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Overgrowth syndromes: review of clinical and molecular aspects and tumour risk

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*Abstract (170 words)*

Overgrowth syndromes are a heterogeneous group of rare disorders characterised by generalized or segmental excessive growth, commonly associated with additional features. They are caused by either genetic or epigenetic defects, affecting factors involved in cell proliferation and/or the regulation of epigenetic marks. Some of these conditions are associated with neurological anomalies, such as macrocephaly, cognitive impairment, or autism. Overgrowth syndromes are frequently associated with an increased risk of cancer (embryonic tumours during infancy or carcinomas during adulthood), but with a highly variable prevalence. Given this risk, syndrome-specific tumour screening protocols have recently been established for some of these conditions. Certain specific clinical traits make it possible to discriminate between different syndromes and orient molecular explorations, despite clinical overlaps. Recent advances in molecular techniques using next generation sequencing approaches have increased the number of patients with an identified molecular defect (especially patients with segmental overgrowth).

This review will focus on the clinical and molecular diagnosis, tumour risk, and recommendations for tumour screening for the most prevalent generalized and segmental overgrowth syndromes.

## Introduction

Overgrowth syndromes (OGS) are a heterogeneous group of disorders characterised by excessive growth. Overgrowth is usually observed during foetal life (based on ultrasound examinations during pregnancy), resulting in excessive length and/or weight at birth (*i.e.* above the 90<sup>th</sup> percentile or two standard deviations above the mean for the term). However, in rarer cases, overgrowth appears later in life. Initially, OGS included conditions associated with excessive growth of the whole body (generalized overgrowth). More recently, syndromes associated with segmental overgrowth (*i.e.* of one or several parts of the body) have also been included. Most OGS are caused by genetic or epigenetic mechanisms. Foetal and post-natal growth is a complex process involving various factors, including genetic/epigenetic, endocrine, and metabolic factors and trans-placental exchange during pregnancy, as well as exposure to exogenous factors, such as toxics, pollutants, or infections. Overgrowth may be caused by abnormal expression of genes involved in growth control. It may also be the consequence of a metabolic imbalance (*e.g.* children born to diabetic mothers) or the consequence of a high familial growth potential (constitutional tall stature). In the latter two cases, overgrowth is the only symptom, whereas additional signs are usually observed in (epi)genetic OGS. Despite recent progress in the identification of (epi)genetic aetiologies in overgrowth patients, up to 50% of patients with syndromic overgrowth still have no identified molecular anomalies.<sup>1</sup> The disruption of the same molecular factors involved in the control of the cell cycle can lead to excessive cellular proliferation or a tumour when it occurs as a somatic event, and overgrowth or growth retardation when occurring as a germinal event. For example, the *Cyclin D kinase inhibitor 1C* gene (*CDKN1C*, previously called *p57kip2*) is directly involved in the transition from the G1 to S phase of the cell cycle. *CDKN1C* is under-expressed in various types of tumours (which may explain their excessive proliferation) and germinal mutations of *CDKN1C* lead to 1) Beckwith Wiedemann syndrome (BWS) if they involve loss-of-function mutations<sup>2</sup> or 2) Silver-Russell/IMAGe syndrome (two conditions with growth retardation) if they involve gain of function mutations.<sup>3, 4</sup> Furthermore, somatic mutations in oncogenes, anti-oncogenes, or genes of signalling pathways are frequently observed in tumours. More recently, mutations of such oncogenic pathways have been identified in several conditions characterised by either growth retardation or overgrowth, such as the *PLAG1/HMGA2/IGF2* or the *PI3 kinase/AKT/mTOR* pathways.<sup>5, 6</sup> Finally, abnormal epigenetic marks are usually observed in tumours (such as abnormalities in DNA methylation or histone tail modifications, microRNA expression, mRNA processing, etc.). Intriguingly, germinal mutations in factors that are involved in the regulation of epigenetic marks or RNA processing represent many of the molecular defects identified in syndromic overgrowth.<sup>1</sup>

In this review, we will focus on OGS due to genetic or epigenetic mechanisms. It is impossible for this review to be exhaustive, as many (epi)genetic conditions include overgrowth. Although each of these OGS have specific clinical symptoms that make it possible to distinguish between them, they also share common symptoms (aside from excessive growth), especially an increased risk of tumours.

### *Beckwith Wiedemann syndrome (BWS) and isolated lateralized overgrowth (ILO)*

*Clinical aspects:* BWS (MIM #130650) is the most frequent overgrowth syndrome. The prevalence is estimated to be approximately 1/10,500 births, but may be underestimated because of the existence of incomplete phenotypes. ILO<sup>7</sup> (previously called isolated hemihyperplasia, MIM #235000) was initially defined as a specific condition. However, ILO and BWS are now considered to be part of the BW spectrum (BWSp) as they share common molecular mechanisms. BWS was first reported in the 1960s by a pathologist (Dr. J.B. Beckwith) who described fetuses with overgrowth, omphalocele, and adrenal cytomegaly<sup>8</sup>, and a paediatrician (Dr H.R. Wiedemann) who described children with an association of omphalocele, macroglossia, and gigantism, namely EMG syndrome.<sup>9</sup> Aside from the cardinal features initially described (macrosomia, macroglossia, omphalocele, lateralized overgrowth [LO]), children with BWS usually present a typical facial gestalt, including midface hypoplasia, infraorbital creases and prominent mandible, ear creases/pits, and facial *naevus flammeus*. This condition has been associated with many other congenital anomalies, such as hypoglycaemia/hyperinsulinism, cardiac or nephron-urological malformations, cleft palate, and polyhydramnios.<sup>10</sup> A higher prevalence of preeclampsia in pregnancies of affected fetuses has been reported.<sup>11</sup> Several scoring systems have been proposed to define BWS, with varying sensitivity/specificity concerning the identification of molecular defects.<sup>12, 13</sup> Such scoring systems are usually based on major symptoms (including macroglossia, macrosomia, and omphalocele) and some of the other symptoms associated with BWS. However, there is a high prevalence of incomplete presentations, and a very low rate of “false negative” patients (*i.e.* patients carrying a molecular defect which will not be tested because of a negative clinical score) is needed. Thus, a new scoring system has been defined that includes cardinal (scoring two points) and suggestive features (scoring one point) (Table 1).<sup>12</sup> In this scoring system, features are considered to be cardinal when frequently observed and specific to BWS. The other criteria are considered to be suggestive, because they are less frequent or less specific. For example, macroglossia and LO are considered to be cardinal features and macrosomia at birth and the occurrence of an embryonic tumour are considered to be suggestive. The purpose of this score is to define 1) patients for whom a molecular test should be indicated and 2)

patients with a clinical diagnosis of “classical” BWS, whether a molecular defect has been identified or not. In addition to classical BWS, the BW spectrum (BWSp) includes both patients who fulfil the clinical criteria for “classical” BWS (irrespective of the identification of a molecular defect) and those who do not, but for whom an 11p15 molecular defect has been detected.<sup>12</sup> Concerning the clinical management of patients with BWSp, an international expert consensus group has established 50 recommendations which have been recently published.<sup>12</sup>

Molecular mechanisms: Approximately 80% of patients with a clinical diagnosis of BWS have a molecular defect within the 11p15 region (Figure 1). This region includes imprinted genes (*i.e.* genes which exhibit monoallelic and parent-of-origin specific expression). The telomeric domain of 11p15 contains the *IGF2* gene, which promotes foetal growth and is expressed only from the paternal allele. The monoallelic expression of *IGF2* is controlled by an imprinting centre (IC) called *H19/IGF2:IG-DMR* (or IC1), which is methylated on the paternal allele only. The centromeric domain of 11p15 contains the *CDKN1C* gene (a cell-cycle inhibiting factor), which is expressed only from the maternal allele. Expression of *CDKN1C* is controlled by an IC called *KCNQ1OT1:TSS-DMR* (or IC2), which is methylated on the maternal allele only.<sup>14</sup> Approximately 60% of BWS patients show abnormal methylation at either IC1 (gain of methylation on the maternal allele, IC1 GOM) (5-10%) or IC2 (loss of methylation on the maternal allele, IC2 LOM) (50%). Paternal segmental uniparental disomy (UPD) of 11p15 [upd(11)pat] is observed in approximately 20% of BWS patients. Loss-of-function mutations of *CDKN1C* are observed in 5-10% of BWS patients, but represent the most frequent mechanism in familial cases of BWS.<sup>2, 10</sup> Rare rearrangements of the 11p15 region, such as paternal duplications, have been reported.<sup>15</sup> Finally, patients with whole genome paternal UPD have been reported, with a high risk of developing tumours, including as adults.<sup>16</sup> Epigenetic defects and UPD usually occur after fertilization and are therefore diagnosed as somatic events in a mosaic state, whereas mutations in *CDKN1C* or chromosomal rearrangements usually occur as germinal events. The recurrence risk is low in cases of an epigenetic defect or UPD. On the other hand, the recurrence risk can be as high as 50% in cases of an inherited *CDKN1C* mutation or 11p duplication, depending on the gender of the transmitter.<sup>12</sup> More recently, genetic defects within ICs have been reported, which underlie gain/loss of methylation. Approximately 20% of patients with IC1 GOM carry a mutation/deletion in the OCT4/SOX2 binding sites within the IC1. OCT4 and SOX2 are pluripotency factors which are necessary for the protection of the maternal IC1 from *de novo* methylation after fertilization. The recurrence risk in siblings can be up to 50% in cases of deletion/mutation within imprinting centres, depending on the gender of the transmitter.<sup>17, 18</sup>

BWSp can be suspected in a foetus during pregnancy, and methylation studies of 11p15 in amniotic fluid are sometimes indicated to distinguish between BWS and other conditions with

more severe complications. However, prenatal studies can lead to false negative results because of the usual mosaicism observed in BWS, especially if the rate of mosaicism is low.<sup>19</sup> Therefore, clinical and molecular geneticists should be aware of such a possibility, and prenatal molecular testing should take into account the benefit of a positive prenatal diagnosis of BWS *versus* the possible complications of the prenatal sampling of amniotic fluid and the possibility of a false negative result.

Approximately 25% of BWS patients carry epigenetic defects at other imprinted loci, in addition to the 11p15 locus. These have been called “multilocus imprinting disturbances” (MLID), which have been observed in other imprinting disorders, such as Silver-Russell syndrome<sup>20</sup>, transient neonatal diabetes mellitus<sup>21</sup>, and pseudo-hypothyroidism.<sup>22</sup> The involvement of MLID in the clinical presentation of the patients is still unclear, but several studies suggest a more severe phenotype (especially in terms of cognitive development) in cases of MLID.<sup>23</sup> Very recently, mutations in *NOD-like receptor PYD* (NLRP) factors have been identified in mothers with recurrent miscarriages and children with imprinting disorders, including patients with BWS.<sup>24-26</sup> However, screening for mutations in the mothers of affected children is not currently recommended as a routine diagnostic procedure, as such events rarely occur.<sup>12</sup>

Assisted reproductive technologies (ART): The mechanisms that lead to epigenetic defects are generally unknown (apart from rare deletions/mutations within the ICs, or mutations in NLRP factors), giving rise to the hypothesis of an environmental mechanism. The link between imprinting disorders and ART was suggested in the 2000s, with the reporting of an increased frequency of pregnancies obtained after ART in patients with BWS or Angelman syndrome<sup>27-29</sup>. Intriguingly, Angelman patients conceived after ART often show a LOM at the differentially methylated region of the PWS/AS locus of chromosome 15q11-q13, whereas LOM is observed in less than 5% of Angelman patients conceived naturally. In BWS, Mussa *et al.* very recently determined a relative risk of 10.7 of being born after ART<sup>30</sup>. Most BWS patients conceived after ART show IC2 LOM, whereas this mechanism occurs in only 50% of BWS patients who are naturally conceived. To date, no specific technology or aetiology of infertility has been shown to be involved in the occurrence of epigenetic defects, and further studies are needed to decipher the mechanisms that link subfertility, ART, and imprinting defects.

### *Simpson-Golabi-Behmel syndrome (SGBS)*

SGBS (MIM # 312870) is a rare X-linked disorder which was first reported by Simpson *et al.*<sup>31</sup> and subsequently described by Golabi and Rosen and Behmel *et al.*<sup>32, 33</sup> Since then, there have been a number of case reports but few clinical reviews.<sup>34-36</sup> The exact prevalence has not

been precisely evaluated. In their review, Tenorio *et al.* mentioned that 250 patients had been reported in the literature, but they included patients for whom misdiagnosis was possible, as no confirmation by molecular analysis was performed. However, it is possible that SGBS may still be underdiagnosed due to the unfamiliarity of clinicians with the phenotype. In 2013, Cottureau *et al.* reported the clinical description of 42 male patients and reviewed 63 published cases, all molecularly confirmed.<sup>35</sup> An update of this review, with 18 additional patients tested in our two laboratories (Tours and Trousseau, Paris) and 29 new descriptions in the literature, has led to the identification of 152 male cases with a known mutation, a series for which the frequency of the clinical features has been estimated. There is increasing evidence that some carrier females are symptomatic, usually to a lesser degree than males, but there are no statistical data available in the literature. Large-scale X inactivation studies to understand the underlying mechanism of this phenotypic expression have not yet been performed.

SGBS is caused by mutations in the *GPC3* gene (which maps to Xq26). This gene encodes GPC3, a 70 kDa core protein of 580 amino acids. GPC3 is one of the six known mammalian glypicans which share a heparan sulphate glycan chain and regulate WNT, Hedgehog, fibroblast growth factor, and bone morphogenetic protein signalling. GPC3 itself negatively regulates cell proliferation by inhibiting Hedgehog<sup>37</sup> and modulating WNT signalling pathways.<sup>38</sup> A recent review of the molecular data shows that most of the 86 distinct *GPC3* mutations identified to date are unique and 82% are inherited.<sup>39</sup> Most are large rearrangements (43%), mostly deletions, followed by truncating point mutations (frameshift or nonsense mutations) dispersed throughout the entire gene, and predicted to result in a loss of function. Missense mutations are rare and the two which were functionally characterized impaired GPC3 function by preventing GPC3 cleavage and transport to the cell surface, respectively. No genotype-phenotype correlation has been identified to date.

SGBS has a recognizable clinical picture and should be correctly clinically diagnosed in most cases. Overgrowth is usually detected prenatally and often associated with polyhydramnios. Macrosomia at birth is the most frequent finding (86%)<sup>39</sup>, and is often associated with macroglossia (78%) and visceromegaly (nephromegaly in 61% and hepatomegaly in 46% of cases), whereas postnatal overgrowth occurs in only slightly more than half the patients (58%). Similarly, macrocephaly is present in more than half of the patients at birth (57.5%), but less than half during postnatal life (43%). A height or occipitofrontal circumference (OFC) in the normal range in adulthood therefore does not exclude the diagnosis of SGBS. Patients with SGBS generally have a weight that is appropriate for their stature. They do not appear to be at a high risk of neonatal hypoglycaemia, as this complication has been reported in only eight patients. In most cases (95%), SGBS patients have a particular facial appearance which may be very similar to that of BWS in young patients. However, they are distinguishable by the



presence of a midline groove of the tongue or lower lip and ear pits/grooves are less frequent (17%). In addition, supernumerary nipples are frequent (59%) and hand anomalies (broad and/or short hands, brachydactyly, mild cutaneous finger syndactylies, and nail dysplasia of the index finger) are suggestive. Among OGS, SGBS is distinct, as it includes a constellation of congenital malformations, amongst which genitourinary malformations are the most frequent (73%) and diaphragmatic hernia (30%) the most suggestive. Umbilical hernia/diastasis recti, renal dysplasia, and heart defects are each observed in approximately one third of patients and cleft lip and/or palate in approximately one quarter. It is noteworthy that omphalocele has never been reported. Skeletal anomalies are also frequently observed (50%), including chest deformity (pectus excavatum), as the most suggestive, and rib and vertebral body anomalies, whereas postaxial polydactyly of the hands is infrequent (15%), although it is considered to be a hallmark of the syndrome. As in BWS, neonatal hypotonia and a delay in motor and language development are possible, but intellectual disability is probably rarer than mentioned in the literature, although no precise study has been performed on the subject. However, speech problems, accentuated by a cleft palate and/or macroglossia, are frequent and many patients experience difficulties in school.

#### *Other syndromes with general overgrowth*

Aside from BWS and SGBS, other syndromes with generalized overgrowth have been described, which usually include abnormal intellectual development. Most are caused by genetic defects in genes that are involved in the regulation of epigenetic marks, such as DNA methylation or histone modification.<sup>1</sup> Most of these genes are also altered as somatic events in cancers. This reinforces the hypothesis that alteration of the expression/activity of factors that control physiological cell proliferation can lead to abnormal growth (overgrowth or growth retardation) or cancer.

Sotos syndrome (SS, MIM #117550) is a frequent cause of overgrowth syndrome. Patients usually present with excessive birth length (whereas birth weight is less affected), excessive postnatal growth, and advanced bone age. The OFC is usually high at all ages. Jaundice, hypotonia, and poor feeding are frequent in neonates. A delay in achieving early developmental milestones, particularly motor skills, is common. Most patients with SS have some degree of intellectual impairment, ranging from mild to severe learning disability. Cognitive development is usually more impaired than in BWS and SGBS, with very frequent learning disabilities.<sup>40</sup> Furthermore, patients often exhibit behavioural problems and symptoms of autism spectrum disorder.<sup>41</sup> Up to 50% of patients experience seizures. Patients often present with typical facies, with a long face, large forehead with sparse frontotemporal hair,

down-slanting palpebral fissures, malar flushing, and a typical long and prominent chin. SS can be associated with several malformations, including those of the heart, kidney, and brain. Skeletal signs can also be present, mainly scoliosis (up to 50%), but also flat feet and genu varum/valgum, possibly linked to the hyperlaxity commonly observed.<sup>42</sup> SS is mainly caused by mutations/deletions of the *NSD1* gene.<sup>43</sup> *NSD1* encodes a histone-methyltransferase protein (methylation of H3K36), and is therefore involved in the control of epigenetic marks and gene transcription. These mutations usually occur as a *de novo* event.<sup>44</sup>

Mutations in *SETD2*, *DNMT3A*, or *APC2* have also been described in patients with Sotos-like syndrome. *SETD2* encodes a histone-methyltransferase protein controlling the methylation of H3K36, like *NSD1*. *DNMT3A* encodes a DNA methyltransferase that can bind H3K4me0 histone and thus is also involved in transcriptional control. To date, three individuals with *SETD2* mutations and a Sotos phenotype have been described in the literature.<sup>45, 46</sup> Mutations in this gene have also been published for patients described as “autists”; these data are part of a study of very large “autism” cohorts by NGS (for example<sup>47-49</sup>). In comparison, 27 patients with a Sotos-like phenotype have been reported to harbour *DNMT3A* mutations.<sup>46, 50-52</sup> Patients with mutations in the *DNMT3A* gene are also reported to have Tatton-Brown-Rahman syndrome (TBRS, MIM #615879). Patients with Sotos-like syndrome and *SETD2* mutations have facial signs highly reminiscent of SS, whereas patients with TBRS have a quite different facial morphology, even if the other symptoms clearly mimic SS.<sup>50</sup> Finally, one mutation of *APC2* has been reported in two siblings with overgrowth and intellectual disability and a phenotype compatible with SS.<sup>53</sup> *APC2* is involved in brain development and is a downstream target of *NSD1*. Interestingly, *Apc2* deficient mice show a large OFC, cerebral anomalies, and abnormal behaviour, but no overgrowth.<sup>53</sup>

More recently, deletions/mutations in the *NFIX* gene have been identified in patients with a Sotos-like phenotype, which has been referred to as Malan syndrome (MS, MIM #614753).<sup>54</sup> Patients with MS often have a slightly elevated length and OFC at birth, and a facial aspect close to that of SS. Ocular abnormalities, pectus excavatum, and scoliosis have been reported, defining an intermediate phenotype between SS and Marfan syndrome.<sup>55, 56</sup> Learning disabilities are almost universal, and can be from moderate to severe.<sup>55</sup> Mutations in *NFIX* have also been identified in the Marshall-Smith syndrome (MSS, MIM #602535). This different phenotype may be explained by different types/locations of mutations. Mutations/deletions of *NFIX* leading to haploinsufficiency or loss of the ability to bind DNA lead to MS, whereas mutations with a dominant-negative effect lead to MSS.<sup>56</sup>

The growth phenotype in Weaver syndrome (WS, MIM #277590) is usually comparable to that of SS, with a high birth length and large OFC, a tall postnatal stature, and advanced bone age.

However, the facial gestalt is usually different, with large fleshy eyes and specificity concerning the chin, as patients with WS often have microretrognathism and a horizontal crease of the chin.<sup>57</sup> Other clinical features are almond-shaped palpebral fissures, widely-spaced eyes, and a broad forehead, with the phenotype becoming less evident with age, umbilical hernia, and soft doughy skin. Suggestive features are camptodactylies of the fingers and toes. Bone age is often greatly advanced (even more so than in SS patients), but without advanced tooth eruption. Patients with WS may have poor coordination, abnormal tone, and a hoarse low cry in infancy. Cognitive development is usually impaired in WS, but intellect varies widely, from nearly normal to severely impaired. WS is caused by mutations within the *EZH2* gene, which also encodes a histone methyltransferase, and is therefore associated with the regulation of gene transcription. More recently, whole exome sequencing allowed the identification of mutations in the *EED* (a co-factor of *EZH2*) gene in patients with a Weaver-like phenotype.<sup>58-</sup>

60

Perlman syndrome (MIM #267000) was first described in the 1970-1980s<sup>61</sup>. Children with Perlman syndrome have a phenotype close to that of BWS, with foetal overgrowth, but organomegaly (and especially nephromegaly) is usually very significant. Affected children are usually hypotonic, with neurodevelopmental delay, and have facial dysmorphisms (prominent forehead, broad and flat nasal bridge, inverted V-shaped upper lip, and low-set ears). Mortality is high in new-borns, because of renal dysplasia, and more than half of the children who survive after birth will develop Wilms' tumour (WT). Homozygous mutations in the *DIS3L2* gene were identified in Perlman syndrome children in 2012.<sup>62</sup> *DIS3L2* encodes an exoribonuclease, which has a major role in controlling the degradation of a number of coding and noncoding RNAs.<sup>63</sup> *DIS3L2* also has a role in the regulation of mitosis and cellular proliferation, as the protein is also involved in the exosome machinery.

*PTEN* mutation-related syndromes: *PTEN* is a key negative regulator of the PI3K/AKT/mTOR signalling pathway (see below). Patients harbouring constitutional mutations of *PTEN* can present with various phenotypes, which have been grouped into the *PTEN* hamartoma tumour syndrome. This includes Cowden syndrome (MIM #158350) and Bannayan Riley Ruvalcaba syndrome (BRRS, MIM #153480). Gastrointestinal hamartomatous polyposis, mucocutaneous papillomatous papules, and penile freckling are very frequent, associated with vascular or lymphatic malformations. Concerning growth, patients usually have macrocephaly but a normal stature. Developmental delay and/or autism may be observed, particularly in BRRS, for which intellectual disability is observed in 50% of patients.

*Syndromes with segmental overgrowth*

Aside from BWS, several pathological conditions include segmental overgrowth. This includes congenital lipomatous overgrowth with vascular, epidermal, and skeletal anomalies, (CLOVES, MIM #612918), megalencephaly, capillary malformation (MCAP, MIM #602501), Klippel-Trenaunay syndrome (KTS, MIM #149000), and others (fibroadipose hyperplasia, macrodactyly, etc.), all grouped under the term of PROS (PIK3CA-related OGS). Segmental OGS also include Proteus syndrome (MIM #176920) or hypoinsulinemic hypoglycaemia with hemihypertrophy (HIHGHH). These two latter syndromes usually include cutaneous and/or vascular malformations, which lead to segmental overgrowth of part of the body.

Mutations (which usually lead to a gain of function of the protein) in oncogenic pathways have been identified in these syndromes. The PI3K-AKT-mTOR signalling pathway has been implicated for years in tumorigenesis and is therefore a target for cancer therapy.<sup>64</sup> Mutations in the *PIK3CA* gene were initially described in KTS, a condition that associates capillary and vascular malformations and overgrowth. More recently, the spectrum of *PIK3CA* mutations has been broadened, as somatic mutations in *PIK3CA* have been identified in CLOVES/MCAP. The same mutation “hot spots” have been identified in both syndromes with segmental overgrowth and cancer (for example p.Glu542Lys, p.Glu545Lys, and p.His1047Arg in *PIK3CA*). Mutations in these syndromes are usually observed in a mosaic state and may therefore be undetectable in circulating blood cells and only observed in tissues, with variable rates of mosaicism.<sup>65, 66</sup> Deep-targeted NGS approaches are highly performant tools to detect such somatic mutations, especially for mutations with low mosaicism undetectable by Sanger sequencing.<sup>65, 67</sup> Constitutional *PIK3CA* mutations (*i.e.* non-mosaic mutations which are detectable in circulating blood cells) have also been reported. In the latter case, generalized overgrowth is usually observed, including diffuse megalencephaly, with some symptoms that can overlap with those of BWS, such as omphalocele, hypoglycaemias, and organomegaly<sup>65</sup>.

Germline mutations of the *PTEN* gene have been identified in patients with several conditions that include overgrowth (Cowden and Bannayan Riley Ruvalcaba syndromes, see before). Somatic loss-of-function mutations of *PTEN* have been identified in many types of tumours (review in <sup>64</sup>). More recently, mosaic mutations of *PTEN* have been identified in some cases of segmental overgrowth. Proteus syndrome has been described in patients with segmental overgrowth with a lipomatous cerebriform aspect of the hyperplastic tissues. Rare patients have also been described with segmental overgrowth and HIHGHH. Proteus and HIHGHH syndromes have been linked to mutations of *AKT1*<sup>68</sup> and *AKT2*<sup>69</sup>. The involvement of germline mutations of *PTEN* in Proteus syndrome is uncertain, as some patients carrying *PTEN* mutations may have been misdiagnosed as having Proteus syndrome.<sup>70, 71</sup> Mutations of other members of the PI3K-AKT-mTOR pathway (*AKT3*<sup>72</sup>, *CCND2*<sup>73</sup>, and *PIK3R2*<sup>72</sup>) have been identified in rare conditions which include megalencephaly, including megalencephaly

polymicrogyria polydactyly hydrocephalus (MPPH) syndrome, reinforcing the predominant role of the PI3K/AKT/mTOR pathway in the control of tissue growth and cerebral development.

#### *Tumour risk in overgrowth syndrome and tumour screening (Table 2)*

OGS are usually associated with an increased risk of tumours. This prevalence is only slightly higher than that associated with other conditions, such as Li-Fraumeni syndrome (linked to *P53* mutations) or *BRCA1* and *BRCA2* gene mutations, despite a higher prevalence of tumours in children with OGS than those of the general population. International consensus statements are needed to assess the specific issue of the indication and modality of tumour screening.

The association between OGS and increased tumour risk has been particularly well described in BWS. Sporadic embryonic tumours, such as WTs, adrenocortical carcinomas (ACCs), and hepatoblastomas, highly express *IGF2*<sup>74-76</sup>, and molecular defects of the 11p15 region are frequently observed in sporadic WTs and ACCs (loss of heterozygosity or imprinting)<sup>75, 77, 78</sup>. The overall tumour risk is estimated to be approximately 7% in BWS, but the prevalence of tumours is extremely variable, depending on the molecular mechanism. Indeed, the prevalence may be as high as 20% and 12% in IC1 GOM and upd(11)pat, respectively, whereas the prevalence is approximately 2% in patients with IC2 LOM.<sup>79-81</sup> Given the histotypes, the prevalence of WT is particularly high for patients with IC1 GOM or upd(11)pat, whereas WTs are anecdotic in patients with IC2 LOM or *CDKN1C* mutations. Finally, patients with a classical presentation of BWS, but no identified molecular defect, also have an increased risk of tumours (especially WT). This observation led to international recommendations for tumour screening that are stratified depending on the molecular aetiology, with abdominal ultrasound scans only recommended for the “high risk” groups, including patients with a clinical diagnosis of BWS and no identified molecular defect.<sup>12</sup> The international recommendations vary from the recommendations of the North American Association For Childhood Cancer Research (AACR), which recommends screening for all patients, irrespective of the molecular cause, considering a lower threshold to trigger tumour screening.<sup>82</sup>

In SGBS, the risk of developing an embryonal tumour was evaluated by several authors to be 10%, but included only three cases (one hepatocarcinoma, one gonadoblastoma, and one neuroblastoma) with no molecular analysis.<sup>83</sup> We reviewed 152 cases with a *GPC3* mutation and found one foetus with nephroblastomatosis, five patients with WT, six with hepatoblastoma, and one with medulloblastoma. The occurrence of leukaemia in one patient may have been coincidental, as a risk for hematological malignancies would not be expected in SGBS, given the absence of *GPC3* expression in white blood cells. This leads to an overall

frequency of 8.5%, but the small size of the sample hampers precise determination of the frequency. To date, there is no consensus concerning tumour screening in SGBS. Lapunzina *et al.* suggested tumour surveillance with abdominal ultrasound and measuring serum alpha fetoprotein and urinary catecholamine levels.<sup>83</sup> However, no evaluation of such a procedure has been reported. In the absence of a genotype-phenotype correlation, it may be advisable to perform at least a clinical and abdominal ultrasound surveillance until the age of seven years, as in BWS, while awaiting further studies.

Somatic mutations affecting the *NSD1* or *EZH1* gene have been identified in various types of tumours, suggesting a tumour suppressor role. Concerning germinal mutations in SS and WS, the prevalence of tumours is relatively low (probably less than 5%). This includes neuroblastoma, teratoma, acute leukaemia, and small-cell lung cancer.<sup>57, 84</sup> No tumour screening has been recommended by the AACR for patients with SS or WS because of the relatively low risk and varying tumour type.<sup>84</sup> No tumour has yet been associated with Malan syndrome. The prevalence of WT in patients with Perlman syndrome is very high (up to 64% of patients who survive beyond the neonatal period).<sup>85</sup> No consensual recommendation has been made regarding tumour screening. However, some experts recommend abdominal screening with ultra sound scans, as in children with BWS, given the very high prevalence of WT.<sup>83</sup> Further studies are needed to determine the optimal age at which such screening should be performed.

Patients with *PTEN* mutations have a very high risk of malignant tumours, especially those of the breast, kidney, thyroid, skin, or endometrium, with a penetrance of approximately 75%.<sup>86</sup> These tumours usually occur during adulthood, with the exception of thyroid carcinomas, which may be present during childhood.<sup>87</sup> Given the very high risk of malignant tumours, specific screening protocols have been proposed, including ultrasound screening for thyroid carcinoma from the age of seven years<sup>88</sup> and screening for colorectal, mammary, and endometrial carcinomas and melanomas during adulthood.<sup>89</sup> Disruption of *PTEN* or proteins from the *AKT* family is frequently observed as a somatic event in various types of tumours (mostly carcinomas).<sup>6</sup> Concerning somatic mutations of *PTEN* or *AKT1* in Proteus syndrome, various types of tumours have been reported, most of which are benign. Screening has not been recommended given the large spectrum of tumours, in terms of tumour type and the age of occurrence.<sup>90</sup>

WT or nephroblastomatosis has been reported in patients with *PIK3CA* mutation-related syndromes,<sup>66, 91</sup> but the prevalence of tumours associated with *PIK3CA* mutations has not been accurately assessed. However, given the reported tumours, caution should be advised, and abdominal tumour screening has been suggested.<sup>92</sup>

### *Clinical overlap between overgrowth syndrome and molecular investigations (Figure 1)*

Despite specific traits for each syndrome, OGS often share clinical symptoms (Table 3). This is particularly true for BWS and SGBS patients who can share macroglossia, macrosomia, umbilical hernia, hypoglycaemia, and the same spectrum of embryonic tumours.

Neonatal or postnatal macrosomia is a common finding among OGS. However, a gradient can be observed for postnatal growth for SS, SGBS, and BWS, with SS children being the tallest and BWS patients often being of normal or slightly above-average height. OFC can also distinguish between these three syndromes, as SS patients often have a very large OFC, whereas the OFC is only slightly elevated in SGBS children and usually within the normal range for BWS children. Neurocognitive development is often impaired in SS, and usually normal or only slightly impaired in SGBS and BWS patients. LO has been described in BWS, but not SS or SGBS. However, it can also be observed in patients with *PTEN*, *PIK3CA*, or *AKT1* mutations.

Physicians experienced in the clinical diagnosis of OGS may easily distinguish between these conditions for patients with a classical presentation. However, some patients can present with incomplete or non-classical phenotypes. Several studies showed that a molecular overlap may be observed between OGS, as some patients with an initial clinical diagnosis of BWS may have mutations in the *NSD1* gene, and patients with an initial clinical diagnosis of SS may have molecular anomalies within the 11p15 region.<sup>93, 94</sup> The same observation has been made between BWS and SGBS (unpublished data from the Trousseau Molecular Laboratory, Paris).

Concerning molecular diagnosis, methylation studies at 11p15 detect methylation defects (IC1 GOM or IC2 LOM) or upd(11)pat, confirming BWS, but do not detect mutations in *NSD1*, *GPC3*, or *CDKN1C*. On the other hand, Sanger or next generation sequencing (NGS), based on gene panels or whole exome sequencing, can detect mutations of these genes, but cannot detect methylation defects at 11p15. If BWS is suspected (especially if LO is present), molecular investigations should include methylation studies of 11p15, as these techniques will allow the detection of a molecular defect in approximately 70-75% of patients. If the result is negative, physicians should re-examine the clinical presentation of the patients for further analyses and consider alternative diagnoses. In the presence of LO, mosaicism should be considered and methylation studies on an alternative tissue should be considered.<sup>12</sup> In the absence of LO, most laboratories have developed NGS-based approaches to look for mutations in the genes which have been implicated in OGS. If the initial clinical diagnosis was that of SGBS or SS, NGS should be considered first, as it will detect point mutations/small

deletions. If the result is negative, either 1) MLPA (which is usually specific for one locus) or array technologies, which can detect rearrangements, such as large deletions/duplications or 2) methylation studies of 11p15 (especially for patients with SGBS) should be considered, depending on the clinical presentation. BWS is unlikely if the patient shows intellectual disability associated with overgrowth (except for those with severe perinatal complications, such as severe persistent hypoglycaemia or very preterm birth). In this case, NGS approaches could be performed first, as they will lead to the identification of a molecular defect in up to 50% of the patients (the most prevalent one being *NSD1*).<sup>1</sup>

Concerning segmental overgrowth, distinguishing between the different conditions can sometimes be challenging. Although a diagnosis of MCAP syndrome may be quite simple in cases of segmental overgrowth associated with megalencephaly, the phenotype can be mild in some cases of *PIK3CA* mutations and mimic BWS. For example, patients can present with only LO and vascular malformations of the face, which are also observed in BWS. If segmental overgrowth is associated with brain malformations, molecular studies should initially include NGS of peripheral blood leucocytes and/or hyperplastic tissues. These anomalies are usually present in a mosaic state, and thus ultradeep NGS techniques are needed (those able to detect levels of mutant alleles as low as 1%). Such techniques allow identification of a molecular defect in up to 66% of patients, with a much better rate of detection of these anomalies in the hyperplastic tissue than in circulating blood or buccal swab cells.<sup>67</sup>

### *Conclusion*

OGS are mainly caused by the (epi)genetic disruption of several factors involved in cell proliferation and/or the regulation of gene expression (regulation of epigenetic marks or transcriptional/post-transcriptional processes). Anomalies in the same genes/pathways are often observed in tumours, which may explain the increased tumour risk in OGS. A clinical overlap is observed. However, distinguishing between these conditions is necessary because of its impact on 1) tumour surveillance (which should be stratified depending on the molecular anomaly); 2) the indication of the molecular test (*i.e.* methylation analysis or gene sequencing of circulating blood cells or hyperplastic tissue); and 3) genetic counselling (depending on the result of the molecular test). A detailed clinical description is thus necessary, with particular attention to the OFC and the evaluation of cognitive development. Recent advances in molecular biology have increased the frequency of the identification of molecular defects in such patients, including somatic (epi)mutations in syndromes associated with segmental overgrowth. Consensus meetings involving international experts should be established, in collaboration with patient associations, to redefine these conditions (taking into consideration



the molecular defects in the recently identified factors) and establish guidelines concerning the molecular diagnosis and clinical management of these rare diseases.

#### Figure legends

Figure 1. Representation of the 11p15 region in humans. pat: paternal allele, mat: maternal allele, DMR: differentially methylated regions. The blue and red boxes represent paternally and maternally expressed genes, respectively, and the grey boxes the silenced alleles. The black and white “lollipops” represent methylated and unmethylated DMRs, respectively.

Figure 2. Proposed molecular testing strategy for overgrowth syndromes. \*Consider *CDKN1C* sequencing depending on the family history. \*\*Preferentially, a multiple-gene panel for OGS. \*\*\*Patients may present with distinguishable phenotypes (see main text) and a candidate-gene approach may be indicated if single gene Sanger sequencing is used. OFC: occipitofrontal circumference, OG: overgrowth, NGS: next generation sequencing, GOM: gain of methylation, LOM: loss of methylation, UPD(11)pat: paternal uniparental disomy of chromosome 11

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	Cardinal features	Suggestive features
Clinical findings	Macroglossia Exomphalos Lateralized overgrowth Hyperinsulinism, lasting > 1 week and requiring escalated treatment	Birth weight $\geq$ +2 SDS Umbilical hernia or diastasis recti Facial naevus simplex Polyhydramnios or placentomegaly Ear creases or pits Transient hypoglycaemia, lasting < 1 week Nephromegaly and/or heptomegaly
Tumours	Multifocal and/or Bilateral Wilms' tumour or nephroblastomatosis	Neuroblastoma, rhabdomyosarcoma, unilateral Wilms' tumour, hepatoblastoma, adrenocortical carcinoma, pheochromocytoma
Pathology findings	Adrenal cortex cytomegaly Placental mesenchymatous dysplasia Pancreatic adenomatosis	

Table 1: consensus scoring system proposed for Beckwith-Wiedemann syndrome.<sup>13</sup> Cardinal features score 2 points each, and suggestive features score 1 point each. A clinical score of at least two points indicates a molecular study of the 11p15 region. A clinical score of 4 points or more define a clinical diagnosis of Beckwith Wiedemann syndrome.

Syndrome	Prevalence	Type of tumour	Screening program	REF
<b>Recommendations based on consensus meetings</b>				
<b>Beckwith Wiedemann syndrome</b>	7%*	Wilms' tumour Hepatoblastoma Neuroblastoma	Abdominal USS each 3 months until 7 years for the high risk groups*	Brioude
<b>Sotos syndrome</b>	3%	Neuroblastoma Teratoma	No screening	Vilani
<b>Weaver syndrome</b>	Unknown	Hodgkin ALL Neuroblastoma	No screening	Vilani
<b>PTEN related hamartoma tumour syndrome</b>	Up to 75%	Thyroid, breast, kidney, endometrium carcinomas	Thyroid USS from the age of seven years Screening for melanoma, mammary, endometrial and colorectal carcinomas at adult age	Schultz, Daly
<b>Recommendations with no consensus</b>				
<b>Simpson-Golabi- Behmel syndrome</b>	8%	Wilms' tumour Neuroblastoma Hepatoblastoma	Abdominal USS each 3 months until 7 years Urine catecholamines Serum Alpha-foetoprotein	Tenorio J Orpha Rare Dis 2014
<b>Perlman syndrome</b>	Up to 40%	Wilms' tumour	Abdominal USS each 3 months until 7 years	Lapunzina 2005
<b>PIK3CA mutations</b>	unknown	Wilms' tumour	No screening or Abdominal USS each 3 months until 7 years	
<b>Malan syndrome</b>	Not reported		No screening	

Table 2: reported recommendations for tumour screening in overgrowth syndromes. Legend: USS: ultrasound scan

\* depending on the molecular subtype. Note that this program differs from the North American Association for Cancer Research (reference 80) who recommended abdominal screening for any patient with BWS.

	<b>Simpson Golabi Behmel</b>	<b>Sotos</b>	<b>Weaver</b>	<b>Malan</b>	<b>Perlman</b>	<b>Beckwith Wiedemann</b>	<b>PTEN related hamartoma tumour</b>	<b>PIK3CA related</b>	<b>Proteus</b>
OMIM	#312870	#117550	#277590	#614753	#267000	#130650	#158350 #153480	#612918 #149000 #602501	#176920
Gene(s)	<i>GPC3</i>	<i>NSD1</i>	<i>EZH2</i> <i>EEP</i>	<i>NFIX</i>	<i>DIS3L2</i>	<i>IGF2*</i> <i>CDKN1C*</i>	<i>PTEN</i>	<i>PIK3CA</i>	<i>AKT1</i> <i>PTEN</i>
Overgrowth	Global overgrowth						Segmental overgrowth		
	+	++	++	++	++	+	+ / ++	+ / ++	+ / ++
Head circumference	0/+	++	++	++	0/+	0	++	++	0
Cognitive impairment	0/+	+ / ++	+ / ++	+ / ++	+ / ++	0**	+ / ++	+ / ++	0

Table 3: clinical description of the most common overgrowth syndromes. Legend: 0: absent/normal. + Mild. ++ Severe

\* *IGF2* and *CDKN1C* are two imprinted genes mapped at 11p15.5. Abnormal methylation at imprinting centres within 11p15.5 (IC1 or IC2) represents the main molecular mechanism of BWS (see main text)

\*\* at the exclusion of patients with severe neonatal complications (prematurity or hypoglycaemia)