***Short Report***

**Discrepant molecular and clinical diagnoses in**

**Beckwith-Wiedemann and Silver-Russell syndromes.**

D.J.G. Mackay1, J. Bliek2, M.P. Lombardi2, S. Russo3, L. Calzari3, S. Guzzetti 3, C. Izzi4, A . Selicorni5, D. Melis4, I.K. Temple1, E.R. Maher6, F. Brioude7, I. Netchine7, T. Eggermann8

1 Faculty of Medicine, University of Southampton, Southampton SO17 1BJ, UK and Wessex Regional Genetics Laboratory, Salisbury SP2 8BJ, UK

2 Department of Clinical Genetics, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

3 Medical Cytogenetics and Molecular Genetics Laboratory, Centro di Ricerche e Tecnologie Biomediche IRCCS, Istituto Auxologico Italiano, Milan, Italy

4 Prenatal Diagnosis Unit, Department of Obstetrics and Gynecology, ASST Spedali Civili of Brescia, Brescia, Italy

5Pediatric Unit, ASST Lariana Como, Como, Italy

6 Department of Pediatrics, University "Federico II", Napoli, Italy

6 Department of Medical Genetics, University of Cambridge and NIHR Cambridge Biomedical Research Centre and Cancer Research UK Cambridge Centre, Cambridge Biomedical Campus, Cambridge, UK

7 Sorbonne Université, INSERM, UMR 938, Centre de Recherche Saint-Antoine (CRSA), APHP Hôpital Trousseau, 75012 Paris, France

8 Institute of Human Genetics, University Hospital, Technical University of Aachen, Aachen, Germany

Correspondence should be addressed to:

Prof Dr rer. nat. Thomas Eggermann

Institute of Human Genetics

University Hospital, RWTH Aachen

Pauwelsstr. 30

D-52074 Aachen

Phone: +49 241 8088008

Fax: +49 241 8082394

Email: teggermann@ukaachen.de

**Abstract**

Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS) are two imprinting disorders associated with opposite molecular alterations in the 11p15.5 imprinting centers. Their clinical diagnosis is confirmed by molecular testing in 50-70% of patients. The authors from different reference centers for BWS and SRS have identified single patients with unexpected and even contradictory molecular findings in respect to the clinical diagnosis. These patients clinically do not fit the characteristic phenotypes of SRS or BWS, but illustrate their clinical heterogeneity. Thus, comprehensive molecular testing is essential for accurate diagnosis and appropriate management, to avoid premature clinical diagnosis and anxiety for the families.

**Keywords:** Silver-Russell syndrome – Beckwith-Wiedemann syndrome – molecular testing – unexpected results

**Introduction**

BWS and SRS are congenital imprinting disorders, associated with oppositely altered parent of origin-specific expression of two neighbouring clusters of imprinted genes on Chr11p15.5 [1](Fig1). SRS affects ~1:50,000 individuals, with characteristic features including pre- and postnatal growth restriction, relative macrocephaly and prominent forehead, early feeding difficulties, and body asymmetry [2]. BWS, or the recently-described Beckwith-Wiedemann Spectrum (BWSp) affects ~1:10,500 individuals, and its clinical features include macroglossia, anterior abdominal wall defects, prenatal and/or postnatal overgrowth, tumour predisposition and lateralised overgrowth [3]. Due to their clinical heterogeneity, for both syndromes clinical scoring systems are a prerequisite for a more directed diagnostic protocol and clinical management [2, 3].

Over 50% of SRS cases are caused by loss of paternal allele methylation (LOM) of Imprinting Centre 1 (IC1 or H19/IGF2: IG-DMR, whereas gain of maternal allelic methylation at IC1 (GOM) can be identified in 5-10% of BWS cases. However, in BWS 50% of cases show loss of maternal allele methylation of ‘Imprinting Centre 2’ (IC2 or KCNQ1OT1:TSS-DMR). Sequence variants in *CDKN1C* and *IGF2*, as well as copy number variants or mosaic segmental uniparental disomy affecting chromosome 11p15.5, are also associated with BWS and SRS. In 10% of SRS patients, maternal uniparental disomy of chromosome 7 can be detected. Mosaic methylation disturbances of IC1 and IC2 are frequent [2, 3] with strong differences in distribution between different tissues [4], thereby challenging genetic testing and probably leaving several patients without molecular diagnosis. A significant fraction of children with IC1 and/or IC2 LOM have multi-locus imprinting disturbances (MLID), i.e. aberrant imprinting marks at additional imprinted loci (for review: [5]).

To identify the major molecular changes, first-line testing for BWS and SRS is recommended to include DNA methylation analysis of IC1 and IC2 [6]. In fact, the majority of patients exhibit the disease-specific (epi)mutations in 11p15, but single individuals referred with symptoms consistent with BWS or SRS show molecular changes inconsistent with the clinical diagnosis, or even consistent with its molecular ‘mirror’, thus posing challenges for interpretation, diagnostic reporting and genetic counselling.

Here we describe selected cases from different European laboratories where apparent ambiguities have arisen in BWS/SRS diagnosis, to offer a precedent for interpretation and reporting. By considering the clinical data and the reason for referral and the molecular findings, we suggest to categorise these cases into three groups. Examples for each category are presented in Table 1.

**A. Clinical referral of BWS or isolated asymmetry; molecular diagnosis of IC1 LOM.**

In three cases (patients 1, 2, 6), the initial clinical suspicion of BWS based on some key features according to the recent consensus guidelines[3] had to be revised after molecular diagnosis of a IC1 LOM. As this finding is the characteristic epimutation for SRS, two of the patients (patients 1, 2) were clinically reevaluated, but did not fulfil the clinical Netchine-Harbison Score (NHS) for SRS (1 out of 6 items each; [2]. Interestingly, two of the patients showed more or less normal growth. In the majority of patients with IC1 LOM, asymmetry was the major symptom provoking molecular testing (for example, cases 8-16).

Asymmetry is one of the key features of both BWS and SRS, but it can be difficult to clinically distinguish hemihypertrophy from hemihypotroply / hemiatrophy, particularly if other clinical features are lacking. Isolated lateralised overgrowth (ILO) in the presence of an 11p15 molecular anomaly is within the BWSp. ILO is sufficient to prompt BWS testing [3], and some European diagnostic laboratories have historically logged all cases of ILO for BWS first-line testing by 11p15 DNA methylation analysis. According to current consensus guidelines, isolated asymmetry is insufficient to warrant SRS testing [2]. Thus, in cases referred solely for asymmetry, identification of a molecular defect normally associated with a clinical diagnosis of SRS may be unexpected, but it is not discrepant.

**B. Clinical features of SRS (with or without asymmetry); molecular diagnosis consistent with BWS.**

Some individuals with growth restriction, with or without additional SRS features, were referred for SRS diagnosis but received molecular diagnosis consistent with BWS – in the majority, IC2 LOM. Molecular SRS testing is commonly requested as an exclusion diagnosis for growth-restricted children, and in these cases, parallel testing of IC1 and IC2 occasionally diagnoses IC2 LOM. Our data confirm that IC2 LOM in BWS is not strongly associated with overgrowth [3], but that in some cases it is associated with growth restriction (FR, UK unpublished data), which when associated with body asymmetry can prompt initial clinical diagnosis of SRS. Growth restriction associated with IC2 LOM may expand the clinical spectrum of BWSp.

**C. Clinical referral for diagnosis of SRS or BWS; molecular diagnosis of MLID.**

Of eight postnatal referrals with MLID, six had clinical diagnoses of SRS and two of BWS, which may reflect: (a) ascertainment bias for referrals meeting specific clinical criteria; (b) the relative likelihood of imprinting disturbance restricting rather than enhancing growth; (c) mosaic LOM in different tissues, with the critical imprinting disturbance eluding detection in the tissue analysed [4]. Two cases were ascertained prenatally. One case (Patient 34) was referred for 11p15.5 methylation testing after detection of omphalocele and vacuolated placenta, with normal growth parameters. MS-MLPA analysis revealed LOM of IC1, IC2 and the GNAS/GNAS-AS locus. Another case (Patient 35) was ascertained with omphalocele, shortened humeri and mesenchymal placenta, and showed LOM of IC2, *GRB10* and *MEST* loci [7]. To our knowledge these are the first reported prenatal diagnoses of MLID.

MLID is detectable in ~25% of BWS and 10% of SRS cases with IC2 or IC1 LOM, respectively, being mosaic by nature, may elude detection in diagnostic samples. Because MLID may result from underlying genetic changes, and may alter genetic counselling and perinatal as well as clinical management [7], it should be considered in individuals with discrepant molecular and clinical diagnoses.

**Conclusion**

The compilation of data from patients with unexpected molecular findings confirms the urgent need to apply comprehensive molecular tests targeting different imprinted loci to identify unexpected and/or overlapping molecular changes, and thereby to contribute to the discovery of the causative (epi)mutations in patients with unspecific phenotypes. As these examples show, the application of clinical scoring systems and the clinical evaluation can be a major prerequisite for a more directed diagnostic testing strategy, but some patients might be missed if the decision about molecular testing is strictly based on fulfilment of clinical criteria. We want to emphasize that in patients with inconclusive clinical features the communication of a clinical diagnosis should be delayed until molecular confirmation is available because a premature diagnosis might cause anxiety to the families.

The discrepancy between clinical and molecular features of BWS and SRS is an infrequent occurrence. Though objective numbers are lacking, these cases probably represent ≤1% of diagnostic referrals. Prompt, sensitive and comprehensive molecular testing is essential for accurate diagnosis, appropriate management and genetic counselling, for these as for all imprinting disorders.

**List of abbreviations**

BWS Beckwith-Wiedemann syndrome

GOM Gain Of Methylation

IC1 Imprinting Control region 1

IC2 Imprinting Control region 2

ILO Isolated Lateralised Overgrowth

LOM Loss Of Methylation

MLID Multi Locus Imprinting Disturbance

MS-MLPA Methylation Specific Multiplex Ligation-dependent Probe Amplification

SRS Silver-Russell Syndrome

**Declarations**

**Ethics and consent of participate**

All participants gave a written informed consent to participate in research studies. The study has been approved by the ethical committee of the University Hospital Aachen, Germany (EK-302-16).

**Availability of Data and Material**

All data and samples are stored in the contributing institutions, for availability of specific patients the authors should be contacted.

**Competing interests**

The authors declare that they do not have any competing interests.

**Funding**

The authors were members of the former COST Action BM1208.

**Author´s contributions**

All authors provided samples and data, and were involved in the interpretation of data. DJGM and TE draftet the paper.

**Acknowledgement**

Not applicable

**References**

[1] Soellner L, Begemann M, Mackay DJ, Grønskov K, Tümer Z, Maher ER, et al. Recent Advances in Imprinting Disorders. Clin Genet. 2017;91:3-13.

[2] Wakeling EL, Brioude F, Lokulo-Sodipe O, O'Connell SM, Salem J, Bliek J, et al. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. Nat Rev Endocrinol. 2016; doi: 10.1038/nrendo.2016.138.

[3] Brioude F, Kalish JM, Mussa A, Foster AC, Bliek J, Ferrero GB, et al.. Expert consensus document: Clinical and molecular diagnosis, screening and management of Beckwith-Wiedemann syndrome: an international consensus statement. Nat Rev Endocrinol. 2018. doi: 10.1038/nrendo.2017.166.

[4] Azzi S, Steunou V, Tost J, Rossignol S, Thibaud N, Das Neves C, et al. Exhaustive methylation analysis revealed uneven profiles of methylation at IGF2/IC1/H19 11p15 loci in Russell Silver syndrome. J Med Genet. 2015;52:53-60.

[5] Sanchez-Delgado M, Riccio A, Eggermann T, Maher ER, Lapunzina P, Mackay DJG, Monk D. Causes and consequences of multi-locus imprinting disturbances in humans. Trends Genet. 2016;32:444-455.

[6] Eggermann K, Bliek J, Brioude F, Algar E, Buiting K, Russo S, et al. EMQN best practice guidelines for the molecular genetic testing and reporting of chromosome 11p15 imprinting disorders: Silver-Russell and Beckwith-Wiedemann syndrome. Eur J Hum Genet. 2016;24:1377-87.

[7] Soellner L, Begemann M, Degenhardt F, Geipel A, Eggermann T, Mangold E. Maternal heterozygous NLRP7 variant results in recurrent reproductive failure and imprinting disturbances in the offspring. Eur J Hum Genet. 2017;25:924-929.

[8] Russo S, Calzari L, Mussa A, Mainini E, Cassina M, Di Candia S, et al. A multi-method approach to the molecular diagnosis of overt and borderline 11p15.5 defects underlying Silver-Russell and Beckwith-Wiedemann syndromes. Clin Epigenetics. 2016;8:23.

[9] Murphy R, Mackay D, Mitchell EA. Beckwith Wiedemann imprinting defect found in leucocyte but not buccal DNA in a child born small for gestational age. BMC Med Genet. 2012;13:99.

[10] Turner CL, Mackay DJ, Callaway JL, Docherty LE, Poole RL, Bullman H, et al. Methylation analysis of 79 patients with growth restriction reveals novel patterns of methylation change at imprinted loci. Eur J Hum Genet. 2010;18:648-55.

[11] Tee L, Lim DH, Dias RP, Baudement MO, Slater AA, Kirby G, et al. Epimutation profiling in Beckwith-Wiedemann syndrome: relationship with assisted reproductive technology. Clin Epigenetics. 2013;5:23.

[12] Docherty LE, Rezwan FI, Poole RL, Turner CLS, Kivuva E, Maher ER, et al. Mutations in NLRP5 are associated with reproductive wastage and multi-locus imprinting disorders in humans. Nat Commun. 2015;6:8086.

[13] Begemann M, Spengler S, Kanber D, Haake A, Baudis M, Leisten I, et al. Silver-Russell patients showing a broad range of IC1 and IC2 hypomethylation in different tissues. Clin Genet. 2011;80:83-8.

[14] Begemann M, Rezwan FI, Beygo J, Docherty LE, Kolarova J, Schroeder C et al. Maternal variants in NLRP and other maternal effect proteins are associated with multilocus imprinting disturbance in offspring. J Med Genet. 2018 Mar 24. pii: jmedgenet-2017-105190. doi: 10.1136/jmedgenet-2017-105190.

**Tables and Figures**

**Figure 1: Schematic of 11p15 region indicating common imprinting disturbances (DNA methylation imbalances) associated with BWS and SRS.**

Filled lollipops: methylated Imprinting Control Region (IC); empty lollipops: unmethylated IC; hairpins:microRNA; Filled oblongs: coding genes; outline oblongs: noncoding RNA; red denotes genes expressed from maternal allele; blue denotes genes expressed from paternal allele; grey denotes genes not expressed from the allele shown

**Table 1:** Cases with reported discrepancy between clinical referral and molecular diagnosis of BWS or SRS. (1 in the majority of cases, MS-MLPA based kits were used for diagnostic purposes. 2 clinical details given at the referral of DNA samples for molecular testing. Not given: no additional clinical information provided at referral. Abbreviations: BW: birth weight; ICSI: intra-cytoplasmic sperm injection; IUGR: intrauterine growth restriction; LOM: loss of methylation; Nk: not known (clinical data not reported or molecular analysis not performed); PNGR: postnatal growth restriction; MLID: multi-locus imprinting disturbance; OFC: occipitofrontal circumference; AFP: alpha-foetoprotein; WG: weeks of gestation)