Isolated- and Beckwith-Wiedemann syndrome related- lateralised overgrowth (hemihypertrophy): clinical and molecular correlations in 94 individuals

Isolated lateralised overgrowth

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Abstract

Background: The congenital imprinting disorder, Beckwith-Wiedemann syndrome (BWS) is associated with variable clinical features including hemihypertrophy/lateralised overgrowth (LO) and embryonal tumor predisposition. BWS-associated (epi)genetic alterations occur in a subset of patients with isolated LO (ILO), leading to the concept of BWS spectrum disorder (BWSp). We investigated the relationship between clinical features and molecular diagnostic results in a cohort with LO using the BWSp international consensus group (BWSICG) clinical scoring system.

Methods: Clinical/molecular findings in 94 previously-unreported patients with LO referred for BWSp molecular studies were reviewed retrospectively. The BWSICG score was assigned and diagnostic rate calculated.

Results: BWSp-associated (epi)genetic alteration was identified in 15/94 (16%). The molecular diagnostic rate by MS-MLPA (blood DNA) for BWS-related molecular findings in patients with LO was positively correlated with the BWSICG score. 3/48 with ILO had a molecular alteration. No individuals with ILO had developed an embryonal tumor at last follow up.

Conclusion: Among a cohort of individuals with LO referred for BWSp molecular testing, the BWSICG score correlated with diagnostic yield. The embryonal tumor risk in children with ILO and negative molecular testing appeared very low, however longer- and more complete follow up is required to better define tumor risks in this group.

Introduction

Lateralised overgrowth (LO), previously known as hemihypertrophy or hemihyperplasia, is defined as asymmetric regional body overgrowth due to an underlying abnormality of cell proliferation without any other underlying diagnosis(1). LO can occur as part of a syndrome -most commonly Beckwith-Wiedemann syndrome (BWS)- or as an isolated phenomenon(2). Isolated LO (ILO, OMIM # 235000) is defined as 'lateralized overgrowth in the absence of a recognized pattern of malformations, dysplasia, or morphologic variants'(2). Differential diagnoses for syndromic LO include BWS, Neurofibromatosis type 1 and segmental overgrowth conditions caused by activating *PIK3CA* variants. Some cases of ILO are considered a mild presentation of BWS(2).

BWS (OMIM # 130650) is a congenital imprinting disorder, characterised by overgrowth, a variety of congenital anomalies and a predisposition to childhood tumors, the most common of which is nephroblastoma (Wilms tumor, WT) and the second most common is hepatoblastoma(3). WT is a rapidly-growing embryonal cancer of the kidney. Most WT occur in otherwise normal children, but a subset occurs in individuals with an underlying genetic- or epigenetic cause. More than 50 individual disorders have been associated with predisposition to WT. Examples include WAGR-, Denys-Drash-, Sotos-, Perlman-, Edwards-, Frasier-, Bloom- Li-Fraumeni- and Simpson-Golabi-Behmel syndromes(4). The prevalence of BWS has been reported as 1 in 26,000(5).

BWS is associated with multiple genetic and epigenetic mechanisms that cause altered function / expression of imprinted genes located within one of the imprinted gene clusters at chromosome 11p15.5. The most frequent molecular findings are loss of methylation (LOM) on the maternal chromosome at KCNQ1OT1:TSS differentially methylated region (DMR) (also known as imprinting centre 2 (IC2)), found in ~50% of individuals with BWS, and mosaic paternal uniparental disomy (patUPD) for a variable region of chromosome 11 (which always includes the 11p15.5 imprinted gene cluster) in ~20% of cases(6),(7),(8). Less frequently, gain of methylation (GOM) on the maternal chromosome at the H19/IGF2:IG-DMR (also known as imprinting centre 1 (IC1)) (in ~5% of cases) and germline loss of function variants in the maternally-expressed growth suppressor *CDKN1C* (5%-10% of sporadic- and ~40% of familial

cases) are identified(8). Maternally inherited translocations / inversions also occur rarely (1%)(8). As well as patUPD, many cases with epimutations at KCNQ1OT1:TSS-DMR are mosaic(9) and this can cause a varying degree in clinical presentation (e.g. just a single feature of BWS, such as LO) with negative molecular testing(8).

Recently, it was suggested by an international group of clinicians and researchers (the BWS International Consensus Group; BWSICG) that individuals with a florid BWS phenotype and those with ILO who have similar molecular findings to those described in BWS should be viewed as being part of a spectrum (BWS spectrum disorder; BWSp) and that all patients diagnosed with BWSp should be managed according to guidelines developed by the BWSICG(10). To aid the diagnosis of BWSp and selection of patients for molecular testing, the BWSICG proposed a clinical scoring system(10). According to the BWSICG recommendations, any patient with LO -whether isolated or combined with other clinical features of BWSp- reaches the threshold of the clinical scoring system at which molecular testing is indicated(10). We therefore decided to review the literature and investigate the relationships between the BWSICG score, molecular findings and presence of embryonal tumors in a cohort of patients with LO who were referred for molecular testing.

Methods

Patient cohort

A group of individuals referred for molecular testing for BWSp between 1st January, 2014 and 19th March, 2018 at the West Midlands Regional Genetics Laboratory was reviewed to identify (from the clinical referral information and medical notes) those in whom LO was present and detailed clinical features of BWSp were recorded on a standardised form (see questionnaire in supplementary information). Cases were discussed with their responsible clinician to ensure that that the diagnosis of LO was correct and those with documented- and / or alternative diagnoses were excluded (alternative diagnosis in the excluded group were: Klippel-Trénaunay syndrome (clinical diagnosis) (n=1) and pathogenic variants in *NSD1* (Sotos syndrome) (n=1), *PIK3CA* (n=1), *HRAS* (n=1) and *KRAS* (n=1)).

The BWSICG score(10) was calculated for each included individual(10). Where it was not clear whether or not hypoglycaemia was prolonged and due to hyperinsulinism (scores 2) or transient and self-limiting (scores 1), a score of 1 was assigned. Where data regarding the birth weight or gestation of birth (required to score for macrosomia) were missing, this feature was not scored (n=8).

Molecular studies

MS-MLPA was performed as described previously(7). In brief, DNA was extracted from peripheral blood lymphocytes (or, in one case, on a buccal sample because the result of analysis of DNA from lymphocytes was equivocal) and tested using the MRC-Holland BWS/RSS ME-030 methylation-specific multiplex ligation-dependant probe amplification (MS-MLPA) kit according to manufacturer's recommendations in the NHS diagnostic laboratory. This technique enables patients with a KCNQ1OT1:TSS-DMR imprinting defect (who have LOM at KCNQ1OT1:TSS-DMR and unaltered methylation at H19/IGF2:IG) to be distinguished from those with an H19/IGF2:IG imprinting defect (GOM at H19/IGF2:IG and unaltered methylation at KCNQ1OT1:TSS) or patUPD (GOM at H19/IGF2:IG and LOM at KCNQ1OT1:TSS). Germline *CDKN1C* testing was not routinely performed but when a positive result was available, the

information was included. The study was performed as a service evaluation project and/or REC approved research study (Molecular Pathology of Human Genetic Disease approved by South Birmingham Research Ethics Committee). In some cases, i.e. patients who consented to further testing (n=17), additional molecular testing was performed (DNA from 16 blood samples and one buccal sample) by microsatellite marker analysis or SNP microarray, as these techniques are more sensitive for the detection of low level mosaic patUPD(10). For microsatellite dosage analysis, patient and parental samples were genotyped at four polymorphic loci in chromosome 11p15 (D11S1984, TH, D11S1318 and D11S1923) and the ratio of paternal to maternal alleles in the patient was calculated. Ratios over 1.3 in two or more markers were considered indicative of patUPD. If parental samples were not available, SNP microarray analysis was undertaken with the Affymetrix Cytoscan™ 750K array, which includes 200,000 SNP probes that were interrogated for evidence of loss of heterozygosity on the short arm of chromosome 11.

Results

Description of Cohort

We identified 94 patients with LO and suspected to have BWSp referred for BWS molecular testing in a time window of just over 4 years. 50 were male, 44 were female and their median age was 2.3 years (range 0-36.9 years). Amongst patients with ILO (n=48), the median age was 2.5 years (range 0.8–10.4 years).

Clinical scores

The median BWSICG score was 2 (range 2-9) (see Figure 1). The number with ILO (BWSICG score=2) was 48/94 (51.1%). The most common clinical features in addition to LO were macroglossia (n=14, 14.9%), macrosomia (n=12, 12.8%) and facial flammeus naevus (n=11, 11.7%).

[Insert figure 1]

Tumors

No patients with ILO developed an embryonal tumor. Only one patient (1.1%) developed a tumour and this was a bilateral WT. The BWSICG score for this patient was 5 and the additional feature apart from LO and tumors was facial flammeus naevus. The molecular result for this individual was negative.

Molecular testing and molecular diagnostic rates

MS-MLPA analysis or sequencing identified a BWSp-associated methylation defect in 15/94 patients (16.0%). patUPD and KCNQ1OT1:TSS hypomethylation was identified in 9/94 (9.6%) and 6/94 (6.4%) individuals, respectively. No patients had an H19/IGF2:IG-DMR epimutation and 0/5 had a *CDKN1C* pathogenic variant. Of those with ILO, 3/48 (6.3%) had a positive molecular result (two patUPD, one KCNQ1OT1:TSS hypomethylation).

The molecular diagnostic rate (MDR) for BWS-related chromosome 11p15.5 molecular findings (in blood) was positively correlated with the BWS consensus score, Spearman rank

correlation coefficient r= 0.943 P=0.0048 (see Figure 2). (BWSICG score = 2, molecular diagnostic rate (MDR) = 6.25% (3/48); BWSCS = 3, MDR = 5.6% (1/18); BWSCS = 4, MDR = 15.4% (2/13); BWSCS = 5, MDR = 33.3% (2/6); BWSCS = 6 or 7, MDR = 50% (2/4); BWSCS = 8 or 9, MDR = 100% (5/5).

[Insert Figure 2]

Further testing

Samples from 17 patients, with negative molecular results, whose files were available locally and who / whose parents consented to further testing underwent further analysis by microsatellite marker analysis, in an effort to detect low level mosaic patUPD. The results are presented in table 1.

[Insert table 1]

Discussion

Among this cohort of individuals with LO, we found that the likelihood of a detecting a molecular finding diagnostic of BWSp was positively correlated with the BWSICG clinical score. The overall diagnostic yield in individuals with LO using standard MS-MLPA analysis (on DNA from blood) was 16.0% but 6.25% in individuals with ILO.

BWSp is a mosaic disorder, i.e. some cells of the body contain a DNA with epigenetic alterations whilst others contain DNA with a normal methylation pattern. The clinical spectrum observed in BWSp is likely due to the postzygotic epigenetic alterations in most cases of BWSp, with an earlier change resulting in more clinical features compared to a late change(11). It seems likely that the findings reflect a correlation between higher levels of epigenetic alterations in individuals with higher BWSICG scores, compared to those with non-isolated ILO (BWSICG score=2).

The median BWSICG score of 2 in all patients with LO in this study is lower than the median calculated using data from a previously reported cohort examined using the same clinical features questionnaire(7) (median score=5). This discrepancy is probably due to an increased recognition that LO can be the only sign of BWSp and associated increased numbers of requests for molecular testing for BWSp in patients with ILO.

We note that Duffy *et al*. (2019)(11) found molecular diagnostic rates increased in three categories of BWSp defined by BWSICG scores (classical, atypical, ILO = 91.3%, 84.9% and 43.9%, respectively). The higher diagnostic rates reported by Duffy *et al.* (2019) likely reflect the use of more sensitive testing methods (including the use of tissue sampling) and possibly differences in patient characteristics(11).

Regarding incidences of tumors, it was difficult to compare our results to those in the medical literature because analysis of data from studies of patients with LO and the risk of embryonal tumors was hindered since these papers did not necessarily distinguish between patients with ILO and syndromic LO. In a retrospective review of 250 patients with ILO over a 10 year period, 3/250 (1.2%) developed at least one abdominal tumor(12). Comparing tumor likelihood to molecular result was also hampered because older papers (including that of Dempsey-Robertson *et al*. (2012)(12)) did not include molecular testing results. Dumoucel *et*

al. (2014)(13) reported that 12 of 295 (4%) patients with WT had ILO which, in most cases, was discovered after the diagnosis of WT was made. Six of these 12 underwent molecular testing and all were negative(13), which was consistent with the observation in our study, where the only patient with a tumor had a negative molecular result.

A key recommendation of the BWSICG consensus report(10) was that individuals with ILO and a positive molecular finding of BWS should be diagnosed as having BWSp and managed as for patients who have a diagnosis of classical BWS. One area of difficulty in managing children with BWS/BWSp has been in relation to tumor risks and tumor surveillance, such that there are differences between clinical practice in the United States of America and Europe. The American Association for Cancer Research childhood cancer predisposition workshop (an international committee of geneticists, oncologists, radiologists, and genetic counsellors) reviewed published data on children with syndromic WT and made recommendations for screening for WT and hepatoblastoma for patients in the United States of America(14). They proposed a threshold for recommending screening in BWS / BWSp of $\underline{\geq}$1% tumor risk (though the threshold outside the USA is usually higher(14)) and proposed that all patients with confirmed or suspected BWS -including those with ILO- should undergo surveillance every 3 months from the time of diagnosis to age 7 years. Duffy *et al*.(11) also advocated screening for a ≥ 1% tumor risk.

However, BWSp is a molecularly heterogeneous disorder and the embryonal tumor risk differs between different molecular subgroups, being highest in those with patUPD(6),(15),(16),(17) and H19/IGF2:IG GOM(6),(16),(3), and lowest in those with KCNQ1OT1:TSS LOM(6),(3) or *CDKN1C* mutations(6),(3). Accordingly, many centres within Europe, and the BWSICG, have recommended that tumor surveillance in BWSp is to higher risk subgroups and not offered to children with BWSp caused by KCNQ1OT1:TSS LOM (although it should be noted that the overall embryonal tumor risk in this group is 2.6%(14)). Whilst recommending that children with ILO and a chromosome 11p15.5 abnormality should be screened according to their molecular subgroup, the BWSICG did not make consensus recommendations for children with ILO who had a negative molecular test. However, a prior UK consensus recommended that cases of ILO without detectable molecular finding did not require surveillance(18). Our findings would appear to suggest that the tumor risk in such patients is low and screening may not be necessary but there are some caveats to this observation, which could be

addressed by further studies. Firstly, MS-MLPA is not as sensitive as some other diagnostic techniques for detecting low level patUPD mosaicism and in some instances of mosaicism, testing more than one tissue source (e.g. buccal cells, skin, pancreatic cells) of DNA increases the diagnostic yield(19). A limitation of this study is that the analysis was almost all carried out on DNA from blood and this may be the reason why many patients in this study did not receive a positive molecular result. It would be helpful to gather multicentre data in which all participants had undergone a common molecular diagnostic protocol as recommended by the BWSICG(12). Secondly, children presenting with embryonal tumours are likely to have undergone a more rigorous search for clinical signs and this could have introduced ascertainment bias. Indeed LO may, in some cases, become more apparent with age and it is possible therefore that it might be diagnosed in some children only after they have presented with an embryonal tumor. Thirdly - and importantly – at the time of most recent assessment, many of our cohort (80/94, 85.1%) had not reached an age at which the development of an embryonal tumour was highly unlikely (>7 years) and we cannot exclude that some of the younger ones with ILO might develop a tumour at a later date.

The finding that the results of further tests with SNP microarray or microsatellite marker analysis on samples of patients who had a negative MS-MLPA result were also negative demonstrates that MS-MLPA analysis was not likely to fail to identify the abnormality in this study.

In summary, we found a positive correlation between the BWSICG clinical score and a 11p15.5 MS-MLPA-detected methylation anomaly in 94 individuals with LO referred for BWSp molecular testing. A positive molecular result (on testing blood DNA) was seen in 16% of all patients with LO. Our findings suggest that the risk of embryonal tumors in individuals with ILO is very low. However, to increase the accuracy of risk estimates for these rare complications, we recommend further studies of large cohorts of molecularly tested children with LO using the BWSICG score.

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Captions

Table 1: BWSICG scores and molecular results from patients undergoing further testing.

Figure 1: number of individuals with each BWSICG score.

Figure 2: Percentage with molecular findings according to BWSICG score. (UPD = uniparental disomy, IC2 LOM = KCNQ1OT1:TSS epimutation).