Published in final edited form as:
Diabetologia. 2013 January ; 56(1): 218–221. doi:10.1007/s00125-012-2766-z.

Hypoglycaemia following diabetes remission in patients with 6q24 methylation defects – expanding the clinical phenotype

S.E. Flanagan[1], **D. J. G. Mackay**[2], **S. A. W. Greeley**[3], **Timothy J. McDonald**[1], **V. Mericq**[4], **J. Hassing**[5], **E. J. Richmond**[6], **W.R. Martin**[7], **C Acerini**[8], **A.M. Kaulfers**[9], **D.P. Flynn**[10], **J. Popovic**[10], **M.A. Sperling**[10], **K. Hussain**[11], **S. Ellard**[1], and **A.T. Hattersley**[1]

[1]Institute of Biomedical and Clinical Research, University of Exeter Medical School, UK

[2]Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, UK

[3]Kovler Diabetes Center, The University of Chicago, Chicago, IL USA

[4]Institute of Maternal and Child research, Faculty of Medicine, University of Chile, Casilla 226-3, Santiago, Chile

[5]Pediatric Endocrinology, Rockwood Clinic, Spokane, WA, USA

[6]Pediatric Endocrinology, National Children's Hospital, San José, Costa Rica

[7]Pediatric Endocrinology, Sacred Heart Children's Hospital, Spokane, WA, USA

[8]Department of Paediatrics, University of Cambridge, Cambridge, UK

[9]Pediatric Endocrinology, University of South Alabama, Mobile, AL, USA

[10]Division of Pediatric Endocrinology, Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh School of Medicine, Pittsburgh Pa 15224, USA

[11]Department of Endocrinology, Great Ormond Street Hospital for Children NHS Trust, London, and The Institute of Child Health, University College London, UK

Keywords

Chromosome 6q24; Hypoglycaemia; Transient neonatal diabetes; Uniparental Disomy

Methylation defects at chromosome 6q24 are the most common cause of Transient Neonatal Diabetes (TNDM), accounting for 70% of all cases [1, 2]. Those affected have impaired insulin secretion as shown by very low birth weights (median <1st centile) and a diagnosis of diabetes usually at, or shortly after birth, (range 0–4 weeks) [1–3]. In the majority of patients the diabetes remits by a median age of 13 weeks, although for many there is a relapse later in life [3]. Loss of methylation at the chromosome 6q24 locus results from one of three mechanisms 1) paternal uniparental disomy (UPD) (~40% of cases) 2) a paternal duplication (~32%) or 3) loss of methylation without a structural chromosome defect (~28%) (reviewed in [2]). The TNDM critical region on chromosome 6q24 encompasses *PLAGL1*, a tumour suppressor gene, and *HYMAI*, a non-coding RNA of unknown function [2]. The underlying

Corresponding author: Dr Sarah E. Flanagan, University of Exeter Medical School, Barrack Road, Exeter, EX2 5DW, UK, Sarah.Flanagan@pms.ac.uk, Telephone: +44 (0) 1392 406778, Fax: +44 (0) 1392 406767.

Contribution statement

SEF and DJGM performed molecular genetic testing. SEF and ATH were responsible for the conception of the study and drafted the manuscript. SEF, DJGM, SAWG, TJM, VM, JH, EJR, WRM, CA, AMK, DPF, JP, MAS, KH, SE and ATH analysed data and revised the manuscript. All authors approved the final version.

mechanism(s) by which loss of methylation, and hence over expression, of *PLAGL1* and/or *HYMAI* cause TNDM is not known.

We report the novel clinical observation of hypoglycaemia following diabetes remission in 6/43 (14%) patients with 6q24 TNDM. This is in keeping with the prevalence of extra-pancreatic features of patients with 6q24 TNDM such as macroglossia (35%) and umbilical hernia (14%) [2]. Five of the patients have paternal UPD and one patient has a paternal duplication [4]. No differences in diabetes was observed between patients with and without hypoglycaemia as shown by the age at diagnosis (1 week vs 4 days, p= 0.87) and age at remission (14 vs 21 weeks, p= 0.41). The median birth weight was however higher in the 6 patients with hypoglycaemia compared to the 37 patients where hypoglycaemia had not been reported (−1.83 vs −3.14 SDS, p = 0.026) which is in keeping with increased insulin secretion *in utero*. Inactivating *ABCC8* and *KCNJ11* gene mutations, the most common cause of HH were excluded by sequence analysis [5].

Hypoglycaemia (blood glucose <2.6 mmol/L) was diagnosed at a median age of 33.5 weeks which was within 2–22 weeks of remission. The clinical characteristics are provided in table 1. Presentation of hypoglycaemia was variable; in 3 cases (patients 1, 5 and 6) hypoglycaemia was noted on blood measurement following hospital admissions for self limiting viral illnesses. Three cases (patients 2, 3 and 4) were symptomatic with lethargy and shakiness that improved with feeding.

All 6 patients required treatment for hypoglycaemia and only one did not require long term treatment (table 1). In 4 patients, diazoxide was given (5–15mg/kg/day) with 3 continuing to require treatment 1, 2 and 4 years later. One patient requires overnight bolus feeds to prevent hypoglycaemia 2 years after diagnosis and has only recently started diazoxide therapy.

The hypoglycaemia may result from excess insulin secretion. In 5 of the 6 patients serum insulin was measured at the time of hypoglycaemia: in 3 cases insulin was found to be inappropriately high. In the remaining two patients insulin was below the assay limit of detection on a single measurement but blood ketones were inappropriately suppressed (<0.2nmol/L) at the time of hypoglycaemia suggestive of an insulin mediated action. In 4 patients growth hormone and cortisol levels were assessed and deficiency of these hormones was ruled out as a cause of hypoglycaemia. Further investigations are required to confirm that hyperinsulinism is the mechanism of hypoglycaemia in these patients.

The high prevalence of hypoglycaemia in our cohort (14%) when compared to an incidence of hyperinsulinaemic hypoglycaemia (HH) of 0.002% means that it is very likely that the hypoglycaemia is a direct consequence of the chromosome 6q24 abnormality [6]. The mechanism for the hypoglycaemia is currently not understood.

In order to investigate the possibility of a recessively acting mutation unmasked by UPD, we undertook exome sequencing in 3 patients (patients 1, 2 and 3). We analysed all genomic regions corresponding to the NCBI Consensus Coding Sequence database captured by the Agilent's SureSelect Human All Exon Kit (v1). Paired-end sequencing was performed on an Illumina GAII and all variants identified in the minimum shared region of UPD (29.9kb as defined by Affymetrix SNP6.0 analysis, flanking SNPs rs13220827 and rs6931065) were called using GATK. No genes harbouring a novel non-synonymous variant were identified in more than one individual. This excludes coding mutations in the captured exons but does not exclude a causal mutation in a non-coding or regulatory region. The presence of a paternal duplication in one patient suggests that the hypoglycaemia is more likely to be a direct consequence of the methylation defect rather than the chromosome abnormality causing it. However, it is noteworthy that the 5 patients with UPD had a more severe

phenotype as demonstrated by the long term requirement of diazoxide or overnight bolus feeds when compared to the patient with the duplication who had episodic hypoglycaemia. Studies on larger numbers of patients are required to determine whether this observation reflects a genotype/phenotype relationship.

The reason for the remission of diabetes in patients with 6q24 TNDM is not known. Studies in mice with paternal inheritance of a transgene show an increase in the number of pancreatic beta cells prior to diabetes remission [7] and it is possible that in the patients there is an 'overshoot' of this process and consequently beta cell hyperplasia. This however on its own cannot explain why the beta cells inappropriately secrete insulin despite hypoglycaemia. Whilst it is known that mutations in *ABCC8*, *KCNJ11* and *HNF4A* can cause transient congenital HH and later onset diabetes, there have not been any reports where HH develops following diabetes remission [5, 8]. Further studies are required to establish the cause of the severe defect in the regulation of insulin secretion in these patients.

In conclusion, the identification of hypoglycaemia in 14% of patients with 6q24 TNDM provides further evidence for the key role of the chromosome 6q24 locus in the regulation of insulin secretion and glucose homeostasis. It is important to be aware of the increased risk of hypoglycaemia in the months following remission of diabetes in patients with a chromosome 6q24 methylation defect.

Acknowledgments

S.E.F. was the Sir Graham Wilkins, Peninsula Medical School Research Fellow. A.T.H. and SE are employed as core members of staff within the NIHR funded Peninsula Clinical Research Facility.

References

1. Gardner RJ, Mackay DJ, Mungall AJ, et al. An imprinted locus associated with transient neonatal diabetes mellitus. Hum Mol Genet. 2000; 9:589–596. [PubMed: 10699182]
2. Mackay DJ, Temple IK. Transient neonatal diabetes mellitus type 1. Am J Med Genet C Semin Med Genet. 2010; 154C:335–342. [PubMed: 20803656]
3. Flanagan SE, Patch AM, Mackay DJ, et al. Mutations in ATP-sensitive K+ channel genes cause transient neonatal diabetes and permanent diabetes in childhood or adulthood. Diabetes. 2007; 56:1930–1937. [PubMed: 17446535]
4. Mackay DJ, Temple IK, Shield JP, Robinson DO. Bisulphite sequencing of the transient neonatal diabetes mellitus DMR facilitates a novel diagnostic test but reveals no methylation anomalies in patients of unknown aetiology. Hum Genet. 2005; 116:255–261. [PubMed: 15635480]
5. Kapoor RR, Flanagan SE, James C, Shield J, Ellard S, Hussain K. Hyperinsulinaemic hypoglycaemia. Arch Dis Child. 2009; 94:450–457. [PubMed: 19193661]
6. Otonkoski T, Ammala C, Huopio H, et al. A point mutation inactivating the sulfonylurea receptor causes the severe form of persistent hyperinsulinemic hypoglycemia of infancy in Finland. Diabetes. 1999; 48:408–415. [PubMed: 10334322]
7. Ma D, Shield JP, Dean W, et al. Impaired glucose homeostasis in transgenic mice expressing the human transient neonatal diabetes mellitus locus, TNDM. J Clin Invest. 2004; 114:339–348. [PubMed: 15286800]
8. Pearson ER, Boj SF, Steele AM, et al. Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. PLoS Med. 2007; 4:e118. [PubMed: 17407387]

Table 1

Clinical characteristics and genetic results for 6 patients with transient neonatal diabetes and symptomatic hypoglycaemia.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Sex	Male	Female	Male	Female	Female	Female
Birth weight (gestation)	2013g (37 wks)	1130g (30 wks)	2100g (38 wks)	2841g (39 wks)	1700g (38 wks)	1500g (33 Wks)
Birth weight (SDS score)	−2.19	−1.15	−2.40	−0.85	−3.33	−1.47
Extra pancreatic features	No	No	Macroglossia and umbilical hernia	No	No	Ventricular septal defect, thrombocytopenia, anaemia, macroglossia
Diabetes						
Age at diagnosis (weeks)	0.14	0.14	4	2	1	1
Blood glucose at presentation (mmol/L)	16	25	21	38	37	13
Insulin Requirement (U/Kg/day)	0.40	0.40	0.68	0.66	0.38	2.40
Age of remission (weeks)	26	29	9	26	17	1.14
Hypoglycaemia						
Age at initial presentation of hypoglycaemia (weeks) [1]	39	43	22	28	3 episodes with intercurrent illness at 39, 43 & 56 wks	5
Blood glucose at initial presentation[a] **(mmol/L)**	2.4	2.3	1.9	2.2*, CGM[b]	1.5*, 2.4* and 1.9	1.9
Blood insulin at time of hypoglycaemia[a,c] **(pmol/L)**	13.9	18.7	Not detected	Not detected	Not measured	62.5
C-peptide at time of hypoglycaemia[a] **(pmol/L)**	<30	Not measured	Not measured	67	Not measured	206
Treatment (Dose)	10% IV Dextrose, Diazoxide (10mg/kg/day)[d]	Diazoxide (5mg/kg/day)	Diazoxide (15mg/kg/day)	Diazoxide (5mg/kg/day)	Diet, 5% IV dextrose (1 occasion)	IV dextrose and bolus feeds; continuous overnight feeds until aged 112 days.
Age at remission of hypoglycaemia	Ongoing at 6 yrs	3 yrs	Ongoing at 2 yrs	Ongoing at 2.9 yrs	Dextrose infusion for 12 hours aged 56 weeks	Overnight feeds still required at 2.6 yrs
6q24 mechanism	Paternal UPD	Paternal UPD	Paternal UPD	Paternal UPD	Paternal duplication	Paternal UPD

[a] Hypoglycaemia is defined as blood glucose <2.6mmol/l. An asterisk (*) denotes glucose measured on a capillary blood glucose monitor; all others done in a laboratory.

[b] Patient 4 was confirmed to have low blood glucose values using continuous glucose monitoring (CGM).

[c] Patient 3 and Patient 4 (after overnight 13 hr fast) had suppressed ketone levels indicative of hyperinsulinism

[d] Diazoxide treatment was commenced at the age of 28 months following the detection of hyperinsulinaemic hypoglycaemia in this patient.

UPD = Uniparental Disomy; IV = Intravenous