Progress toward understanding vascular malformations

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Publication date
2003

Capillary malformations: beyond port-wine and strawberries, toward a neural malformation ?

Mascagni (ca. 1785)

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Submitted for publication

Introduction

Capillary malformations (port-wine stains) are the most common vascular malformations occurring in 0.3% to 2.1% of newborns (1-3). The majority of capillary malformations (CM) are found on head and neck region, with 85% occurring in a unilateral, dermatomal distribution (4,5). The V2 dermatome is most commonly involved (57%); while in lesions where more than one dermatome is involved, the V2 is involved with V1 and / or V3 in 90% of cases (4). CM may also be part of a complex vascular anomaly e.g. Sturge-Weber- or Klippel-Trenaunay syndrome, but this paper will focus only on the non-syndromal capillary malformations (2,6). The mainstay of treating CM is flashlamp-pumped pulsed dye laser treatment (PDL), but a significant minority of these patients will achieve a suboptimal response. (7,8).

For an accurate diagnosis and subsequent optimal treatment, the pathogenesis has to be known. We want to provide an overview of our current understanding of the pathogenesis of capillary malformations. We first summarize some relevant issues of normal skin anatomy, and then compare this to the pathology involved in capillary malformations. In the subsequent discussion some theories about the etiology will be mentioned, and although this discussion will not focus on pulse dye laser *per se*, it is inevitable to include some information in a discussion about capillary malformations. We further summarize the most important parts of the increased molecular knowledge applicable to capillary malformations.

Normal skin anatomy

Findings by Johnson et al suggest that the major vascular organization of the dermis is defined in the first trimester of pregnancy (9). By 14 weeks of development the reticular dermis is distinguishable from the papillary dermis (10). At this stage the dermis is also invaded by the downward invagination of developing epidermal appendages.

The cutaneous microcirculation is organized in two horizontal plexuses (11-13) (Figure 1). The most superficial plexus is located 1 – 1.5 mm below the skin surface and is located at the junction of the papillary and reticular dermis and is called the subepidermal plexus (papillary plexus). The deep plexus is located at the dermis-subcutaneous border and is called the deep dermal plexus (reticular plexus). Pearl and Johnson have shown that a subcutaneous vascular plexus is located between the dense and loose adipose subcutaneous tissue and appears to be the homologue to the panniculus carnosus in lower mammals (14). The subcutaneous vascular

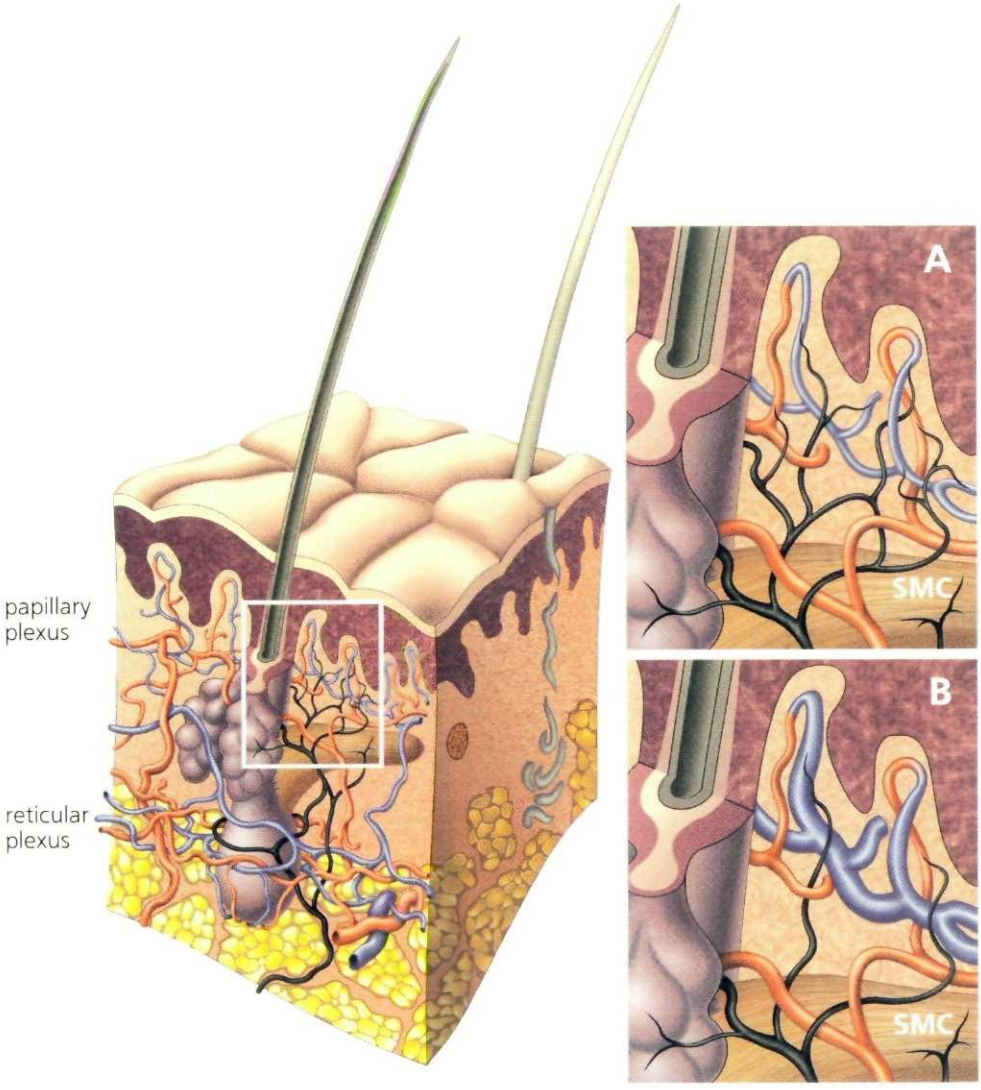

Figure 1:
Section of normal skin on the left defining the papillary (subepidermal) vascular plexus and the reticular (deep dermal) plexus. (red = arteries, blue = veins, black = nerves) On the right side of the picture is an enlargement of part of the skin with (A), demonstrating the normal skin, and (B) the skin in capillary malformations. (SMC = smooth muscle cell = arrector pili muscle of hair follicle). In (B) the decreased nervous innervation to the vasculature, with normal nervous innervation to the SMC is demonstrated. The dilated vasculature in response to the decreased innervation is also demonstrated in the capillary malformation (B).

network is best developed in the face, while the least numerous subcutaneous network is located in the lower extremities (14). The subcutaneous vascular plexus is divided into a deep, middle and superficial vascular plexus (15,16), but this discussion will only focus on the dermal circulation. The reticular plexus is formed by perforating vessels from the underlying muscle and subcutaneous tissue, and gives rise to arterioles and venules to form the papillary plexus (11). The reticular plexus also gives separate lateral tributaries to the sweat glands and hair bulbs and may pass deeply to supply adipose tissue. There seem to be some direct connections between the ascending arterioles and descending venules (arteriovenous fistulas). The papillary plexus receives branches from al three plexuses of the subcutaneous vessel system (15). The capillary loop arises from terminal arterioles from the papillary plexus and has an ascending limb, an intrapapillary loop (with a hairpin turn) and a descending loop that connects to the postcapillary venules (11,12). *Each* dermal papilla has its own single capillary loop. The endothelial tube at the crest and the intrapapillary descending limb is 1–1.5μm wider than the ascending limb. The characteristic of an arterial capillary is the homogenous appearing basement membrane material in the vessel wall. The character of the descending limb changes at the border of dermal papilla. The endothelial tube becomes wider and the basement membrane material in the wall loses its homogenous appearance and develops multilayers (multilaminated basal lamina layer is characteristic of venous vessels) before it connects to the papillary plexus. Bridged fenestrations are normally found in capillaries involved in rapid exchange of molecules between the vascular system and tissues e.g. choroid plexus of the brain and renal glomeruli. In healthy skin bridged fenestrations are limited to venous capillary loops adjacent to the eccrine sweat glands and dermal papilla of the hair.

Some of the differences between the vessels of the upper dermis and the lower dermis are summarized in Table A (11,13,17). The deeper dermal arterioles have elastic tissue in their wall, while several layers of smooth muscle cells also arround them. Some of the ultrastructural differences between the vessel components of the microcirculation are summarized in Table B (11-13,17-21). The terminal arterioles have a single smooth muscle cell layer, while the larger ascending arterioles have two distinct smooth muscle cell layers *over* a discontinuous elastic layer (18). The wings (the most lateral parts) of the smooth muscle cells in the terminal arterioles

Table A:
Some differences between the two dermal vascular plexuses

	Papillary plexus	**Reticular plexus**
Vessel diameter	25 μm	50 μm
Wall thickness	4 -5 μm	10 - 16 μm
SMC / PC layer	1 – 2	4 – 5
Position of bundles collagen	periphery of vessel	subendothelial position

Table B:
Some ultrastructural differences between the vessel components of the microcirculation (SMC = smooth muscle cells, PC = pericytes, EC = endothelial cells)

	Larger arteriole	Terminal arteriole	Arterial capillary	Venous capillary	Postcapillary venules	Larger venules
Vessel diameter	17 -26 µm	17-12 µm	10-12µm	10-12 µm	12-35 µm	35 - 50 µm
Wall thickness	?	?	2 - 3 µm	2 - 3 µm	3.5 - 5 µm	?
Basement membrane	Homogenous	homogenous	homogenous	Multilayer	multilayer	multilayer
Bridged fenestrations	-	-	-	+ (limited to eccrine sweat glands + dermal papilla of hair)	-	-
Elastin layer	+ (between EC and SMC)	+ (at periphery of vessel)	-	-	-	-
SMC or PC	SMC	SMC	PC	PC	PC	PC
SMC / PC layers	2	1	1	1	2-3	More PC (number not stated)
SMC / PC wings		Totally surround and even overlap	Average of 80% of EC encircled, never overlap	Average 80% encircled, never overlap	Average 80% encircled, never overlap	PC's more randomly placed
SMC / PC interdigitations on EC	++	++	+++	+++	+++	++
Tropomyosin and alpha-actin in SMC/PC	+++	+++	+	+	+++	+++

often overlap and totally surround the endothelial cells.

Smooth muscle cell/ endothelial cell interdigitations do occur, but less than in the case of pericytes and endothelial cells. As the diameter of the arteriole decrease from 26ìm, the elastic fibers gain a more peripheral position in the vessel wall. At 15ìm diameter the elastic fibers have disappeared and only a thin sheath divides the wall and the surrounding veil cell. At a diameter of 10 - 12ìm this elastic sheath disappears; this is the beginning of the capillary bed. Smooth muscle cells are not found below the 15ìm diameter level. Here the endothelial cells are surrounded by pericytes. Most vessels in the papillary plexus are postcapillary venules. Pericytes form tight junctions with endothelial cells through breaks in the basement membrane. Pericytes differ from smooth muscle cells by being thinner, lacking dense bodies, and having fewer myofilaments, but they do have proteins e.g. tropomyosin and alpha-actin for contraction (11,19-21). The basic structure of the postcapillary venule in the papillary plexus is similar to the larger venules at the reticular plexus, with the exception of vessel diameter and number of endothelial cells. The postcapillary venules have 2 - 3 layers of pericytes surrounding them, while venous capillaries have one layer of pericytes. At the border of the dermis and the subcutaneous tissue are valve containing veins. These veins have smooth muscle cells, elastic fibers and a homogenous basement membrane, but they do not have an internal elastic lamina (which are characteristic features of arteries).

The skin has a rich nervous supply. On reaching the dermis, nerve fasiciculi branch extensively to form a deep reticular plexus (22,23). This plexus serves the dermis, including most hair follicles (arrector pili), sweat glands and larger arterioles. From this plexus many fasiciculi pass to ramify in the papillary plexus at the border of the papillary and reticular dermis. Branches pass more superficially from this plexus terminating on reaching the basal epidermis, or in relation to encapsulated receptors. Sensory nerves are derived from the neural crest (posterior root ganglia), while the motor fibres are derived from cells of the sympathetic ganglia. The guidance of the developing axon by for example neurotrophins, has been a subject of considerable differences in opinions (22,24-27). In the development of peripheral nerves, axonal outgrowths precedes migration of Schwann cells. These two soon form a functional unit of migration, the nerve fibre, whose progress is guided by local conditions. It is likely that axonal growth and guidance depend on a fine balance of cell surface and extracellular matrix molecules (28). One of the most accepted theories is that growth cones are guided by some form of attraction called the neurotropism or chemotropism hypothesis (29). For example in the developing epithelium of the mouse face, studies have indicated that a chemoattractant originates at the epithelium that lures sensory afferents of the trigeminal system (30).

Capillary malformations (port-wine stain) anatomy

It has been suggested that CM are congenital lesions confined to the upper dermis (31-34). With advancing age capillary malformations undergo progressive ectasia resulting in the typical red-to-purple lesions (31,35). Ectasia of vessels was found to begin at age 10, with histologic findings in younger children revealing a normal vasculature with regard to number and size of blood vessels (36).

By staining endothelium with the PAL-E antibody the pathology of capillary malformations has been attributed to represent capillaries and / or medium sized venules or small veins (35). Braverman et. al. studied a capillary malformation in a 60-year old male by light and electron microscopy and by 3-dimensional reconstruction from photomicrographs of 1-im sections (32). The vascular dilatations were found to represent only post-capillary venules. In the papillary dermis vessels consist of terminal arterioles, arterial and venous capillaries and postcapillary venules, with the postcapillary venules being the majority of the vessels in this layer. Electron microscopy by Schneider et al revealed ectatic venules, in the subepidermal plexus and the first third of the reticular layer, while only a few patients had their subepidermal capillaries widened slightly (37).

Barsky et al have shown that CM consists of increased number of ectatic vessels. The walls of these vessels are thin, while the lining endothelium cells are flat (31). Several studies have proven that the endothelial cells lining the ectatic channels function normally (35,38). Immunofluorescence studies of the three major constituents of the vessel wall, fibronectin, factor VIII and collagenous basement membrane-type IV collagen, did not show abnormalities in the vessel wall (39). It has been proven in a three-dimensional histological reconstruction of capillary malformations, that multiple clusters of small diameter (10 - 50mm) blood vessels occur (40). Smoller and Rosen showed that the density of the pericapillary neurons is abnormally low (41). Only 17% ± 10% (mean ± SD) of vessels studied in capillary malformations were associated with nerves. In normal skin 75% ± 11% of vessels coursed 0.03mm of a nerve ($p<0.005$). They further found no difference in vessel numbers in CM and normal skin. A significant reduction in the response of CM to both vasoconstrictor and vasodilator stimuli has been demonstrated suggesting a reduction in autonomic control (42). It has been demonstrated that CM show occasional nerve fibres and that there is a deficit in both symphathetic and sensory innervation only in connection to the dilated vessels (43).

Histology has revealed that thickening of the post-capillary venule wall is a constant feature (37). This was already visible in a six-year old child, but was more obvious in adults. The thickening was caused by laminated basement membrane, amorphous material and collagen fibrils. Immunohistological studies revealed an increased deposition of basement membrane components

such as type IV collagen, laminin and fibronectin (44). This is in contrast to studies performed by Finley et al (31). Electron microscopy by Schneider et al has shown that the pericytes were located in the inner part of the wall. Functionally they did not look more active than normal (37). A striking finding by electron microscopy was that bridged fenestrations were observed in nearly all patients examined.

Discussion

Although our knowledge about CM has substantially increased in the last two decades, there are still many unanswered questions. A combination of molecular and histopathological information will be needed to ultimately identify the involved pathology and the subsequent etiology. With our current quest to understand pathological conditions like cancer and diabetes mellitus, molecular research in the mechanisms of angiogenesis and vasculogenesis has provided us with useful information about the etiology of vascular malformations (review ref. 45). By defining the regional pathology involved, the relational anatomy of all the associated structures (such as nerves and muscles) becomes important. It is possible that not only vessels are involved in the pathology of vascular malformations, but that the surrounding structures maybe involved as well. In capillary malformations the decreased nerve innervation is a good example.

The treatment of CM has significantly improved with the flashlamp-pumped pulsed tunable dye laser (PDL) (47). By observing the results of PDL, information is gained that could inform us about the pathogenesis of CM. The optimal parameters for laser treatment of CM have been derived theoretically by making use of computer models (48-50). It is clear that for a certain combination of wavelength, pulse duration and radiant exposure, only a limited range of blood vessel size can be injured optimally (49,50). PDL with a wavelength of 585 nm and a pulse duration of 450 is can provide optimal thermal damage with minimal energy. In normal skin PDL with 7 J/cm^2 penetrates the skin approximately 1.5 mm in depth, only reaching the most superficial vessels (51). Higher radiant exposure eg. 10 J/cm^2 can penetrate to a depth of 2.5 mm. These penetration values are in normal skin, and is expected that this is less for capillary malformations. Larger vessels require more fluences because a larger area is heated and the absorption of light in blood prevents the blood in the centre of the vessel lumen to be involved in the heating process (8). Smaller blood vessels require higher fluences as well because the amount of energy lost by conduction of heat becomes a much greater fraction of the absorbed energy. This theory has been confirmed by histochemically by Hohenleutner et al (52). Thus the depth and the diameter of the vessels in capillary malformations will influence the response to PDL, this without

even considering other factors such as melanin content and the blood flow (8). By using the appropriate wavelength and supplying enough energy within the thermal relaxation time of the target chromophores (oxyhaemoglobin) it is possible to specifically damage the vasculature and the surrounding perivascular tissue (47). Despite findings from our institute indicating that children do not need less PDL sessions, it is often suggested in the literature that children have fewer PDL treatments than adults and that better treatment results are achieved with children (8, 53,54). It has been shown that lesions located in the centrofacial region respond less favorably to PDL (clearing 70.7%) than other head and neck capillary malformations (clearing 82.3%) (55,56). Lesions located in the V 2 dermatome in adults and children respond less favorably than other lesions on the head and neck. Centrofacial regions were defined as lesions located on the medial part of the cheek and the upper cutaneous lip and nose. Lesions located on the periorbital region, the neck and temple responded best to the PDL.

The slight differences in skin thickness observed between different parts of the body, are not sufficient to explain the differences in response on treatment with PDL. Structural characteristics such as the orientation of the dermis, the density of the vessels, nerves, adnexae and fibrous proteins all likely play a role in the response pattern (55). The central part of the face is characterized by embryonic fusion planes and close-set rigid sebaceous follicles embedded in a dense fibrous stroma which may also influence the result (55,57). The angiosome concept (discussed later) may shed some light on this topic.

Fiskerstrand et al examined 30 biopsies of patients with capillary malformations before PDL treatment (58). In 16 patients with a good response the vessels were significantly more superficially located. Poor responders had significantly smaller vessels. They further suggested that vessel diameter mainly determines the color of the capillary malformation. Pink lesions have the smallest diameter vessels and purple lesions the largest. Vessel depth was partly correlated with the color with red lesions being more superficially located than purple and pink lesions. It seems that pink capillary malformations have a poor response to PDL due to the small vessel size and deep location, while red lesions have a better response due to the superficial lesions (58,59). This is in contrast to results from Kane et al (60). They stated that flat, pink capillary malformations cleared the quickest, while red-purple, nodular lesions cleared the slowest. It is thus unclear if clinical characteristics have any predictive value (7). Making use of reflectance spectra for visible light from normal skin and capillary malformation skin, it has been indicated that the redness seen in capillary malformations depends on both the concentration of dermal blood as well as how it is distributed (61). By using a videomicroscope Motley et al, and later Eubanks et al, identified that when lesions had ectasia isolated to the vertical capillary loops (type 1 lesion), the response to treatment was better than when lesions also had ectasia in the horizontal superficial

ring pattern (type 2 lesion) (62,63). Type 3 lesions are a combination of type 1 and 2 and are likely to respond poorly to PDL. By using high-resolution ultrasound Troilius et al have recently shown that the depth of capillary malformations only correlates to some extend to the response of the capillary malformation has to PDL treatment (64).
Overgrowth is often associated with certain forms of CM and may indicate that those lesions have a component outside the dermis. Skeletal overgrowth may occur particularly in the maxillary region (65). There seems to be a correlation between the diameter of the subcutaneous vessels and the diameter of the subdermal vessels (14). Pearl et al have indicated that with the subcutaenous vascular plexus seen in the head and neck area, the larger arteries and concomitant veins were seen every 1 cm, while in the lower extremity they were seen every 8 - 12 cm. This compares to the locations of the most and the least concentration of CM.

Findings have suggested that the pathogenesis of CM is at least partly described by an abnormal neural regulation process (41). It has been postulated that the lack of sympathetic nerves to regulate blood flow is the cause of the progressive vascular ectasia. It has been suggested that the pathology lies in maturation of the cutaneous sympathetic innervation. CM lack not only sympathetic innervation, but also sensory innervation (43). Sensory neuron peptides produce, transport and release neuropeptides at the peripheral site. Substance P is an example of a neuropeptide known to stimulate smooth muscle cell growth. It is thus possible for CM to be a disease related to trophic effects of peripheral nerves. The dilated vessels in the dermis were found to have defective innervation, while other structures in the skin like sweat glands and hair follicles showed normal density of nerve fibers (figure 1). The nerve bundles were often seen to pass the ectatic channels without giving off any branches (43).
The lack of innervation of capillary malformation vessels can result in gradual ectasia of these vessels in response to increased perfusion. This may explain the progress of the disease from childhood to adult age (8). The larger venules have pericytes that are more randomly placed, with the pericytes not interdigitating with as many endothelial cells as in the post-capillary venule. These venules also have valves at the border with the subcutaneous tissue, which could contribute to the vessels becoming ectatic. It seems that capillary malformations are confined to vessels surrounded by pericytes and not smooth muscle cells, but further studies should investigate this. Historical histological studies performed by Miescher and Schnyder have shown that in young persons the vessels in CM had a lake-like distribution in the upper dermis, but that with increasing age the ectasias also involved the deeper dermal plexus and subcutaneous vessels (36,46). Normally bridged fenestrations occur only in venous capillaries. They are thin plates in the shape of discs that occur within the plasma membrane of venous capillary endothelial cells. They represent

areas where molecular exchanges are taking place at an accelerated rate between the circulation and the immediate surrounding tissues. In normal skin they are found in the venous capillaries surrounding sweat glands and hair follicles. There is little literature available about the role these bridged fenestrations play in CM. They are probably caused by increased passage of vesicles through the endothelial cells (34). Increased pressure on the walls by stasis may play a role. The consequence of bridged fenestrations is an increased permeability with perivascular deposition of amorphous exudates, which might influence the PDL result.

It has been suggested that the developing vascular plexus is normally followed by accompanying nerves, based on observations made in the forelimb of quail embryos by Taylor et al (69). Both nerves and blood vessels seem to undergo a highly stereotypic sequence of development. The close spatial relationship between nerves and blood vessels either suggest a high degree of developmental interdependence or shared patterning mechanisms. It is still not clear whether peripheral nerves use the blood vessels as a substrate for path finding in the limb bud or whether neural crest cells guide the developing vascular tree (69,70). The endothelial cells and axonal growth cues are guided by differential adhesivity cues provided by the extracellular matrix (ECM) of the enviroment which they invade, and both may respond in a chemotactic or trophic manner to cues provided by diffusible factors (71,72). The blood vessels and nerves appear to be inhibited to invade the same particular region, including the presumptive dermis (71,73). In the quail forelimb blood vessels are present at day 2,5 (corresponds to 3,5 weeks of human gestation) and that nerves are visible at day 4 (corresponds to 4,5 weeks in humans) (69). The definitive neurovascular anatomical pattern was established by day 7,5 (corresponding to 8 weeks in the human embryo). Martin et al suggest that nerves do not use blood vessels as pathways along which they develop (71). They suggested that nerves and blood vessels follow the same route during embryology, but that they respond independently to the same mescenchymal cues. Recently they made the interesting observation that normal wound healing is impaired in the absence of nerves (74). The mechanisms of invasion by endothelial cells (blood vessels) and axons (peripheral nerves) are similar: both have filopodia, while obstructions in front of the development are dissolved by secreting proteases (71).

Taylor and Palmer introduced the angiosome concept over a decade ago (66). They considered the human body to be composed anatomically of three-dimensional blocks of tissue supplied by particular source arteries. Most of these anatomic territories span between skin and bone, but some are completely submerged beneath the skin surface like the vertebral angiosome in the head and neck (67). In the extremities these angiosomes are arranged in longitudinal sectors,

but in the head and neck the angiosomes are often irregular and sometimes convoluted (68). The 13 angiosomes of the head and neck are supplied by branches of the internal carotid, external carotid and subclavian arteries. Blood supply to the skin follows the connective tissue network. Where tissues are fixed and rigid, the vessels travel within / close to these skin areas, emerging from around the fixed skin margins to subsequently radiate long distances in skin areas where the tissues are mobile (67). In the head and neck the skin of the face is attached in a circle around the skull base, zygomatic arches, orbits and the root of the nose. At the alar base, the anterior border of the masseter muscle and along the lower border of the mandible, the skin is tethered (67). At these fixed skin margins the main skin perforators pierce the fascia from their source arteries. They then radiate in the mobile skin. In the neck the main perforators emerge from source arteries where the skin is attached along the anterior and posterior borders of the sternocleidomastoid muscle, over the anterior border of the trapezius muscle and along the hyoid bone above the clavicula and the sternum below. When we look at the areas previously described as areas not responding so well to PDL treatment like the centrofacial area or the angle of the jaw, these areas seem to correspond well to the areas where the skin is fixed and where the skin perforators are located. This explanation is not applicable everywhere (eg. periorbital and neck) but it should be interesting to describe the areas not responding well to treatment to the angiosomes and to study whether any correlation can be identified.

The hair follicles and sweat glands in CM have a normal vascularity and nervous innervation (sympathetic cholinergic) when compared to the ectatic vessels (43). It has been proven that the reticular vascular plexus in the dermis gives separate lateral tributaries to the sweat glands and hair bulbs (11). By 14 weeks of development the dermis is invaded by the downward invagination of developing epidermal appendages (9). At this stage the two dermal plexusses are also distinguishable. Due to economical reasons the hair follicles have been studied in more detail in sheep than in humans. The primary elements of vascularization at the bursae pili start by day 78 of prenatal development (75). They receive their vascularization from the subepidermal vascularization. El-Bab et al have shown that in sheep the hair papillae only start to receive their vascularizing vessels by the 104'th day of gestation (75). There are separate vessels from the subepidermal vascular plexus supplying the hair papillae.
In humans most hairfollicles have an arrector pili muscle, excluding the follicles on the face (23). Hairfollicles also have sensory innervation; free nerve endings around the hairfollicles. In CM there is decreased innervation to the pericytes in the postcapillary venules, while the innervation of the smooth muscles of the hairfollicels are not affected. Skin appendages eg. sweat glands and sebaceous glands are ectodermal in origin and are innervated by cholinergic sympathetic

fibers, while dermal blood vessels are mesodermal from origin and innervated by sympathetic adrenergic fibres (23). It is possible that the peri-endothelial support cells release growth factors to attract the axons that differ between these groups. It is likely that by gaining information about the difference in innervation of pericytes and smooth muscle cells, that more information about CM will also be gained. It seems that the pathologic process involved in capillary malformations occurs before the invagination of the ectodermal appendages (week 14), because the innervation of structures (eg. smooth muscle cell of hairfollicles) developing after this time seem normal. There seems to be a "window of opportunity" for the capillary malformations to occur if the developing nervous system is susceptible (by genetic factors?) to infections, lack of certain vitamins, mechanical or other factors. With more than 80% of capillary malformations occurring on the face, it seems that there is a specific period when the developing neurons in the face are more susceptible resulting in an "abnormal" development.

The first description of familial multiple nevi flammei was presented in 1949 by Shelley and Livingood (76). Since then there have been a few descriptions of families with multiple capillary malformations, suggesting a possible autosomal dominant inheritance with variable expression (45). Although a hereditary basis underlaying the development of capillary malformations is suggested, they clearly represent a minority of the total cases of capillary malformations seen. It is likely that genes implicated in these familial cases may be involved somatically in the more common sporadic cases. Recently a locus for an autosomal dominant disorder in a three-generation family has been mapped to chromosome 5q13-22 (77). It has been proven that the vascular endothelium and the surrounding support cell reciprocally influence each other, and it is likely that any disruption in the cellular physiology of either cell type can result in dysfunction (78). Although the candidate interval (5q13-22) at present is large, further studies and refinement of this region and mutation analysis of the described genes will determine if any of these loci are responsible for the phenotype.

Recent papers have informed us about the role pericytes play in the control of the developing vasculature (78,79). There seems to be a delicate reciprocal interaction between pericytes and endothelial cells. The endothelial cell further has several synthetic properties which are important in the endothelium's interaction with vasoactive amines of mast cells and nerves (80). A defective endothelial function may result in a defective neurite outgrowth. Although we have seen that endothelial cells are histologically normal in CM's, we know very little about their protein synthesizing function which may in theory influence pericyte development. During vasculogenesis endothelial cells are derived from mesoderm. Embryonic data suggest that these initial enothelial

tubes may be responsible for the subsequent development of the peri-endothelial support cells (78). The exact mechanisms by which endothelial cells recruit pericytes during vessel formation is unclear. Since there appears to be a tight control between the number of endothelial cells and mural cells, it is likely that multiple sites of control exist (78,79). Pericytes have more points of contact on endothelial cells than smooth muscle cells with the subsequent chance of problems arising at the pericyte site being bigger (11). Potential regulators include soluble factors acting in a paracrine and/or autocrine fashion, gap junctions, adhesion molecules, mechanical forces secondary to blood flow and blood pressure, as well as homotypic (enothelial cell - endothelial cell, mural cell - mural cell) and heterotypic (enothelial cell-mural cell) cell interactions. The anatomy of the smooth muscle cell and pericytes in relation to the endothelial is such, that decreased innervation in both will cause dilatation much faster on the venous side than on the arterial side.

Endothelial cells recruit mesenchymal cells via the elaboration of factors such as platelet-derived growth factor (PDGF), heparin-binding epidermal growth factor (HB-EGF) or basic fibroblast growth factor (bFGF) (78,80) (figure 2). The mural cell precursors migrate to the endothelial cells where they make contact with each other. Interactions between these cells lead to activation of transforming growth factor-beta 1 (TGF-b 1) (80). TGF-b 1 induces the mesenchymal cells to express pericyte markers and inhibits endothelial cell proliferation. Growing endothelial cells synthesize PDGF and HB-EGF which are potent stimulators of pericyte proliferation (83). TGF-b 1 is located in our candidate gene region (5q13-22), and pericyte growth has been shown to be inhibited by TGF-b (83). The tyrosine kinase receptor, TIE-2 binds the growth factor angiopoietin-1 and is specifically expressed in vascular endothelial cells (84). Angiopoietin-1 mutant embryos also have abnormal vasculature architecture due to the failure of endothelial cells to recruit pericyte and smooth muscle cell precursors to the developing vessel wall (85). Other possible genes located in the 5q13-22 region are the transcription factor myocyte enhancer factor-2 C (MEF2C), FER gene and the EFNA5 protein. In a study performed by Lin et al targeted deletions of the mouse MEF2C gene resulted in severe vascular abnormalities (86). Endothelial cells were present and able to differentiate but failed to organize normally in a vascular plexus. Peri-endothelial support cells failed to differentiate in the mutant embryos. The vascular defects in MEF2C mutant embryos resemble those in mouse mutants lacking VEGF and Flt-1 and suggest that MEF2C is required either directly or indirectly for VEGF signaling during vasculogenesis. Drake et al have proven that the deletion of the MEF2C gene resulted in defects that were accompanied by a reduction in angiopoietin-1 and VEGF mRNA production, indicating that MEF2C is required for expression of important endothelial-directed cytokines (87).

Although many factors have been identified that influence the nerve growth cone growth, it is still unclear how the growth direction is specified (69). Contact guidance mechanisms have to operate parallel with neurotropism, and physical cues in the pathway will probably also play a role (23). There seem to be a variety of attractive and repulsive molecules expressed within the extracellular tissues as substrate bound or diffusible gradients (24-27). The possible role of contact inhibition in developmental processes has also been investigated. Culture experiments have indicated that when chick peripheral sensory neurons are confronted, their mobility is inhibited and that their growth cone will collapse (23,88). F-actin accumulates in the lamellae and at the sites of contact with the target cells (28). It has been suggested that the increased F-actin concentration may be responsible for the attractive guidance. During development both motorneuron and sensory axons choose the correct peripheral nerves from the onset (88,90). It also appears that the motorneuron axons influence the pathfinding of the sensory axons (89). It is suggested that a target-derived growth factor causes axons to grow towards the source of the factor (91). In experiments in which muscle-less limbs were created, muscle nerves did not form (92). Martin et al have also proven that the early removal of the skin results in the absence of the cutaneous nerves that normally supply the denuded region (71).

Several neurotrophic factors have been identified that influence the turnover of vertebrate neurons and only some will be mentioned (24-27). Nerve growth factor (NGF) has been identified to have an in vitro and in vivo influence on nerve cell growth (93). Antibodies to NGF caused the death of neuronal subsets at times when they reached their peripheral targets, and added NGF rescued the neurons that would otherwise die. Several other neurotrophins have been identified of which neurotrophin-3 and NT-4/5 have been identified by molecular cloning (94,95). NT-3 has been shown to be essential for the normal development of atria, ventricles and cardiac outflow tracts, suggesting the wider function of this neurotrophin (96). Other growth factors to influence the growth and survival of neurons include the *fibroblast growth factors* (FGF) (97). There is increasing evidence that the neurite outgrowth also depends on the presence of electric fields, originating in the neural tube itself and in the skin. McCaig et al have proven that in vitro the growth cone behavior is severely influenced by the presence of electric fields (98). It is subsequently possible that neurotrophins and endogenous electric fields also interact in vivio. The EFNA 5 protein is also located in the candidate area (5q13-22) and it has been suggested that this protein may be involved in axon guidance (99). Arregui et al have further indicated that the FER protein is involved in neurite outgrowth (100). Also located in our candidate region on chromosome 5 are neurogenin (Ngn) and NeuroD. Both are able to activate neurogenesis, but it is suggested that Ngn expression precedes that of NeuroD (review, 101).

It is possible that peri-endothelial support cells release mediators / growth factors that causes

axons to grow towards these pericytes. A defective release could subsequently cause a defective nervous innervation. Further studies on CM's should also be directed at the innervation of the dermal vasculature. Although histological studies have demonstrated that the ectatic part involved in CM is confined to the venular part, we know little about the real innervation of the arteriolar part and further research will have to clarify this aspect.

Conclusions

Since the pathology involved in CM seems to be located in the post-capillary venules and small venules, it seems that our definition of port-wine stains being capillary malformations is wrong. One important pathological characteristic detected in "capillary malformations" so far is the decreased neural innervation. It thus seems legible to describe these lesions as a neural malformation as well. Maybe the description of port-wine stains being a venular / neural malformation is the best description to date. It is even possible that all the vessel deformation is secondary to the neurological pathology, and that port-wine stains are pure neural malformations with vascular dilatation being secondary. Only time will tell. Since the biological classification of vascular malformations by Mulliken and Glowacki has been of enormous value to bring at least some clarity in these issues, we suggest continuing using this system until a new universally accepted classification will arise from further histological and molecular studies. With the common developments of the vascular system, nervous system and lymphatic system it seems logical that future investigations on capillary malformations will concentrate on the "genesis" as a whole and not only on vasculogenesis and angiogenesis.

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