The European Molecular Genetics Quality Network

EMQN Pilot External Quality Assessment Scheme for Beckwith-Wiedemann syndrome (SRS) (2015)

SCHEME REPORT

Authorisation/Approval

EMQN is a UKAS accredited provider of EQA services – this scheme is NOT covered under the scope of its accreditation. This document has been authorised / approved on behalf of EMQN by:

Simon Patton (EMQN Director) on 02 June 2015

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02 June 2015

Dear Colleague,

Thank you for participating in the EMQN pilot EQA scheme for **Beckwith-Wiedemann Syndrome** testing. This document is the final report summarising the results of the scheme- your Individual Laboratory Report (ILR) is available from your website account. The key findings raised by the scheme this year are shown in the table below. For more detailed information about the scheme, please see the remainder of the document.

KEY FINDINGS FROM SCHEME

CATEGORY	COMMENTS
Genotyping	1. **Case 1 (mosaic UPD11pat):** Five laboratories returned genotyping errors. Three laboratories correctly detected the hypomethylation of ICR2, but not the hypermethylation of ICR1; as a result, isolated ICR2 hypomethylation was reported rather than UPD11. One laboratory identified hypermethylation of ICR1 but not hypomethylation of ICR2, giving the same incomplete diagnosis. One laboratory correctly identified the hypomethylation of ICR2 and hypermethylation of ICR1, but attributed it to a duplication of 11p15, where in fact no copy number change was present. 2. **Case 2 (ICR2 hypomethylation):** Four laboratories gave genotyping errors. One laboratory reported hypermethylation of ICR1 additionally to hypomethylation of ICR2. One laboratory reported hypermethylation of CDKN1C, which is not a described cause of BWS. One lab reported a methylation abnormality of ICR2 without stating what nature of abnormality, another did not clearly indicate whether or not copy number data were analysed in its MLPA analysis. 3. **Case 3 (ICR1 hypermethylation with underlying deletion of ICR1):** Five laboratories returned genotyping errors. Two laboratories identified the ICR1 deletion but not ICR1 hypermethylation, whereas three identified the hypermethylation but not the deletion. One laboratory reported that its genotyping was performed using an MLPA kit for chromosome 17p, which made its genotyping confused and uninterpretable.
Interpretation	**This was a pilot EQA scheme and therefore interpretation was assessed, but not assigned a mark.** 4. **Case 1:** Interpretation was particularly compromised for those laboratories that did not correctly detect UPD11pat, or did not detect ICR1 hypermethylation at all. ICR1 hypermethylation, whatever its underlying cause, is associated with an increased risk of childhood cancer, and therefore incomplete genotyping puts patients at risk of missing screening. It is a cause of concern that 16 laboratories did not counsel that UPD11pat is associated with an increased risk of some childhood cancers including Wilms' tumour. Nine laboratories did not state that the recurrence risk in other offspring was low. 5. **Case 2:** Twenty-four laboratories did not counsel that ICR2 hypomethylation is associated with increased risk of some childhood cancers. Twelve did not state that ICR2 hypomethylation has low recurrence risk. One requested further testing for UPD11pat, despite this being unnecessary in the case of ICR2 hypomethylation. 6. **Case 3:** Three laboratories returned misleading interpretative comments (including suggestion of autosomal dominant transmission of BWS, and that paternal transmission of the deletion would lead to SRS). Nine laboratories did not calculate recurrence risk in future offspring due to transmission of the deletion, which is concerning since all but three labs detected the deletion; likewise, six laboratories did not request parental samples to determine whether the deletion was de novo. Nineteen laboratories did not counsel the increased risk of childhood cancer (and thus recommend tumour surveillance).
Nomenclature	7. It is EMQN policy to use HGVS mutation nomenclature – this includes use of RefSeq's.
Reporting	8. In general, we only had very few deductions in this field. However, we do ask that laboratories try to be concise in with reports and highlight the important message; write reports that clinicians will also understand, i.e. give clear information and omit redundancies. When giving references from the literature, make sure that they are up-to-date and precise. 9. One laboratory prepared remarkably long and impenetrable reports which might not be helpful to a clinician..
Clerical Accuracy and Patient Identifiers	10. The majority of laboratories did not number pages (as in, Page M of N); this is inadvisable where lost pages may lead to lost interpretation. 11. Several laboratories did not state patient genders; one stated a wrong gender, one spelt a patient's name two different ways in different parts of the report, and another omitted a patient's date of birth. The patient´s name and page numbers should be given on every page of a report (e.g. page 1 / 2 of

	report….). This is according to ISO 15 189. Reports should be restricted to one page whenever possible – and it usually is possible!

STRUCTURE OF THE SCHEME

Three cases were chosen to represent the range of molecular anomalies and interpretative situations often encountered in BWS diagnosis. DNA samples were prepared from lymphoblastoid cell lines and lyophilised. The genotypes were validated independently by two laboratories on the same batch of DNA. We took particular care that the DNA from cultured lymphoblastoid cells faithfully recapitualted the DNA methylation of the primary cells. Diagnostic requests appropriate for the clinical scenario were dispatched with the samples.

CASES AND EXPECTED GENOTYPES

CASE	PATIENT NAME	DATE OF BIRTH (dob)	REASON FOR REFERRAL	CONFIRMED RESULT
1	Sophia VILLERS	29/07/2014	Sophia Villers is referred from neonatology with hemiphypertrophy of the right leg, bilateral ear creases, marked and increasing macroglossia, and hypoglycaemia.	Dosage analysis: normal Methylation analysis 11p: hypermethylation of ICR1, hypomethylation of ICR2 (microsatellite analysis: over-representation of paternal alleles) Diagnosis:BWS due to UPD11pat
2	Viktor MALACKY	28/08/2014	Viktor Malacky is referred neonatally with a clinical suspicion of BWS based on the following signs: macrosomia, exomphalos, hypotonia and bilateral ear creases.	Dosage analysis: normal Methylation analysis: hypomethylation of ICR2, Diagnosis:BWS due to ICR2 hypomethylation
3	Karl BONNER	04/07/2014	Please test for BWS in Karl Bonner, who was born at 38 weeks gestation with a birthweight of 4.95kg, macroglossia and naevus flammeus, and rapidly developed hyperinsulinaemic hypoglycaemia.	Dosage analysis: Microdeletion of ICR1 on 11p (maternal origin) Methylation analysis 11p: hypermethylation of ICR1 Diagnosis:BWS due to ICR1 deletion of mat origin and consequent hypermethylation of ICR1

NB: there is currently no standardised nomenclature for the loci affected by methylation disturbance in BWS, nor for the nature and degree of methylation disturbance. Until such standardisation exists, a variety of synonyms remain acceptable, such as: ICR1 = H19; ICR2 = LIT1 = KCNQ1OT1 = KvDMR; hypermethylation = gain of methylation; hypomethylation = loss of methylation.

ASSESSMENT

The team involved in helping to organise and assess the scheme results were as follows:

COLLEAGUE	COUNTY	ROLE
Deborah Mackay	United Kingdom	Scheme Organiser
Irene Netchine	France	Assessor
Karen Groenskov	Denmark	Assessor
Thomas Eggermann	Germany	Assessor

The assessment consisted of three categories: genotyping accuracy, correct biological and clinical interpretation of results and patient identifiers/clerical accuracy. For assessment, in addition to the genotype, we expected a biological interpretation of the observed nucleotide change and we further review the clinical interpretation given. Clinical interpretation refers to the immediate consequence of the observed mutation for the patient and her family. The reporting format and style were reviewed and not marked. The full score for genotyping and patient identifiers/clerical accuracy categories was 2.00 marks. **This was a pilot EQA scheme and therefore interpretation was assessed, but not assigned a mark.**

CASE	CATEGORY	CRITERIA	MARKS
1	**Genotyping**	**Correct genotype**	2.00
		Deductions: Critical genotyping error	-2.00
		Incomplete genotype	-1.00
		Genotype mis-positioned or mis-called (e.g. incorrect base/amino acid detected)	-0.50
		Error in HGVS nomenclature which could be mis-interpreted	-0.50
		Not correctly using HGVS nomenclature (for either nucelotide or protein)	-0.50
		RefSeq missing / incorrect / inconsistent	-0.50
		RefSeq version number missing / incorrect / inconsistent	-0.25
	Biological and clinical Interpretations	Missing: this result confirms a clincial diagnosis of BWS If testing includes microsatellite analysis Missing: the molecular cause of BWS is UPD11pat (segmental / mosaic)	Comment only
		If testing does not include microsatellite analysis Missing: the DNA methylation pattern, in the absence of a duplication of 11p, is consistent with UPD11pat	

		Comments: UPD11pat is associated with increased risk of some childhood cancers including Wilms' tumour	
		Missing: risk of recurrence is low	
		Missing: details of testing methodology	
		Missing: recommend referral of patient and family to clinical genetics	
	Patient identifiers and clerical accuracy	See standard deductions below.	2.00

2	**Genotyping**	**Correct genotype**	2.00
		Deductions: Critical genotyping error	-2.00
		Incomplete genotype	-1.00
		Genotype mis-positioned or mis-called (e.g. incorrect base/amino acid detected)	-0.50
		Error in HGVS nomenclature which could be mis-interpreted	-0.50
		Not correctly using HGVS nomenclature (for either nucelotide or protein)	-0.50
		RefSeq missing / incorrect / inconsistent	-0.50
		RefSeq version number missing / incorrect / inconsistent	-0.25
	Biological and clinical Interpretations	Missing: this result confirms a clincial diagnosis of BWS	Comment only
		Missing: the molecular cause of BWS is hypomethylation of ICR2 paternal allele	
		Missing: risk of recurrence is low	
		Missing: include details of testing methodology	
		Missing: hypomethylation of ICR2 is associated with increased risk of some childhood cancers including hepatoblastoma. No case of nephroblastoma has been reported.	
		Missing: recommend referral of patient and family to clinical genetics	
		Comments: risk of multi-locus imprinting disturbance may be mentioned and further testing suggested	
	Patient identifiers and clerical accuracy	See standard deductions below.	2.00

3	**Genotyping**	**Correct genotype**	2.00
		Deductions: Critical genotyping error	-2.00
		Incomplete genotype	-1.00
		Genotype mis-positioned or mis-called (e.g. incorrect base/amino acid detected)	-0.50
		Error in HGVS nomenclature which could be mis-interpreted	-0.50
		Not correctly using HGVS nomenclature (for either nucelotide or protein)	-0.50
		RefSeq missing / incorrect / inconsistent	-0.50
		RefSeq version number missing / incorrect / inconsistent	-0.25
	Biological and clinical Interpretations	Missing: this result confirms a clincial diagnosis of BWS	Comment only
		Missing: the molecular cause of BWS is hypermethylation maternally-derived allele of ICR1	
		If gene dosage analysis (MS-MLPA or long-range PCR of ICR1) is performed Missing: the molecular cause of BWS is a deletion on the maternally-derived allele of ICR1	
		Missing: request for parental (maternal) blood to determine whether the deletion is de novo	
		If gene dosage analysis (MS-MLPA or long-range PCR of ICR1) is not performed Missing: the methylation pattern observed is consistent with a duplication on the maternally-derived allele.	
		If gene dosage analysis (MS-MLPA or long-range PCR of ICR1) is not performed Missing: copy number analysis in the proband and parents (mother) is recommended to confirm presence of a duplication and whether it is inherited or de novo	
		Missing: Hypermethylation of ICR1 is associated with increased risk of some childhood cancers including Wilms' tumour	
		Missing: recommend referral of patient and family to clinical genetics	
		Comments: include details of testing methodology	
		Missing: calculation of recurrence risk.	
	Patient identifiers and clerical accuracy	See standard deductions below.	2.00

STANDARD DEDUCTIONS		
	Standard deductions for patient identifiers and clerical accuracy	*Points were deducted for:* • Incorrect DOB (any error) (-1.00) • Spelling errors in patient name (-0.50) • Incorrect or missing patient gender (-0.50)

		Minor points (not leading to deduction of mark) • Date of referral / arrival noted • Missing - title of the report • Missing - identity of the laboratory performing the analysis and issuing the report • Missing - full date of the report • Missing - page numbers indicating the total number of pages (essential when multiple pages are used) • Missing - name and address of the physician referring the patient • Signature of the report by two authorised persons • Laboratory reference • Reason for referral restated • Clear and concise report

PARTICIPATION

This year, 42 laboratories from 19 countries registered for the scheme; all returned reports. The participating countries are shown in the Appendix (figure 1).

RESULTS

Overall quality was satisfactory and we had the pleasure to see some excellent reports. Most often, the genotypes were correctly found, reported and interpreted in their clinical context. No marks were deducted for interpretation.

METHODOLOGY

Thirty two (75%) laboratories used methylation-specific MLPA. Other methods reported included MLPA (not reported to be methylation-specific), high-resolution melting, methylation-specific PCR and allele-specific methylated multiplex real-time quantitative PCR (see Figure 2). There was some concern that DNA quality led to difficulties in this pilot scheme. Five laboratories did not submit reports due to difficulties with genotyping. Three further laboratories did not submit reports for case 1, and one for case 3. Therefore we conclude that no sample presented insuperable difficulties to all laboratories. However, DNA preparation will be re-addressed in the coming year and the pilot repeated, to attempt to secure higher success rates with the samples.

APPEALS PROCEDURE

Performance criteria do not apply and there is no appeals procedure against the marking as this is a pilot EQA scheme. Please remember that the primary aim of this EQA is to be educational, not punitive, and that we are trying to assist laboratories in their continuous efforts towards a higher quality of service.

CONFIDENTIALITY

The fact that your laboratory participates in EMQN schemes is not confidential. However, the raw data and performance scores are. Your laboratory information is confidential between you and the EMQN office (and in exceptional circumstances the Scheme Organiser and Management Board). Only your laboratory's allocated unique EMQN reference number will identify its scores if published within this summary report.

FINAL COMMENTS

Finally, the assessors wish to cordially thank the participants for their hard work, prompt returns and their co-operation during this exercise. We have seen a quite high technical standard of mutation analysis and we have had the pleasure to review some excellent and many good reports. We hope that labs will take on board any comments made by the assessors to help improve the scores in future schemes. Regular participation is associated with improved interpretation performances. We therefore encourage all labs to participate every year and we look forward to your participation in the 2016 scheme that will again be announced by the EMQN office in Manchester. Registration will be through the EMQN web site as before.

With our best wishes,
Yours

Deborah Mackay, Karen Gronskov, Irene Netchine and Thomas Eggermann,

APPENDIX

Figure 1: *Scheme participation*

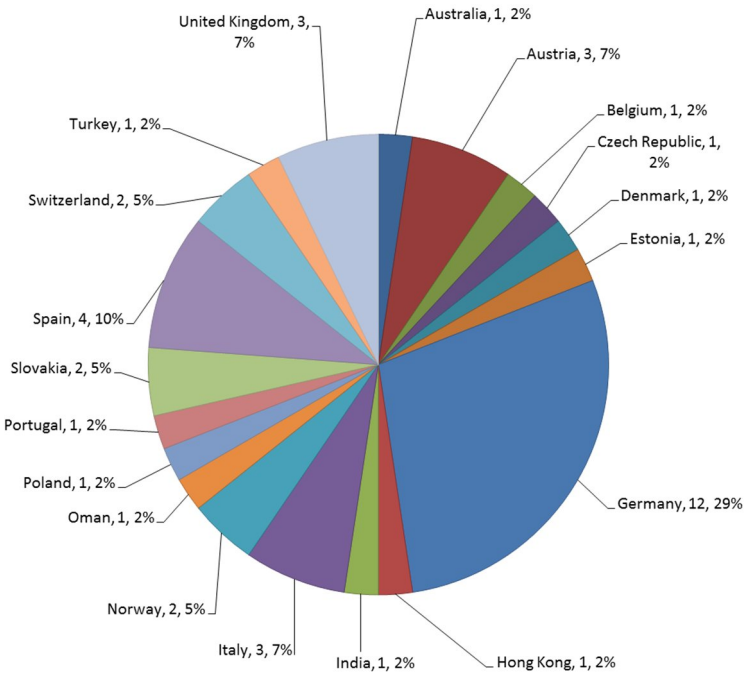
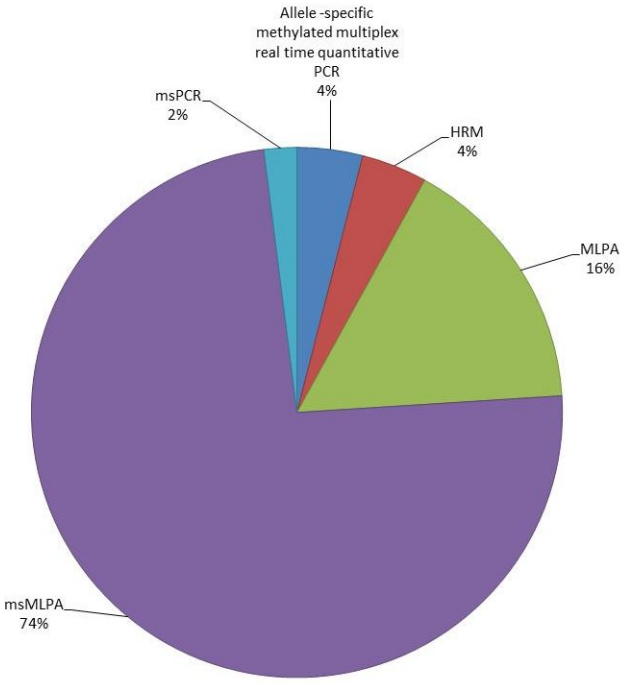


Figure 2: *Methods used* [1]



[1] This figure represents a summary of all the different methods used in the scheme. It DOES NOT depict the combination of methods used by different labs in their testing approach for this disease indication.

Table 1: *Mean genotyping, interpretation and patient identifiers / clerical accuracy results*

	AVERAGE SCORES PER CASE		
	Genotyping	**Interpretation**	**Patient identifiers / Clerical accuracy**
Case 1	1.54	Not marked	1.97
Case 2	1.94	Not marked	1.97
Case 3	1.59	Not marked	1.97
Mean	**1.69**	**Not marked**	**1.97**

Table 2: *Genotyping error rates*

	GENOTYPING ERRORS PER CASE		
	No. of cases completed	**No. of errors**	**Error rate (%)**
Case 1	42	5	11.9
Case 2	42	4	9.5
Case 3	42	5	11.9
Total	**126**	**14**	**11.1**

References:

- *None*