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Glycated Albumin in Patients with Neonatal Diabetes Mellitus Is Apparently Low in Relation to Glycemia Compared with That in Patients with Type 1 Diabetes Mellitus

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Key Words
Neonatal diabetes mellitus · Glycated albumin · Blood glucose · Type 1 diabetes mellitus · Hemoglobin A_{1c}

Abstract
Background: Although glycated albumin (GA) is a useful glycemic control marker in neonatal diabetes mellitus (NDM), there has been no report comparing GA levels between NDM patients and non-diabetic infants. Moreover, GA in NDM patients may be apparently low in relation to glycemia due to the assumed elevation of albumin metabolism in neonates. ***Methods:*** We compared GA levels between 6 patients with NDM and 18 non-diabetic infants or 14 patients with type 1 diabetes mellitus (T1DM). Mean blood glucose (MBG) was calculated on the basis of self-monitoring of blood glucose for 1 month before GA measurement. ***Results:*** GA in NDM patients was significantly higher than that in non-diabetic infants (22.0 ± 5.8 vs. 10.2 ± 1.4%; $p < 0.0001$), and GA levels significantly correlated with MBG in both NDM and T1DM patients. However, GA in NDM patients was significantly lower than in T1DM patients (25.8 ± 5.3%; $p = 0.0046$), whereas MBG in NDM patients was significantly higher than in T1DM patients (233 ± 79 vs. 183 ± 41 mg/dl; $p = 0.0006$). ***Conclusion:*** GA levels in NDM patients were apparently low in relation to glycemia. Therefore, reference values for infants should be used for assessing the GA level in NDM.

Introduction

Glycation of various proteins is known to be higher in diabetic patients than in non-diabetic subjects and some of these proteins are thought to be involved in the onset and progression of chronic diabetic complications [1]. Of these glycated proteins, glycated albumin (GA) is used as a glycemic control marker as well as hemoglobin A_{1c} (HbA_{1c}) [2]. Because the half-life of serum albumin (14 days) is shorter than that of erythrocytes, GA reflects plasma glucose (PG) levels over a shorter period than HbA_{1c} [3]. Given these characteristics, GA also appears to be a useful glycemic control indicator at the start of diabetes therapy [4]. In addition, GA has the advantage of not being influenced by abnormal Hb metabolism [5]. Therefore, in hemolytic anemia [6], renal anemia [7, 8] and late pregnancy [9, 10], where HbA_{1c} levels do not accurately reflect the glycemic control status, GA has been reported to be useful. However, GA levels are altered in

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patients with albumin metabolism disorders. For example, in nephrotic syndrome [11], hyperthyroidism [12] and with glucocorticoid administration in which albumin metabolism increases, GA is apparently low. In contrast, in liver cirrhosis [13] and hypothyroidism [12] where albumin metabolism is decreased, GA is apparently high.

Neonatal diabetes mellitus (NDM) is a type of diabetes caused by a single gene abnormality in which insulin dependence develops within 6 months of birth [14]. NDM is clinically classified into transient NDM, in which treatment is no longer required a few months after onset, or permanent NDM, in which lifelong treatment is required. HbF is the major Hb produced during the fetal period. In fact, immediately following birth, HbF accounts for 80–90% of Hb, whereas HbA accounts for only 10–20%. Thereafter, HbF is gradually replaced by HbA, and by 6 months of age, most of the Hb produced is HbA [15].

We previously reported that GA is useful as a glycemic control marker in NDM whereas HbA_{1c} cannot be used in NDM because of the influence of age-related changes in HbF [16]. However, there was no report comparing GA levels between NDM patients and non-diabetic infants. Moreover, it has been suggested that albumin metabolism is increased in healthy neonates [17, 18], which may result in apparently low GA levels in relation to glycemia in NDM patients. To examine them, we compared the GA levels between NDM patients and non-diabetic infants or patients with type 1 diabetes mellitus (T1DM).

Subjects, Materials and Methods

Study Patients

We enrolled 6 NDM patients, 18 non-diabetic infants and 14 T1DM patients. NDM patients were 5 males and 1 female, aged 231 ± 97 days. The age at onset of diabetes was 33 ± 22 days. Five of these 6 NDM patients have been reported previously [16]. Non-diabetic infants were 9 males and 9 females, aged 103 ± 60 days (range 30–190). T1DM patients were 8 males and 6 females, aged 14.6 ± 4.9 years. All T1DM patients exhibited diabetic ketoacidosis at the diagnosis of diabetes and were positive for anti-glutamic acid decarboxylase antibody or anti-insulinoma-associated protein 2 antibody. The mean duration of diabetes was 7.9 ± 4.7 years, and all patients were treated with insulin injections. Diabetic patients with renal disease, liver cirrhosis, thyroid disease, or who were receiving glucocorticoid administration, which are all factors that can affect GA levels, were excluded.

In both the NDM and T1DM patients, the mean blood glucose (MBG) level was calculated from preprandial self-monitoring of blood glucose (SMBG) for 1 month prior to GA measurement. In the NDM patients, we measured GA every month after onset of diabetes up to a mean of 227 ± 104 days. In the T1DM patients, we measured GA every month during the previous 1-year period. The frequency of SMBG measurements was 3.8 ± 2.2 counts/day/person in the NDM patients and 3.7 ± 0.5 counts/day/person in the T1DM patients; there were no significant differences between the groups. The number of GA data was 5.7 ± 2.7 counts/person (total 34 counts) in the NDM patients and 6.4 ± 3.4 counts/person (total 89 counts) in the T1DM patients. In non-diabetic infants, GA and PG in the randomly collected blood samples were also assessed.

This study was approved by the Ethics Committee at Asahikawa Medical University. All patients and/or the patient's guardians provided written informed consent.

Laboratory Methods

Serum GA was determined by an enzymatic method using an albumin-specific protease, ketoamine oxidase, and an albumin assay reagent (Lucica GA-L; Asahi Kasei Pharma, Tokyo, Japan) [19, 20]. GA was hydrolyzed to amino acids by an albumin-specific proteinase and then oxidized by ketoamine oxidase, producing hydrogen peroxide, which was measured quantitatively. Albumin was measured in the same serum sample using a new bromocresol purple method. Serum GA levels were calculated as the percentage of GA relative to total albumin. Data from SMBG were obtained using a blood glucose meter.

Statistical Analysis

All data are shown as means ± SD. For statistical analyses, the Mann-Whitney U test was used to compare GA levels between the NDM patients and non-diabetic infants or the T1DM patients because the distribution of these parameters was not Gaussian in these populations. The Student t test was used to compare MBG (or PG) levels between the NDM patients and non-diabetic infants or the T1DM patients. To analyze correlations between 2 parameters, univariate regression analyses were performed. All analyses were performed using SPSS version 16.0 (SPSS, Chicago, Ill., USA). p values were calculated, and the level of significance was set at $p < 0.05$.

Results

In both the NDM and T1DM patients, GA significantly positively correlated with MBG ($r = 0.778$, $p < 0.0001$ for NDM vs. $r = 0.515$, $p < 0.0001$ for T1DM; fig. 1). However, the regression line in the NDM patients was shifted downward as compared with that of the T1DM patients ($y = 0.057x + 8.8$ vs. $y = 0.067x + 13.6$). MBG of the NDM patients was significantly higher than PG of the non-diabetic infants (233 ± 79 vs. 92 ± 9 mg/dl; $p < 0.0001$; fig. 2a), and GA of the NDM patients was also significantly higher than that of non-diabetic infants (22.0 ± 5.8 vs. 10.2 ± 1.4%; $p < 0.0001$; fig. 2b). MBG of the NDM patients was significantly higher than that of the T1DM patients (183 ± 41 mg/dl; $p = 0.0006$; fig. 2a), but GA in the NDM patients was significantly lower than that of the T1DM patients (25.8 ± 5.3%; $p = 0.0046$; fig. 2b).

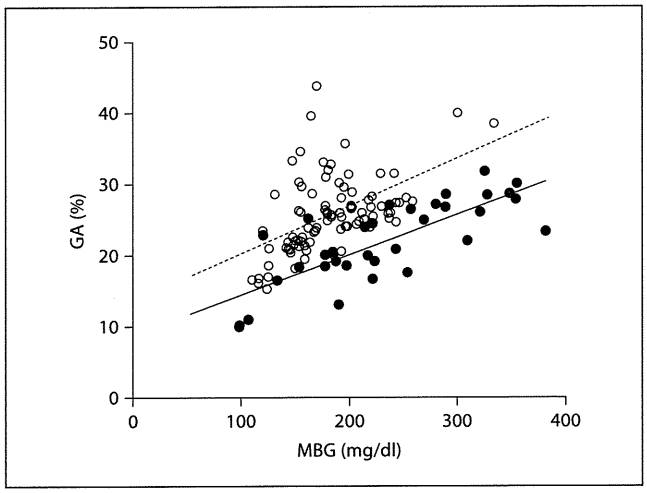

Fig. 1. Correlations between GA and MBG in the NDM patients (●) or the T1DM patients (○) are shown. In both the NDM and T1DM patients, GA significantly and positively correlated with MBG ($r = 0.778$, $p < 0.0001$ for NDM vs. $r = 0.515$, $p < 0.0001$ for T1DM).

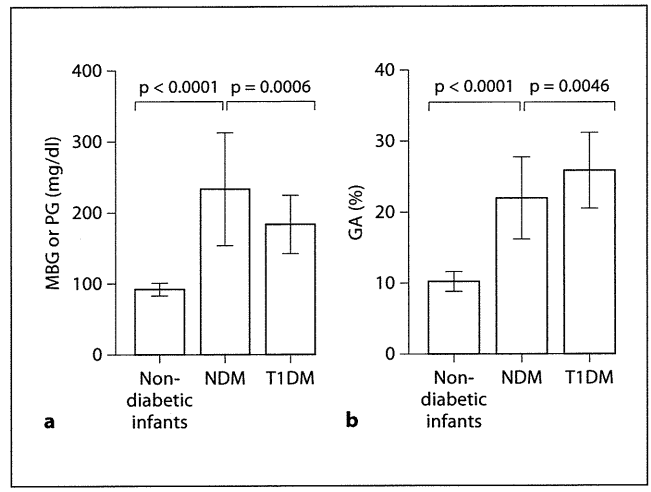

Fig. 2. MBG (or PG) and GA in the non-diabetic infants, the NDM patients and the T1DM patients. The NDM patients were compared to the non-diabetic infants or the T1DM patients with regard to levels of MBG (or PG) (**a**) and GA (**b**). Data represent the mean ± SD.

Discussion

We previously reported that GA, which is not influenced by HbF, is useful as a glycemic control marker for NDM patients [16]. On the other hand, there has been no evidence that GA is really high in NDM patients. In this study, we firstly demonstrated that GA in NDM patients was significantly higher than that in non-diabetic infants, confirming that GA is a useful glycemic control marker. However, several reports have suggested that the albumin metabolism is elevated during the neonatal period [17, 18]. Therefore, we hypothesized that GA is low in relation to glycemia in NDM patients, and we examined this by comparison with the GA level in T1DM patients. As a result, we demonstrated that GA was significantly lower in NDM than in T1DM patients whereas MBG was significantly higher in NDM than in T1DM patients. These results indicated that GA in infancy is regulated by something else not just glucose.

Albumin metabolism as well as blood glucose is known to be a regulating factor for GA [2]. During the fetal and neonatal development, weight increases rapidly in parallel to protein gain [18]. Protein gain results from a difference between protein synthesis and breakdown, i.e. albumin turnover, which is quantified using stable ^{15}N- or ^{13}C-labeled amino acids as biologic markers [18]. When protein levels change, both protein synthesis and breakdown follow the same trend [21, 22]. These tracer studies on whole protein metabolism have confirmed that the more rapid the expected growth and protein gain, the higher the rates of protein synthesis and breakdown [18, 21]. Although no direct evidence of increased albumin catabolism has been reported in neonates, increased albumin biosynthesis has been shown in a dynamic study using an isotope [17]. Furthermore, at the age of 1 year, the protein synthesis rate is reportedly more than twice as fast as that in adults [18]. Therefore, increased albumin metabolism in infancy leads to lower levels of GA in relation to glycemia in NDM patients. To the best of our knowledge, the present study is the first to report this finding; namely, GA is apparently low in relation to glycemia in NDM patients.

Although Abe et al. [23] reported GA reference values for infants, in their study, GA was measured using HPLC rather than by enzymatic method. The use of enzymatic methods rather than HPLC is known to provide lower GA values [19], and currently, GA is most commonly measured by enzymatic methods. Actually, we demonstrated that GA in non-diabetic infants was 10.2 ± 1.4% (n = 18), which is lower than in the previous report with 16.1 ± 3.0% (n = 16) [23]. Consequently, the GA values in the non-diabetic infants demonstrated in this study are useful for currently assessing the GA in NDM. However, a large number of infants will be necessary to establish the age-related reference range of GA in the future.

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ARTICLE

Glycated albumin but not HbA_{1c} reflects glycaemic control in patients with neonatal diabetes mellitus

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Abstract
Aims/hypothesis It is difficult to use HbA_{1c} as an indicator of glycaemic control in patients with neonatal diabetes mellitus (NDM) because of high levels of fetal haemoglobin (HbF) remaining in the blood. In this study, glycated albumin (GA), which is not affected by HbF, and HbA_{1c} were compared to evaluate whether they reflect glycaemic control in patients with NDM.
Methods This study included five patients with NDM. Age at diagnosis was 38±20 days. Insulin therapy was started in all patients, and levels of GA, HbA_{1c} and HbF were measured monthly for 6 months. One-month average preprandial plasma glucose (aPPG) was calculated using self-monitoring of blood glucose.
Results Plasma glucose and GA were elevated (29.7±13.1 mmol/l [n=5] and 33.3±6.9% [n=3], respectively) but HbA_{1c} was within normal limits (5.4±2.6% [35.5±4.9 mmol/mol]; n=4) at diagnosis. With diabetes treatment, aPPG (r=−0.565, p=0.002), GA (r=−0.552, p=0.003) and HbF (r=−0.855, p<0.0001) decreased with age, whereas HbA_{1c} increased (r=0.449, p=0.004). GA was strongly positively correlated with aPPG (r=0.784, p<0.0001), while HbA_{1c} showed no correlation with aPPG (r=0.221, p=0.257) and was significantly inversely correlated with HbF (r=−0.539, p=0.004).
Conclusions/interpretation GA is a useful indicator of glycaemic control in patients with NDM, whereas HbA_{1c} is influenced by age-related changes in HbF and does not accurately reflect glycaemic control.

Keywords Fetal haemoglobin · Glycated albumin · HbA_{1c} · Neonatal diabetes

Abbreviations

1,5-AG	1,5-Anhydroglucitol
aPPG	Average preprandial plasma glucose
GA	Glycated albumin
HbA	Haemoglobin A
HbF	Fetal haemoglobin
NDM	Neonatal diabetes mellitus
JDS	Japan Diabetes Society

Professor K. Fujieda, who supervised this research, died on 19 March 2010 before publication of this work.

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NGSP	National Glycohaemoglobin Standardization Program
PH	Pancreatic hypoplasia
PNDM	Permanent NDM
pUPD6	Paternal uniparental disomy of chromosome 6
TNDM	Transient NDM

Introduction

Neonatal diabetes mellitus (NDM) is a type of diabetes that results from a single gene abnormality as a result of which insulin dependence develops within 6 months of birth [1]. NDM is clinically divided into transient NDM (TNDM), in which treatment is no longer required a few months after diagnosis, and permanent NDM (PNDM), in which lifelong treatment is required. TNDM is mainly caused by the overexpression of an imprinted region of chromosome 6q24 with paternal expression (i.e., paternal uniparental disomy of chromosome 6 [pUPD6], paternal 6q24 duplication, or methylation defect of maternal 6q24). In most cases, diabetes occurs within 1 week of birth, with remission in an average of 3 months [2, 3]. PNDM is mainly caused by an abnormality of the *KCNJ11* gene that encodes the Kir6.2 subunit of the ATP-sensitive potassium channel of pancreatic beta cells, with onset at a mean age of 7 weeks [3, 4].

Glycation of various proteins is known to occur at higher rates in individuals with diabetes compared with those without diabetes. Some of these glycated proteins are thought to be involved in the onset and progression of chronic diabetic complications [5]. Of these glycated proteins, HbA_{1c} is widely used as an indicator of glycaemic control [6]. Since the erythrocyte lifespan is approximately 120 days, HbA_{1c} levels reflect the plasma glucose levels over the preceding 2–3 months. However, HbA_{1c} levels are affected in some diseases that shorten the erythrocyte lifespan, such as haemolytic anaemia and renal anaemia, which are associated with lowered levels of HbA_{1c} [7]. Furthermore, the accuracy of some HbA_{1c} analysis methods is affected by the presence of variant haemoglobins [8]. Thus, HbA_{1c} levels may not accurately reflect glycaemic control in these situations [8]. In addition to variant haemoglobin, some assays and calculation methods for estimating HbA_{1c} indicate that HbA_{1c} levels appear low when fetal haemoglobin (HbF) levels are high [9, 10]. HbF is the main haemoglobin present during the fetal period and accounts for 80–90% of haemoglobin just after birth, whereas haemoglobin A (HbA) accounts for only 10–20% [11]. After birth, HbF is gradually replaced by HbA; by 6 months of age, most of the haemoglobin present is HbA. Therefore, if HbA_{1c}, a glycosylation product of HbA, is reported as a percentage of total haemoglobin, the influence of HbF in neonates leads to apparently low HbA_{1c}. It is therefore difficult to use HbA_{1c} as an indicator of glycaemic control in neonates. Glycation of any type of haemoglobin can be detected by affinity chromatography [12]. However, glycated haemoglobin levels appear to be low when HbF levels are high, because of the lower glycation rate of HbF compared with that of HbA [10]. Consequently, none of the current glycaemic indicators have been useful for patients with NDM; plasma glucose analysis is therefore currently used for the diagnosis and treatment of NDM.

Glycated albumin (GA) is also used as an indicator of glycaemic control [13]. Because the half-life of serum albumin is shorter than that of erythrocytes, GA reflects plasma glucose levels over a shorter period of time [14]. Thus, use of GA has been advocated for monitoring short-term changes in glycaemic control [15]. In addition, because GA is not affected by haemoglobin metabolism, it is a useful indicator of glycaemic control in patients with haemolytic anaemia [16]. Moreover, we have reported that, because GA is not affected by HbF, it can be used as an indicator of glycaemic control in neonates, when measured in umbilical cord blood [17]. Therefore, GA and HbA_{1c} were compared in this study to evaluate their utility in monitoring glycaemic control in patients with NDM.

Methods

Study patients This study included five patients with NDM (PNDM, 4; TNDM, 1) who were referred to the Department of Pediatrics, Asahikawa Medical University (Table 1). Their gestation period was 39±1.7 weeks, and their birthweight was 2,069±601 g. The birthweight SD score corrected for gestational age in Japanese babies [18], was low (−2.5±0.9 SD). Age at diagnosis was 38±20 days. Insulin therapy was started in all patients immediately after diagnosis. HbA_{1c} and GA were measured in four and three patients, respectively, at the time of diagnosis. Three patients were referred immediately to the department, and the other two patients were referred later. Age at referral was 58± 11 days. After referral, HbA_{1c}, GA and HbF were measured monthly for 6 months after diagnosis. Based on the results of self-monitoring of blood glucose (3.8±2.3 times/day) before feeding, average preprandial plasma glucose (aPPG) levels were calculated for a 1 month period.

The *KCNJ11* gene analysis was performed by direct sequencing in all patients [3]. Heterozygous mutations were identified in three patients (Patient 1, p.V59M; Patient 4, p. R201C; and Patient 5, p.F35V). Patient 2 had pancreatic hypoplasia (PH). Patient 3 exhibited TNDM and insulin therapy was discontinued at 5 months of age. We performed microsatellite marker analysis of chromosome 6 [3] on Patient 3, confirming that the patient had TNDM due to

Table 1 Characteristics of the patients with neonatal diabetes in this study

Patient	Sex	Type	Aetiology	At birth			At diagnosis					Age at enrolment (days)	Assay for HbA_{1c}
				Gestation (weeks)	BW (g)	BW (SDS)[a]	Age (days)	Plasma glucose (mmol/l)	HbA_{1c} (%)	HbA_{1c} (mmol/mol)	GA (%)		
1	Male	PNDM	*KCNJ11*	41	2,582	1.9	53	47.5	8.9	73.8	40.8	53	G8
2	Female	PNDM	PH	37	1,353	3.7	58	19.4	5.1	32.2	31.7	58	8160
3	Male	TNDM[b]	pUPD6	37	1,514	2.8	19	17.6	2.6	4.9	NA	75	G8
4	Male	PNDM	*KCNJ11*	39	2,258	2.5	14	24.8	NA	NA	NA	57	8160
5	Male	PNDM	*KCNJ11*	39	2,640	1.4	45	39.2	4.9	30.1	27.3	45	G8
Mean ± SD				39±1.7	2,069±601	2.5±0.9	38±20	29.7±13.1	5.4±2.6	35.5±4.9	33.3±6.9	58±11	

BW, birthweight; G8, Tosoh HLC-723G8; 8160, ADAMS-HA-8160; NA, not available

[a] SD score (SDS) based on the average Japanese baby with correction for gestational age [18]; [b] Remission at 5 months of age

pUPD6. All steps of this study, including DNA analysis, were approved by the Ethics Committees at Asahikawa Medical University, and the study complied with the ethical guidelines of the Helsinki Declaration as revised in 2000. All patients' parents provided written informed consent.

Laboratory methods Plasma glucose at diagnosis was determined using a standard laboratory assay. HbA_{1c} levels were determined by use of the following two methods: HPLC method using HLC-723G8 (Tosoh, Tokyo, Japan) for Patients 1, 3, and 5, and ADAMS-A1C HA-8160 (Arkray, Kyoto, Japan) for Patients 2 and 4. HbA_{1c} is calculated as a percentage of total haemoglobin. The value for HbA_{1c} (%) was estimated as a National Glycohaemoglobin Standardization Program (NGSP) equivalent value (%), calculated by the formula HbA_{1c} (%) = HbA_{1c} (Japan Diabetes Society: JDS) (%) +0.4%. This calculation takes into consideration the relationship between HbA_{1c} (JDS) (%), measured by the previous Japanese standard substance and measurement methods, and HbA_{1c} (NGSP) [19]. Normal control HbA_{1c} (NGSP) levels for adults are in the range of 4.7–6.2%.

Serum GA was determined by an enzymatic method using an albumin-specific protease, ketoamine oxidase, and an albumin assay reagent (Lucica GA-L; Asahi Kasei Pharma, Tokyo, Japan) [20]. GA was hydrolysed to amino acids by an albumin-specific proteinase and then oxidised by ketoamine oxidase, producing hydrogen peroxide, which was measured quantitatively. Serum GA levels were calculated as the percentage of GA relative to total albumin. Normal control serum GA levels for adults are in the range 11.0–16.0%. Albumin was measured in the same serum sample using a new bromocresol purple method (Lucica GA-L; Asahi Kasei Pharma). Normal control serum albumin levels for adults are in the range 39–49 g/l with this method.

Statistical analysis Results are expressed as means ± SD. Simple regression analyses were used to assess the associations between continuous variables. All analyses were performed using SPSS version 16.0 (SPSS, Chicago, IL, USA). p values were calculated and the level of significance was set at $p<0.05$.

Results

At the time of diagnosis, plasma glucose and GA were markedly elevated in our study group (29.7±13.1 mmol/l [n=5] and 33.3±6.9% [n=3], respectively) (Table 1). On the other hand, HbA_{1c} was elevated only in patient 1; it was within normal limits in patients 2 and 4 and was low in patient 3. The mean HbA_{1c} was 5.4±2.6% (35.5±4.9 mmol/mol) (n=4).

Figure 1 depicts the clinical course of patient 1 from the time of diagnosis. Patient 1, a boy born at 41 weeks of gestation weighing 2,582 g (−1.9 SD) at birth, exhibited poor feeding for several days before the first evaluation and was admitted to the local hospital at 53 days of age because he was 'not doing well'. Laboratory tests at the initial evaluation showed the following: PG, 47.5 mmol/l; GA, 40.8%; HbA_{1c}, 8.9% (73.8 mmol/mol); HbF, 22.2%; serum C-peptide level, <0.06 nmol/l; and pH, 6.826 on arterial blood gas analysis. Diabetic ketoacidosis was diagnosed, and insulin therapy was started immediately. With insulin therapy, his general condition improved and his aPPG and GA levels decreased. However, despite treatment for diabetes, HbA_{1c} levels gradually increased until 5 months of age. After that point, HbA_{1c} levels decreased. HbF levels decreased over time.

Figure 2 depicts the clinical course (in other words, the relationship between diabetes treatment and age [days]) for aPPG, GA and HbA_{1c} in all patients. aPPG was significantly inversely correlated with age as expected, given the course of treatment for diabetes (r=−0.565, p=0.002). Similarly, GA was also significantly inversely correlated with age (r=-0.552, p=0.003). On the other hand, HbA_{1c} was significantly positively correlated with age (r=0.449, p=0.004). HbF also decreased with age, but at 7 months of age (215±18 days), the value (3.2±1.3%) was still higher than normal (<2%).

Next, we evaluated whether GA or HbA_{1c} reflected glycaemic control in NDM patients. GA was strongly positively correlated with aPPG (r=0.784, $p<0.0001$) (Fig. 3). However, HbA_{1c} showed no correlation with aPPG (r=0.221, p=0.257), and HbA_{1c} was significantly inversely correlated with HbF (r=−0.539, p=0.004) (Fig. 3).

Discussion

At the time of diagnosis, the mean HbA_{1c} was within normal limits for all patients with NDM. In the neonatal

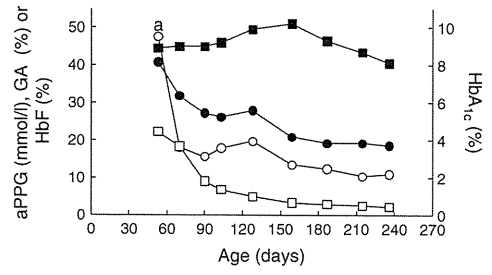

Fig. 1 Clinical course of Patient 1. The time courses of aPPG (white circles), GA (black circles), HbA_{1c} (black squares), and HbF (white squares) since diagnosis of diabetes in Patient 1 are shown. [a]Plasma glucose at diagnosis. To convert values for HbA_{1c} in % to mmol/mmol, subtract 2.15 and then multiply by 10.929

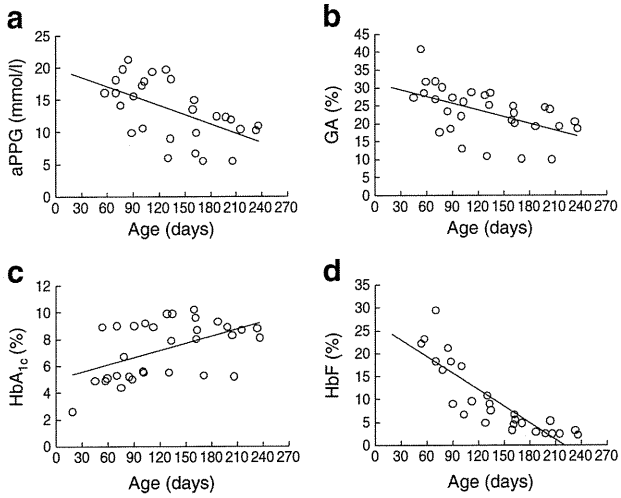

Fig. 2 Time courses of aPPG (**a**), GA (**b**), HbA_{1c} (**c**) and HbF (**d**) for five patients with NDM. (**a**) $r=-0.565$, $p=0.002$; (**b**) $r=-0.552$, $p=0.003$; (**c**) $r=0.449$, $p=0.004$; (**d**) $r=-0.855$, $p<0.0001$

period, the level of HbA_{1c} is low [17] if HbA_{1c} is calculated as a percentage of total haemoglobin, because HbF is the main haemoglobin and HbA accounts for only a small percentage of the total haemoglobin. In addition, most erythrocytes containing HbA in newborns are newly formed after birth and have a relatively short lifespan, which can add to the apparent low value of HbA_{1c}. As a result, HbA_{1c} was measured to be relatively low at the time of NDM diagnosis. However, HbA_{1c} increased despite a decrease in aPPG levels after diabetes treatment. This paradoxical phenomenon may be explained in two ways. First, HbA levels increase as HbF decreases with age. Second, the lifespan of erythrocytes containing HbA increases with age. In patients with NDM, HbA_{1c} levels did not correlate with aPPG, but inversely correlated with HbF. Our findings therefore confirmed that HbA_{1c} is not suitable as a glycaemic control marker for patients with NDM when, as in this study, HbA_{1c} levels are measured by HPLC.

Our study was limited in that we did not compare HbA_{1c} levels obtained from a variety of methods. However, measurements using immunoassay and enzymatic assays tend to yield similar results, because they are both specific assays for HbA_{1c} and do not measure HbF glycosylation products [17]. Although affinity methods can measure total glycated haemoglobins (including both HbA and HbF), glycated haemoglobin levels tend to be measured as low when levels of HbF are high. This is because the glycation rate for HbF, which does not have a glycation site at the N-terminal valine of the gamma chain, may be lower than the glycation rate of HbA [10]. In fact, we found that glycated haemoglobin, measured by an affinity method, did appear low in cord blood [17]. Therefore, these methods should not be used for the assessment of glycation levels in samples from infants. On the other hand, quantification by some HPLC methods (other than those used in this study) showed almost similar HbA_{1c} level in samples with normal to higher than 20% HbF content [10]. These methods were, unfortunately, unavailable to us. There is a possibility that HbA_{1c} may be a useful marker if it is calculated as HbA_{1c}/(total haemoglobin − HbF) in order to eliminate the influence of high HbF levels in neonates. Although we did not examine this issue, the relationship between HbA_{1c}/(total haemoglobin − HbF) and plasma glucose or GA should be examined in future.

Fructosamine and 1,5-anhydroglucitol (1,5-AG), neither of which are affected by haemoglobin metabolism, have also been used as indicators of glycaemic control in adults [21, 22]. However, because 1,5-AG is mainly ingested through food and almost no 1,5-AG is ingested during the neonatal period, serum 1,5-AG levels are undetectable in neonates [23]. Moreover, since fructosamine measures the amount of glycosylated protein, it is influenced by serum protein concentrations [24, 25]. Consequently, low fructosamine levels have been reported during the neonatal period, since serum proteins are low [25]. Moreover, total IgG in neonates, which is derived from maternal IgG, gradually disappears during the first 6–8 months of life. At the same time, the rate of infant IgG synthesis increases [26]. As a result, total IgG usually reaches a low point at 3–4 months of

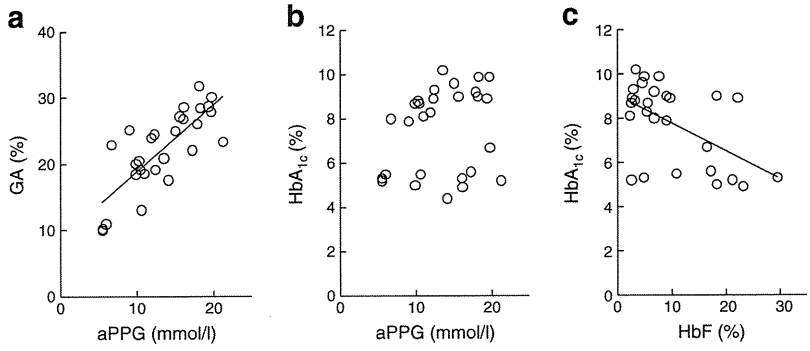

Fig. 3 Correlation of GA (**a**) and HbA_{1c} (**b**) with aPPG and correlation of HbA_{1c} with HbF (**c**) in five patients with NDM. (**a**) $r=0.784$, $p<0.0001$; (**b**) $r=0.221$, $p=0.257$; (**c**) $r=-0.539$, $p=0.004$

age. Because IgG—one of the major components of total protein—is easily glycated [27], the alteration of IgG in infancy may be a large contributor to alterations of fructosamine levels.

GA quantification is not affected by haemoglobin metabolism, nor is it affected by serum albumin concentrations, except in the case of high albumin metabolism, such as that which occurs in nephrotic syndrome [13], since GA is measured against serum albumin as a ratio [13, 16]. In fact, GA is much less affected by serum total protein concentration than is fructosamine [25]. In addition, Shima et al. reported that GA levels are not influenced by the concentration of total serum proteins [28]. In this study, GA was high at the time of NDM diagnosis. With diabetes treatment, GA decreased along with aPPG. Moreover, GA showed a strong positive correlation with aPPG. Based on these results, GA appears to be a very useful marker for both NDM diagnosis and treatment.

GA better reflects short-term changes in plasma glucose than does HbA_{1c} [14, 15]. In patients with fulminant type 1 diabetes, a subtype of type 1 diabetes in which the progression from normoglycaemia to hyperglycaemia accompanied by ketoacidosis is extremely rapid [29], HbA_{1c} levels at onset are normal or only slightly high [30]. On the other hand, GA and GA/HbA_{1c} ratios are already high at disease onset [31]. Therefore, in patients with NDM, in whom hyperglycaemia occurs within a short period from birth to diagnosis, GA is already high at the time of NDM diagnosis—similarly to those individuals with fulminant type 1 diabetes. Moreover, since GA is a shorter-term glycaemic control marker, it may be more useful than a longer-term marker for patients with NDM; it is desirable to monitor glycaemic control changes more frequently in neonates.

We previously reported that the GA values in umbilical cord blood were 9.4±1.1%, which was low compared with the reference values in adults. This may reflect low glucose levels or increased albumin metabolism in the fetus [17]. In this study, GA in infants with NDM was found to be higher than the normal reference values for adults. Abe et al. [25] reported GA reference values for infants, but in that study, GA was measured using HPLC. The use of enzymatic methods, rather than HPLC, is known to provide lower GA values [20], but GA is most commonly measured using an enzymatic method. In the future, GA reference values for infants, measured using enzymatic methods, need to be established.

In conclusion, this study has shown that GA can be a useful indicator of glycaemic control in patients with NDM. These findings will need to be further confirmed in a larger number of patients with NDM.

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S.S. and M.K. researched data, contributed to the discussion, wrote the manuscript, and reviewed/edited the manuscript. S.A., A.N., K.W., K.O., S.H. and A.R.S. researched data and drafted the manuscript. H.T., K.M. and Y.T. contributed to the discussion, and drafted and reviewed/edited the manuscript. K.F. supervised the research. S.S., M.K., S.A., A.N., K.W., K.O., S.H., A.R.S, H.T., K.M. and Y.T. approved the final version.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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Short Report

Partial paternal uniparental disomy of chromosome 6 in monozygotic twins with transient neonatal diabetes mellitus and macroglossia

Suzuki S, Fujisawa D, Hashimoto K, Asano T, Maimaiti M, Matsuo K, Tanahashi Y, Mukai T, Fujieda K. Partial paternal uniparental disomy of chromosome 6 in monozygotic twins with transient neonatal diabetes mellitus and macroglossia.
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Transient neonatal diabetes mellitus (TNDM) usually develops within the first few weeks of life and resolves at a median age of 3 months. In most of the cases, TNDM is caused by the over-expression of a paternally expressed imprinted *PLAGL1* locus on chromosome 6q24. The most frequent manifestation other than TNDM is intrauterine growth retardation (IUGR), and in some cases macroglossia. We investigated monozygotic twins who had macroglossia without IUGR. Both of the twins developed insulin-dependent hyperglycemia within the first week of life, which subsequently resolved. DNA profiling with polymerase chain reaction amplification was performed for polymorphic microsatellite markers of chromosome 6. The six informative markers, located between 6p24 and 6q15, showed normal biparental inheritance. However, the six distal informative markers, located between 6q23.2 and the 6q telomeric region, showed the absence of a maternal allele and the presence of a single paternal allele. The monosomy of the 6q telomeric region was not confirmed by chromosome banding showing 46, XX. These findings provide further evidence that partial paternal uniparental disomy of chromosome 6 (pUPD6) causes TNDM. The phenotypes other than diabetes observed in patients with partial pUPD6 may differ from those observed in patients with complete pUPD6.

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Key words: macroglossia – monozygotic twin – partial paternal uniparental disomy 6 – transient neonatal diabetes

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Transient neonatal diabetes mellitus (TNDM) is a rare disorder, in which insulin-dependent hyperglycemia usually develops within the first few weeks of life and resolves at a median age of 3 months with possible relapse as permanent diabetes mellitus in later childhood (1). Most patients with TNDM are born with intrauterine growth retardation (IUGR) and they sometimes exhibit macroglossia (2, 3). About 70% of the cases of TNDM are caused by over-expression of a paternally expressed imprinted *PLAGL1* locus on chromosome 6q24. This is because of three genetic causes: paternal uniparental disomy of chromosome 6 (pUPD6), paternal duplication of 6q24, and loss of maternal methylation of the differential methylated region at 6q24 (1). On the other hand, approximately 20% of the patients with TNDM have activating mutations in the *KCNJ11* and *ABCC8* genes that encode for the ATP sensitive potassium (K_{ATP}) channel (1).

Partial uniparental disomy (UPD) is a rare genetic abnormality. It is defined as UPD of a part of a chromosome (interstitial or telomeric) together

with biparental inheritance of the rest of this chromosomes' pair and a normal karyotype (4).

To date, there has been no report on TNDM due to partial pUPD6 although one case of neonatal diabetes mellitus due to partial pUPD6 has been reported; in that report, the type (i.e. TNDM or PNDM) was not confirmed because the patient died at 14 days of age (5).

Here, we describe the cases of a pair of female, monozygotic twins with TNDM due to partial pUPD6. IUGR was not observed in either of the twins, but they exhibited macroglossia. The identical phenotype between the monozygotic twins confirmed that partial pUPD6 is the genetic cause of TNDM.

Materials and methods

Case report

The patients were a pair of female monozygotic twins who were conceived after *in vitro* fertilization–embryo transfer. Monochorionic–diamniotic twin pregnancy resulted from the implantation of a single blastocyst. The twins were born at 29 weeks of gestation because of threatened premature delivery, and were the first children of their parents. The birth weight and length of twin 1 were 1472 g [+0.6 standard deviation (SD)] and 42 cm (+0.8 SD), and those of twin 2 were 1127 g (−0.8 SD) and 38 cm (−0.3 SD), respectively. The SD score was based on the data derived from a Japanese population (6). Both twins had macroglossia, and received mechanical ventilation for 5 days after birth because of respiratory distress syndrome. Intravenous feeding followed by tube feeding was initiated after birth. The blood glucose (BG) levels at birth were 2.8 and 1.3 mmol/l for twins 1 and 2, respectively. However, routine laboratory analysis revealed hyperglycemia without ketonuria at 2 days of age (twin 1: BG level, 12 mmol/l; twin 2: BG level, 15.5 mmol/l). Regular insulin was administered, and the BG levels decreased rapidly to within the normal range. However, persistent hyperglycemia developed at 12 days of age in twin 1 and 4 days of age in twin 2, and insulin was administered. Thereafter, the BG levels gradually decreased, and insulin administration was stopped at 29 days of age for twin 1 and 22 days of age for twin 2. At 1 year of age, euglycemia was maintained, and glycosylated hemoglobin levels were 4.9% for twin 1 and 4.7% for twin 2.

Microsatellite analysis of chromosome 6

The analysis was performed according to a previous report (2). Briefly, genomic DNA was extracted from the peripheral blood of the twins and both parents. DNA profiling with polymerase chain reaction (PCR) amplification was performed for polymorphic microsatellite markers by using the ABI Prism Linkage Mapping Set version 2 (Applied Biosystems, Foster City, CA). PCR products were electrophoresed by using the ABI Prism 310 Genetic Analyzer and analyzed by using GeneScan (Applied Biosystems). This study has been approved by the Ethical Review Board of Asahikawa Medical College. Written consent was obtained from the patients' parents.

Results

Microsatellite analysis confirmed that the twins were monozygotic. The six informative markers (D6S1574, D6S309, D6S470, D6S422, D6S1610, and D6S462), located between 6p24 and 6q15, showed normal biparental inheritance. However, the six distal informative markers (D6S292, D6S1569, D6S1577, D6S264, D6S1697, and D6S281), located between 6q23.2 and the 6q telomeric region, showed the absence of a maternal allele and the presence of a single paternal allele, indicating paternal uniparental isodisomy within this region (Table 1). The two microsatellite markers (D6S434 located at 6q21 and D6S287 located at 6q22.31, respectively) are heterozygous with different sizes indicating that these may be biparental. Mosaicism was not proven in all the regions. The monosomy of the 6q telomeric region was not confirmed by chromosome banding, which showed a normal karyotype (46, XX).

Discussion

To the best of our knowledge, only one case of neonatal diabetes due to partial pUPD6 has been reported to date; however, in that case, the type of neonatal diabetes (i.e. TNDM or PNDM) was not determined because the patient died at 14 days of age. Therefore, our study provides evidence that partial pUPD6 causes TNDM. In the present case, partial pUPD6 may be localized between markers D6S287 and D6S292, which map to 6q22.31 and 6q23.2, respectively. Our findings obtained in monozygotic twins with identical clinical courses provide valuable information to elucidate for clinical and genetic aspects of TNDM due to partial pUPD6. Furthermore, the same diabetic phenotype in the twins was a clinically important observation and confirmed the association between partial pUPD6 and TNDM. Moreover, differences in clinical manifestation exist between partial pUPD6 and either complete pUPD6 or paternal duplication

Table 1. Results of the analysis of microsatellite markers of chromosome 6 in the twins and their parents[a]

Locus	pter–qter	Mb from tel	Father	Twin 1	Twin 2	Mother	Result
D6S1574	6p25.1	6.01	1,2	2,3	2,3	1,3	Biparental
D6S309	6p24.3	8.22	1,2	2,4	2,4	3,4	Biparental
D6S470	6p24.2	10.63	2,3	1,3	1,3	1,2	Biparental
D6S289	6p23	15.3	2,3	2,3	2,3	1,3	Uninformative
D6S422	6p22.3	20.37	1,3	1,4	1,4	2,4	Biparental
D6S1610	6p21.2	39.26	2,2	1,2	1,2	1,3	Biparental
D6S460	6q14.1	90.58	2,3	2,2	2,2	2,2	Uninformative
D6S462	6q15	90.93	1,3	2,3	2,3	2,2	Biparental
D6S434	6q21	102.4	2,3	2,3	2,3	1,3	Uninformative
D6S287	6q22.31	119.5	1,2	1,2	1,2	1,1	Uninformative
D6S262	6q23.1	131.7	1,2	2,2	2,2	2,2	Uninformative
D6S292	6q23.2	136.3	3,4	4,4	4,4	1,2	Paternal UPD
D6S1569	6q23.3	139	1,4	1,1	1,1	2,3	Paternal UPD
D6S308	6q23.3	141.2	1,2	1,1	1,1	1,2	Uninformative
D6S1654	6q24.3	149.5	1,2	1,1	1,1	1,3	Uninformative
D6S1577	6q25.2	155.4	1,2	1,1	1,1	3,4	Paternal UPD
D6S1581	6q25.3	160.2	2,2	2,2	2,2	1,2	Uninformative
D6S305	6q26	162.1	2,2	2,2	2,2	2,3	Uninformative
D6S1599	6q26	162.8	1,1	1,1	1,1	1,1	Uninformative
D6S264	6q27	166.7	1,2	2,2	2,2	1,1	Paternal UPD
D6S1697	6q27	167.8	2,2	2,2	2,2	1,1	Paternal UPD
D6S281	6q27	169.9	1,2	1,1	1,1	2,3	Paternal UPD
D6S446	6q27	170.5	2,2	2,2	2,2	1,2	Uninformative

[a]The location of the genetic markers on the physical maps was established according to the information contained in the National Center for Biotechnology Information (NCBI) database.

of this region (7, 8). In addition to TNDM, most of the patients with complete pUPD6 or paternal duplication exhibit IUGR and in some cases macroglossia (2, 7, 8). There were no significant differences in the clinical presentation between patients with complete pUPD6 and those with paternal duplication (Table 2). IUGR is thought to be, in part, the consequence of the reduction in the secretion of insulin, which acts as a fetal growth factor; this finding is supported by knockout mice studies in which insulin action is impaired (9). In fact, patients with neonatal diabetes due to other causes such as activating mutations in the K_{ATP} channel also exhibit IUGR. However, a previous study showed that the mean birth weight was lower in patients with the 6q24 abnormality than in those with K_{ATP} channel mutations (10). Therefore, the 6q24 abnormality by itself may cause IUGR. On the other hand, macroglossia is seldom observed in patients with K_{ATP} channel mutations (2). Therefore, macroglossia is thought to occur in patients with 6q24 abnormalities. Very similar to complete pUPD6, the twins in this study manifested TNDM (7, 8) (Table 2). However, the twins did not have IUGR, but had macroglossia. Why did the present twins develop normally? One reason is that the twins were born prematurely, that is, before the major growth period in the last trimester, which is influenced by insulin (11). However, some previous reports have described the occurrence of IUGR due to pUPD6 in patients who were born prematurely (2, 12, 13). In fact, fetal pancreas can produce insulin before the second trimester in humans, and insulin may serve as a potent anabolic signal (11). These findings together suggest that the normal growth in the present twins may not necessarily be due to their premature birth. Likewise, in the previously reported case of neonatal diabetes due to partial pUPD6, the patient also had normal birth weight and length and macroglossia (5) (Table 2). These observations support the hypothesis that partial pUPD6 is not associated with IUGR but with macroglossia. Interestingly, an apparent partial isodisomy at 6q25-qter, which resulted in homozygous expansion of a CAG repeat coding for glutamine domain in the TATA-binding protein, was described in a patient with spinocerebellar ataxia type 17 (14). This patient exhibited neither TNDM nor dysmorphic features. Furthermore, the present monozygotic twins with partial pUPD6 exhibited identical phenotypes. Clinical variability of a particular condition is often observed in many monozygotic twins due to genetic/epigenetic and prenatal environmental post-zygotic mechanisms (15). However, similar to our case, Nielsèn previously described the case of the concordant phenotype in monozygotic twins with pUPD6 (12). Kant et al. also reported

Table 2. Comparison for phenotype between partial pUPD6 and pUPD6 or paternal duplication (Pat dup)

Reference	Number of subjects		Median age of TNDM		IUGR (birth weight <10% tile) (%)	Macroglossia (%)
			Onset (day)[a]	Recover (week)[a]		
This paper	2 (twin)	Partial pUPD6	9 (6, 12)	7 (7, 7)	0 (0)	2 (100)
Das et al. (9)	1	Partial pUPD6	2	No (dead at 14 days)	0 (0)	1 (100)
Temple et al. (12)	11	pUPD6	3 (1–31)	15 (5–60)	11 (100)	3 (27)
	9	Pat dup	3 (1–19)	14 (5–24)	8 (89)	1 (11)
Cave et al. (11)	1	pUPD6	13	25	1 (100)	1 (100)
	6	Pat dup	5 (2–30)	10 (2–144)	6 (100)	0 (0)
Suzuki et al. (2)	7	pUPD6	8 (0–11)	8 (3–23)	6 (86)	3 (43)
	4	Pat dup	6 (2–8)	14 (2–35)	2 (50)	2 (50)

IUGR, intrauterine growth retardation.
[a]Values inside the parentheses denote the range of age.

the monozygotic triplets, two of whom exhibited TNDM due to the maternal imprinting defect of the TNDM differentially methylated region, whereas one of whom did not exhibit either TNDM or the imprinting defect (16). These observations suggested that the penetrance of the phenotype due to overexpression of a paternally expressed imprinted *PLAGL1* locus on chromosome 6q24 may be high.

From a genetic perspective, it is important to understand the mechanism underlying the formation of partial pUPD in monozygotic twins. Two postulated mechanisms are known to cause partial UPD. One is a post-zygotic recombination error and the other is a meiotic/mitotic mechanism (4). In the former mechanism, mosaic partial UPD would exist either because of the persistence of both daughter cells or because of somatic recombination during a later cell division. In fact, mosaicism for paternal UPD of the chromosomal segment at 11p15-pter is found in approximately 10–20% of cases with Beckwith–Wiedemann syndrome (17). Although we did not show mosaicism in other tissues, blood DNA analysis revealed the presence of only the paternal allele of 6q24-qter in the present twins, suggesting that post-zygotic recombination was rather unlikely. Moreover, mosaicism may be unlikely in the present case because the twins had identical phenotypes. Exceptionally, mosaicism may not be detected if the opposite UPD is lost either because it occurred very early during mitosis and underwent subsequent splitting in embryonic and extraembryonic tissues or because of cell lethality due to maternal isodisomy. However, the latter possibility could be excluded in our case because survival after complete maternal UPD6 has been reported in two cases (18). Accordingly, partial pUPD6 in the present twins may occur due to a meiotic/mitotic mechanism, in which non-disjunction with meiotic recombination was followed by a mitotic crossing-over between one paternal and one maternal chromatid, subsequently leading to trisomy rescue (4). The time at which mitotic recombination occurred is narrowed between fertilization and twin formation in the present monochorionic diamniotic twins. Similar to our case, mosaicism was not identified in a previously reported patient with neonatal diabetes due to partial pUPD6 (5). In addition, mosaicism was not observed in a patient with Silver–Russell syndrome due to partial maternal UPD at 7q31-qter (19), whereas a patient due to paternal UPD of chromosome 7 was not lethal (20). Therefore, not only post-zygotic recombination error but also a meiotic/mitotic may be involved in the formation of partial UPD (4).

In conclusion, the identical phenotypes observed between the monozygotic twins provide evidence of the association between partial pUPD6, in the distal portion of the 6q chromosomal region, and TNDM. Phenotypes other than diabetes, such as IUGR and macroglossia, observed in patients with partial pUPD6 may differ from those observed in patients with complete pUPD6. The findings of this study also provide valuable evidence to support the hypothesis that a meiotic/mitotic mechanism leads to partial UPD.

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Conflicts of interest

The authors declare no conflict of interest.

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Original Article

HLA-class II and class I genotypes among Japanese children with Type 1A diabetes and their families

Sugihara S, Ogata T, Kawamura T, Urakami T, Takemoto K, Kikuchi N, Takubo N, Tsubouchi K, Horikawa R, Kobayashi K, Kasahara Y, Kikuchi T, Koike A, Mochizuki T, Minamitani K, Takaya R, Mochizuki H, Nishii A, Yokota I, Kizaki Z, Mori T, Shimura N, Mukai T, Matsuura N, Fujisawa T, Ihara K, Kosaka K, Kizu R, Takahashi T, Matsuo S, Hanaki K, Igarashi Y, Sasaki G, Soneda S, Teno S, Kanzaki S, Saji H, Tokunaga K, Amemiya S and The Japanese Study Group of Insulin Therapy for Childhood and Adolescent Diabetes (JSGIT). HLA-class II and class I genotypes among Japanese children with Type 1A diabetes and their families.
Pediatric Diabetes 2012: 13: 33–44.

Objective: To determine the HLA-DRB1, DQB1, DPB1, A, C, and B genotypes among Japanese children with autoimmune type 1 diabetes.
Methods: Four hundred and thirty patients who were GADAb and/or IA-2Ab-positive (Type 1A) were recruited from 37 medical centers as part of a nationwide multicenter collaborative study. DNA samples from 83 siblings of the children with Type 1A diabetes and 149 parent–child trios were also analyzed. A case-control study and a transmission disequilibrium test (TDT) were then performed.
Results: The susceptible and protective DRB1 and DQB1 alleles and haplotypes were confirmed. DPB1 alleles unique to the Japanese population and those common to multiple ethnic groups were also present. A linkage disequilibrium (LD) analysis showed both susceptible and protective haplotypes. The TDT did not reveal any alleles that were transmitted preferentially from the mother or father to children with Type 1A. Homozygosity for DRB1*09:01-DQB1*03:03 and heterozygosity for DRB1*04:05-DQB1*04:01 and DRB1*08:02-DQB1*03:02 were associated with an extremely high risk of Type 1A. A comparison of children with Type 1A and their parents and siblings suggested a dose effect of susceptible DRB1-DQB1 haplotypes and an effect of protective alleles on immunological pathogenesis. DRB1*09:01 appeared to be strongly associated with an early onset in preschool children with Type 1A diabetes.
Conclusions: This study demonstrated the characteristic association of HLA-class II and class I genes with Type 1A diabetes among Japanese children. A TDT did not reveal the genomic imprinting of HLA-class II and class I genes in Type 1A diabetes.

Genetic and environmental factors are thought to be responsible for differences in the incidence of type 1 diabetes among different ethnic groups. The contribution of the HLA-DRB1, DQA1, and DQB1 genes to susceptibility to autoimmune type 1 diabetes (Type 1A) has been well described (1, 2). Several genome scans for linkage to type 1 diabetes have been performed, and these studies have indicated that a gene or genes in the HLA region (insulin-dependent diabetes mellitus 1) at 6p21 has or have

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the strongest impact on disease risk (2, 3). In addition, the independent effects of HLA-DPB1, A, and B have also been demonstrated (4, 5).

The incidence of childhood-onset type 1 diabetes mellitus in Japan is very low (1.4–2.2/100 000 individuals per year) compared with Caucasian populations, especially in Europe (Sardinia, Finland, Sweden, and the UK) and Canada (20/100 000 per year) (6). The risk for siblings of individuals with type 1 diabetes is similar between Caucasians (about 6%) and Japanese (3.8%) (7, 8). These results suggest the existence of both a different set of immunogenetic mechanisms in Japanese patients with type 1 diabetes and a common pathogenesis with Caucasian patients.

The genetic effects of HLA-DRB1 and DQB1 in Japanese patients with type 1 diabetes reportedly differ from those in Caucasian patients (9–15). In Caucasian populations, a predisposition to type 1 diabetes is mostly associated with the DRB1*03:01-DQA1*05:01-DQB1*02:01 and/or DRB1*04:01-DQA1*03:01-DQB1*03:02 haplotypes, whereas the DRB1*15:01-DQB1*06:02 haplotype confers strong protection against the disease. In the Japanese population, three characteristic haplotypes confer susceptibility to type 1 diabetes: DRB1*04:05-DQB1*04:01, DRB1*08:02-DQB1*03:02, and DRB1*09:01-DQB1*03:03. Furthermore, two haplotypes confer protection: DRB1*15:01-DQB1*06:02 (which is common among Caucasians), and DRB1*15:02-DQB1*06:01 (which is characteristic of the Japanese population) (11–15).

HLA-DPB1 alleles are not generally recognized as major contributors to type 1 diabetes. However, an increased risk associated with allele DPB1*02:02 and *03:01 and a decreased risk associated with allele *04:02 have been reported in a number of ethnic groups (4, 5, 16–19). The association of DPB1*02:01 with Japanese childhood-onset type 1 diabetes has been reported by Nishimaki et al. (20), but the number of subjects in this study was relatively small.

This study is the first nationwide multicenter collaborative study for genetic factors in Japanese children with type 1 diabetes and their families. The objective of this study was to determine the genetic characteristics of both HLA-class II (DRB1, DQB1, and DPB1), and class I (A, C, and B) genotypes among Japanese children with Type 1A diabetes and to compare these characteristics with both control data and data obtained from the parents and siblings of the children with Type 1A diabetes. We also studied the diabetes-associated allelic transmission rates from mothers and fathers to children with Type 1A diabetes in the Japanese population.

Methods

Subjects

We recruited 497 Japanese children with type 1 diabetes from 37 medical centers throughout Japan between February 2008 and February 2009. The patients were divided into two groups: Type 1A (GADAb and/or IA-2Ab-positive at diagnosis and/or at registration in this study) and Type 1B (GADAb and IA-2Ab-negative). Type 1A accounted for 430 patients (158 boys and 272 girls) who were 0.8–16.4 years old (mean ± SD, 7.6 ± 3.7 years) at the time of diagnosis. Type 1B accounted for 67 patients (28 boys and 39 girls) who were 0.1–15.1 years old (6.2 ± 4.4 years) at the time of diagnosis. In this study, we focused on children with Type 1A diabetes. Type 1B diabetes may have heterogeneous pathogenetic mechanisms, and some cases of Type 1B have been shown to have a particular monogenic cause, such as mutations in the insulin gene (*INS*), *KCNJ11*, or *ABCC8*. Furthermore, the number of subjects with Type 1B diabetes was too small to obtain a sufficient power in the case-control study.

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Clinical data for all the type 1 diabetes children were obtained. The diagnosis of type 1 diabetes was based on both clinical features and laboratory data. All the patients with Type 1A diabetes were ketosis-prone, lacked endogenous insulin secretion, and required insulin injections at the time of diagnosis based on the 1999 Japan Diabetes Society criteria. The HbA1c levels at the time of diagnosis were 11.9 ± 2.6% among the patients with Type 1A diabetes. The insulin dose at the time of study registration was 1.1 ± 0.3 units/kg/day among the patients with Type 1A diabetes. Eighty-three siblings of 66 children with Type 1A diabetes and 148 father and mother pairs of 149 children with Type 1A diabetes (149 parent–child trios) were recruited. The control data for the HLA allele and haplotype frequencies were based on previously reported data for 1216 subjects in a general Japanese population (21) and a study of 159 families with 561 subjects (22).

This study was approved by the institutional ethics review board of the Tokyo Women's Medical University, the National Research Institute for Child Health and Development, and each of the clinics or hospitals affiliated with a study collaborator. Written informed consent was obtained from the parents or guardians and/or the participants.

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HLA typing

Genomic DNA was extracted from whole blood samples. HLA typing was performed using a Luminex Multi-Analyte Profiling system with a WAKFlow HLA typing Kit (Wakunaga, Hiroshima, Japan), as described elsewhere (23). Briefly, highly polymorphic exons 2 and 3 of the HLA-A, -B, and -C genes and exon 2 of the HLA-DRB1, -DQB1, and -DPB1 genes were amplified using the primer pairs included with the kit. Each polymerase chain reaction product was hybridized using sequence-specific oligonucleotide probes that were complementary to the allele-specific sequences.

Statistical analysis

All the statistical analyses were performed using the R statistical environment, version 2.9.1 (http://www.r-project.org/). The Fisher exact test was applied to a two-by-two contingency table, and the corrected p values (Pc), equivalent to the p values multiplied by the number of comparisons for each locus or haplotype, were determined. A Pc value <0.05 was considered statistically significant.

The study had a sufficient power (more than 0.98) to detect an odds ratio (OR) = 2.0 for an allele frequency of 0.1 in the case-control study comparing DRB1, DQB1, DPB1, A, C, and B between the children with Type 1A diabetes ($n = 430$) and the control data.

The frequency of HLA haplotypes was estimated using the maximum likelihood method (24) or the PHASE program (25). Relative linkage disequilibrium (RD) was calculated as the linkage disequilibrium (LD)/|Dmax| for the relative assessment of LD (22). |Dmax| was the absolute value of the maximum LD for the haplotype.

Results

Association of HLA-DRB1, DQB1, and DPB1 with Type 1A diabetes

In the case-control study, the susceptible alleles associated with Type 1A diabetes in Japanese children were DRBI*09:01 ($Pc < 10^{-29}$; OR, 3.00), DRBI*04:05 ($Pc < 10^{-20}$; OR, 2.60), DRBI*08:02 ($Pc < 10^{-12}$; OR, 3.11), DQBI*03:03 ($Pc < 10^{-26}$; OR, 2.80), DQBI*04:01 ($Pc < 10^{-16}$; OR, 2.32), DQBI*03:02 ($Pc < 10^{-12}$; OR, 2.34), DPBI*02:01 ($Pc < 10^{-2}$; OR, 1.49), and DPBI*03:01 ($Pc < 0.05$; OR, 1.92). The protective alleles were DRBI*15:02 ($Pc < 10^{-21}$; OR, 0.09), DRBI*15:01 ($Pc < 10^{-16}$; OR, 0.06), DRBI*08:03 ($Pc < 10^{-14}$; OR, 0.14), DRBI*04:06 ($Pc < 10^{-3}$; OR, 0.23), DQBI*06:01 ($Pc < 10^{-36}$; OR, 0.11), DQBI*06:02 ($Pc < 10^{-19}$; OR, 0.00), DQBI*03:01 ($Pc < 10^{-11}$; OR, 0.29), DPBI*09:01 ($Pc < 10^{-8}$; OR, 0.25), and DPBI*04:02 ($Pc < 10^{-2}$; OR, 0.57) (Table 1).

The susceptible HLA-DRB1-DQB1 haplotypes associated with Type 1A diabetes in Japanese children were DRB1*09:01-DQBI*03:03 ($Pc < 10^{-20}$; OR, 3.05), DRB1*04:05-DQBI*04:01 ($Pc < 10^{-10}$; OR, 2.33), DRB1*08:02-DQBI*03:02 ($Pc < 10^{-11}$; OR, 5.41), and DRB1*04:05-DQBI*03:02 ($Pc < 10^{-11}$). The protective HLA-DRB1-DQB1 haplotypes were