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Arterial dysgenesis and limb defects: clinical and experimental examples

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Abstract

Limb malformations are amongst the most common and visible birth effects. Causes have been purported to include genetic aberrations as well as teratogens, such as thalidomide. Here we review the evidence for vascular disruption in the genesis of limb malformations through abnormal arterial transitioning and from events such as amniocentesis, uterine constriction, and through teratogen exposure. We use several clinical and experimental examples and highlight the need to understand more about the role the vascular system plays in the molecular mechanisms underpinning normal limb development.

Blood vessel formation and development

Blood vessels are essential for embryogenesis as well as for normal physiological function throughout life. In the embryo the first primitive vessels are formed by vasculogenesis, where endothelial cell precursors congregate and form vascular tubes for blood cells to pass through. This initial network is expanded upon through angiogenesis, where new vessels form through the proliferation and migration of endothelial cells and vascularize previously avascular tissues (Figure 1) [1]. Many molecules are involved in angiogenesis and one of the main triggers of angiogenesis is hypoxia which stimulates the expression of Vascular Endothelial Growth Factor to cause the proliferation and migration of endothelial cells into previously avascular tissue [1-3]. In the rapidly developing early embryo, angiogenesis occurs in the majority of tissues to ensure good oxygen and nutrient supply and the removal of waste products. Vessels can recruit a smooth muscle coating which stabilizes the vessel and also maintains blood pressure, and until the loss of the smooth muscle coat, the vessel then undergoes quiescence (Figure 1).

Limb development and vascularisation of the developing limb

In the developing human embryo, the upper limbs form around day 26 (approx. week 3.7) after fertilization followed 24hrs later by the lower limbs [4]. The limbs grow out from the body under the control of specialized signaling regions, specifically the Zone of Polarising Activity (ZPA) and the Apical Ectodermal Ridge (AER). These two regions in particular signal to the mesenchyme to induce mesenchymal cell proliferation and limb outgrowth as well as control differentiation of the bones and tissues of the limbs (Figure 2A) [1, 5]. As the limbs grow out from the body, the multitude of tissues including muscle, tendon, nerve and bones of the limb differentiates in a proximal to distal manner, that is the humerus/femur is specified and laid down before the radius/fibula and ulna/tibia and finally the digits (Figure 2A). The vessel patterns change accordingly (Figure 2B). Vessel regression in the areas where the long bones and the future digits form occurs and this process also marks a change in the vessel pattern to the adult state (Figure 2B). By around day 56 (approx. week 8) human limbs are fully complete with fingers/toes etc.

The molecular and morphological events that underlie limb development and some limb malformations are becoming clearer and have largely been ascertained from animal models particularly the chicken and mouse (Figure 2A) [5-8]. Yet, the role of the rapidly changing vasculature in the molecular and mechanistic events underlying limb development are largely unknown.

Most of our understanding of limb vascularization has come from animal model systems and studies in human embryos/fetuses from the beginning of the 20th century [9, 10]. There are some

differences in the arterial vascularization of the upper and lower limbs. Following the initiation of limb development, the main artery supplying the upper limb buds is the subclavian artery and from this a capillary network rapidly forms (via angiogenesis) throughout the outgrowing limb bud (Figure 2B) [1, 3, 11]. As limb development proceeds the subclavian artery name changes to become the brachial artery in the upper limb. The brachial artery itself later gives rise to the ulnar and radial arteries in the forearms. In the lower limbs, arterial vascularization also proceeds through angiogenesis but is a little more complicated. Initially primitive arteries from the umbilical artery traverse the forming limb bud as the axial and external iliac arteries. The external iliac artery eventually becomes the femoral artery, supplying the upper leg. The axial artery traverses the outgrowing leg bud to supply the distal regions of the leg bud and further divides into the fibular (peroneal) arteries (Figure 3). As the leg bud develops, there is further splitting of the femoral artery to produce further arterial supply to the forming bones of the limbs (Figure 3).

In addition, there is also vessel regression as well as formation of composite arteries. The fibular (peroneal artery), which primarily provides the blood supply to the fibula, is one such composite artery; it contains four elements/segments of three earlier embryonic arteries, which have undergone repeated rounds of angiogenesis and stabilization to achieve the adult configuration (Figure 3) [9, 10, 12].

Embryonic to adult arterial transition

Elegant analyses in human embryos have demonstrated that, in early limb buds, a capillary network is evident. As development proceeds, the vascular pattern differentiates into defined vessels initially in the proximal tissue and then finally in the distal tissue, where the digits form last (Figure 3) [9, 10, 13-16]. Approximately five weeks after fertilization, single axial arteries of both the upper and lower extremities begin the transition to more complex arterial patterns (Figure 3). The arterial transitions coincide with the long bone transitions from mesenchymal primordia (anlage) to chondral and then osseous structures. Arterial alterations occur prior to and during the organization of the muscles, tendons and nerves but after specification/patterning of the limb is completed [17]. The upper and lower human limb embryonic arterial transitions have been completed prior to the 8^{th} week of development, resulting in the adult arterial pattern (Figure 3) [9, 10 13, 14, 18].

In the chicken embryo the transition of the arterial pattern of the limbs from embryonic to mature state has occurred by approximately day 8 of development. As in the the human embryo, the arterial transitions occur during and after the specification and patterning of the bony elements has occurred. The arterial transition likely allows the bones of the limb to form correctly and in the correct positions [11, 19]. Much of our understanding of the processes of limb vascularization has come from studying chicken limb development [1, 2, 11, 19]. Unlike vessels in other regions of the chicken embryo, the early developing limb vascular network remains devoid of vascular smooth muscle cells, which lend protection and support to the vessels. Not until relatively late in limb development, after the adult vascular pattern has started to become established are vascular smooth muscle cells seen upon limb vessels (Figure 2B) [11, 20]. Indeed, the brachial artery starts to recruit smooth muscle first, in chicken embryos from around HH St25 (E5; about 2.5 days after limb development has started, which is relatively late in development as the limbs buds are well advanced in outgrowth) (Figure 2A). Other vessels only start recruiting smooth muscle much later in development [11, 20]. By comparison, vessels in

other regions of the embryo, including the head, contain vascular smooth muscle coats by these stages [1, 20, 21]. Smooth muscle protects the vessel and prevents angiogenesis. In order for angiogenesis to re-occur, the smooth muscle coat needs to be lost [1]. Since the developing limbs are rapidly growing and changing, the developing limb requires a vasculature that adapts to the changing environment quickly; thus, a smooth muscle coat would be an obstacle to early development. Absence of the smooth muscle coat presumably permits rapid changes in limb outgrowth to occur. However, this also makes the vessels more susceptible to potential injury and damage [1, 20].

Embryonic to adult arterial transition failure and limb defects

Whilst there may be a variation of limb arterial branching patterns in up to 20% of normal adult humans [13], a failure of correct vessel patterning or a failure of the embryo to adult vascular transition has been associated with limb defects in humans [12, 22-27] and in animal models [20, 28-31].

Indeed, through radiographic and pathologic analyses of dysmorphic human lower limbs, the combined associations of reduction or absence of the proximal femur [22, 32], the fibula [33] and midline metatarsals [23] suggests the possibility of a temporal and anatomic linkage of the skeletal changes to failure of embryonic arterial transition, when the long bones are first forming [9, 10, 12, 15, 18]. Arteriography of a series of such affected limbs further revealed abnormal arterial patterns, which have demonstrated persistence of primitive embryologic arteries, absence of one of the normal adult arteries and failure of formation of the plantar arch of the foot [12, 32-35].

For example, some cases of congenital fibular deficiency (Figure 4A-C) [12] and radial aplasia [36] have been described indicating the vascular pattern of the limbs appears to be intermediate between an embryonic and adult state, suggesting the long bones might not form or might form incorrectly due to the misplacement or mis-positioning of the vessels. Furthermore, the foot is typically supplied with three arteries, the posterior tibial, anterior tibial and the fibular (peroneal) (Figure 3B). In the debilitating condition clubfoot, where the foot points downward, toes are turned inward and the bottom of the foot, that would normally be walked on is twisted inward, the anterior tibial artery is most consistently reduced in length in the most severely deformed limbs and absent in the distal foot. The lack of alternative arterial pathways has been proposed to explain why the foot ‘clubs’ in such cases (Figure 4D) [17, 24, 35, 37, 38]. In contrast, in cases of the congenital vertical talus deformity, where the foot is pointed upwards, rigidly, causing the toes to point up and out with loss of the arch of the foot, the posterior tibial artery is most consistently significantly reduced or sometimes missing [24, 34, 35, 38]. Similarly, arteriography of the limbs of children with proximal femoral focal deficiency (PFFD; congenitally short limb), where the femur may be significantly reduced in length and can vary morphologically from just a distal remnant of the femur to an almost normal sized femur, has revealed distinctly consistent arterial changes in a majority of the affected limbs [32]. The changes may include reduction of the diameter and length of the femoral arteries and/or preservation of the primitive sciatic (ischiadic) artery [32].

An abnormal arterial pattern in the developing limb could put the limb at risk for subsequent malformation as, for example, there may be a reduced potential for collateral vascular circulation

resulting in tissue damage or loss. Conditions that antagonise blood flow through the remaining arteries could then lead to tissue damage. This tissue damage could, in turn, interfere with the developmental specification or differentiation of limb structures, leading to bony and/or soft tissue abnormalities [12, 17, 24, 26, 33, 37].

Timing of arterial dysgenesis

Limb development and arterial transition occur between 5 and 8 weeks of human embryonic development. This coincides with several periods of very rapid growth, considering that the crown rump length of 10 mm at 5½ weeks grows to 20 mm at 7 weeks, thus effectively doubling in length over a ten day period. The next most significant period of growth occurs between 7 and 8 weeks, over a seven day period, when the embryo enlarges from a crown rump length of 20 mm to a crown rump length of 30 mm. That is a 50% enlargement over a seven day period [4, 39]. During these times, the outgrowth of the upper and lower extremities is rapidly progressing, after the specification of the limbs has begun and while the embryonic arterial alterations are occurring [4, 12, 17, 37, 39]. Since the critical period for the initiation of bone development, beginning as mesenchymal condensations, occurs subsequent to the fifth week of embryonic life, coinciding with the time that the adult arterial pattern of vessels is emerging, it is possible that both the arterial and skeletal systems simultaneously are mutually vulnerable to teratogenic insults. Thus, the maldevelopment of the bones and the embryonic arterial pattern may be intimately related [23, 33]. The evidence of embryonic arterial dysgenesis in association with a variety of skeletal malformations and deletions has provided an explanation for the timing, pathogenesis and distribution of some limb malformations.

Indeed, comparisons of the estimates of the upper femoral vs. lower leg dysmorphologies suggest that the estimated timings of the respective arterial embryonic dysgeneses are associated with the skeletal dysmorphologies observed. These findings are consistent with a progressively more distal model of arterial development, after the completion of limb specification [9, 10, 12, 17, 18, 23, 32, 37]. Indeed, the critical time for connection between the femoral artery and the sciatic (ischiadic) artery in the femoral region of the lower extremity has been estimated to occur between the 12 and 14 mm stages of embryonic development (approximately week 6) (Figure 3B) [9, 10, 32]; the critical time for arterial changes of the more distal part of the lower extremity in the tibial and fibular region has been estimated to occur between the 19 and 22 mm stages of embryonic development (approximately week 7) (Figure 3B) [12, 23]. Hence many limb malformations may be interpreted as post-specification events, which occur after the bony elements have been specified but which are then damaged, diminished or radiologically absent through failure or loss of appropriate vascularization resulting in smaller or missing bones. More elementally, such careful dissections and/or detailed imaging of human malformed limbs has revealed that such limb malformations possess an arterial pattern typical of embryonic stages and/or missing or misplaced vessels consistent with a failure of correct vascularization of the involved bone [12, 17, 22, 23, 32, 33, 34, 35, 37]. Failure of arteries to develop to vascularize the condensing cartilage precursor of the long bones will have intuitively resulted in the loss or reduction in the length of the long bone (Figure 4). For example, late arterial invasion of the tibia has been linked to the shortening and bending of the bone due to a delayed formation of the perichrondral ring, which encases the diaphysis (growth plate) of the long bones [33, 40]. Likewise, in limbs with PFFD, a significantly reduced arterial supply to the femur has been identified that is linked to femoral shortening [32]. Further, the distal 2/3 of the fibula is supplied

by the fibular (peroneal) artery, whilst the proximal 1/3 is supplied by the anterior tibial artery; thus, loss of either one of these vessels during fibula differentiation might result in fibula deficiency or diminution [9, 10, 12]. Finally, in the upper limb, loss of the radial artery has been purportedly linked to the underlying cause of radial aplasia (loss of the radius) [36].

The failure of arteries to vascularize their respective bony precursors could explain many limb abnormalities including long bone loss, long bone bending as well as changes to digital patterns due to the loss of or shortening of the long bones. Given the arterial transition occurs as the bones have been specified and are starting to condense, the damage is thus, post-specification.

What could cause the blood vessels to not form correctly, or fail to transit to the adult state or simply mispattern to cause limb anomalies?

Molecular changes resulting in vascular disruption

We know there are many human (and animal) limb defects associated with genetic mutations and signaling changes [6, 7]; perhaps these are caused in early development before specification of the bony elements or perhaps the resulting signaling changes alters vascularization of the developing limbs and the arterial transition and branching events? For example, in some patients with Holt-Oram Syndrome, a condition caused by a mutation in the gene Tbx5, upper limb reduction ranging in severity from loss of the thumb to phocomelia is seen [41]. A reduced peripheral vasculature in the affected limb/s has been reported in some patients [42]. Whether this is secondary to the limb malformation or could actually be responsible is unclear.

Furthermore, recent work in animal models does suggest that failure of the forming bones to receive the correct vascularization at the right time does result in bone outgrowth failure. For example, a study where the neurotrophic factor, TrkA, essential to direct innervation and promote vascularization of the outgrowing bone, when knocked out in mouse embryos result in smaller bones in the limb due to a failure of vascularization of the bone [43]. Moreover, molecular signals essential for blood vessel formation and identity, such as the Ephrins, members of the Notch signaling pathway and members of the VEGF signaling pathway, are also essential for proper bone vascularisation [44, 45]. Thus, if molecular signals fail to induce correct vascularization of the forming bones, those bones do not form correctly.

Trauma during pregnancy leading to arterial dysgenesis or vascular disruption

-Occlusion of vessels

Pregnancy significantly increases the chances of thrombotic events, frequently due to changes in blood coagulation whilst pregnancy is ongoing to prevent excess blood loss during pregnancy and childbirth [46]. However, this could also increase the chance of thrombotic events in the embryo. Some studies have proposed that vessel occlusion in the embryo through thrombi through the placenta or from the heart or injured tissue could result in tissue damage leading to malformation in humans [25, 27, 47, 48] and in animals [29, 31, 49].

Indeed, one study in chicken embryos demonstrated that following occlusion of the carotid arteries severe brain anomalies such as anencephaly could be induced [49]. The study simply demonstrates that the carotid arteries were missing and tissue distal to the occlusion never formed [49]. This is an example of downstream tissue loss after vascular obstruction.

-Hemorrhage

Hemorrhage early in embryogenesis has also been linked in the genesis of malformations [50-52]. Indeed localized hematomas have been observed in spontaneously aborted human fetuses, without determination that the hematoma was the cause of the fetal death [53]. In addition focal hematomas or hemangiomas have been observed on the forehead of thalidomide damaged babies [51, 54, 55]. Hemorrhage induction in rat embryos through injection of drugs like aspirin [56] or through amniocentesis [57] has been shown to result in limb defects in rat fetuses, where increased uterine contractions in a smaller uterine space are purported to have caused damage, specifically via hemorrhaging, in the forming hand and footplates of the embryos; this can result in constriction bands, loss of digits and acrosyndactyly [57]. Similarly, in chicken embryos, after vessels were physically injured resulting in hemorrhage, embryonic development was impaired [52]. The hemorrhage most likely initiated a failure of differentiation of mesoderm and interfered with signaling processes, resulting in such dysmorphologies.

These events can result from external influences such as drug exposure (see next section) or through trauma, even from procedures that are seemingly simple. As, for example, amniocentesis, a method of analysis of the chromosome karyotype of the embryo/fetus involves aspirating amniotic fluid. It can lead to uterine constriction due to the reduction in amniotic fluid volume, resulting in damage to the limbs. Moreover, chorionic villus sampling, also used as a prenatal diagnostic tool, has been linked to terminal transverse limb malformations through disruptive vascular events, through hemorrhaging or cell death/tissue loss and/or hemangioma formation [58]. Although these events rarely occur, and most procedures occur without untoward consequences, these studies do highlight the need to monitor pregnancies without reduction of amniotic fluid to minimise damage to the extremities if uterine constriction does occur. In addition, placental damage itself from vascular insufficiency or from trauma to the uterus could result in thrombi that occlude vessels or cause hemorraging in the placenta restricting blood flow to the embryo potentially resulting in growth retardation of embryonic tissues [58].

Drugs taken during pregnancy leading to vascular disruption

-Thalidomide

Of course, environmental influences can also cause vascular anomalies in embryos, these include exposure to drugs like phenytoin, cocaine and valproate [58-60]. Perhaps the most infamous are thalidomide and more recently, misoprostol.

Thalidomide has been shown to be antiangiogenic in rabbit and rodent cornea assays [61, 62] and in chicken and zebrafish embryos [21, 63-66]. Indeed, the drugs antiangiogenic action is widely viewed as part of the key in the drugs teratogenic actions where the drug may target angiogenic, smooth muscle negative vessels resulting in their loss, or preventing them to transition to an adult state as well as then resulting in changes in molecular signaling pathways and ultimately tissue loss/malformation [67- 69].

It is of further interest to note that several studies in thalidomide survivors (children and adult) with limb anomalies, have reported cardiovascular malformations and vascular changes and/or missing vessels in the limbs which might help explain the damage [70, 71].

The hypothesis is that thalidomide induces vessel loss or may interfere with the vascular transition which could lead to localized cell death resulting in missing or lost tissue, resulting in bony elements being shorter or lost [67, 68]. As the time sensitive window of thalidomide embryopathy is day 20-36 after fertilization (day 34-50 after the last menstrual cycle), when vascular transition of the limbs is occurring and when proximal bony elements are condensing and starting to differentiate (Figure 2A), inhibition of vascularization could well result in the deficiency of or shortening of the forming long bones which could vary depending on the arterial alterations occurring at that time [67, 68].

-*Misoprostol*

Misoprostol is a synthetic analog of Prostaglandin E1 and used to prevent gastric side effects caused by nonsteroidal anti-inflammatory drugs. It also causes endometrial bleeding and uterine contractions and so has been used as an abortificient, particularly in Brazil [58, 72]. Sadly, it has been shown to cause birth defects in children when the drug was used improperly [58]. The injuries observed in such affected infants appears similar to the damage seen in thalidomide damaged children [73] with limbs, facial and internal organs all affected. Moreover, the drug is thought to cause vascular disruption in tissues likely due to increased uterine contractions that Misoprostol induces (as part of the abortifacient process) resulting in hypoperfusion of the embryo/fetus and blood entrapment/hemorrhaging [58, 74, 75].

Several other agents, including Valproic acid, can also cause vascular anomalies during embryonic development. In chicken embryos valproate exposure produces a range of defects similar to those in humans including neural and cardiac defects [76] and limb reduction defects [77]. Furthermore, recent work demonstrated that popular anti-cancer antiangiogenic agents, including Sunitinib, Sorafenib, Everolimus are also teratogenic and can cause a range of defects including limb anomalies [78].

Conclusion

Blood vessels in the developing embryonic limbs change rapidly to accommodate the outgrowth of the limbs and the differentiation processes needed to allow the complex combinations of tissues to form. The morphology of the bony pattern of the limbs evolves as vascular pattern is transforming to a mature, adult-like state. Noxious events which prevent the proper arterial transitioning, can prevent vessels from correctly invading the forming precursors of the bones resulting in a variety of defects. Similarly, injury resulting from hemorrhage, uterine constriction, physical damage, drug exposure, molecular signalling failure or interference could also result in such vascular changes. Such loss or inhibition of vessels or disruption of blood flow results in poorly perfused tissues and bones leading to deficiency and/or malformation. As arterial transition and bone formation are occurring at similar time points in embryonic development, such vascular damage could explain a variety of limb anomalies.

Our understanding of the normal mechanisms underlying and controlling angiogenesis and how the developing vasculature influences normal limb development, particularly the underlying molecular signaling events, remains unclear. Indeed, recent models of pattern formation in limb development have seemed solely focused on molecular mechanisms [8, 79, 80] yet largely ignore

the developing vasculature of the limbs which clearly fulfills an essential role in limb development.

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Figure Legends

Figure 1

Vasculogenesis and Angiogenesis

Endothelial cell precursors coalesce to form a clump of cells which then form a lumen to form the primitive vessels in a process termed **vasculogenesis** – this is the de novo formation of the first vessels. The vessels undergo **angiogenesis** following signal stimulation which result in endothelial cell proliferation and migration. Once vessels have reached their target they can recruit smooth muscle cells to become stable and to mature. In order to undergo further angiogenesis the vascular smooth muscle coat needs to be shed. Some of the signalling molecules involved in angiogenesis and vessel maintenance are detailed.

Figure reproduced with permission from N. Vargesson, Vascularisation of the developing chick limb bud: role of the TGFbeta signalling pathway. J. Anat. 202 (2003) 93-103. [1]

Figure 2

Limb development and limb vascularisation

A.Generalised schematic of limb development. In the early limb bud Fgf8 is released from the the apical ectodermal ridge (AER) and signals to the zone of polarising activity (ZPA) to produce Shh which then establishes and maintains a feedback loop to allow mesenchymal cell proliferation and limb bud outgrowth. As limb development proceeds differentiation of the bones of the limb starts in a proximal to distal manner with the humerus forming first. The AER-ZPA feedback loop is maintained and induces many other signals involved in limb formation and patterning including Hox genes which also play a role in patterning the different bony elements.

B.Vascularisation of the developing chicken embryo forelimb. Upper panels are ink injected limb buds at different stages of development to highlight the forming and rapidly changing vasculature. The numbers refer to the stage of development of the embryo: 18 = day 2.5; 23 = day 4; 26 = day 5; 30 = day 7. The limb bud grows out rapidly and the vasculture changes rapidly to accommodate the outgrowth. Arrows demarcate the avascular zone between the angiogenic vessels and the limb tip. Bottom panel are confocal microscope images of limbs at the equivalent stages as the corresponding upper panel showing an antibody stain for vascular smooth muscle actin. This highlights that the subclavian artery is the first and only artery to recruit smooth muscle at around day 5 of development and by day 7 of development, the whole of the subclavian artery and the proximal parts of the radial and ulnar arteries have smooth muscle but very few other vessels are smooth muscle positive at this point. The recruitment of smooth muscle to the subclavian artery coincides with the visualisation of the humerus proximally. The recruitment of smooth muscle to the radial and ulnar arteries coincides with the appearance of the radius and ulna condensations. Vascular smooth muscle negative vessels are more angiogenic and permit rapid change to accommodate the outgrowing limb bud, however

they are also weaker and more susceptible to teratogenic insults. The black asterisk in the handplate denotes vessel regression in the future areas where the digits will condense and form.

Figure A reproduced with modification from N. Vargesson, Thalidomide-induced teratogenesis: history and mechanisms. Birth Defects Res C Embryo Today. 105 (2015) 140-156. [68]

Figure B reproduced with permission from N. Vargesson, Vascularisation of the developing chick limb bud: role of the TGFbeta signalling pathway. J. Anat. 202 (2003) 93-103. [1]

Figure 3

Embryo to adult arterial transition

A Upper limb arterial transition. The vessel capillary plexus network seen in the very early limb bud around Stage 12 (30 days) disappears and differentiates into the adult pattern following the appearance of the skeletal elements.

Stage's refer to the Carnegie staging system of human embryo development (O'Rahilly and Muller, 1987). Stage 12, 30 days (approx. 4.3 weeks); Stage 13, 32 days (approx. 4.6 weeks); Stage 14, 33 days (approx. 4.7 weeks); Stage 15, 36 days (approx. 5.1 weeks); Stage 17, 41 days (approx. 5.9 weeks); Stage 18, 44 days (approx. 6.3 weeks) and Stage 21, 51 days (approx. 7.3 weeks).

s, subclavian artery; a, axillary artery; b, brachial artery; r, radial artery; u, ulnar artery; ai, anterior interosseous; H, humerus; U, ulna; R, radius.

B Lower limb arterial transition. 12mm stage (approx. 5.4 weeks), 14mm stage (approx 5.7 weeks), 18mm stage (approx. 6.3 days), 22mm stage (approx. 6.8 weeks), Adult stage (after 7th week).

12mm stage of development a single-axis artery traverses the leg bud. By 14mm two arteries (TP and PPS) originate posteriorly and the RPC branches anteriorly off the Interossea artery. By 18mm the anterior tibial artery develops as a continuation of the RPC. A branch (the RC) develops off the PPS and anastomoses with the interossea artery. Five arteries now traverse the forming leg. At 22mm stage of development there is regression of the distal part of the peronea posterior superficialis and of the proximal part of the interossea artery. Note the presence of the fibular (peroneal) artery (PE) which has formed from parts of the Axial, PPS, RC arteries. By the 7^{th} week the adult pattern has emerged with the regression of the popliteal profunda.

Embryonic arteries: PP popliteal profunda; PS popliteal superficialis; PPS peronea posterior superficialis; I interossea; RC ramus communicans inferior; RPC ramus perforans cruris; IS ischiatic; TP tibialis posterior superficailis; P popliteal; PT posterior tibial; PE peroneal; AT anterior tibial; RT recurrent tibial.

Muscles: MP popliteus; MTP tibilis posterior; MFH flexor halluces.

Figure A reproduced with permission from M. Rodriguez-Niedenfuhr, G. J. Burton, J. Deu, J. R. Sanudo, Development of the arterial pattern in the upper limb of staged human embryos: normal development and anatomic variations. J. Anat. 199 (2001) 407-417. [13]

Figure B reproduced with permission from E. M. Levinsohn, D. R. Hootnick, D. S. Packard Jr. Consistent arterial abnormalities associated with a variety of congenital malformations of the human lower limb. Invest Radiol. 26 (1991) 364-373. [12]

Figure 4

Altered/missing arterial patterns in legs with abnormalities

Drawings of arterial patterns in A. Normal Limb; B. Limb with skeletal dysplasia with abnormally short fibula – the anterior tibial and fibular (peroneal) arteries are absent and functional embryonic arteries (I, PRS, RC) remain; C. Limb with skeletal dysplasia and absent fibula with midline metatarsal dysplasia. Anterior tibial and fibular (peroneal) arteries are absent. Functional embryonic arteries (I, RC) remain; D. Clubfoot, the anterior tibial artery is reduced. A functional embryonic artery (PPS) is present.

These drawings are summary diagrams made following dissection of human tissues to analyse the vessel pattern changes and the causation of the deformity [12, 17, 23, 33, 37].

Figure A-D from D. R. Hootnick, D. S. Packard Jr, E. M. Levinsohn, R. Constantine, Postoperative necrosis in Clubfoot: recent findings and review. Orthopedics International Edition 1992; 1 (1): 48-68. Reprinted with permission from SLACK Incorporated [81]

Figure 1

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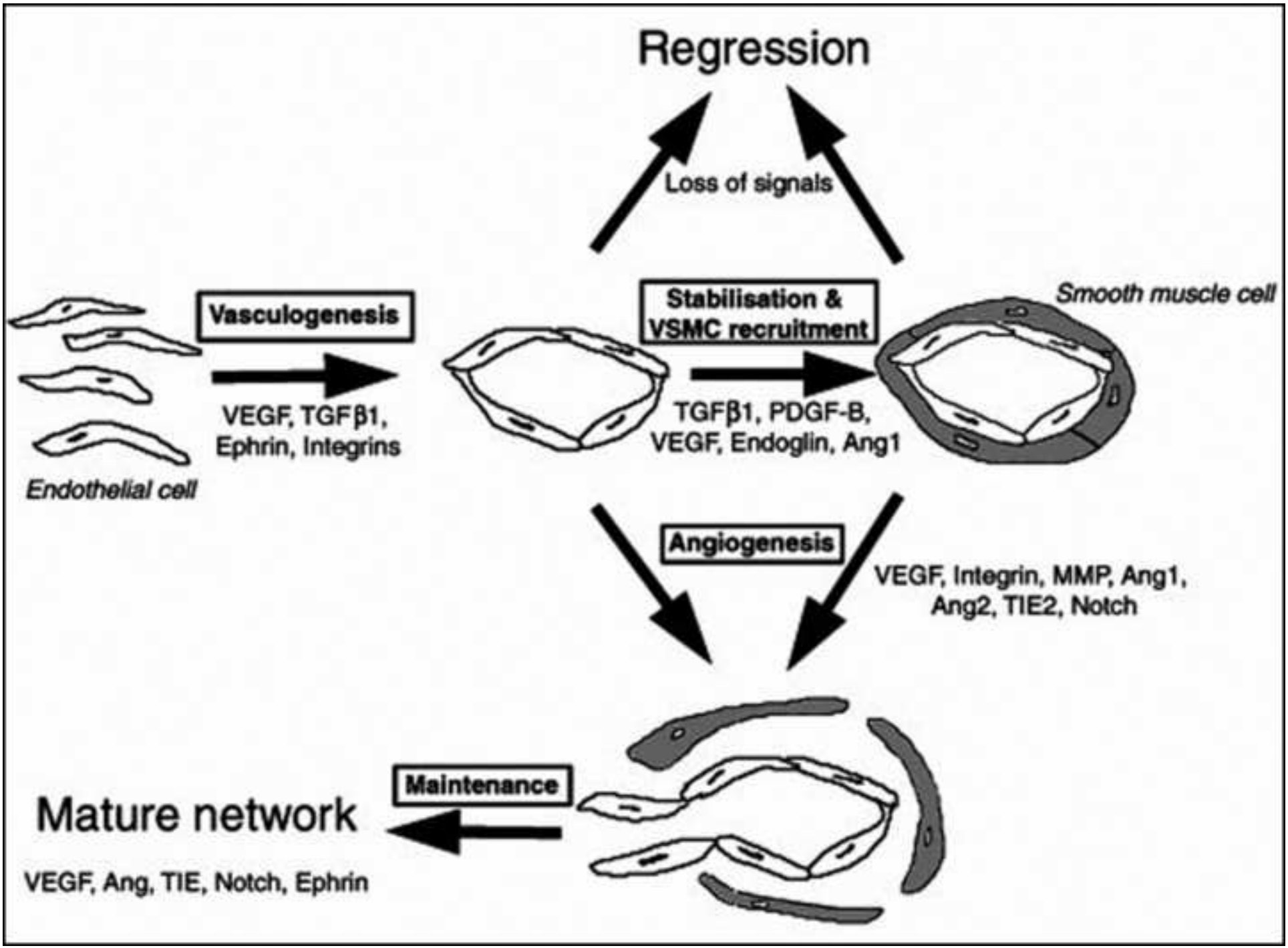

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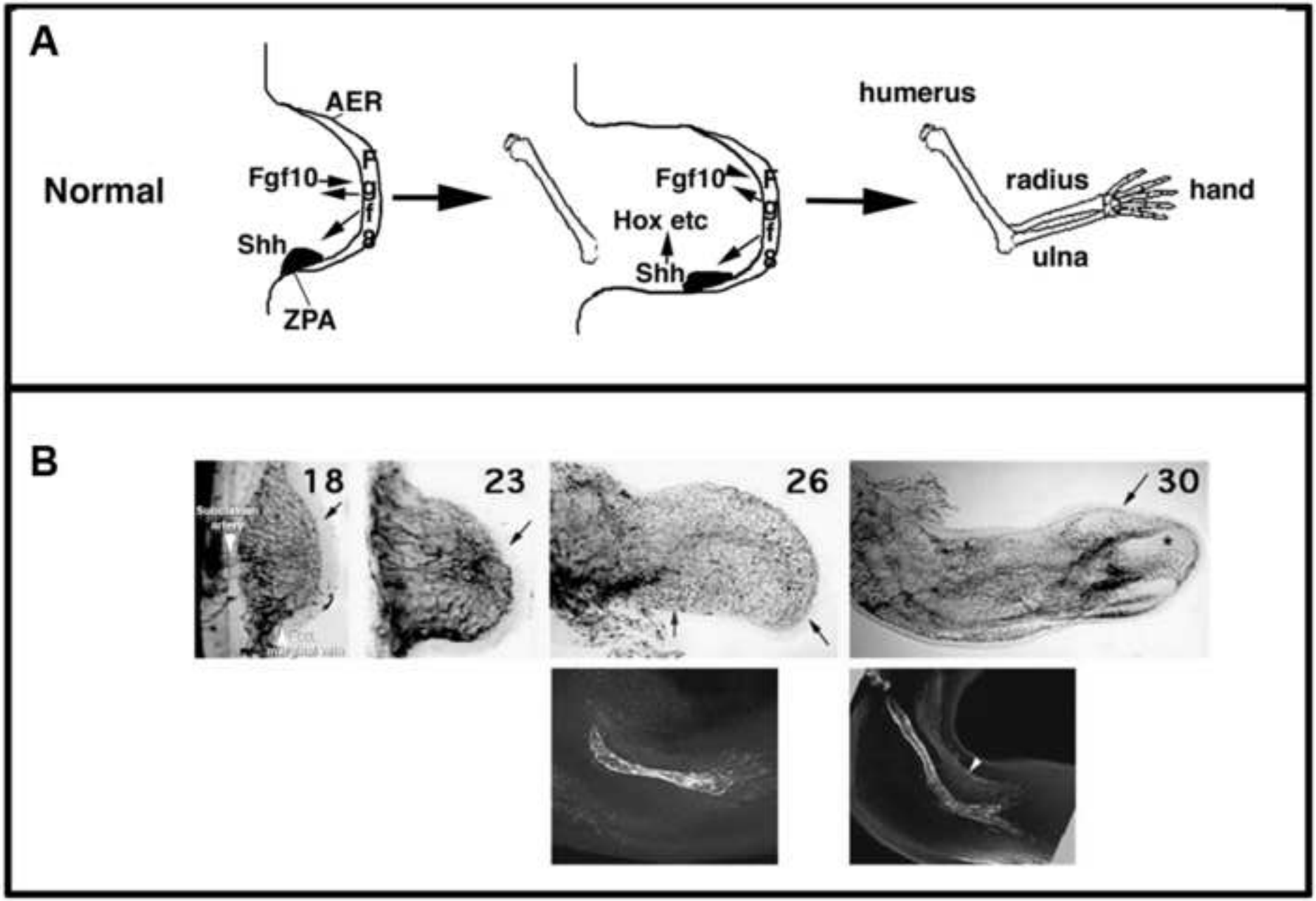

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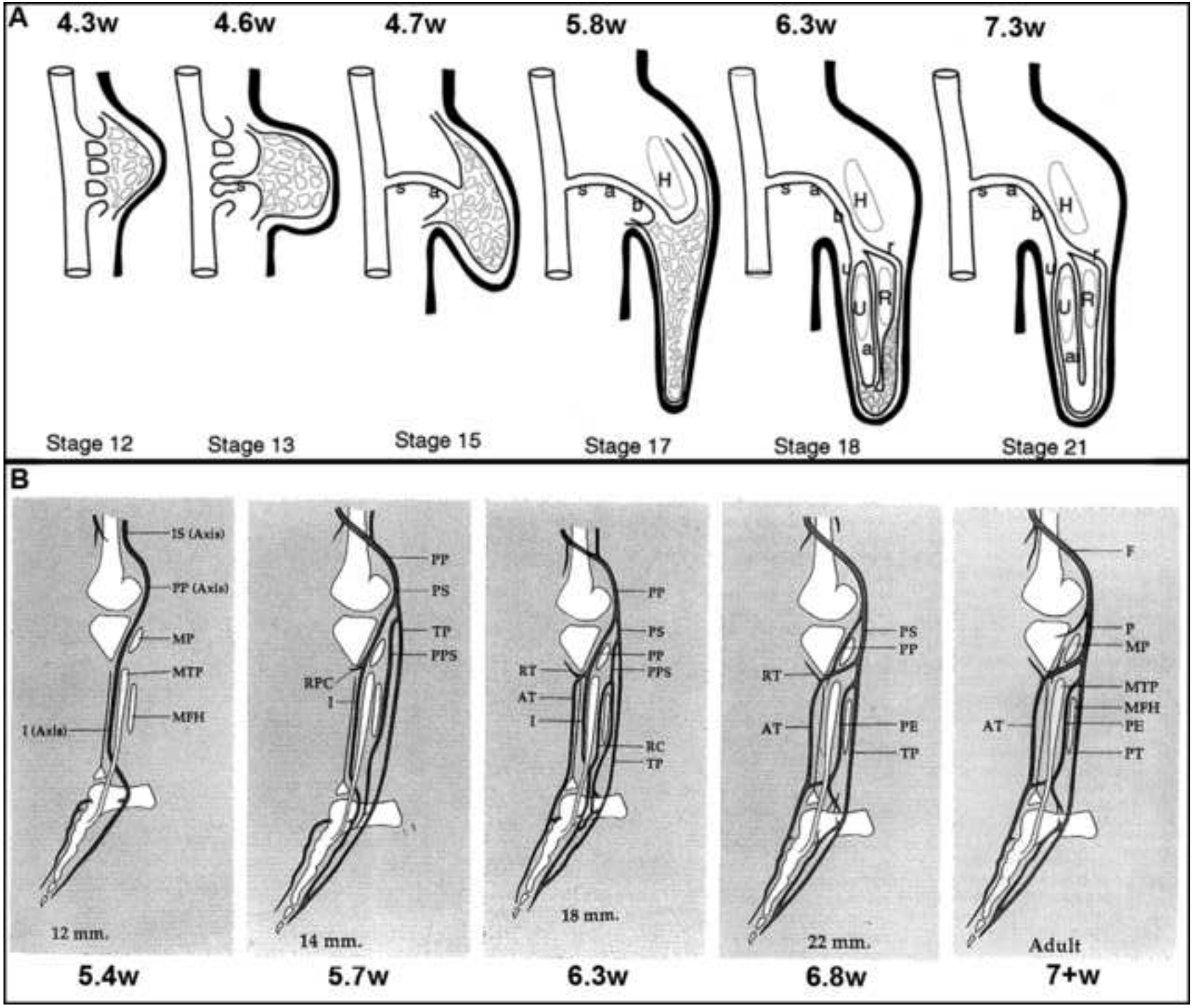

Figure 4

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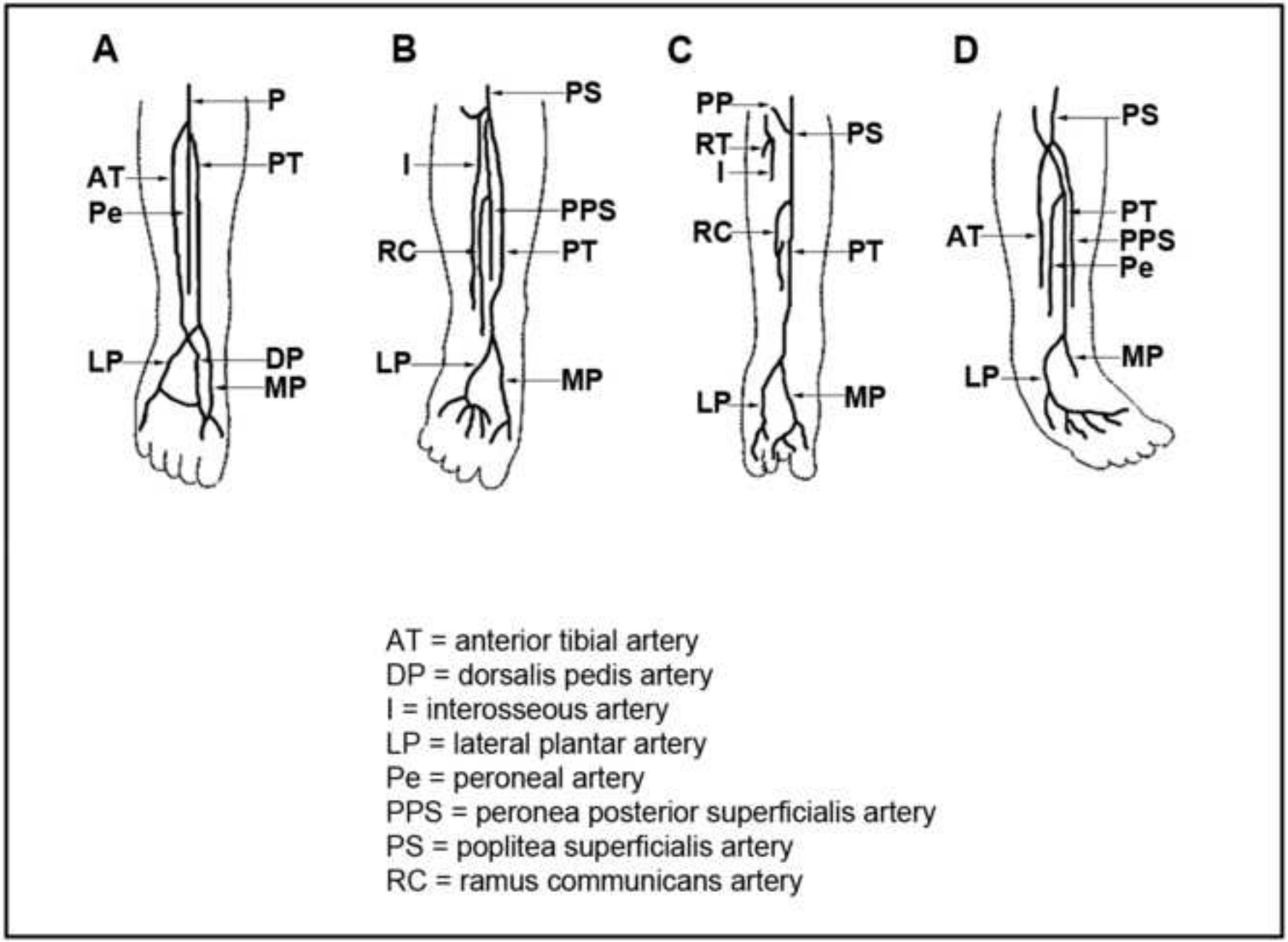

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