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ARTICLE

Clinical presentation of 6q24 transient neonatal diabetes mellitus (6q24 TNDM) and genotype–phenotype correlation in an international cohort of patients

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Abstract
Aims/hypothesis 6q24 transient neonatal diabetes mellitus (TNDM) is a rare form of diabetes presenting in the neonatal period that remits during infancy but, in a proportion of cases, recurs in later life. We aim to describe the clinical presentation of 6q24 TNDM in the largest worldwide cohort of patients with defined molecular aetiology, in particular seeking differences in presentation or clinical history between aetiological groups.
Methods One-hundred and sixty-three patients with positively diagnosed 6q24 TNDM were ascertained from Europe, the Americas, Asia and Australia. Clinical data from referrals were recorded and stratified by the molecular aetiology of patients.
Results 6q24 TNDM patients presented at a modal age of one day, with growth retardation and hyperglycaemia, irrespective of molecular aetiology. There was a positive correlation between age of presentation and gestational age, and a negative correlation between adjusted birthweight SD and age of remission. Congenital anomalies were significantly more frequent in patients with paternal uniparental disomy of chromosome 6 or hypomethylation of multiple imprinted loci defects than in those with 6q24 duplication or isolated hypomethylation defects. Patients with hypomethylation had an excess representation of assisted conception at 15%.
Conclusions/interpretation This, the largest case series of 6q24 TNDM published, refines and extends the clinical phenotype of the disorder and confirms its clinical divergence from other monogenic TNDM in addition to identifying previously unreported clinical differences between 6q24 subgroups.

Keywords Chromosome 6q24 · DNA methylation · Epigenetics · Imprinting · Imprinting disorder · Neonatal diabetes · Transient neonatal diabetes mellitus

Abbreviations

ART	Assisted reproductive technology
HIL	Hypomethylation of multiple imprinted loci
Non-ZFP57-HIL	No *ZFP57* mutation with hypomethylation of multiple imprinted loci
TNDM	Transient neonatal diabetes mellitus
UPD6pat	Paternal uniparental disomy of chromosome 6

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ZFP57-HIL *ZFP57* mutation with hypomethylation of multiple imprinted loci

Introduction

Transient neonatal diabetes mellitus (TNDM) is a clinically defined form of neonatal diabetes mellitus that presents soon after birth, undergoes spontaneous remission during infancy but may relapse to a permanent form of diabetes mellitus in childhood or adolescence [1]. While 26% of TNDM patients have mutations of *KCNJ11* (OMIM 601374 [http://omim.org; accessed 04 May 2012]), *ABCC8* (OMIM 600509), *INS* or *HNF1B*, almost 70% (OMIM 601410) have genetic and epigenetic aberrations at the TNDM locus on chromosome 6q24, causing overexpression of two imprinted genes, *PLAGL1* and *HYMAI* [2, 3]. The three reported causes of *PLAGL1* and *HYMAI* overexpression are: (1) paternal uniparental disomy of chromosome 6 (UPD6pat); (2) paternally inherited duplication of 6q24 (duplication) and (3) maternal hypomethylation of the differentially methylated region (DMR) at 6q24. In a proportion of patients the hypomethylation appears to be purely epigenetic, without any detectable underlying genetic cause, and exclusively affects the DMR in TNDM. In other cases, hypomethylation of multiple imprinted loci (HIL) is observed, with a portion of these cases associated with genetic mutations of *ZFP57* (OMIM 612192; ZFP57-HIL) [4, 5].

The rarity of TNDM (1:200,000 to 1:400,000 live births) poses challenges for data collection about clinical features, outcome and management. Until now the clinical features of 6q24 TNDM have been defined in small case studies, some including patients without a molecularly confirmed diagnosis [6, 7]; therefore trends in birthweights, presentation, remission and clinical features, particularly comparing different 6q24 TNDM aetiologies, has been limited by low statistical power. Here we describe the clinical presentation of the largest worldwide cohort of confirmed 6q24 TNDM cases, the majority of whom have not been previously reported, which enables us for the first time to quantify genotype–phenotype correlations.

Methods

Patients Patients positively diagnosed with 6q24 TNDM at the Wessex Genetics Service (www.wrgl.org.uk; accessed 11 September 2012) were ascertained to be from Europe, the Americas, Asia and Australia but ethnicity was not recorded. They were identified through the British Paediatric Association Surveillance Unit, British Diabetic Association, or after referral by endocrinologists, clinical geneticists and paediatricians to either the Peninsula Genetics Service or the Wessex Genetics Service. As part of the diagnostic process referring physicians completed a clinical questionnaire recording: conception, pregnancy history, gestation, birthweight, age of presentation and remission, treatment and number and nature of congenital abnormalities (electronic supplementary material [ESM] questionnaire). Consent to include clinical data in the referral was obtained by the referring physician.

Genetic analysis DNA was extracted from whole blood using standard procedures. Methylation-specific PCR was used to detect hypomethylation of the 6q24 locus, followed by microsatellite analysis to discriminate UPD6pat from isolated hypomethylation at 6q24, as described [4]. Extent of paternal duplication was not routinely determined since it was incidental to molecular diagnosis of TNDM, and extent of uniparental disomy could not always be definitively determined where microsatellite data were uninformative. Samples with 6q24 hypomethylation but not UPD6pat were tested for hypomethylation at other imprinted loci and for *ZFP57* mutations, as described [5].

Data handling and analysis Information from referral questionnaires was recorded on an in-house clinical database. Birthweight, gestation and sex were used to calculate adjusted birthweight standardised deviation scores (SDS) using the LMSgrowth application (version 2.76. www.healthforallchildren.co.uk/; accessed 4 May 2012). Statistical calculations were performed using SPSS (version 19: http://spss-mac.en.softonic.com/mac, accessed 4 May 2012).

Results

One-hundred and sixty-three patients with a molecular diagnosis of TNDM were analysed: 87 (53%) male and 76 (47%) female. Sixty-six (41%) had UPD6pat, 54 (33%) paternal 6q24 duplication and 43 (26%) maternal 6q24 hypomethylation. Of hypomethylation patients 18 (11%) were isolated, 12 (7%) were non-ZFP57-HIL, 12 (7%) were ZFP57-HIL and 1 (1%) were unclassified due to insufficient sample for complete analysis; because standard molecular diagnostic methods did not unequivocally determine the extent of either UPD6 or chr6 duplication, these patients were not further subclassified.

The majority of patients in our cohort were born small for gestational age, with a mean weight and adjusted birthweight SD of 2,001 g and −2.5 respectively (Table 1). Forty of the 133 patients for whom data were available were born at <37 weeks of gestation (30.1%), an incidence significantly higher than in the general population (e.g. the 6.2% quoted by the UK Office of National Statistics) [p=0.02, paired t test; www.ons.gov.uk/ons/publications/re-reference-tables.html?edition=tcm%3A77-50818 (archived)] or the global incidence of 9.6% estimated by the World Health Organization (www.who.int/bulletin/volumes/88/1/08-062554/en/; accessed 13 November 2012).

Table 1 Clinical features of the 6q24 TNDM patients, divided according to aetiology

Clinical feature	Total no. of cases	6q24pat duplication	UPD6pat	6q24 hypomethylation			
				Combined	Isolated	Non-ZFP57-HIL	ZFP57-HIL
Current age (years)							
n	155	52	63	40	16	12	12
Mean	13.1	16.2	11.5	11.5	11.8	13.3	10.3
SD	11.7	15.0	9.6	9.6	7.5	8.6	8.6
Range	1–53	2–53	1–41	1–34	3–28	1–34	1–29
Gestation (weeks)							
n	133	41	54	38	15	11	11
Mean	37.8	38.0	37.3	38.3	38.6	37.6	38.8
Mode	40	40	37	40	40	40	40
SD	2.7	2.5	2.8	2.7	2.3	3.2	2.9
Birthweight (g)							
n	143	47	57	39	15	11	12
Mean	2,001	2,005	1,956	2,064	1,968	2,139	2,098
SD	417	420	433	391	298	577	300
Adjusted birthweight SD							
n	131	41	54	36	14	10	11
Mean	−2.5	−2.6	−2.4	−2.5	−2.8	−2.3	−2.5
SD	1.0	1.1	0.9	1.2	1.0	1.4	1.3
Age of presentation (days)							
n	146	48	59	39	15	11	12
Mean	8	8	7	11	9	7	18
SD	12	9	10	17	9	12	25
Mode	1	1	1	1	1	1	2
Median	4	5	2	8	4	2	7
Age at remission (months)							
n	121	37	50	34	11	11	11
Mean	4.5	3.8	4.8	4.6	4.2	3.9	6.0
SD	5.8	3.9	7.8	4.1	4.2	2.4	5.3
Mode	2	1	2	1	1	1	4
Median	3	3	2	3	2.5	3	4
No. of congenital abnormalities							
n	134	42	55	37	13	12	12
0	64	29	21	14	7	2	4
1	33	7	14	12	6	3	3
2	22	3	14	5	0	5	1
3	7	3	3	1	0	0	1
4	6	0	3	3	0	2	1
5	2	0	0	2	0	0	2
Mean	0.98	0.52	1.15	1.27	0.46	1.73	1.83

n, no. of patients available

The cohort presented with hyperglycaemia at a modal age of 1 day, but at markedly greater median and mean ages (4 and 8 days, respectively; Table 1, ESM Fig. 1). Likewise, the modal age of remission was 2 months but the median and mean ages were 3 and 4.5 months, with the longest recovery recorded at 48 months. Age of presentation with diabetes was significantly correlated with gestational age (r=0.244, p=0.005). In addition, age of remission was negatively correlated with adjusted birthweight SD (r= −0.188, p=0.046, ESM Table 1). The removal of the 48-month outlier increased this significance further (r= −0.199, p=0.036, data not shown).

The most commonly reported congenital abnormalities were macroglossia and umbilical hernia, in 54/123 (44%) and 24/114 (21%) of patients, respectively. Less frequently reported congenital abnormalities included dysmorphic facial appearance 21/114 (18%), renal tract abnormalities (duplex kidneys, hydronephrosis, dilated renal pelvis and vesicoureteral reflux) 11/117 (9%), cardiac anomalies (ductus arteriosus, tetralogy of Fallot, atrial–septal defects and persistent foramen ovale) 10/114 (9%), clinodactyly, polydactyly, nail and short finger abnormalities 9/116 (8%) and hypothyroidism 4/103 (4%). No other significant congenital abnormalities were observed in our modest sample size (Table 2).

Congenital abnormalities occurred significantly less frequently in the 6q24 duplication subgroup, at 0.52/patient compared with 1.15 (p=0.032) and 1.27 (p=0.017) for UPD6pat and hypomethylation subgroups, respectively (Table 1, ESM Table 2). The duplication subgroup had reduced frequency across several congenital abnormalities (Table 2). Within the hypomethylation subgroup, patients with hypomethylation confined to 6q24 also had reduced frequency of congenital abnormalities, averaging 0.46/patient, compared with those with non-ZFP57-HIL or ZFP57-HIL who had an average of 1.73 and 1.83 congenital abnormalities, respectively (Table 1). Notably, macroglossia was the only anomaly recorded in the isolated hypomethylation subgroup (ESM Table 3).

Of 65 cases with data on conception (16 duplication, 23 UPD6pat, 26 maternal 6q24 hypomethylation), four were conceived after assisted reproductive technology (ART). All were hypomethylation patients, three non-ZFP57-HIL and one unclassified. The recorded incidence of ART in the hypomethylation subgroup was 15%.

Discussion

In this study we gathered information from clinicians worldwide on patients molecularly diagnosed with 6q24 TNDM at the Wessex Genetics Service. This is the largest cohort reported to date, containing more extensive clinical details than previous studies, and permits statistical analysis of 6q24 TNDM at presentation.

The principal findings of this study were: (1) the previously unreported relationship between age of presentation of 6q24 TNDM and gestation and the relationship between age of remission and adjusted birthweight SD; (2) the reduced frequency of congenital abnormalities among duplication and isolated hypomethylation patients; and (3) the elevated incidence of ART (15%; 4/26) within the hypomethylation group. Previous observations on severe intrauterine growth retardation and mean age of remission were confirmed [7].

Table 2 Congenital anomalies in patients with 6q24 TNDM divided by genetic abnormality subgroup

Congenital abnormality	Total no. of cases	Genetic abnormality		
		Duplication at 6q24	UPD6pat	6q24 hypomethylation
Macroglossia				
No. present (%)	54 (43.9)	10 (28.6)	24 (46.2)	20 (55.6)
Total	123	35	52	36
Umbilical hernia				
No. present (%)	24 (21.1)	2 (5.6)	15 (33.3)	7 (21.2)
Total	114	36	45	33
Renal tract abnormality				
No. present (%)	11 (9.4)	3 (8.1)	3 (6.5)	5 (14.7)
Total	117	37	46	34
Hand abnormality				
No. present (%)	9 (7.8)	1 (2.9)	5 (10.4)	3 (8.8)
Total	116	34	48	34
Cardiac abnormality				
No. present (%)	10 (8.8)	2 (5.9)	4 (8.7)	4 (11.8)
Total	114	34	46	34
Facial dysmorphism				
Present (%)	21 (18.4)	4 (11.1)	10 (22.7)	7 (20.6)
Total	114	36	44	34
Hypothyroidism				
Present (%)	4 (3.9)	0 (0)	3 (7.3)	1 (3.2)
Total	103	31	41	31

These findings underline the lower birthweight and earlier presentation in 6q24 TNDM that is caused by potassium channel mutations (<1st vs 12th centile, and <1 week vs 4 weeks, respectively). However, the relatively low birthweight previously reported in duplication patients [8] was not supported by this study.

While birthweight (adjusted for gestation) was normally distributed, the ages of presentation and remission of diabetes were markedly skewed, with modes at 1 day and 2 months, but means of 8 days and 4.5 months. The limited clinical data available and the wide variety of healthcare settings in which these patients were treated makes it uncertain whether these variations represent primary variations in clinical history, or variations in diagnosis and management (e.g. delayed recognition of hyperglycaemia or delayed withdrawal of exogenous insulin). TNDM symptoms such as dehydration and failure to thrive are non-specific, so delayed diagnosis in full-term neonates may simply reflect a delay in recognition of neonatal diabetes among other potential diagnoses. The negative correlation between adjusted birthweight SD and age of remission of 6q24 TNDM may be accounted for by earlier remission in the subset of patients with residual insulin secretion and therefore higher birthweight. The correlation of gestation with age at presentation may reflect the prompt testing of blood glucose in premature babies. The high prevalence of preterm birth (30% <37 weeks gestation) may reflect early medical intervention to deliver infants on detection of growth restriction; detailed assessment of clinical history is required to determine whether there is an underlying trend to prematurity.

Stratified analysis of aetiological subgroups was limited by low patient numbers, but some interesting observations emerged. The increased incidence of congenital anomalies in UPD6pat and the largely consanguineous ZFP57-HIL cases may reflect the potential for unmasking of recessive traits among affected individuals. The increased prevalence of congenital abnormalities in the non-ZFP57-HIL group is hitherto unreported, probably because of the extreme rarity of these patients, and may stem from gene dysregulation at other loci affected by their wide-ranging epimutations. The ART frequency observed in the hypomethylation patients, though of limited power due to low cohort size, is in keeping with the incidence of ART in Beckwith–Wiedemann syndrome (4–10%) and is significantly higher than levels in the normal population [9].

In conclusion, 6q24 TNDM may be distinguished from other types of neonatal diabetes by birthweight, with congenital malformations indicating an aetiological subgroup. Emerging genotype–phenotype relationships may predict prognosis for patients in the future. Since TNDM is a relatively newly defined disorder, generally diagnosed in infancy, and therefore the majority of patients are not yet adults, long-term follow-up remains rare (e.g. [10]), but TNDM registries have been established in the UK and USA to aid this process (www.soton.ac.uk/geneticimprinting and http://monogenicdiabetes.uchicago.edu/neonatal-registry/; accessed 11 September 2012).

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Contribution statement IKT, JPHS and DJGM made substantial contributions to the conception and design of the study, LD, AL, EH, LH, SEF, S. Ellard, ATH and DJGM to acquisition of data and LD, SK and S. Ennis to analysis and interpretation of data. LD and SK drafted the manuscript and other authors critically revised it; all authors approved the final version.

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