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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: Beckwith–Wiedemann Syndrome

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Beckwith–Wiedemann syndrome; BWS (exomphalos–macroglossia–gigantism syndrome/EMG syndrome; Wiedemann–Beckwith syndrome/WBS).

1.2 OMIM# of the disease

130650.

1.3 Name of the analysed genes or DNA/chromosome segments

11p15.5:
Imprinting Control Region 1 (ICR1/IC1: i.e. H19, IGF2).
Imprinting Control Region 2 (ICR2/IC2/KvDMR1: i.e. CDKN1C, KCNQ1, KCNQ1OT1/LIT1).

1.4 OMIM# of the gene(s)

ICR1: H19, 103280; IGF2, 147470; ICR2: CDKN1C, 600856; KCNQ1OT1/LIT1, 604115; KCNQ1, 607542.

1.5 Mutational spectrum

#11p15.5 (based on data from the literature (for review [1,2]) and diagnostic cohorts from the authors):

ICR1 hypermethylation	5–10%
ICR2 hypomethylation	50–60%
UPD(11p15)pat	20–25%
CDKN1C point mutations[3,4]	1.3–5% in sporadic cases, 20–40% in familial cases
Duplication of paternal chromosome 11p15.5	1–2%
ICR1 microdeletions (for review[5])	<1%
ICR2 microdeletions[6]	<1%
Multilocus methylation defect in cases with ICR2 hypomethylation[7,8]	up to 25%
Mosaic genome-wide paternal uniparental disomy[9,10]	up to 1.4%

1.6 Analytical methods

(MS: methylation-specific)

MS-MLPA; MS-Southern-blot; MS-PCR; MS-single nucleotide primer extension (SNuPE); allele-specific methylation multiplex (ASMM) – RT-QPCR; MS high-resolution melting (HRM)[11]; MS-pyrosequencing; short tandem repeat marker typing; molecular karyotyping (SNP array, aCGH); qPCR; conventional cytogenetics; FISH with region-specific probes.

1.7 Analytical validation

Parallel analysis of negative and positive controls. Determination of methylation and copy number reference ranges in unaffected individuals.

1.8 Estimated frequency of the disease

(Incidence at birth (birth prevalence) or population prevalence. If known to be variable between ethnic groups, please report):

1:13.700–17.000

1.9 Diagnostic setting

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comment:
Prenatal testing may be requested occasionally in cases of familial chromosomal rearrangements affecting chromosomes 11p15 or in case of familial CDKN1C point mutations. Prenatal diagnostic testing for BWS may be requested after detection of exomphalos, mesenchymal dysplasia or macroglossia and macrosomia by ultrasonography. Prenatal testing for genomic disturbances (duplications and point mutations) can be offered without limitations, but the prenatal

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application of methylation-specific tests is questionable due to the insufficient knowledge about the prenatal setting of the ICR1 and ICR2 imprinting marks and the mosaic pattern of these anomalies. More work in this area is needed.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives	C: False negative
			B: False positives	D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A + C)
			Specificity:	D/(D + B)
Negative	C	D	Positive predictive value:	A/(A + B)
			Negative predictive value:	D/(C + D)

2.1 Analytical sensitivity
(proportion of positive tests if the genotype is present)

11p15.5	ICR1 hypermethylation	>90%*
	ICR2 hypomethylation	>90%*
	UPD(11p15)pat	>90%*
	CDKN1C point mutations (mostly maternal)	>95%°
	Aberrations/microdeletions in chromosome 11p15.5	nearly 100%
	Mosaic genome-wide paternal uniparental disomy	>90%*

*due to their mosaic distribution some molecular defects might escape detection. The detection rate is significantly influenced by the tissue tested and by the sensitivity of the diagnostic method, but the relative analytical sensitivity of different methods has not yet been formally compared.
°~100% in the case of *de novo* point mutations, but low-level mosaicism might escape detection by the standard sequencing techniques.

2.2 Analytical specificity
(proportion of negative tests if the genotype is not present)

Nearly 100%.

2.3 Clinical sensitivity
(proportion of positive tests if the disease is present)

The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if quantification can only be made case by case.

In 70–80% of cases, there is evidence that clinical BWS can be present without currently known molecular alteration or that mutations are detectable in patients not meeting the threshold diagnostic criteria for BWS.

The sensitivity might be enhanced if tested in different tissues (eg., fibroblasts or mouth wash DNA in addition to DNA from leucocytes).

2.4 Clinical specificity
(proportion of negative tests if the disease is not present)

The clinical specificity depends on variable factors such as age or family history. In such cases, a general statement should be given, even if a quantification can only be made case by case.

Nearly 100%.

2.5 Positive clinical predictive value
(life-time risk of developing the disease if the test is positive)

Up to 100%, but depends on the type of the alteration and on the sex of the transmitting parent. Furthermore, the phenotype can be very mild in positive cases. In the case of CDKN1C point mutations, clinically unaffected mothers have been reported (probably because these mothers inherited the mutation from their father[12,13]), but functional proof of pathogenicity for some variants is lacking. In the case of monozygotic twins, clinical discordance is common with only one twin being affected by BWS. However, both frequently display ICR2 hypomethylation of leukocyte DNA, because they share foetal circulation. In such circumstances, other accessible tissues (skin fibroblasts and buccal cells) should be tested to demonstrate a normal ICR2 11p15 methylation in the non-affected twin.

2.6 Negative clinical predictive value
(Probability of not developing the disease if the test is negative)

Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:
100% (caveat: for epimutations, heterogeneity cannot be excluded because the causative genetic factors are unknown)

Index case in that family had not been tested:
1% (caveat: difficult to estimate)

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected

As BWS is clinically heterogeneous and the clinical diagnosis is sometimes difficult, the identification of a molecular aberration in 11p15 excludes other (genetic) overgrowth syndromes with different recurrence risks and different prognoses. A phenotypic overlap with other overgrowth syndromes exists mainly for Sotos, Simpson–Golabi–Behmel, Weaver and Perlman syndromes.

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☐ (continue with 3.1.4)	
Yes	☒	
	Clinically	☒
	Imaging	☐
	Endoscopy	☐
	Biochemistry	☐
	Electrophysiology	☐
	Other (please describe):	

A clinical examination may suggest the diagnosis even if genetic testing cannot confirm it.

3.1.2 Describe the burden of alternative diagnostic methods to the patient

The clinical diagnosis is not associated with additional invasive procedures for the patient.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

Owing to the clinical heterogeneity, the clinical diagnosis can sometimes be doubtful. The molecular prove of a 11p15 epimutation/mutation precludes further investigations for overgrowth.

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☐	
Yes	☒	
	Therapy (please describe)	*Certainty of diagnosis aids medical management. Indeed, the treatment of hypoglycaemia, abdominal wall repair, endotracheal intubation, tongue reduction surgery, and further surgical procedures should be based on symptoms rather than on the genetic diagnosis.*
	Prognosis (please describe)	*Relatively good, but a significantly increased risk for neoplasia has to be considered*
	Management (please describe)	*Neoplasias should be treated according to standard paediatric oncology protocols. However, the need for surveillance in some molecular subtypes is controversial and some national guidelines do not advocate screening in all situations, whereas others currently continue with screening as a precaution. A consensus on tumour surveillance is currently in discussion, and national guidelines need harmonisation.*

Suggested neoplasia screening for BWS patients age 0–4 years is abdominal ultrasound (US) every 3 months, which should detect nephroblastomas, hepatoblastomas as well as rare neuroblastomas, adrenocortical carcinomas and rhabdomyosarcomas. For patients aged 4–8 years, a renal US every 3 months is recommended as a sufficient imaging method for detecting Wilms Tumour (WT).

However, the risk of WT varies according to the molecular subtype[2,14,15] and some centres adjust screening protocols to reflect this[16], for example, in the case of ICR1 hypermethylation, dup(11p)pat and UPD(11p15)pat, screening may be more intensive; AFP screening is advised and scanning may continue until adolescence. Current evidence from cohort studies suggests that ICR2 hypomethylation and CDKN1C mutations are not associated with increased risk for WT, though increased risk for hepatoblastoma cannot be discounted. In some centres, patients with these molecular findings do not have US every 3 months, but have clinical examination every 3 months and targeted imaging if clinical symptoms appear, whereas in other centres, AFP levels are measured in these patients every 6–12 weeks until the age of 4 years (95% of the reported BWS-associated hepatoblastomas developed by this age). In some centres there is no surveillance.

Caveat: AFP values tend to be increased in BWS patients both with and without hepatoblastoma, and many centres think the test is not a good screening test[17,18]. Note that, if molecular screening is negative in a patient with the clinical diagnosis of BWS with hemihypertrophy, the patient should still be screened as if affected and considered at risk for neoplasia especially if a nephromegaly is detected by US.

In case of a genome-wide paternal uniparental disomy, longer tumour surveillance might be necessary.

3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history
(To be answered if in 1.9 'B' was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?
If the test result is positive (please describe)

If the test result is negative (please describe)

BWS can be difficult to diagnose after early childhood when growth can be normal. Mosaicism can mean that external clinical features are minimal. If the test is positive, it is important to consider the relatively high risk for neoplasia in the case of UPD(11p15)pat or ICR1 hypermethylation. The risk of developing cancer in adulthood is unknown.

It has been shown that assisted reproductive technology (ART) enhances the risk for BWS recurrence.[19,20]

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?
Such an individual may continue to have unnecessarily high anxiety about the risk of neoplasia or not know of the risk. Anxiety may also include concern about transmission to the next generation. Genetic testing might provide further clarity.

3.3 Genetic risk assessment in family members of a diseased person
The majority of molecular alterations in BWS occurs sporadically (aberrant ICR1 and ICR2 methylation, UPD(11p15)pat). However, single families have been reported with ICR1 and ICR2 microdeletions, chromosomal rearrangements of 11p15 and CDKN1C point mutations, wherein a risk of up to 50% can be estimated. Multilocus methylation defects have been identified in a family with two sibs[21], and were published to be associated with mutations within the NALP2. Thus, it is difficult to provide an accurate risk assessment due to the unknown nature of some of the underlying genetic and environmental factors and the risk of having a second affected child cannot be ruled out.

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?
Yes (caveat: predisposing genetic and environmental factors as the cause for some epimutations are the subjects of ongoing research).

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
Yes.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Yes.

3.4 Prenatal diagnosis
To be answered if in 1.9 'D' was marked.

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?
In the case of genomic alterations, that is, ICR1 and ICR2 microdeletions, chromosomal aberrations and CDKN1C mutations, prenatal testing can be considered.

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe).

The identification of a mutation or epimutation allows a more precise delineation of a recurrence risk for the patient and his or her family as well as providing important information concerning tumour-risk stratification.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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