Blood and lymphatic vessel formation is a vital dynamic process that when dysregulated can lead to excessive or abnormal formation of the vasculature. Vascular malformations are often associated with bony and/or soft tissue hypertrophy (overgrowth) that may occur regionally or involve the whole body. These overgrowth syndromes can be categorized based on the type of vascular malformation present (Table 2.1). The purpose of this review is to provide an overview of the features, pathogenesis, and molecular mechanisms involved in overgrowth syndromes associated with vascular malformations.

Overgrowth Syndromes Associated with Slow-Flow Vascular Malformations

**Congenital Lipomatous Asymmetric Overgrowth, Vascular Malformation, Epidermal Nevus, and Skeletal Anomalies (CLOVES) Syndrome**

CLOVES syndrome is a sporadic disorder characterized by truncal lipomatous masses, vascular malformations, and cutaneous and acral/musculoskeletal anomalies [1, 2]. Its delineation came from a group of patients with overlapping clinical...
characteristics who did not meet the specific diagnostic criteria for Proteus syndrome (Table 2.2). The dysregulated adipose tissue, scoliosis, and enlarged bony structures in CLOVES are not progressive or structurally distorting like those seen in Proteus syndrome [1], and the overgrowth in CLOVES syndrome is congenital, “ballooning” in nature, grows proportionately with the patient, and often affects both feet [1, 3].

In 2012, Kurek and colleagues reported their finding of activating mutations in PIK3CA as the cause of CLOVES syndrome [4]. This group used massively parallel sequencing of DNA or RNA recovered from affected tissue from individuals with CLOVES syndrome who had undergone surgical resection of lipomatous overgrowth or vascular malformation with lipomatous overgrowth. Mutations were not detected in blood or saliva DNA from individuals who had mutations in their affected tissue. This supports Happle’s theory of paradominant inheritance of a single gene to explain the occurrence of both familial and sporadic cases [5–7]. This could occur if individuals heterozygous for a defective gene were phenotypically normal, and only those with a somatic mutation resulting in loss of heterozygosity of the gene would develop the syndrome. Such a mechanism would result in a clonal population of cells homozygous or hemizygous for the mutation and could explain the mosaic pattern of lesions in KTS (Fig. 2.1). Happle suggests that homozygous expression of the genetic defect would likely be incompatible with life [7], which would explain the sporadic and apparent non-Mendelian inheritance pattern.

Interestingly, the PIK3CA mutations discovered in CLOVES syndrome have been detected in several types of cancer [8]. This finding also underscores the importance of the PI3K-AKT pathway (Fig. 2.2) in overgrowth syndromes known to result from related activating mutations (Table 2.1).

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**Table 2.1 Syndromes associated with vascular malformations**

<table>
<thead>
<tr>
<th>Syndromes associated with slow-flow lesions</th>
<th>Syndromes associated with fast-flow lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klippel-Trenaunay syndrome (KTS)</td>
<td>PTEN hamartoma tumor syndromes (PHTS)</td>
</tr>
<tr>
<td></td>
<td>• Cowden syndrome</td>
</tr>
<tr>
<td></td>
<td>• Bannayan-Riley-Ruvalcaba syndrome (BRRS)</td>
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<tr>
<td></td>
<td>• SOLAMEN syndrome</td>
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<tr>
<td>Cutis marmorata telangiectatica congenita (CMTC)</td>
<td>Capillary malformation-arteriovenous malformation (CM-AVM) syndrome</td>
</tr>
<tr>
<td>Megalencephaly-capillary malformation (MCAP)</td>
<td>Parkes Weber syndrome (PKWS)</td>
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<tr>
<td>Macrocephaly-capillary malformation (M-CM) syndrome</td>
<td></td>
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<tr>
<td>CLOVES syndrome</td>
<td></td>
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<tr>
<td>Proteus syndrome</td>
<td></td>
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<tr>
<td>Beckwith-Wiedemann syndrome</td>
<td></td>
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</tbody>
</table>
Table 2.2  Diagnostic criteria for Proteus syndrome

<table>
<thead>
<tr>
<th>Diagnostic criteria for Proteus syndrome</th>
<th>Specific criteria (category signs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>For diagnosis: general criteria (mandatory)</td>
<td>Either one from A or</td>
</tr>
<tr>
<td>Mosaic distribution of lesions</td>
<td>Two from B or</td>
</tr>
<tr>
<td>Progressive course</td>
<td>Three from C</td>
</tr>
<tr>
<td>Sporadic occurrence</td>
<td>Manifestations</td>
</tr>
</tbody>
</table>

**A.**  
1. Connective tissue nevus

**B.**  
1. Epidermal nevus  
2. Disproportionate overgrowth (one or more)  
   - Limbs  
   - Arms/legs  
   - Hands/feet/digits  
   - Skull  
   - Hyperostoses  
   - External auditory meatus  
   - Hyperostosis  
   - Vertebrae  
   - Megaspondylodysplasia  
   - Viscera  
   - Spleen/thymus  
3. Specific tumors before end of second decade  
   - Bilateral ovarian cystadenomas  
   - Parotid monomorphic adenoma

**C.**  
1. Dysregulated adipose tissue (either one)  
   - Lipomas  
   - Regional absence of fat  
2. Vascular malformations (one or more)  
   - Capillary malformation  
   - Venous malformation  
   - Lymphatic malformation  
3. Facial phenotype  
   - Dolichocephaly  
   - Long face  
   - Minor downslanting of palpebral fissures and/or minor ptosis  
   - Low nasal bridge  
   - Wide or anteverted nares  
   - Open mouth at rest

Adapted from Biesecker et al. [9]

**Proteus Syndrome**

Proteus syndrome is a rare sporadic disorder characterized by soft tissue and bony hypertrophy of the hands and feet, hemihypertrophy, cranial hyperostosis, dysregulated adipose tissue, vascular malformations, and connective tissue and epidermal nevi. There are strict diagnostic criteria for Proteus syndrome, listed in Table 2.2
Fig. 2.1 *Representation of somatic mosaicism.* Mosaic distribution of lesions in vascular anomalies and associated overgrowth syndromes is postulated to occur via a post-zygotic mutation resulting in a subpopulation of cells with the mutation among a population of normal cells with no mutation.

Fig. 2.2 *Schematic of the PI3K/AKT pathway.*
Diagnosed patients are required to meet both general and specific criteria, with the general characteristics of mosaic distribution of lesions, progressive clinical course, and sporadic occurrence mandatory criteria. The vascular malformations associated with Proteus syndrome are not well characterized, although clinical observation suggests that slow-flow lesions (capillary, venous, and lymphatic malformations) similar to KTS may occur.

In 2011, Lindhurst et al. found a mosaic activating mutation in \( AKT1 \) associated with Proteus syndrome [10]. The p.Glu17Lys mutations were detected in affected lesion tissue, but not in unaffected tissue samples from the same individual. Like CLOVES syndrome, this finding supports Happle’s original hypothesis of somatic mosaicism that is lethal in the non-mosaic state [5, 6]. This idea is substantiated by the mosaic pattern of lesions in Proteus syndrome as well as the report of discordance in identical twin pairs [1].

**Klippel-Trenaunay Syndrome (KTS)**

KTS is characterized by regional overgrowth of soft tissue and bony structures with associated superficial capillary malformations, varicose veins, and/or venous malformations. Lymphatic malformations occur in about 11% of patients [11]. The cutaneous capillary stain and venous malformations are often noted at birth and are associated with limb hypertrophy. Involvement of lower extremities is typical; however, 10–15% of cases involve both the upper and lower extremities [12]. Affected limbs are commonly longer with an increased circumference when compared with the unaffected limb. The limb length discrepancy may be slight or dramatic and may become prominent with age, though progression is unpredictable. Limb enlargement tends to slow or stop once the child’s growth cycle is complete [11]. Macrodactyly, syndactyly, and hypertrophy of the thorax, pelvis, abdomen, head, or neck can also occur [13].

On histological exam, the capillary malformations (CMs) in KTS consist of an increased number of abnormal ectatic capillaries with flat endothelial cells (ECs) in the papillary dermis [14]. The lymphatic malformations typically accompanying the CMs display dilated lymphatic vessels filled with lymph fluid [14], whereas the venous malformations can include aneurismal dilation, aplasia, hypoplasia, duplication, or anomalous vessels [13].

The origin of KTS is unknown, and most cases are sporadic in nature. However, Aelvoet, Jorens, and Roelen reported familial cases of KTS suggesting a multifactorial pattern of inheritance [15]. Additionally, there have been reports of de novo chromosomal abnormalities in three different KTS patients [16–18], although no candidate genes have been verified in larger KTS populations. These reports suggest that genetic factors may contribute to the development and pathogenesis of KTS. Given the overlapping clinical characteristics between KTS and other overgrowth syndromes such as CLOVES syndrome and Proteus syndrome, it is possible that KTS also results from mutations in components of the PI3K-AKT pathway. To date, no such mutations have been reported.
Cutis Marmorata Telangiectatica Congenita (CMTC)

CMTC is a low-flow vascular malformation characterized by a fixed coarsely reticulated vascular pattern on the skin that is usually noted at birth and can have a localized or generalized distribution. CMTC is associated with congenital anomalies in 27–50% of cases [19, 20]. These anomalies are more often found in generalized cases of CMTC. The most frequent anomaly is limb hypoplasia [12]. However, other growth abnormalities can also be associated with CMTC such as limb hypertrophy, skull asymmetry, and syndactyly [19–22].

Histologic studies have been inconsistent with some studies reporting dilated capillaries in the dermis and subcutaneous tissue, while others were unable to detect a vascular anomaly [12]. Picascia and Esterly reported sparse dermal perivascular lymphocytic infiltrates and swelling of endothelial cells [19]. The cause of CMTC is unknown, and it appears to occur sporadically. Several theories have been presented to explain this disorder. One case series suggested a common dysmorphogenic environmental agent in the pathogenesis [23], while others propose genetic mosaicism in which the lethal gene survives via partial or mosaic expression [24, 25]. Once again, Happle’s theory of paradigmatic inheritance has also been proposed for CMTC [26].

Megalencephaly-Capillary Malformation (MCAP)

MCAP, previously known as Macrocephaly-Capillary Malformation (M-CM) syndrome, is a rare sporadic disorder distinct from cutis marmorata telangiectatica congenita (CMTC), although the vascular pattern can appear similar. MCAP syndrome is characterized by a CMTC-like vascular malformation in association with macrocephaly and developmental delay. While MCAP syndrome resembles the vascular malformation in CMTC, the skin lesion in MCAP syndrome is a reticulated capillary malformation rather than a true CMTC, as there is no ulceration or cutaneous atrophy associated with the lesion [27]. Often, a midline facial capillary malformation is reported, with other associations including hydrocephalus, connective tissue defects, toe syndactyly, frontal bossing, and hemihypertrophy [27, 28].

Recently, Riviere and colleagues discovered de novo germline and post-zygotic mutations in PIK3CA associated with MCAP [29]. These mutations, like those found in PIK3CA in Proteus syndrome, are activating mutations that result in elevated PI3K-AKT pathway activity (Fig. 2.2) [29].

Beckwith-Wiedemann Syndrome

Beckwith-Wiedemann is a syndrome characterized primarily by gigantism, macroglossia, and omphalocele [30], but may also include posterior helical ear pits, hypoglycemia, a predisposition to tumors, and facial capillary malformations. The
capillary malformations are usually centrofacial and virtually identical to, but more persistent than, salmon patches (nevus simplex).

Most cases of Beckwith-Wiedemann syndrome are sporadic, but there have also been reports of autosomal dominant inheritance within families. Cytogenetic analysis has demonstrated dysregulation of a region on chromosome 11p15 composed of two distinct domains of imprinted genes regulated by two imprinting centers [31, 32]. (Epi)genomic alterations in this region have been associated with approximately 80% of Beckwith-Wiedemann cases [33]. Within these domains, overexpression of key imprinted genes has been identified to contribute to disease pathogenesis, IGF2 and H19 in domain 1 and LIT-1 and p57\textsuperscript{Kip2} in domain 2. The molecular alterations in domain 1 only occur in 5% of Beckwith-Wiedemann patients, whereas imprinting defects within domain 2 account for roughly 50% of molecular defects in affected individuals [33]. Rare mutations have been identified in families with autosomal dominant inheritance and primarily involve the p57\textsuperscript{Kip2} gene. It has been suggested that p57\textsuperscript{Kip2} mutations are related to embryonal tumor types like rhabdomyosarcoma and hepatoblastoma, whereas upregulation of IGF2 has been more commonly associated with Wilms’ tumor [32]. Paternal uniparental disomy (UPD) involving both domain 1 and domain 2 is found in approximately 20% of Beckwith-Wiedemann cases [33]. Given that these individuals with UPD exhibit somatic mosaicism of lesions, it is possible that this molecular defect is caused by a post-zygotic event [33].

Overgrowth Syndromes Associated with High-Flow Vascular Malformations

\textit{PTEN Hamartoma Tumor Syndromes (PHTS)}

The PHTS is a spectrum of autosomal dominant hamartomatous disorders with phenotypic variability caused by germline mutations of the tumor suppressor gene phosphatase and tensin homolog deleted on chromosome 10 (PTEN) [34–40]. Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS) are the most commonly reported syndromes within the PHTS spectrum [34–40]. A variety of vascular malformations have been reported in patients with BRRS or CS [41, 42]. These lesions are most frequently deep vascular anomalies that are almost all fast-flow with unusual characteristics including multifocal distribution, musculoskeletal location, ectopic adipose tissue, and drainage into dilated veins [43]. In addition to vascular anomalies, both BRRS and CS are also characterized by lipomatosis, macroglobuloma, and hamartomatous lesions involving the skin, mucous membranes, GI tract, thyroid, breast, brain, and genitourinary system. A more recently described syndrome classified as part of the PHTS spectrum is the segmental overgrowth, lipomatosi, arteriovenous malformation, and epidermal nevus (SOLAMEN) syndrome [44]. This syndrome is distinct from CS and BRRS as it involves complex congenital dysmorphisms including segmental overgrowth and epidermal nevi in
addition to the other associated phenotypes. While similar to Proteus syndrome, individuals with SOLAMEN syndrome do not meet the strict diagnostic criteria for Proteus [9, 44].

The PHTS disorders are associated with germline mutations in the tumor suppressor gene PTEN. Although CS and BRRS are often differentiated by clinical characteristics, investigations have been unable to determine specific genotype-phenotype correlations. A single mutation in PTEN has resulted in both CS and BRRS within a family [45]. This suggests that CS and BRRS actually represent one condition with a phenotype continuum that could be influenced by host factors or additional somatic mutations. It has been shown that SOLAMEN syndrome is also associated with a germline mutation in PTEN; however, analysis of lesional tissues demonstrated a second molecular event at the PTEN locus resulting in deletion of the remaining wild-type allele [44].

**Capillary Malformation-Arteriovenous Malformation (CM-AVM)**

CM-AVM is a hereditary disorder characterized by multifocal cutaneous capillary malformations in association with high-flow vascular malformations, such as AVMs or arteriovenous fistulas (AVFs). These high-flow lesions are often found in the skin, subcutaneous tissue, bone, muscle, and brain. The capillary malformations tend to present as small pink to red macules often surrounded by a pale halo and may be widely distributed cutaneously. There have also been reports of larger solitary capillary malformations associated with CM-AVM [46].

CM-AVM is an autosomal dominant disorder caused by loss-of-function mutations in the RASA1 gene [47]. The molecular mechanisms by which these mutations lead to the localized vascular phenotype are unknown, although they likely involve the loss of inhibition of RAS p21 by the RASA1 protein. RAS p21 is a protein that controls cellular growth, proliferation, survival, and differentiation, indicating a potential role in the vascular overgrowth. The variable location within the skin, subcutaneous tissue, bone, muscle, and brain and intrafamilial presentations may suggest a second molecular event (second hit) is necessary.

**Parkes Weber Syndrome (PKWS)**

Parkes Weber syndrome is the association of high-flow lesions (AVM or AVF) with capillary malformation(s) and overgrowth of the affected extremity. Lymphatic malformations and lymphedema may also be present [12]. It seems that PKWS is a clinically and etiologically heterogeneous disorder, as RASA1 mutations have been found to occur in affected individuals with multifocal capillary malformations but not
those with a solitary lesion [48]. Like CM-AVM syndrome, a second-hit mechanism may be involved in affected tissue, although this mechanism has not been demonstrated in PKWS.

Vascular Malformations and Associated Overgrowth: Etiology

Tissue overgrowth results from dysfunction of processes that control apoptosis, cellular proliferation, and cell growth. One signaling pathway that contributes to this regulatory process is the phosphoinositide-3-kinase (PI3K)-AKT pathway (Fig. 2.2). Numerous animal studies provided evidence that disruption of the PI3K/AKT pathway results in disordered growth [49–55]. In addition, advances in the field of vascular anomalies’ research have led to the discovery of numerous human diseases of abnormal growth that are associated with defects in the pathway. Some of the first examples in human disease were the PHTS disorders, which result from loss-of-function mutations in the PTEN gene that negatively regulates the PI3K/AKT pathway. PTEN is a dual phosphatase protein that negatively regulates the PI3K/AKT pathway, a pathway essential to cellular growth and survival. When PTEN function is disrupted, the activation of this pathway increases. It is possible that the germline PTEN mutation with a subsequent second hit in somatic tissue could be a result in the various forms of overgrowth seen in PHTS, with the type and extent of overgrowth determined by the timing of the event and the cell type affected. However, this theory has yet to be validated. Additional evidence demonstrating the importance of this pathway came from the identification of RASA1 mutations in CM-AVM syndrome and PKWS. Ras positively regulates signaling of pathways involved in cellular growth and proliferation, including the PI3K/AKT pathway. RASA1 functions through inhibition of Ras, thus loss of RASA1 function results in loss of Ras inhibition and uncontrolled pathway activation.

More recently, activating mutations in PIK3CA were found to be associated with Proteus syndrome and MCAP. PIK3CA encodes the 110-kD catalytic alpha subunit of PI3K, which is activated upon tyrosine kinase receptor ligand binding. Activated PI3K converts phosphatidylinositol (3,4)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-triphosphate (PIP3) and subsequently leads to the translocation and phosphorylation of PDK1, which then phosphorylates AKT. AKT then exerts its downstream effects on PI3K-AKT pathway constituents. This occurs more directly in the case of CLOVES syndrome, as the activating mutations in this syndrome are found in AKT1 [4]. Though mutations in the PI3K-AKT pathway were identified, the mechanisms and understanding related to variable phenotypic presentation in individuals with the same mutation are still unclear. While genetic causes have not yet been delineated for some other overgrowth syndromes, the potential of PI3K-AKT pathway involvement is clear and warrants further investigation.

Overgrowth syndromes associated with vascular malformations represent a spectrum of disorders with variable clinical presentations and molecular associations
that are challenging to diagnose, manage, and treat clinically. Although phenotypically heterogeneous, a common characteristic among this spectrum is the mosaic nature of the overgrowth and the presence of more than one affected cell lineage [56]. This suggests that the molecular insult responsible for these syndromes could be explained by Happle’s theory of a post-zygotic somatic mutation that is lethal in a germline state [6]. The evidence provided by several groups who identified somatic activating mutations associated with overgrowth syndromes supports Happle’s theory, as the somatic changes resulted in altered expression of PI3K pathway components detectable only in the lesion tissue of affected individuals. The variable clinical phenotypes and severity of the lesions are likely to be determined by the timing, location, and nature of the secondary event, though this has not been confirmed [47, 56]. Future studies to investigate this relationship as well as the potential that the PI3K-AKT pathway offers for therapeutic development will likely yield opportunities for intervention in individuals with overgrowth syndromes associated with vascular malformations.

References


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