**Temple syndrome as a result of isolated hypomethylation of the 14q32 imprinted DLK1/MEG3 region**

Tracy A Briggs1, Kemi Lokulo-Sodipe2,3, Kate E Chandler1, Deborah J G Mackay2, I Karen Temple2,3.

1. Manchester Centre for Genomic Medicine, St Mary's Hospital, University of Manchester, Manchester M13 9WL, UK

2. Academic Unit of Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK

3. Wessex Clinical Genetics Service, University Hospital Southampton NHS Foundation Trust, Princess Anne Hospital, Southampton, UK

**Running heads**

Title: Temple syndrome secondary to epimutation

Authors: Briggs *et al*.

**Corresponding author**

I Karen Temple, Wessex Clinical Genetics Service, Princess Anne Hospital, Coxford Road, Southampton, Hampshire, SO16 5YA, UK; ikt@soton.ac.uk

**Abstract**

We present a Caucasian female, who was diagnosed at 13 years of age with Temple syndrome (formerly referred to as ‘maternal UPD 14 phenotype’) due to an epigenetic loss of methylation at IG-DMR/MEG3-DMR at the chromosome 14q32 imprinted locus. Clinical features were typical and included intra-uterine growth retardation (IUGR), low birth weight, hypotonia, and poor feeding in the neonatal period; and failure to thrive and developmental delay – particularly in relation to speech – in early childhood. Premature puberty, with short stature and truncal obesity, but normal intelligence, were the key features in teenage years.

To-date only six patients with Temple syndrome due to an epigenetic error have been described and the aetiology of the methylation defect is currently undetermined. In view of a tendency towards central obesity, patients are at potential risk of early-onset type 2 diabetes mellitus, as well as cardiovascular disease and they therefore require appropriate monitoring.

**Key words**

Temple syndrome

Epigenetics

Imprinting

Chromosome 14q32

**Introduction**

Temple syndrome is caused by altered imprinted gene expression at chromosome 14q32. There are three underlying causes of Temple syndrome, which have clear clinical overlap, consisting of pre- and postnatal growth retardation, poor feeding, hypotonia, joint laxity, motor delay, premature puberty, and obesity. In the majority (over 75% of reported cases to date) maternal uniparental disomy of chromosome 14 (UPD14mat) is the underlying molecular aetiology [Temple et al., 1991]. However, more recently, patients with Temple syndrome secondary to a paternal deletion at 14q32 [Buiting et al., 2008; Kagami et al., 2008] or isolated loss of methylation mutation at the differentially methylated regions on 14q32 [Mitter et al., 2006; Temple et al., 2007; Buiting et al., 2008; Hosoki et al., 2008; Zechner et al. 2009] have been described, both appearing to be of relatively equal frequency.

A cluster of imprinted genes have been identified at chromosome 14q32.2, including those which are paternally expressed; *DLK1*, *RTL1* and *DIO3*, in addition to maternally expressed non-coding RNAs, *MEG3* (also known as *GTL2*), *RTL1as*, *MEG8* and numerous C/D box small nucleolar RNAs and microRNAs. The locus is controlled by a paternally methylated intergenic differentially methylated region (IG-DMR) and a secondary DMR termed *MEG3*-DMR. The clinical phenotype is thought to result from a loss of expression of the paternally expressed genes, *DLK1* and *RTL1* [Kagami et al., 2008].To-date only six patients have been described which result from an isolated methylation loss affecting this region [Mitter et al., 2006; Temple et al., 2007; Buiting et al., 2008; Hosoki et al., 2008; Zechner et al. 2009]. We describe a further patient to bring this syndrome to the attention of health professionals. We compare the phenotype to all patients reported to date, to highlight the key features and management issues in Temple syndrome.

**CLINICAL report**

We present a Caucasian female born to healthy, non-consanguineous parents. She was their second child and her elder brother was fit and well. There was no significant extended family history.

The proband was conceived naturally, when her mother was 20 and her father 30 years old. The early antenatal period was uneventful, however intra-uterine growth retardation (IUGR) was noted at 20 weeks’ gestation and continued subsequently in the pregnancy, necessitating induction of labour at 38 weeks. At delivery APGAR scores were good, but birth weight was low at 2007g (<0.4th centile)(see Figure 2) and hypotonia was noted. There were feeding difficulties, with a poor suck, which necessitated a brief re-admission at 15 weeks of age, although nasogastric tube feeding was not required.

In early childhood she remained persistently below the 5th centile for all growth parameters. Her early motor milestones were delayed and she did not sit unsupported until 14 months, although she walked at 16 months. The predominant concern was in relation to her speech. Despite normal hearing, at two years and six months she only spoke three single words and therefore the first two years of her education were at a language unit. She was toilet trained during the daytime at five years of age and was described as stubborn, but there were no marked behavioural problems. She was noted to have a bilateral esotropia, which was managed with glasses.

At age seven years, with significantly improved speech and no further requirement for extra support, she transitioned to mainstream school. However, she was diagnosed with dyspraxia. Her growth parameters progressed between the 0.4th and 2nd centiles, despite a mid-parental height on the 50th centile. She entered puberty prematurely, with breast development at age six years, pubic hair at seven years and menarche at nine years of age. Subsequently she developed marked acne vulgaris, which was managed with erythromycin with zinc acetate.

On examination at 11 years of age, her height was between the 0.4th and 2nd centile and her head circumference was on the 2nd centile, whilst her weight had increased to the 50th centile, with truncal adiposity noted (Figure 1a). She had subtle dysmorphic features with a high forehead, broad nose with slightly anteverted nares (Figure 1b) and small hands and feet (size 13)(Figure 1c). She had mild pes planus and joint hypermobility, but no scoliosis. Beyond abdominal striae, there were no cutaneous stigmata of disease and systemic examination was normal, with a blood pressure of 117/58 mmHg.

Extensive investigations were undertaken, particularly in early childhood to try to determine the cause of her symmetrical short stature and developmental delay. These tests included Mitomycin C, Fragile X, and cystic fibrosis testing, neuro-metabolic screening, karyotype testing, cerebral computer tomography (CT) scanning and electro-encephalogram (EEG): no abnormalities were detected. Subsequently a microarray-CGH was performed which was normal. However, at the chronological age of 12 years, a bone age of 15 years was reported, and whilst the majority of biochemical and endocrine investigations were normal, mildly elevated androstenedione (8.9 nmol/l; normal range 0-6) and testosterone (2 nmol/l; normal range 0-1.5) levels were noted.

Imprinting studies were then undertaken on a research basis, with parental consent into the research study “Imprinting disorders – finding out why” (IDFOW: Southampton and South West Hampshire Research Ethics approval 07/H0502/85). Targeted testing identified hypomethylation at the *MEG3* differentially methylated region at chromosome 14q32 (methods as described in Temple *et al*.[5](#_ENREF_5)). Methylation analysis was performed at: DIRAS3 (1p31); PLAGL1(6q24); IGF2R (6q27); GRB10 (7p12); PEG10 (7q21); PEG1/MEST (7q32); KCNQ1OT1/H19/IGF2P0 (11p15);  DLK1 (14q32);  SNRPN (15q11); PEG3 (19q32) and NESPAS/GNAS (20q13), only DLK1 *(MEG3)* was abnormally methylated, suggesting an absence of multilocus imprinting disturbance. Subsequent parental studies with multiple polymorphic microsatellite markers showed no evidence of uniparental disomy for chromosome 14(methods as described in Temple *et al*.[5](#_ENREF_5)). No copy number anomaly of 14q32 was detected by MLPA analysis (using kit ME032-A1, MRC-Holland). Whilst segmental UPD cannot be fully excluded, these results suggest that it is unlikely and that Temple syndrome in this patient probably results from a purely epigenetic phenomenon.

In view of the truncal adiposity, baseline cardiovascular and diabetic investigations were undertaken, including cholesterol, triglycerides, IGF-1 (insulin-like growth factor 1) and glucose level. All indices were within normal ranges.

**Discussion**

As described above, Temple syndrome is caused by aberrant expression of imprinted genes at chromosome 14q32, and can be due to maternal UPD 14, a paternal deletion within the locus or loss of methylation at the *DLK1/MEG3* differentially methylated region on chromosome 14. To our knowledge this patient represents only the seventh reported epimutation at the IG-DMR; the first being described in 2007 [Temple et al.]. All epimutation patients are summarised in table I, illustrating the key features and are compared to those with maternal UPD14 and paternal deletions [Ioannides et al. 2014].

In general it is not possible to differentiate between patients with epigenetic aberrations and those with maternal UPD 14 or a paternal deletion. The major features of IUGR, low birth weight, neonatal hypotonia, poor feeding, short stature, with a corresponding low weight initially, but truncal adiposity in later childhood and adulthood are reported in the majority. A review of the literature [Ioannides et al. 2014] failed to identify differences in the subtle facial dysmorphic features, consisting of frontal bossing, a fleshy nasal tip, short philtrum, high palate and micrognathia, between the sub-categories of Temple syndrome.

A tendency towards early adrenarche and puberty, resulting in reduced final height, is also described in all sub-groups of Temple syndrome. In our patient and one previous epimutation report [Buiting et al., 2008] premature puberty was associated with the detection of an advanced bone age, when assessed in the teenage years.

 Our patient report supports the finding that preterm births have not been reported in epimutation cases. Interestingly, in all epimutation patients where speech has been described, there has been early speech delay. This is also seen in all patients with a paternal deletion. In contrast speech delay is reported in only 45% of patients with maternal UPD 14 [Ioannides et al. 2014].

Central adiposity/obesity has been noted in five of seven (71%) epimutation patients, and 50% of maternal UPD patients [Ioannides et al. 2014]. One of the two epimutation patients, who did not display this phenotype was 2 years and 2 months old at the last follow-up. The youngest affected epimutation patient was reported to have rapid weight gain from 2 years 6 months and the others were between 5 and 8 years old. In view of the tendency to later obesity, these individuals are at potential risk of early-onset type 2 diabetes and cardiovascular disease. We highlight the presence of raised androstendione and testosterone as raised serum androgens are associated with central obesity and insulin resistance. We suggest that long term monitoring should be undertaken and most suitably by a paediatric endocrine team, who may already be involved with these patients for short stature and early onset of puberty.

The ability to distinguish phenotypes in imprinting disorders is challenging as many of the impacts involve subtle abnormalities with fetal and postnatal growth. However, as more patients are described there may be a more consistent phenotype within the epigenetic subgroup. The differential diagnoses of Prader-Willi (because of the early hypotonia) and Russell-Silver syndromes, (because of the marked short stature), were considered in our patient and a number of other reported patients with Temple Syndrome [Mitter et al., 2006; Ioannides et al. 2014].

The mechanism of the epimutation is not yet determined and so genetic risks to relatives is uncertain. While no cases of recurrence have been reported in sibs, it is theoretically possible that some cases are due to an as-yet undiscovered underlying genomic deletion/rearrangement in cis, as has been shown in other imprinting disorders [Bastepe et al, 2005]. In such situations there may be a risk of recurrence. Primary epigenetic errors may be expected to have a low offspring risk, as the methylation mark should be reset in the developing germ cells.

Individuals with Temple syndrome may present to a number of specialists, including paediatric endocrinologists, community paediatricians and geneticists. A high index of suspicion is needed to instigate testing and avoid extensive investigation which might delay diagnosis. With increasing uptake of methylation-based studies, further patients with Temple syndrome secondary to epimutations are likely to be identified. Increased detection will allow for further delineation of the phenotype in this sub-category of patients and we would encourage the reporting of patients with epigenetic aberrations at 14q32 in order to delineate the (epi)genotype-phenotype correlation. Follow-up of known patients will also be important to understand risk factors in adulthood, including but not limited to, the possible cardiovascular and diabetic risks.

**Acknowledgments**

We would like to thank the patient and her family for their cooperation with the preparation of this manuscript. TAB acknowledges the NIHR clinical lecturer programme. KLS is supported by a NIHR Research for Patient Benefit grant.

We acknowledge the support of the Wessex Clinical Research network. DJGM and IKT are members of the COST Action BM1208.

**References**

Bastepe, M., Frohlich, L. F., Linglart, A., Abu-Zahra, H. S., Tojo, K., Ward, L. M., Juppner, H.2005. Deletion of the NESP55 differentially methylated region causes loss of maternal GNAS imprints and pseudohypoparathyroidism type Ib. Nat Genet 37, 25-7.

Buiting, K.,Kanber, D., Martin-Subero, J. I., Lieb, W., Terhal, P., Albrecht, B., Purmann, S., Gross, S., Lich, C., Siebert, R., Horsthemke, B., Gillessen-Kaesbach, G*.* 2008. Clinical features of maternal uniparental disomy 14 in patients with an epimutation and a deletion of the imprinted DLK1/GTL2 gene cluster. Hum mutat 29, 1141-6.

Hosoki, K., Ogata, T., Kagami, M., Tanaka, T. & Saitoh, S. 2008. Epimutation (hypomethylation) affecting the chromosome 14q32.2 imprinted region in a girl with upd(14)mat-like phenotype. Eur J Hum Genet16, 1019-23.

Ioannides, Y., Lokulo-Sodipe, K., Mackay, D.J., Davies, J.H. & Temple, I.K. 2014. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. J Med Genet 51, 495-501.

Kagami, M., Sekita, Y., Nishimura, G., Irie, M., Kato, F., Okada, M., Yamamori, S., Kishimoto, H., Nakayama, M., Tanaka, Y., Matsuoka, K., Takahashi, T., Noguchi, M., Masumoto, K., Utsunomiya, T., Kouzan, H., Komatsu, Y., Ohashi, H., Kurosawa, K., Kosaki, K., Ferguson-Smith, A. C., Ishino, F., Ogata, T. 2008. Deletions and epimutations affecting the human 14q32.2 imprinted region in individuals with paternal and maternal upd(14)-like phenotypes. Nat Genet 40, 237-42.

Mitter, D., Buiting, K., von Eggeling, F., Kuechler, A., Liehr, T., Mau-Holzmann, U. A., Prott, E. C., Wieczorek, D., Gillessen-Kaesbach, G. 2006. Is there a higher incidence of maternal uniparental disomy 14 [upd(14)mat]? Detection of 10 new patients by methylation-specific PCR. Am J Med Genet A 140, 2039-49.

Temple, I.K., Cockwell, A., Hassold, T., Pettay, D. & Jacobs, P. 1991. Maternal uniparental disomy for chromosome 14. J Med Genet 28, 511-4.

Temple, I.K., Shrubb, V., Lever, M., Bullman, H. & Mackay, D.J. 2007. Isolated imprinting mutation of the DLK1/GTL2 locus associated with a clinical presentation of maternal uniparental disomy of chromosome 14. J Med Genet 44, 637-40.

Zechner, U., Kohlschmidt, N., Rittner, G., Damatova, N., Beyer, V., Haaf, T., Bartsch, O.2009*.* Epimutation at human chromosome 14q32.2 in a boy with a upd(14)mat-like clinical phenotype. Clin Genet 75, 251-8.

**Legend**

Figure 1: Clinical images of the proband at age 11 years, demonstrating a degree of truncal obesity (A), a prominent forehead and broad nose, with slightly anteverted nares (B) and small hands (C).