

A patient with multi-locus imprinting disturbance involving hypomethylation at 11p15 and 14q32, and phenotypic features of Beckwith-Wiedemann and Temple syndrome.

Sarah E Grosvenor¹, Justin H Davies¹, Margaret Lever², Julie Sillibourne², Deborah JG Mackay^{1,2}, I Karen Temple^{1,2*}

1 Faculty of Medicine, University of Southampton, Paediatric Endocrinology, Southampton Children's Hospital, UK

2 Wessex Genetics Service, Southampton University Hospitals Trust, Southampton, and Salisbury Hospital NHS Foundation Trust, Salisbury, UK

* To whom correspondence should be addressed: Wessex Clinical Genetics Service, Princess Anne Hospital, Coxford Road, Southampton SO16 5YA, UK: ikt@soton.ac.uk

Main word count: 2132

Abstract count: 116

ABSTRACT

Beckwith-Wiedemann syndrome and Temple syndrome are classical imprinting disorders with non-confluent clinical features. We report here on a patient with clinical features of both syndromes, in whom epimutations were found at the BWS and TS imprinted regions, consistent with multi-locus imprinting disturbance (MLID). This is the first case report of a patient with clinical features of both conditions who was found to have loss of methylation (LOM) of *KCNQ1OT1*: TSS-DMR (ICR2) in the 11p15 imprinted region associated with BWS, and LOM of *MEG3*:TSS – DMR in the 14q32 imprinted region associated with Temple syndrome. The report draws attention to the importance of testing for MLID as a cause of atypical clinical presentations of patients with imprinting disorders.

KEYWORDS

Beckwith-Wiedemann, Temple, Silver-Russell, imprinting, multi-locus imprinting disorder.

INTRODUCTION

Imprinting is a phenomenon by which epigenetic markers such as DNA methylation are maintained from one generation to another, ensuring parent of origin-specific monoallelic expression of genes critical for growth, development, and metabolism (D. J. G. Mackay & Temple, 2017; Monk, Mackay, Eggermann, Maher, & Riccio, 2019). Disturbances to this process are associated with deleterious developmental effects in pregnancy and beyond, including disturbance of pre- and postnatal growth, development, metabolism and behaviour.

Imprinting disorders (IDs) may be caused by *cis*-acting genetic (CNV, UPD, chromosomal rearrangement) or epigenetic errors of disease-associated loci. However, a subset of affected individuals has methylation abnormalities at multiple imprinted loci (multi-locus imprinting disorders, or MLID). In MLID, *cis*-acting genetic changes are not found (Elbracht, Mackay, Begemann, Kagan, & Eggermann, 2020). Implicated instead are environmental, iatrogenic and *trans*-acting genetic factors affecting oocyte development or early developmental epigenetic reprogramming, including *trans*-acting mutations found either in the affected person or their mother (Begemann *et al.*, 2018; D. J. Mackay, Callway JL, Marks SM, White HE, 2008; Sanchez-Delgado *et al.*, 2016).

Determining the prevalence of MLID across imprinting disorders is challenging. Firstly, it is almost always mosaic, which complicates clinical diagnosis; secondly, its heterogeneous phenotype means that patients may not meet the clinical criteria for molecular testing, thus escaping diagnosis; thirdly, comprehensive epigenomic analysis is not routinely performed in individuals with molecular imprinting disturbance, so MLID may be missed. It is therefore probable that cases of MLID, whether or not associated with classical phenotypes, are going undetected. There is currently no standardized protocol for MLID diagnosis, nor has the

impact of a positive diagnosis on clinical management been formally defined (Eggermann *et al.*, 2014).

There is some evidence that individuals with MLID may exhibit a more diverse phenotype than cases with isolated imprinting errors (Poole *et al.*, 2013). Testing a more extensive number of imprinted loci therefore may blur the boundaries between individual imprinting disorders which may have overlapping phenotypes. The concept of a network of imprinted genes that interact with each other further undermines any notion that molecular or clinical findings can be neatly compartmentalised (Arima *et al.*, 2005).

Here we report on a patient with imprinting disturbances at loci associated with two distinct classical imprinting disorders: one at chromosome 11p15 in the imprinted region associated with Beckwith-Wiedemann syndrome (BWS: OMIM #130650), and one at chromosome 14q32, in the imprinted region associated with Temple syndrome (TS: OMIM #616222).

BWS is clinically characterized by macrosomia, macroglossia, visceromegaly, hyperinsulinism, exomphalos, and elevated risk of paediatric tumours; its incidence is estimated as 1:10,500 live births (Mussa *et al.*, 2013). 50% of affected individuals have *KCNQ1OT1*: TSS-DMR LOM, of whom 30% have MLID (Brioude *et al.*, 2018; Elbracht *et al.*, 2020). Temple syndrome is characterized by low birth weight, hypotonia, feeding difficulties, early puberty and short stature (Gillissen-Kaesbach *et al.*, 2018); it has phenotypic overlap with Silver-Russell syndrome (SRS) but is rarer than SRS, with unknown incidence and no agreed clinical scoring system. Only around 12% of cases of TS are due to imprinting error (Ioannides, Lokulo-Sodipe, Mackay, Davies, & Temple, 2014; Kagami *et al.*, 2017), and MLID has rarely been reported in individuals clinically characterised with TS. While several individuals have been described with MLID involving numerous loci, including *KCNQ1OT1* and *MEG3*, (Caliebe *et al.*, 2014, Begemann *et al.*, 2017, Cubellis *et*

al., 2020) to date only one case has been reported where the only disease-associated loci involved were *KCNQ1OT1* and *MEG3* (Bens et al., 2016), for which no clinical data was provided.

CLINICAL CASE REPORT

The child was the first born to non-consanguineous parents. He was conceived naturally when his mother was 37 years of age, one month after she had undergone a fallopian tube cannulation following several years of infertility. The pregnancy was complicated by cholestasis but growth *in utero* was normal. Following an emergency caesarean section for foetal distress he was born at 39 weeks' gestation, weighing 2.73 kg (-1.54 SDS). Concerns were raised regarding his head size; his OFC was not recorded at birth, but in his first year was consistently >98th centile (Figure 1a).

At 8 months of age, he was referred because of macrocephaly, facial and body asymmetry, and naevus flammeus. His right side appeared smaller than his left, but both sides were functionally normal, so it was unclear which was the abnormal side. In addition to facial asymmetry, he was noted to have a prominent forehead, broad nasal tip, and flat nasal bridge (see Figure 2). No macroglossia, exomphalos, or organomegaly were identified. Hands and feet were of normal size.

There was no formal history of feeding difficulties, though his mother described him as a "fussy eater". Despite this, his weight was disproportionate to his height at 15 months (height SDS -1.39, weight SDS +1.22, OFC SDS +3.78 SDS) and 2 years of age (height SDS -0.89, weight SDS +1.66, OFC SDS +3.71 SDS) (Figure 1b).

He sat at 6 months of age and walked independently at 17 months. Mild speech and language delay was evident at age 2.

There was no family history of obvious significance. The mother's sibling had four children, all of whom were healthy. The index case's father had two healthy children from a previous relationship.

The initial differential included hypochondroplasia, Noonan's syndrome and PTEN related disorders. Plain radiographs of the pelvis and leg were normal, as were parathyroid, calcium and phosphate levels. Head ultrasound scan showed no evidence of hydrocephalus.

In view of the macrocephaly, body asymmetry and naevus flammeus, BWS was also suspected, although his BWS score was <4 (Table 1). He exhibited some features atypical of BWS, such as relatively small stature in comparison to most cases with the syndrome, protruding forehead, and relative macrocephaly in infancy; these features are more consistent with other imprinting disorders such as Silver-Russell syndrome. His pre-and postnatal growth were not consistent with SRS, though he scored 3/6 using the Netchine-Harbison scoring system (see Table 1, and assuming head size at birth on similar centiles to those recorded through the first year of life). His relatively short stature and disproportionately high weight were more suggestive of Temple syndrome. Clinical features were assessed using Face2Gene analysis (<https://www.face2gene.com>): this assessment placed Temple syndrome in the top 4 potential syndromes (Figure 2). As his phenotype did not align clearly with a single imprinting disorder, he was tested for MLID at 2 years of age.

MATERIALS AND METHODS

Editorial Policies and Ethical Considerations

The patient was recruited into the research study “Imprinting disorders – finding out why” (IDFOW: Southampton and South West Hampshire Research Ethics approval 07/H0502/85) through the UK Comprehensive Local Research network (www.southampton.ac.uk/geneticimprinting/informationpatients/imprintingfindingoutwhy.page). Written consent was obtained from the parents of the proband, including consent to access medical records and for the publication of patient images.

Molecular studies

Initial determination of DNA methylation was performed on blood-derived DNA by methylation-specific MLPA (MRC-Holland b.v.) according to the manufacturer’s specification, using kits for: Beckwith-Wiedemann / Silver-Russell syndrome (ME030-C3); *GRB10*, *MEST*, *PLAGL1* and *MEG3* (ME032-A1); Prader-Willi / Angelman syndrome (ME028-C1); Pseudohypoparathyroidism (ME031-B2), and MLID (ME034-C1).

Methylation-specific PCR analysis was performed using previously described primers and protocol (Poole *et al.*, 2013): loci analysed were *DIRAS3* Ex2 DMR (*DIRAS3*, 1p31); *PLAGL1* TSS alt-DMR (*PLAGL1*, 6q24); *IGF2R* Int2 DMR (*IGF2R*, 6q27); *GRB10* alt-TSS DMR (*GRB10*, 7q32); *MEST* alt-TSS DMR (*MEST*, 7q32); H19 TSS DMR (H19, 11p15.5); *IGF2* DMR0 (11p15.5); *KCNQ1OT1* TSS DMR (*KCNQ1OT1* or IC2, chr11); *MEG3* TSS DMR (*DLK1* or *MEG3*, 14q32); *SNRPN* alt-TSS DMR (*SNRPN*, 15q11); *IGF1R* Int2 DMR (*IGF1R*, 15q26); *PEG3* TSS DMR (*PEG3*, 19q32); *GNAS-AS1* TSS DMR (*NESPAS/GNAS*, 20q13); *WRB* alt-TSS DMR (*WRB/GET1*, 21q22); *SNU13* alt-TSS DMR (*SNU13/NHP2L1*, 22q13).

Array CGH reported a karyotype 46:XY with benign CNVs only. *PTEN* testing by gene panel and MLPA analysis, and Noonan syndrome testing by gene panel analysis, reported no pathogenic variants. Results of imprinting analysis are shown in Table 2. MS-MLPA detected partial loss of DNA methylation at the *KCNQ1OT1* TSS DMR and *MEG3* TSS DMR, with no copy number change at either locus. MS-PCR confirmed these results, and additionally detected partial hypomethylation of *DIRAS3* Ex2 DMR, *IGF2R* Int2 DMR and *WRB* alt-TSS DMR (*WRB/GET1*), as well as complete hypomethylation of *SNU13* alt-TSS DMR. These results were consistent with a diagnosis of MLID.

DISCUSSION

Previous studies of cohorts found to have hypomethylation affecting multiple loci suggest that clinical phenotypes of patients with MLID do not map neatly onto a single clinical disorder, and the involvement of additional loci may alter the extent or severity of the phenotype (Poole *et al.*, 2013). There is some evidence that the dominant phenotype may be determined by the locus with the most severe methylation abnormality, or its prevalence in a target organ where mosaicism occurs (Azzi *et al.*, 2014). Epigenotypes conferring clinically opposing effects could theoretically “balance” each other in the resultant phenotype, but this has not been proven, and can only be deduced from epigenotype-phenotype correlations.

This is the first clinical case report of an individual with clinical features of two imprinting disorders, BWS and TS, and MLID involving the loci associated with these disorders:

KCNQ1OT1: TSS-DMR (*ICR2*) and *MEG3*:TSS-DMR. Although LOM at these two loci has been described previously (Caliebe *et al.*, 2013, Begemann *et al.*, 2017, Cubellis *et al.*, 2020), the affected patients had MLID involving loci of numerous imprinting disorders, and therefore the contribution of each locus to the phenotype was unclear. Furthermore, clinical features reported were minimal. In the patient reported here, the only disease-associated loci are *ICR2* and *MEG3* and the role, if any, of the other epimutations remains to be found.

The proband has phenotypic features of both BWS and TS (Figure 2, Table 1). Relative macrocephaly is seen in both TS and BWS, which could explain the patient’s excessive head circumference. His BMI was over the 99th centile at age 2: this may be the consequence of BWS in a person with relatively short stature, or conversely, it could reflect excessive food intake for someone with TS. He had umbilical hernia and naevus flammeus, minor signs of BWS (Table 1), but few of the signs of overgrowth classically seen in BWS (for example no

macroglossia, and a normal birth weight). His facial features were more typical of TS than BWS, but his hands and feet were not as small as typically found in TS.

The idea that different phenotypic features could “offset” each other raises intriguing questions about whether the resultant phenotype might not meet clinical scoring criteria, and thereby elude diagnosis. Furthermore, the involvement of additional epigenetic mutations at loci with no known phenotype may contribute to the overall clinical presentation. As an example, our patient walked at 17 months, arguably on the cusp of mild developmental delay, and had confirmed speech and language delay. While this is insufficient to be considered unambiguous evidence of learning difficulties, it is interesting given that a previous cohort of patients with BWS in combination with MLID were more likely to have developmental delay than those with “isolated” BWS (Poole *et al.*, 2013).

There was no evidence of a *cis*-acting genetic defect underlying any of the epimutations detected. The fact that there had been no prior pregnancies, and several years of infertility, raises the possibility that oocyte quality was compromised, either through maternal mutations or environmental factors. Notably, the epigenetic features of the patient suggested that the imprinting errors occurred post-zygotically: both maternally- and paternally methylated germline DMRs were affected, ruling out a developmental defect specific to either the oocyte or sperm, while the patient’s mosaicism was inconsistent with an imprinting error present in the germline or the one-cell zygote. These features have been described in cases of MLID associated with maternal-effect mutations (eg Begemann 2017, Caliebe 2014, Cubellis 2020)

As the core set of tested loci expands and more data are gathered regarding epigenotype-phenotype correlations, the conventional paradigm of a clinical diagnosis confirmed with a molecular test may no longer be the best way to capture patients whose clinical features are nuanced or conflicting. This case illustrates how a molecular diagnosis of MLID can alter the

clinical outcome and inform the health surveillance required for the patient. Clinical features such as excessive weight gain and small stature appear to counterbalance each other, and the patient will be managed for both BWS and TS. Continued surveillance of individuals with MLID will determine whether the affected loci influence their phenotype over the long term.

In summary, we describe a patient with clinical and epigenetic features of both TS and BWS, who illustrates how the clinical phenotype of MLID may depend on the imprinted loci involved. We contend that patients whose emergent phenotype is not associated with classical phenotypes may escape diagnosis, and suggest that such patients may benefit from molecular testing of multiple loci, agnostic to clinical definitions of classical imprinting disorders.

CONFLICT OF INTEREST

The authors state that no conflict of interest exists.

ACKNOWLEDGEMENTS

We would like to thank the patient and his family for their cooperation with the preparation of this manuscript. We are grateful to Dr Gabriella Gazdag, Wessex Genetics Service, for performing Face2Gene analysis. SG acknowledges the support of the MSc Genomic Medicine Programme at Southampton University. NIHR CRN: Wessex, NIHR Wellcome Trust Southampton Clinical Research Facility support the IDFOW study. IKT is supported in part by the Southampton NIHR Biomedical Research Centre, UK (2017-2022. IS-BRC-1215-20004). This work is supported in part by the Child Growth Foundation– grant 519026101, University of Southampton.

DATA AVAILABILITY

Data available on request from the authors.

REFERENCES

- Arima, T., Kamikihara, T., Hayashida, T., Kato, K., Inoue, T., Shirayoshi, Y., . . . Wake, N. (2005). ZAC, LIT1 (KCNQ1OT1) and p57KIP2 (CDKN1C) are in an imprinted gene network that may play a role in Beckwith-Wiedemann syndrome. *Nucleic Acids Research*, 33(8), 2650-2660. doi:<http://dx.doi.org/10.1093/nar/gki555>
- Azzi, S., Blaise, A., Steunou, V., Harbison, M. D., Salem, J., Brioude, F., . . . Netchine, I. (2014). Complex Tissue-Specific Epigenotypes in Russell-Silver Syndrome Associated with 11p15 ICR1 Hypomethylation. *Human Mutation*, 35(10), 1211-1220. doi:10.1002/humu.22623
- Begemann, M., Rezwan, F. I., Beygo, J., Docherty, L. E., Kolarova, J., Schroeder, C., . . . Mackay, D. J. G. (2018). Maternal variants in NLRP and other maternal effect proteins are associated with multilocus imprinting disturbance in offspring. *Journal of Medical Genetics*. doi:<http://dx.doi.org/10.1136/jmedgenet-2017-105190>
- Bens, S., Kolarova, J., Beygo, J., Buiting, K., Caliebe, A., Eggermann, T., . . . Siebert, R. (2016). Phenotypic spectrum and extent of DNA methylation defects associated with multilocus imprinting disturbances. *Epigenomics*, 8(6), 801-816. doi:<http://dx.doi.org/10.2217/epi-2016-0007>
- Brioude, F., Kalish, J. M., Mussa, A., Foster, A. C., Blik, J., Ferrero, G. B., . . . Maher, E. R. (2018). Beckwith wiedemann syndrome: First international consensus regarding diagnosis and clinical management. *Hormone Research in Paediatrics*, 90(Supplement 1), 427-428. doi:<http://dx.doi.org/10.1159/000492307>
- Caliebe A, Richter J, Ammerpohl O, Kanber D, Beygo J, Bens S., ... Siebert R (2014). A familial disorder of altered DNA-methylation. *J Med Genet*, 51(6):407-12. doi: 10.1136/jmedgenet-2013-102149
- Cubellis M. V., Pignata L., Verma A., Sparago A., Del Prete R., Monticelli M., Calzari L., . . . Riccio A. (2020). Loss-of-function maternal-effect mutations of PADI6 are associated with familial and sporadic Beckwith-Wiedemann syndrome with multi-locus imprinting disturbance. *Clin Epigenetics*, 12(1):139. doi: 10.1186/s13148-020-00925-2
- Eggermann, T., Heilsberg, A.-K., Bens, S., Siebert, R., Beygo, J., Buiting, K., . . . Soellner, L. (2014). Additional molecular findings in 11p15-associated imprinting disorders: an urgent need for multi-locus testing. *Journal of molecular medicine (Berlin, Germany)*, 92(7), 769-777. doi: 10.1007/s00109-014-1141-6
- Elbracht, M., Mackay, D., Begemann, M., Kagan, K. O., & Eggermann, T. (2020). Disturbed genomic imprinting and its relevance for human reproduction: Causes and clinical consequences. *Human Reproduction Update*, 26(2), 197-213. doi:<http://dx.doi.org/10.1093/humupd/dmz045>
- Gillessen-Kaesbach, G., Albrecht, B., Eggermann, T., Elbracht, M., Mitter, D., Morlot, S., . . . Beygo, J. (2018). Molecular and clinical studies in 8 patients with Temple syndrome. *Clinical Genetics*, 93(6), 1179-1188. doi:<http://dx.doi.org/10.1111/cge.13244>

- Ioannides, Y., Lokulo-Sodipe, K., Mackay, D. J. G., 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. *Journal of Medical Genetics*, 51(8), 495-501. doi:<http://dx.doi.org/10.1136/jmedgenet-2014-102396>
- Kagami, M., Matsubara, K., Nakabayashi, K., Nakamura, A., Sano, S., Okamura, K., . . . Ogata, T. (2017). Genome-wide multilocus imprinting disturbance analysis in Temple syndrome and Kagami-Ogata syndrome. *Genetics in Medicine*, 19(4), 476-482. doi:<http://dx.doi.org/10.1038/gim.2016.123>
- Kagami M., Nagasaki K., Kosaki R., Horikawa R., Naiki Y., Saitoh S., . . . Ogata T. (2017). Temple syndrome: comprehensive molecular and clinical findings in 32 Japanese patients. *Genet Med*, 19(12):1356-1366. doi: 10.1038/gim.2017.53
- Mackay, D. J., Callway JL, Marks SM, White HE. (2008). Hypomethylation of multiple imprinted loci in individuals with transient neonatal diabetes is associated with mutations in ZFP57. *Nat Genet*, 40:49-951.
- Mackay, D. J. G., & Temple, I. K. (2017). Human imprinting disorders: Principles, practice, problems and progress. *European Journal of Medical Genetics*, 60(11), 618-626. doi:10.1016/j.ejmg.2017.08.014
- Monk, D., Mackay, D. J. G., Eggermann, T., Maher, E. R., & Riccio, A. (2019). Genomic imprinting disorders: lessons on how genome, epigenome and environment interact. *Nature Reviews Genetics*, 20(4), 235-248. doi:10.1038/s41576-018-0092-0
- Mussa, A., Russo, S., De Crescenzo, A., Chiesa, N., Molinatto, C., Selicorni, A., Richiardi, L., Larizza, L., Silengo, M.C., Riccio, A., Ferrero, G.B., 2013. Prevalence of Beckwith-Wiedemann syndrome in North West of Italy. *Am. J. Med. Genet. A* 161, 2481e2486. doi: 10.1002/ajmg.a.36080
- Poole, R. L., Docherty, L. E., Al Sayegh, A., Caliebe, A., Turner, C., Baple, E., . . . Int Clinical Imprinting, C. (2013). Targeted Methylation Testing of a Patient Cohort Broadens the Epigenetic and Clinical Description of Imprinting Disorders. *American Journal of Medical Genetics Part A*, 161(9), 2174-2182. doi:10.1002/ajmg.a.36049
- Sanchez-Delgado, M., Riccio, A., Eggermann, T., Maher, E. R., Lapunzina, P., Mackay, D., & Monk, D. (2016). Causes and Consequences of Multi-Locus Imprinting Disturbances in Humans. *Trends in Genetics*, 32(7), 444-455. doi:<http://dx.doi.org/10.1016/j.tig.2016.05.001>

Figure legends

Figure 1

A: Head circumference chart at 2 - 11 months. B: Growth chart (height and weight) at 15 months and 24 months.

Figure 2

A, B. Clinical images of the proband aged two years, demonstrating facial asymmetry, protruding forehead and macrocephaly. C: clinical features included in Face2Gene analysis, and suggested syndrome outputs.

Figure 1

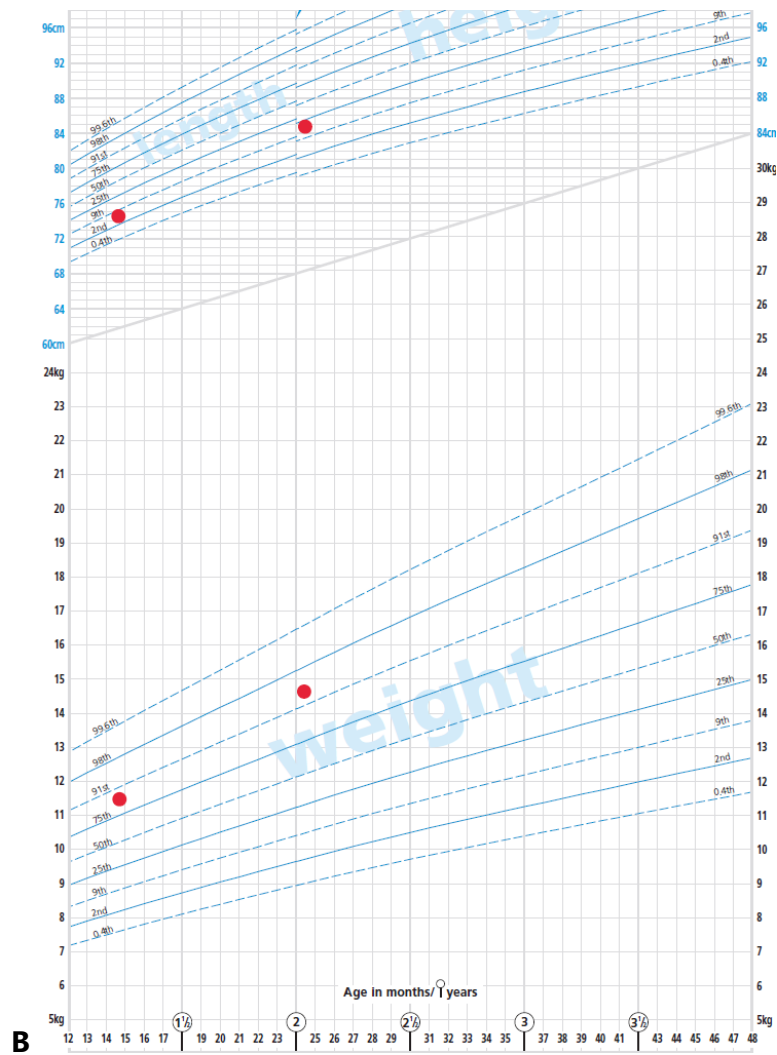
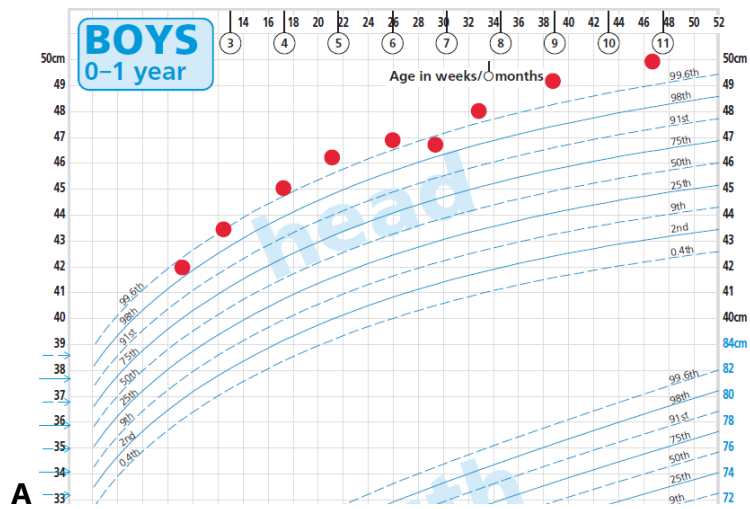




Figure 2C

| Clinical feature input to Face2Gene | Suggested syndrome output |
|---|---|
| Delayed speech and language development Depressed nasal bridge Broad nasal tip Macrocephaly Frontal bossing Prominent forehead | Barth Syndrome Mental Retardation and Autistic features 3-methylglutaconic Aciduria Temple Syndrome Megalencephaly-capillary malformation-polymicrogyria syndrome (MCAP) Atkin-Flaitz Syndrome Noonan Syndrome Clark-Baraitser Syndrome Lubs-type X-linked syndromic intellectual developmental disorder (MRXSL) Craniofacialfrontodigital syndrome Pallister-Killian Syndrome Coffin-Siris Syndrome Silver-Russell Syndrome |