**6q24 Transient Neonatal Diabetes Mellitus (6q24 TNDM) – clinical presentation and genotype phenotype correlation in an international cohort of cases**

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**Keywords**

**Diabetes, transient neonatal diabetes, imprinting, chromosome 6, epigenetics**

**Word count**

Abstract 230

Main text 1602

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**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

ZFP57-HIL ZFP57 mutation with hypomethylation of multiple Imprinted Loci

**Abstract**

Aim: 6q24 transient neonatal diabetes mellitus (TNDM) is a rare form of diabetes presenting in the neonatal period, which 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: 163 patients with positively diagnosed 6q24 TNDM were ascertained from Europe, 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 birth weight 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%.

Conclusion: 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 (6/10): Transient neonatal diabetes mellitus, neonatal diabetes, imprinting disorder, DNA methylation, chromosome 6q24.

**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](#_ENREF_1)].

While 26% of TNDM patients have mutations of *KCNJ11* (OMIM #601374), *ABCC8* (OMIM #600509), *INS* or *HNF1β*, almost 70% (OMIM #601410) have genetic and epigenetic aberrations at the TNDM locus on chromosome 6q24, causing overexpression of two imprinted genes, *PLAGL1* (Pleomorphic Adenoma Gene-Like 1) / ZAC and *HYMAI (*Hydatidiform Mole Associated and Imprinted) [[2](#_ENREF_2), [3](#_ENREF_3)]. The three reported causes of *PLAGL1* and *HYMAI* overexpression are: - paternal uniparental disomy of chromosome 6 (UPD6pat), paternally inherited duplication of 6q24 (duplication) and 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 TNDM DMR. 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](#_ENREF_4),5].

The rarity of TNDM (1:200,000 - 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](#_ENREF_6), 7]; therefore trends in birth weights, 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.

# RESEARCH DESIGN AND METHODS

Patients

Patients positively diagnosed with 6q24 TNDM at the Wessex Genetics Service ([www.wrgl.org.uk](http://www.wrgl.org.uk)) were ascertained from Europe, 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, birth weight, age of presentation and remission, treatment and number and nature of congenital abnormalities. 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 TND, and extent of UPD 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](#_ENREF_4)].

## Data handling and analysis

Information from referral questionnaires was recorded on an in-house clinical database. Birth weight, gestation and gender were used to calculate adjusted birth weight standardised deviation scores (SDS) using the LMSgrowth application (Version 2.76. [http://www.healthforallchildren.co.uk/;](http://www.healthforallchildren.co.uk/) 2011). Statistical calculations were performed using SPSS (version 19).

**RESULTS**

163 patients with a molecular diagnosis of TNDM were analysed: 87 (53%) male and 76 (47%) female. 66 (41%) had UPD6pat, 54 (33%) paternal 6q24 duplication and 43 (26%) maternal 6q24 hypomethylation. Of hypomethylation patients 18 (11%) were isolated, 12 (7%) non ZFP57-HIL, 12 (7%) *ZFP57*-HIL and 1 (1%) unclassified due to insufficient sample for complete analysis); because standard molecular diagnostic methods did not unequivocally determine the extents 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 birth weight SD of 2001g and -2.5 respectively (Table 1). 40 of 133 patients for whom data were available were born at <37 weeks of gestation (30.1%), significantly higher than general population: eg the 6.2% quoted by the UK Office of National Statstics (P=0.02, paired T-test: http://www.ons.gov.uk/ons/publications/re-reference-tables.html?edition=tcm%3A77-50818) or the global incidence of 9.6% estimated by the World Health Organisation (http://www.who.int/bulletin/volumes/88/1/08-062554/en/).

The cohort presented with hyperglycaemia at a modal age of one day, but at markedly greater median and mean ages (four and eight days respectively: Table 1, Supplementary Figure 1). Likewise, the modal age of remission was two months but the median and mean ages were three and 4.5 months, with the longest recovery recorded at 48 months.Age of presentation with diabetes was significantly correlated with gestational age (correlation coefficient = 0.244, p= 0.005). Additionally, age of remission was negatively correlated with adjusted birth weight SD (correlation coefficient = -0.188, p= 0.046,Supplementary Table 1). The removal of the 48 month outlier increased this significance further (coefficient = -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%), clinodactlyly, 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 and Supplementary 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 to those with non ZFP57-HIL or *ZFP57*-HIL with 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 (Supplementary 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 is 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: the previously unreported relationship of age of presentation of 6q24 TNDM with gestation, and age of remission with adjusted birth weight SD, the reduced frequency of congenital abnormalities among duplication and isolated hypomethylation patients, and 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](#_ENREF_6)]. These findings underline the lower birth weight and earlier presentation in 6q24 TNDM than that caused by potassium channel mutations, (<1st vs 12th centile, and <1 week vs 4 weeks, respectively). However, the relatively low birth weight previously reported in duplication patients [[8](#_ENREF_7)] was not supported by this study.

While birth weight (adjusted for gestation) was normally distributed, the age of diabetic presentation and remission were markedly skewed, with modes at 1 day and 2 months, but mean 8 days and 4.5 months. The limited clinical data available and the wide variety of healthcare settings in which these patients are treated makes it uncertain whether these variations represent primary variations in clinical history, or variations in diagnosis and management, eg 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 term neonates may simply reflect a delay in recognition of neonatal diabetes among other potential diagnoses. The negative correlation between adjusted birth weight 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 of 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 is limited by low patient numbers, but some interesting observations emerge. 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 wideranging 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 (between 4 and 10%) and is significantly higher than normal population levels [[9](#_ENREF_8)].

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

**Acknowledgement**

We thank the patients and their relatives who provided the samples for this study, and also the referring clinicians.

**Funding**

LED, AL and EC were funded by grant 08/0003611 from Diabetes UK, and SE and ATH by the Wellcome Trust, and LH was supported by the Hampshire and the Isle of Wight NIHR Comprehensive Local Research Network.

**Duality of interest**

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

**Contribution statement**

IKT, DJGM had substantial contributions to conception and design, LD, AL, EH, LH, SEF, SE, ATH, DJGM, acquisition of data and LD, SK, and SE analysis and interpretation of data. LD and SK drafted the manuscript and other authors critically revised it with all authors approving the final version.

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Clinical features | Total 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 |
| S.D | 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 |
| S.D | 2.7 | 2.5 | 2.8 | 2.7 | 2.3 | 3.2 | 2.9 |
| Birth weight (g) | N | 143 | 47 | 57 | 39 | 15 | 11 | 12 |
| Mean | 2001 | 2005 | 1956 | 2064 | 1968 | 2139 | 2098 |
| S.D | 417 | 420 | 433 | 391 | 298 | 577 | 300 |
| Adjusted birth weight SD | N | 131 | 41 | 54 | 36 | 14 | 10 | 11 |
| Mean | -2.5 | -2.6 | -2.4 | -2.5 | -2.8 | -2.3 | -2.5 |
| S.D | 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 |
| Mode | 1 | 1 | 1 | 1 | 1 | 1 | 2 |
| Median | 4 | 5 | 2 | 7 | 4 | 2 | 8 |
| S.D | 12 | 9 | 10 | 17 | 9 | 12 | 25 |
| Age of 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 |
| Mode | 2 | 1 | 2 | 1 | 1 | 1 | 4 |
| Median | 3 | 3 | 2 | 3 | 2.5 | 3 | 4 |
| S.D | 5.8 | 3.9 | 7.8 | 4.1 | 4.2 | 2.4 | 5.3 |
| Number 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, number of patients available; S.D, standard deviation.

|  |  |  |
| --- | --- | --- |
| Congenital abnormality | Total Cases | Genetic abnormality |
| Duplication at 6q24 | UPD6pat | 6q24 hypomethylation |
| Macroglossia | Present (%) | 54 (43.9%) | 10 (28.6%) | 24 (46.2%) | 20 (55.6%) |
| Total | 123 | 35 | 52 | 36 |
| Umbilical hernia | Present (%) | 24 (21.1%) | 2 (5.6%) | 15 (33.3%) | 7 (21.2%) |
| Total | 114 | 36 | 45 | 33 |
| Renal tract abnormality | Present (%) | 11 (9.4%) | 3 (8.1%) | 3 (6.5%) | 5 (14.7%) |
| Total | 117 | 37 | 46 | 34 |
| Hand abnormality | Present (%) | 9 (7.8%) | 1 (2.9%) | 5 (10.4%) | 3 (8.8%) |
| Total | 116 | 34 | 48 | 34 |
| Cardiac abnormality | 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 |

Table 2: Congenital anomalies in patients with 6q24 TNDM divided by genetic abnormality sub-group.

Supplementary Table 1 – Clinical correlations (Spearman’s Rho)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Gestation | Adjusted birth weight SD | Number of congenital abnormalities | Age of presentation | Age of remission |
| Age of presentation | Correlation Coefficient | .244 | -.016 | -.107 |  | .129 |
| Sig. (2-tailed) | .005a | .855 | .222 |  | .160 |
| N | 131 | 130 | 133 |  | 121 |
| Age of remission | Correlation Coefficient | .070 | -.188 | -.056 | .129 |  |
| Sig. (2-tailed) | .458 | .046a | .557 | .160 |  |
| N | 114 | 113 | 112 | 121 |  |

a Significant p values

Supplementary Table 2 –Pairwise comparison of congenital abnormality frequency using Post Hoc test (Tukey's) test.

|  |  |  |
| --- | --- | --- |
|  | UPD6pat | 6q24 hypomethylation |
| Combined | Non ZFP57-HIL | ZFP57-HIL |
| 6q24pat duplication | Mean Difference | -.622 | -.746 |  |  |
| Significance (p value) | .032a | .017a |
| S.D | .245 | .269 |
| UPD6pat | Mean Difference |  | -.125 |  |  |
| Significance (p value) | .875 |
| S.D | .254 |
| Isolated | Mean Difference |  |  | -1.266 | -1.372 |
| Significance (p value) | .080 | .047a |
| S.D | .566 | .553 |
| Non ZFP57-HIL | Mean Difference |  |  |  | -0.106 |
| Significance (p value) | .982 |
| S.D | .577 |

S.D, standard deviation. a Significant p values

Supplementary Table 3 – Congenital anomalies in patients with 6q24 TNDM divided by hypomethylation sub-group.

|  |  |  |
| --- | --- | --- |
| Congenital abnormality | Total | Genetic abnormality |
| Isolated | Non ZFP57-HIL | ZFP57-HIL |
| Macroglossia | Present (%) | 20 (56.6%) | 6 (46.2%) | 7 (63.6%) | 7 (58.3%) |
| Total | 36 | 13 | 11 | 12 |
| Umbilical hernia | Present (%) | 7 (20.6%) | 0 (0%) | 4 (36.4%) | 3 (25.0%) |
| Total | 34 | 11 | 12 | 12 |
| Renal tract abnormality | Present (%) | 6 (14.3%) | 0 (0%) | 3 (27.3%) | 2 (16.7%) |
| Total | 35 | 12 | 11 | 12 |
| Hand abnormality | Present (%) | 3 (8.6%) | 0 (0%) | 0 (0%) | 3 (25.0%) |
| Total | 35 | 12 | 11 | 12 |
| Cardiac abnormality | Present (%) | 4 (11.4%) | 0 (0%) | 1 (9.1%) | 3 (25.0%) |
| Total | 35 | 12 | 11 | 12 |
| Facial dysmorphism | Present (%) | 7 (20.0%) | 0 (0%) | 3 (27.3%) | 4 (33.3%) |
| Total | 35 | 12 | 11 | 12 |
| Hypothyroidism | Present (%) | 1 (3.1%) | 0 (0%) | 1 (9.1%) | 0 (0%) |
| Total | 32 | 11 | 11 | 10 |

Legend to Supplementary Figure 1.

Box plots showing distribution of (A) age of presentation and (B) age of remission in TNDM patients, stratified by aetiological subgroup. Boxes indicate the distribution of data between the 1st and 3rd quartiles (Q1 and Q3). The line inside each box indicates the median value, and the whiskers extend to 1.5 times the interquartile range of Q1 and Q3. Statistical outliers are marked by asterisks.