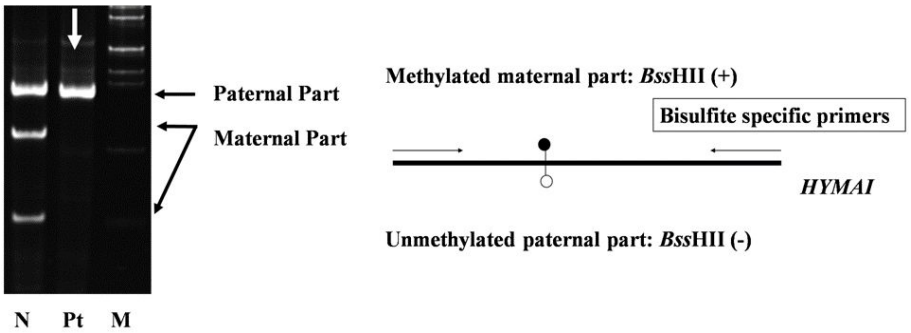

Fig. 2. Methylation pattern of the *HYMAI* gene. Methylation-specific PCR analysis showed that *HYMAI* is expressed only from the paternal allele. Therefore, the region appeared to be differentially methylated, with a paternal unmethylated allele and a maternal methylated allele. The patient (with the paternal methylation defect of chromosome 6) shows only an unmethylated pattern, whereas the normal control exhibits a normal methylation pattern (Lane M: DNA marker-100 bp ladder, Lane N: normal control, Lane Pt: patient).

However, no apparent phenotypic difference was observed among the patients with UPD, duplication of 6q24, and a methylation defect.

Two overlapping imprinted genes have been identified as potential candidates for maternal allele silencing in the chromosome 6q24 locus. The first is *ZAC* (zinc finger protein associated with apoptosis and cell cycle arrest), a zinc-finger transcription factor involved in cell growth and apoptosis. Overexpression of *ZAC* might cause TNDM through its function as a regulator of cell cycle arrest and apoptosis, altering the absolute number and efficiency of the β-islet cells during a critical time of pancreatic development in utero[15]. The second gene in the region is *HYMAI* (imprinted in hydatidiform mole), which encodes an untranslated RNA whose function and role in TNDM is not known [16]. The *HYMAI* gene exhibits allele-specific differential DNA methylation pattern between the two parental alleles at an adjacent CGI and is expressed only from the paternal chromosome[17].

Molecular diagnosis of chromosome 6 anomalies provides a tool to distinguish TNDM from PNDM in the neonatal period. The ability to identify patients with a paternal UPD of chromosome 6 allows the clinician to predict a transient, rather than permanent, course of insulin treatment for diabetes mellitus and also to assure no increased recurrence risk of TNDM in subsequent pregnancies. TNDM accounts for 50-60% of cases of NDM[6] and the clinical course of TNDM is characterized by intrauterine growth retardation, failure to thrive, decreased adipose tissue, glycosuria, dehydration, and hyperglycemia requiring insulin for correction within the first few weeks of life[18, 19].

Differentiation between TNDM and PNDM is currently based entirely on the persistent need for insulin in the permanent form. TNDM cannot be distinguished from PNDM based on clinical features alone; therefore, these cannot aid the clinician in determining the prognosis. It has been suggested that there are several points that differentiate TNDM and PNDM. For example, in TNDM, patients are younger at the time of diagnosis of diabetes and have lower initial insulin requirements[20]. Macroglossia appears to be feature more commonly associated with TNDM than with the PNDM [21]. Patients with TNDM are also more likely to have intrauterine growth retardation and are less likely to develop ketoacidosis and ketonuria than are patients with PNDM[20]. The high rate of intrauterine growth retardation is in keeping with the crucial role of insulin in fetal growth and is most likely caused by the failure of insulin secretion in fetal life, especially during the last trimester of pregnancy[22].

TNDM is a developmental disorder of insulin production that is usually resolved within 6 months. However, more than 50% of patients with TNDM are prone to relapse to a permanent diabetes state (type 2 diabetes) usually around adolescence or as adults[3, 20, 23]. TNDM, as a rare but distinct cause of neonatal diabetes, has a better prognosis compared to many other causes of childhood diabetes. It is the result of an imprinted gene at 6q24 and the genetic cause can be identified in 80% of cases[14]. A differentially methylated CGI was found within the critical region and was methylated only on the maternally inherited chromosome 6 homologs. All of the patients with paternal UPD of chromosome 6 showed a complete lack of methylation at this site[14]. The region was also found to contain eight

CGIs. One CGI was shown to be differentially methylated in normal subjects, such that the paternal CGI was unmethylated and the maternal CGI was methylated[7].

In the study reported here, we found a patient with TNDM whose DNA methylation pattern was identical to that reported for patients with a methylation defect of chromosome 6. MS-PCR analysis is the first step for the molecular diagnosis of TNDM. We can find out the presence or absence of a maternal methylated allele through this method. If no methylated allele is detected, microsatellite analysis of chromosome 6 is required to identify UPD[24]. In Korea, Kwon et al[11] and Cho et al[12] reported TNDM patients caused by UPD through microsatellite analysis. Because methylation defect can be detected by MS-PCR, we can distinguish TNDM from PNDM by using this method before microsatellite analysis. This patient will require long-term follow-up to determine whether recurrence of diabetes mellitus in later life occurs in TNDM patients on a consistent basis[25].

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한 글 요 약

메틸화 특이 PCR로 진단된 거설증을 동반한 일과성 신생아 당뇨병

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일과성 신생아 당뇨병은 6번 염색체의 부친 이체성, 부친으로부터 유래한 염색체 6q24의 중복, *ZAC* 또는 *HYMAI* 유전자의 CpG 섬의 메틸화 결함에 의해 야기된다. 저자들은 고혈당, 거설증, 자궁내성장지연으로 발현되어 부친으로부터 유래된 *HYMA1* 유전자만을 보인 일과성 신생아 당뇨병 1례를 경험하였다. 생후 18일된 여아가 거설증과 반복되는 고혈당으로 입원하였다. 거설증과 함께 큰 대천문, 작은 턱, 두드러진 눈을 보였으며 혈중 포도당 농도는 200-300 mg/dL로 유지되다가 입원 2일 후부터 인슐린 투여 없이도 정상 범위로 유지되었다. 모체로부터 유래된 메틸화된 대립유전자 유무를 확인하기 위하여 말초 혈액으로부터 genomic DNA를 추출하여 bisulfite를 처리한 후, 메틸화 특이 중합 효소 연쇄 반응으로 *HYMAI* 유전자의 일부분을 증폭시키고, 제한 효소 BssHII를 이용한 제한 효소 절편 길이 다형성 (restriction fragment length polymorphism, RFLP) 분석을 이용하여 *HYMAI* 유전자의 메틸화 여부를 확인한 결과, *HYMAI* 유전자의 메틸화 결함을 보여 부친에서 유래된 *HYMAI* 유전자만을 갖고 있음을 확인하였다. *HYMAI* 유전자의 메틸화 검사를 통해 6번 염색체의 각인된 유전자가 증명되었으며 메틸화 결함으로 인해 일과성 신생아 당뇨병이 발생하였다.

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