



Review

Genetics of syndromic and non-syndromic hereditary nail disorders

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The nail is a unique epithelial skin appendage made up of a fully keratinized nail plate. The nail can be affected in several systemic illnesses, dermatological diseases, and inherited nail disorders. Nail dystrophies can present as isolated disorders or as a part of syndromes. Substantial progress has been achieved in the management and diagnosis of nail diseases; however, not much is known about the underlying molecular controls of nail growth. The homeostasis and development of the nail appendage depend on the intricate interactions between the epidermis and underlying mesenchyme, and comprise different signaling pathways such as the WNT signaling pathway. Digit-tip regeneration in mice and humans has been a known fact for the past six decades; however, only recently the underlying biological mechanisms by which the nail organ achieves digit regeneration have been elucidated. Moreover, significant progress has been made in identifying nail stem cells and localizing stem cell niches in the nail unit. More fascinating, however, is the role they play in orchestrating the processes that lead to the regeneration of the digit. Further elucidating the role of nail stem cells and the signaling pathways driving epithelial–mesenchymal interactions in the nail unit might contribute to the development of novel therapeutic tools for amputees.

Conflict of interest

No conflicts of interest to declare.

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The nail is a unique epithelial skin appendage with an elaborate structure comprising a fully keratinized nail plate formed by the germinative epithelium of the nail matrix. The nail is related to the claw or hoof of other vertebrates, and has evolved in primates in parallel with attaining manual dexterity (1). This special design serves to defend the soft tissues of the distal digits against environmental assault and injury. Furthermore, the nail organ facilitates fine tactile manipulation, contributes to the sensory perception of fingertips, provides the ability to scratch and groom, can be used as a natural weapon, and lastly, contributes to esthetic appearance (2).

The nail unit is known to be affected in several systemic illnesses, dermatological diseases, as well as wide variety of inherited nail disorders. The latter are rare and are part of a diverse group of genodermatoses. Nail dystrophies can present as isolated disorders or as

a part of syndromes. However, these genodermatoses affecting the nail are, as of yet, an understudied field with little effort in categorizing these illnesses in a systematic manner. Substantial progress has been achieved in the management and diagnosis of nail diseases, however, not much is known about the underlying molecular controls of nail growth and development. As such, a thorough understanding of the nail unit and its features in the setting of disease is an invaluable tool utilized by both dermatologists and other specialists.

Furthermore, development and homeostasis of skin appendages – including hair, nails, and teeth – depends on the intricate interactions between the epidermis and underlying mesenchyme, and comprises different signaling pathways such as the WNT signaling pathway (3).

Digit-tip regeneration in mice and humans has been a known fact for the past six decades; however, only

recently the underlying biological mechanisms by which the nail organ achieves digit regeneration have been elucidated (4, 5). Moreover, substantial advances have been made in identifying nail stem cells and localizing stem cell niches in the nail unit (3, 5–7). More fascinating, however, is the role they play in orchestrating the processes that lead to digit regeneration. This implies that further elucidating the role of nail stem cells and the signaling pathways driving epithelial–mesenchymal interactions in the nail unit might contribute to the development of novel therapeutic tools for amputees.

In this review, we aim to provide an update on recent discovery of mutations in syndromic and non-syndromic nail dysplasias, latest advances in our understanding of molecular pathways governing nail development and homeostasis, as well as the latest findings on nail stem cells and their contribution to digit regeneration.

Limb embryology

Before embarking on the journey of nail development, it is important to dwell on the formation of the limb. Limb development starts at the end of the fourth week when limb buds appear at the ventrolateral body wall (8). A member of the fibroblast growth factor (FGF) family FGF10 secreted by the lateral plate mesoderm initiates limb outgrowth (9, 10). The forelimbs appear first and the hindlimbs follow 1–2 days later. The ventral ectoderm expresses bone morphogenetic proteins (BMPs) that lead to downstream signaling through the homeobox gene *Msx2* (11). This causes the distal edge of each bud to thicken and form a specialized ectoderm, the apical ectodermal ridge (AER), which interacts with the underlying mesenchyme and induces it to remain undifferentiated. The dorsal ectoderm secretes RFNG O-fucosyltransferase 3-beta-N-acetylglucosaminoyltransferase (RFNG) that confines the position of the AER to the distal edge of the limbs (12). Once the ridge is well formed, it starts expressing FGF4 and FGF8, which maintain the rapid proliferation of the underlying mesenchymal cells, which form the progress zone (13). The undifferentiated zone expresses a member of the AP-2 family of transcription factor, which may be contributing in sustaining FGFs secretion by the AER (8). As the limb grows from proximal to distal, the cells further from this undifferentiated zone are no longer exposed to FGFs, and under the influence of retinoic acid secreted by flank mesenchymal cells start differentiating into muscle and cartilage. As the differentiation front moves distally, the autopod differentiates under the influence of sonic hedgehog (SHH) and HOXA13 (13). Near the AER at the posterior border of the limb lies a cluster of mesenchymal cells which form the zone of polarizing activity (ZPA) (13). These cells secrete SHH responsible for the patterning of the anteroposterior axis of the limb and having the thumb on the anterior side. The dorsoventral axis is regulated by an interplay between the ventral and dorsal ectoderm. The ventral ectoderm produces BMPs which induce the expression of the transcription factor endothelin1 (EDN1) (14). EDN1 suppresses expression of WNT7A in the ventral ectoderm and hence confines it

to the dorsal limb ectoderm. WNT7A in turn leads to the expression of the transcription factor *Lmx1* in the dorsal mesenchyme which directs cells to be dorsal. WNT7A also affects the anteroposterior patterning indirectly by maintaining the SHH expression in the ZPA (14). The final result is that at 6 weeks a paddle-shaped hand and footplates become evident. At day 48, cell death in the AER leads to distinct digital rays; and by day 51 cell death in the interdigital space leads to partition of the digits so that digit separation is complete by day 56 (8).

Nail embryology

Once the fingers are well established, nails begin to form. Nail formation begins at 9 weeks of gestation, whereby mesenchyme condenses in the dorsal distal tip of digits. The overlying epidermis consists of the nail anlage which comprises a deep stratum germinatum (15). At 10 weeks, the dorsal surface of the distal phalanx then develops into the primary nail field, which is the earliest externally visible nail structure in the embryo and the site of the future nail. At this point, the proximal nail field gives rise to the matrix primordium which starts to grow proximally and ventrally leading to invagination of the primary nail field. By the 14th week, the early nail plate emerges, the flanking nail folds are evident and the matrix primordium differentiates into the nail matrix which comprises proliferating keratinocytes. Epithelial keratins begin to be expressed by the keratinocytes dorsal to the matrix which ultimately undergo apoptosis and lead to the deposition of a cornified structure on the nail plate (16). Initially, networks of keratin5 (K5) and K14 and networks of K1 and K10 are expressed and form the intermediate filaments, which establish the cytoskeleton of epithelial cells. Next, cells around the nail matrix move toward the spinous layer and no longer divide. As such they start secreting other structural proteins and enzymes specific of the nail plate. These include soft keratins, K6A and K17, and hard keratins, K31, K33B, K34, K39, K81, K85, and K86 (17, 18). Filaggrin (FLG) aggregates keratins and a number of other proteins, such as loricin, involucrin and proline-rich proteins, are expressed as well as transglutaminases that cross-link those proteins. By the 20th week, the nail plate covers the entire nail unit, and grows in tandem with the digit (15). Development of toenails lags approximately 4 weeks behind fingernails (15).

Molecular pathways in nail development and homeostasis

The molecular mechanisms fundamental for these steps are poorly understood. Recent discovery of mutations in syndromic and non-syndromic nail dysplasias and experiments on mouse models have provided insights into nail development. The mouse nail unit shares major characteristics with the human nail unit and as such can serve as a useful model of nail biology (19). The development of nail and limb bud is closely tied and is dependent on intimate interaction between ectoderm

and mesoderm via different signaling pathways. Table 1 provides a summary of selected congenital syndromic and non-syndromic hereditary nail disorders classified according to the molecular pathway to which the mutated gene belongs.

WNT signaling

The WNT signaling pathway is a pathway that has been highly conserved throughout evolution; it plays a crucial role in the overall embryonic development – including nail morphogenesis – and adult tissue homeostasis. It orchestrates the overall differentiation process and regulates assembly of the cytoskeleton in developing nails. Nineteen WNT proteins have been identified in humans (20). There are different WNT signaling pathways including the canonical Wnt/ β -catenin signaling pathway (21). A recent study showed using conditional knockout mice that catenin beta 1 (CTNNB1) is required for nail differentiation and that WNT activation is required for nail regeneration following amputation (3).

In the absence of WNT signals, a complex of proteins that include adenomatous polyposis coli (APC) and axin target and degrade catenin beta 1. As soon as WNT ligands bind to the complex of coreceptors frizzled (FZD) and the Low density lipoprotein (LDL) receptor-related proteins 5 and 6 (LRP5 and LRP6), catenin beta 1 accumulates and translocates to the nucleus leading to cell response. Zinc and ring finger 3 (ZNR3) and its homologue ring finger protein 43 (RNF43) antagonize WNT signaling by ubiquitinating FZD, leading to endocytosis of the WNT receptor. Binding of R-spondin (RSPO) to leucine-rich repeat containing G protein-coupled receptor 4, 6 (LGR4-6) inhibits ZNR3/RNF43 and enhances WNT signaling (22, 23).

In murine experiments, it was shown that both RSPO3 and RSPO4 are secreted by dermal fibroblasts underlying the digit tip epithelium and bind to their corresponding receptors expressed by stem cells in the nail matrix (24, 25). However, RSPO4 is found exclusively in the mesenchyme underneath the digit tip epithelium whereas RSPO3 is found in a broader region of the digit tip (24). *Rspo3* knockout mice die early during development due to defects in placental development (26). In humans, mutations in *RSPO4* are responsible for onychia/hyponychia congenita (MIM 206800), a rare autosomal-recessive condition in which the absence or severe hypoplasia of all fingernails and toenails is the sole phenotype (25, 27). Although RSPO3 is expressed at the digital tip, it seems that RSPO3 is unable to compensate for RSPO4 in the digital tip in humans (24). This suggests that in all body sites besides the digital tip, RSPO4 may be compensated for by the presence of the other members of the RSPO family. Seventeen mutations in *RSPO4* have been described so far and almost all of these mutations affect the highly conserved exons 2 and 3 which code for two furin-like regions (28). A truncated protein made up of just these two regions was shown to be sufficient for catenin beta 1 stabilization (29). Furthermore, mutations in *FZD6* also result in isolated autosomal recessive nail dysplasia in humans. These

mutations are associated with disturbed formation and attachment of the nail plate leading to onycholysis (30).

WNT7A induces the expression of LMX1 in the dorsal mesenchyme which influences dorsoventral limb patterning (31). Knockout of both *Wnt7a* and *Lmx1b* in mice leads to ventralization of distal limbs (32, 33). LMX1 is a member of the LIM-homeodomain family of transcription factors that plays a crucial role in the normal development of dorsal limb structures, dopaminergic and serotonergic neurons, the anterior segment of the eye, the glomerular basement membrane, and as well as in the preservation of differentiated podocytes in adult kidneys (34). A murine experiment showed using *Lmx1b* knockout mice that LMX1B regulates a large number of extracellular matrix-related soft tissue genes such as collagens and proteoglycans, suggesting a mechanism that involves changes in the extracellular matrix composition to establish limb dorsalization (31). Nail-patella syndrome (or hereditary onycho-osteodysplasia) (MIM 161200) is a rare autosomal dominant due to heterozygous mutation in *LMX1B* gene on chromosome 9q. It is characterized by dystrophic nails, hypoplastic or absent patellae, dysplasia of the iliac horn, and elbows. Almost all affected individuals (96% in one series) have nail dysplasia (35); the dystrophy is usually more marked on the thumb and the degree of dystrophy usually diminishes towards the little finger. Koilonychia and longitudinal striations are the most common finding, followed by anonychia (35). Lunulae anomalies include triangular lunulae or even absent lunulae. So far, no correlation between the range of severity of symptoms and the *LMX1B* genotype has been reported (36, 37). *MSX1* is another WNT-associated transcription factor expressed in mesenchyme subjacent to the epithelium of the nail bed that plays roles in the development of teeth and nails. *Msx1* knockout mouse experiments showed that mesenchymal *MSX1* is required for formation of a normal nail plate; and mutations in *MSX1* gene leads to Witkop syndrome (MIM 189500) (38).

WNT10A plays a pivotal role in the regulation and development of ectodermal appendages. It is upregulated in embryonic skin including in the placodes at follicle morphogenesis (39) and is critical for tooth morphogenesis (40). *WNT10A* nonsense and missense mutations are associated with odonto-onychodermal dysplasia (MIM 257980) or Schöpf–Schulz–Passarge syndrome (MIM 224750), rare syndromes which includes severe hypodontia and nail dystrophy among other ectodermal anomalies (41–45). This highly suggests that WNT10A is essential for the formation of nails.

Other important molecules are catenin beta 1 antagonists such as SOX9, whereby changes in the gene dosage of *SOX9* may disrupt the intricate balance of WNT signaling leading to disruption in digit and nail development (46).

Notch signaling

NOTCH stimulates stem cells in the nail matrix and promotes differentiation of keratinocytes by activating p21 (47). It has been suggested that WNT signaling indirectly

Table 1. Congenital syndromic and non-syndromic hereditary nail disorders

Disease	Gene	Mode of inheritance	Clinical finding	MIM	Reference	
Wnt-related diseases						
Anonychia/hyponychia congenita	<i>RSPO4</i>	AR	Complete absence or severe hypoplasia of all fingernails and toenails	206800	25	
Isolated autosomal-recessive nail dysplasia	<i>FZD6</i>	AR	Claw-shaped nails, onychauxis, onycholysis and hyponychia	614157	30	
Nail-patella syndrome	<i>LMX1B</i>	AD	Dystrophic nails (more severe on the thumb, koilonychias, longitudinal striations, anonychia, triangular lunulae or absent lunulae, hypoplastic or absent patellae, dysplasia of the elbows, and iliac horn)	161200	36	
Wiktop syndrome	<i>MSX1</i>	AD	Thin brittle nails, koilonychias, toenails more severely affected, hypodontia	189500	38	
Odonto-onychodermal dysplasia	<i>WNT10A</i>	AR	Severe hypodontia, nail dystrophy, smooth tongue, dry skin, keratoderma and hyperhydrosis of palms and soles.	257980	41	
Schöpf-Schulz-Passarge	<i>WNT10A</i>	AR	Multiple eyelid apocrine hidrocystomas, palmo-plantar keratoderma, hypodontia, hypotrichosis and nail dystrophy.	224750	41	
Keratin and keratin regulator-related diseases						
Pachyonychia congenita 1			Hypertrophic nail dystrophy, painful palmoplantar keratoderma and blistering, oral leukokeratosis, pilosebaceous cysts (including steatocystoma and vellus hair cysts), palmoplantar hyperhydrosis, and follicular keratoses on the trunk and extremities	167200	68	
PC-K6a	<i>K6A</i>	AD		148041		
PC-K16	<i>K16</i>			148067		
Pachyonychia congenita 2			Follicular keratoses on the trunk and extremities	167210	68	
PC-K6b	<i>K6B</i>	AD		148042		
PC-K17	<i>K17</i>			148069		
Pure hair and nail ectodermal dysplasia	<i>K85</i> <i>K74</i> <i>HOXC13</i>	AR	Onychodystrophy, micronychia, hypotrichosis and brittle hair	602032	62 65 52	
TNF- α pathway-related diseases						
Ectodysplasin-EDAR-EDARRADD signaling pathway						
Anhidrotic ectodermal dysplasia	<i>EDA</i>	XLR	Sparse to absent hair, hypodontia, conical teeth, markedly decreased sweating, characteristic facies	305100	80	
	<i>EDAR</i>	AD		129490		
	<i>EDARADD</i>	AR		224900 229400		
NEMO regulatory pathway			Four stages: inflammatory/vesicular Verrucous Hyperpigmented Hypopigmented/atrophic Alopecia, naildystrophy pegged or missing teeth Neurological abnormalities	308300	80	
Incontinentia pigmenti	<i>NEMO/IKBKGP1</i>	XLD				
Anhidrotic ectodermal dysplasia with immunodeficiency	<i>NEMO/IKBKGP1</i>	XLR		Sparse hair, hypodontia, conical teeth, mildly decreased sweating, recurrent infections	300291	80
Anhidrotic ectodermal dysplasia with osteopetrosis and immunodeficiency	<i>NEMO/IKBKGP1</i>	XL		300301	114	
Anhidrotic ectodermal dysplasia with immunodeficiency	<i>NFKBIA</i>	AD		612132	115	
P63 and p63 regulators related disorder						
Clouston syndrome	<i>GJB6</i>	AD	Hypotrichosis, severe nail dystrophy, and often palmoplantar hyperkeratosis as well as hyperpigmentation of the skin over large joints	129500	79	

Table 1. Continued

Disease	Gene	Mode of inheritance	Clinical finding	MIM	Reference
Popliteal pterygium syndrome	<i>IRF6</i>	AD	Popliteal webbing, cleft lip, cleft palate, lower lip pits, syndactyly, genital anomalies and nail dystrophy	119500	116
Hay–Wells syndrome	<i>TP63</i>	AD	Alopecia, scalp infections, dystrophic nails, hypodontia, ankyloblepharon and cleft lip and/or cleft palate	106260	117
Sonic hedgehog pathway-related disorders					
Pallister–Hall syndrome	<i>GLI3</i>	AD	Nail dystrophy, polydactyly, asymptomatic bifid epiglottis and hypothalamic hamartoma, laryngotracheal cleft with neonatal lethality	146510	118
Ellis Van Crefeld	<i>EVC LBN</i>	AR	Short stature, short limbs, growth retardation, polydactyly and ectodermal defects with cardiac anomalies	225500	119
Telomere-related disorder					
Dyskeratosis congenita	<i>TERC</i>	AD	Classic clinical triad of abnormal skin pigmentation, leukoplakia, and nail dystrophy; bone marrow failure, predisposition to malignancy and pulmonary and hepatic fibrosis	127550	120
Enzymes involved in nail morphogenesis related disorders					
Isolated congenital nail clubbing	<i>HPGD</i>	AD	Digital clubbing is characterized by enlargement of the nail plate and terminal segments of the fingers and toes	119900	92
Hereditary leuconychia/porcelain nails	<i>PLCD1</i>	AD, AR	Leuconychia	151600	93
Other					
Yellow nail Syndrome	<i>FOXC2</i>	AD	Classic triad of yellow dystrophic nails, lymphedema and respiratory tract abnormalities	153300	97
Trichorhinophalangeal syndrome	<i>TRPS1</i>	AD	Growth retardation, craniofacial abnormalities, alopecia and brachyphalangia, nail dystrophies including V-shaped longitudinal nail dystrophies	190350 150230 190351	99

participates in the long-range effect of NOTCH1 on tissue homeostasis in the nail (48).

BMP signaling

MSX2 and FOXN1 are two transcription factors downstream of BMP signaling and upstream of NOTCH1 signaling during nail differentiation and nail bed homeostasis (49). MSX2 and FOXN1 are both involved in regulating hard keratin expression during hair differentiation, distal nail matrix and nail bed homeostasis.

Regulators of keratin and keratin associated genes

HOXC12 and HOXC13 are transcription factors important for development of hair and nail (50). They both belong to the homeobox (HOX) gene family, which encodes evolutionarily conserved transcription factors that serve as regulators of downstream genes involved in cell proliferation and differentiation (51). Interestingly, HOXC gene cluster is found close to the type II keratin gene cluster (52). HOXC13 is an essential transcriptional regulator of a number of hair keratin and

keratin-associated protein (*Kap*) genes (53–55) as well as other genes in hair follicles and or nail units such as *Foxn1*, *Foxq1*, *Dsg4* and *Crisp1* (56).

FOXN1 is primarily expressed in the thymus and skin epithelial cells, where it is critical for survival and differentiation (57). In the hair follicle, the HOXC13 and FOXN1 function using the feed-forward loop (FFL) network motif (58); the so-called HOXC13-FOXN1-keratin/KAP gene regulatory circuit whereby HOXC13 regulates FOXN1 and both together regulate keratin and keratin associated genes (59). Mutations in this gene in mice lead to severe combined immunodeficiency (SCID) and the phenotype is referred as Nude/SCID, due to the combination of athymia with complete hairless. In humans, mutations in FOXN1 lead to nail dystrophy, congenital alopecia and T-cell immunodeficiency. (MIM 601705) (57).

Keratins

Keratins constitute 80% of the dry weight of the nail plate. The spectrum of structural and non-mechanical functions of keratins is constantly increasing (60).

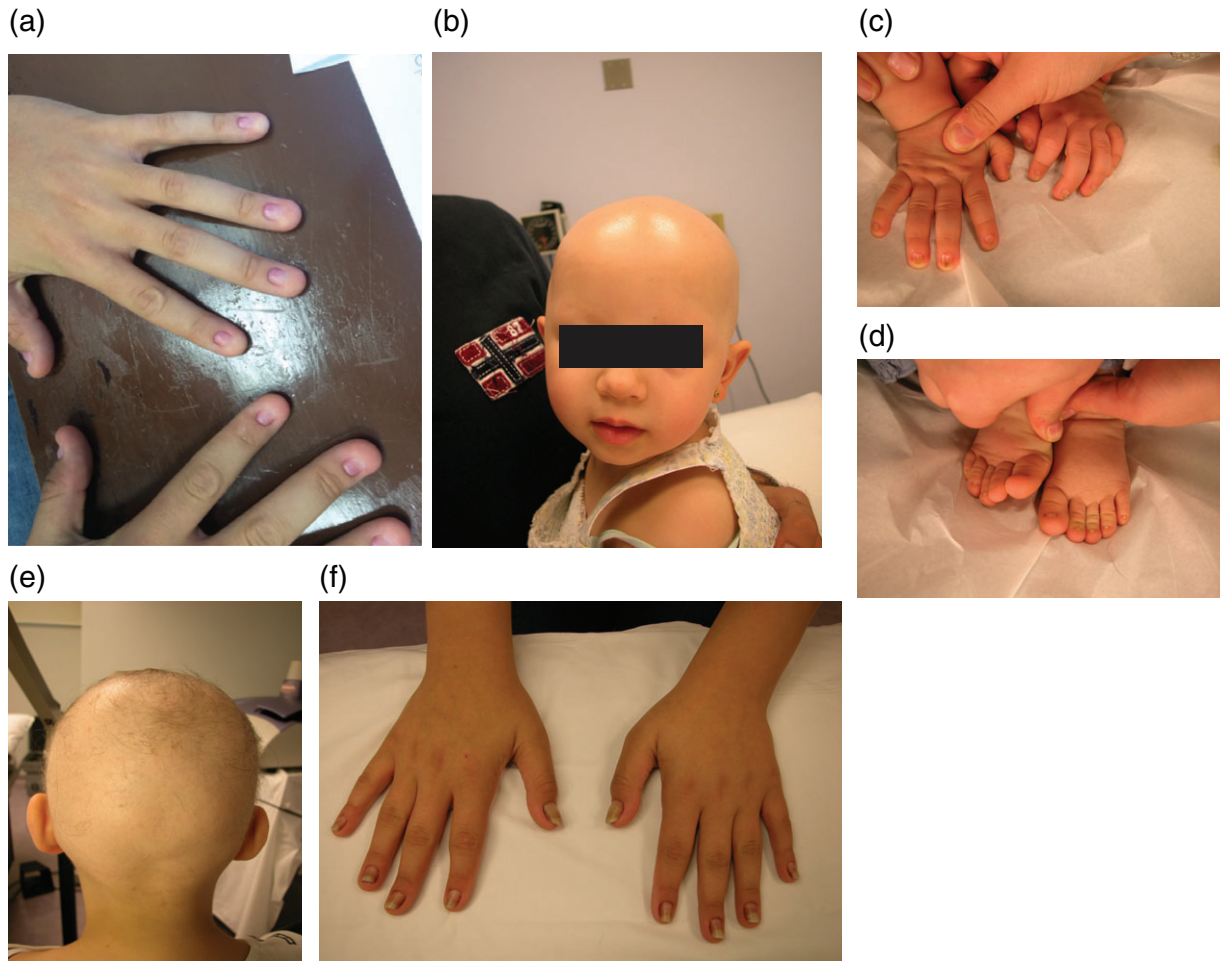


Fig. 1. Clinical pictures a severe hypoplasia of all fingernails in anonychia congenita. (b–d) hypotrichosis, severe hypoplasia in all fingernails and toenails respectively in pure hair and nail ectodermal dysplasia. (e, f) Hypotrichosis and thickened nail plate and nail bed in a patient with Clouston syndrome.

K85 plays a pivotal role in the keratinization of hair and nails in humans. It is found early during nail morphogenesis in the basal compartment and the lower keratogenous zone of the apical and ventral nail matrix (61). It is postulated that dysfunction of K85 disrupts keratin intermediate filament formation leading to abnormal desmosomal assembly in hair and nails (62). Therefore, it is expected that the absence of the protein during hair and nail morphogenesis leads to a severe hair and nail phenotype (63).

Pure hair and nail ectodermal dysplasia (PHNED) (MIM 602032) is a heterogeneous group of rare congenital disorders characterized by onychodystrophy, micronychia, hypotrichosis and brittle hair (Fig. 1b–d). The condition is inherited in an autosomal recessive pattern with mutations of both *KRT85* and *HOXC13* on chromosome 12p11.1–q14.3 (52, 64). A recent study has also identified a homozygous mutation in *KRT74* leading to PHNED (65). K74 is found in the distal digits during mouse claw development (16) and in the inner root sheath of hair follicles (66). As such, it seems that K85 and K74 can be compensated for in the regeneration of skin as opposed to its crucial role for the morphogenesis of hair and nails.

Keratins 6a, 6b, 16 and 17 are upregulated in stress response and wound healing (67). Mutations in these keratins lead to pachyonychia congenita (MIM 167200 and 167210), a rare autosomal dominant disease primarily involving tissues of the ectodermal origin. These mutations cause cytoskeletal fragility and cytolysis with overproduction of keratins to compensate for the mutations (68). Clinical features include dense subungual hyperkeratosis and hypertrophic nail dystrophy, painful palmoplantar keratoderma, oral leukokeratosis and pilosebaceous cysts.

Other keratins that seem to play a minor role in nail development are K81, K83 and K86. Monilethrix is a hereditary hair disorder that presents either monosymptomatically or as a monilethrix syndrome associated with other ectodermal anomalies (69), including a triple combination of monilethrix, koilonychias and keratosis pilaris (70). Features that may be associated include nail anomalies other than koilonychias as well (71). Autosomal dominant monilethrix is usually due to mutations in the type II hair keratins K81 and K86, and less commonly K83. Normal development of the cortex is highly dependent on these three keratins that are highly expressed

within the hair cortex. Autosomal recessive monilethrix is due to mutations in *DSG4*. Desmoglein 4 is the single desmoglein found in the mid-cortex of the hair shaft, where K81, K83 and K86 are present (72).

Type I keratins such as keratin 14 (K14) confer resistance to tumor necrosis factor- α (TNF- α)-induced apoptosis in epithelial tissues including the nails possibly through sequestration of TNF receptor type 1-associated death domain protein (TRADD) (73, 74). Mutations in the E1/V1-encoding region of the *KRT14* gene result in K14 haploinsufficiency, which *in vitro* leads to increased susceptibility of epidermal cells to (TNF- α)-mediated apoptosis (75). Clinically, such mutations lead to Naegeli–Franceschetti–Jadassohn syndrome (NFJS) (MIM 161000) and dermatopathia pigmentosa reticularis (DPR) (MIM 125595), two closely related autosomal dominant ectodermal dysplasia syndromes that present with partial or complete absence of dermatoglyphics, abnormal regulation of sweating, reticulate hyperpigmentation of the skin, palmoplantar keratoderma and nail dystrophy and dental anomalies (only in NFJS) (75, 76).

FZD6 of the WNT signaling pathway also upregulates the transcription of a significant number of epidermal differentiation related genes including, hard keratins (e.g. K86, K81, K34, K31), soft Ks (K6a and K6b), keratin-associated proteins, transglutaminases and late cornified envelope proteins (16), and thus disruption along this signaling pathway will affect several nail-related keratins.

Tumor protein p63 is a major transcription factor that regulates a number of genes expressed in the epidermis (77). During embryogenesis, intercellular communication via gap junctions seems to play a crucial role in skin development as evidenced by the gradual increase in the number of gap junctions (78). Connexin 30 (CX30) is highly expressed in the nail matrix and the nail bed in both postnatal humans and mouse embryos; it is encoded by gap junction protein beta 6 (*GJB6*) (79). Evidence suggests that *GJB6* is a transcriptional target gene of p63 (79). Mutated *GJB6* might interfere with the differentiation and proliferation of those parts of the nail, leading to thickened nail bed and nail plate in patients with Clouston syndrome (MIM 129500) (79) (Fig. 1e,f).

Tumor necrosis factor α (TNF α)-related signaling pathway

Proteins of the TNF α -related signaling pathway are responsible for the signal transduction from ectoderm to mesenchyme during development. They play a critical role in the differentiation of ectoderm-derived structures such as skin, hair, teeth, eccrine sweat glands and nails (80). The pathway is initiated by the WNT/ β -catenin pathway and requires lymphoid enhancer-binding factor-1 (LEF-1) for activation of the ectodysplasin A (EDA) expression (81) and regulation of the expression of ectodysplasin A receptor (EDAR) (82). EDA-A1 and its two amino acids shorter isoform EDA-A2 are type II transmembrane protein that becomes functionally active when the membrane-bound protein product of EDA is cleaved by furin-like proteases and released out

of cells, where it forms trimers (80). EDAR is a type I transmembrane protein which has a potential death domain in its intracellular region (83). When EDA-A1 binds to EDAR it activates it, EDAR then interacts with EDAR-associated death domain (EDARADD) through its death domain. EDAR functions in suppressing the placode inhibitory factors, mainly dickkopf WNT signaling pathway inhibitor 1 (DKK1) (84). EDA-A2 binds to its receptor EDA2R (also known as XEDAR), which does not require a protein adaptor to react with TNF receptor-associated factors (TRAF) proteins. EDARADD interacts with TRAF6 which recruits TAK1-binding proteins, TAB1 and TAB2 (85). These latter activate the TGF β -activated kinase 1 multisubunit complex made up of two kinases, IKK1/ α and IKK2/ β that phosphorylate κ B inhibitors α and β , respectively (86), and IKK γ (NEMO) (87) resulting in the activation of NF- κ B. NF κ B stimulates transcription of target genes (88). The protein products of these genes are crucial for the development of the ectodermal structures including the nail as they interact with many different signaling pathways such as BMP and Shh (80). Mutations in these genes disturb the process of initiation, formation, and differentiation of skin appendages, leading to ectodermal dysplasia syndromes with similar clinical features, which include nail dystrophies (Table 1).

Enzymes involved in nail morphogenesis

Hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD) is the main enzyme of prostaglandin degradation. It resides in the cytosol and catalyses, using NAD (+) as a coenzyme, the oxidation of the 15-hydroxyl group on prostaglandin and related eicosanoids. This is the first step in the catabolism of these molecules and leads to their reversible inactivation (89, 90). Homozygous mutations in HPGD leads to individuals developing primary hypertrophic osteoarthropathy secondary to chronically elevated prostaglandin E(2) levels (91, 92)

Phosphoinositide-specific phospholipase C delta 1 subunit (PLCD1) is a crucial enzyme in phospho-inositide metabolism and is thought to play key roles in different physiological functions. High expression of PLCD1 has been reported in the nail matrix and the nail bed (93). Deactivating mutations in *PLCD1* gene cause autosomal recessive and dominant hereditary leukonychia (94) (MIM 151600); however, the actual causal mechanism remains unknown. It is believed that PLCD1 functions downstream of FOXN1 and regulates hard keratin gene expression necessary for nail differentiation (95). Thus, loss of PLCD1 function may result in abnormal keratinization of the nail plate due to abnormal expression of hard keratins.

Other

Many other genes are of course also involved in the morphogenesis of the nail, such as those coding for

transcription factors. Indeed, *FOXC2* is a member of the forkhead transcription factor that plays a role in a variety of developmental processes including angiogenesis and lymphangiogenesis (96). Mutations in *FOXC2* lead to different lymphedema syndromes with overlapping features including lymphedema-distichiasis syndrome and yellow nail syndrome (97); the latter consists of the classic triad of yellow dystrophic nails, lymphedema, and respiratory tract abnormalities. Yellow discoloration of the nails have been described in patients with lymphedema-distichiasis (97); however, this could be attributed to the lymphedema *per se*. Chronic lymphedema leads to rough thickened opaque nail plates, whereas nail changes seen in the yellow nail syndrome are more specific and consist of an overcurved yellow nail plate that is smooth and translucent (98).

Another important transcription factor is *TRPS1*. A mutation in *TRPS1* leads to trichorhinophalangeal syndrome which consists of craniofacial abnormalities, growth retardation, alopecia, brachyphalangia and nail abnormalities which include V-shaped longitudinal nail dystrophies (99).

Nail stem cells and limb regeneration

Stem cells are multipotent cells that can self-replicate and differentiate into several lineages. Since the discovery two decades ago of the bulge as the stem cell niche, the literature on stem cells of the hair follicle has expanded exponentially (100–102). In contrast, the stem cells in nails have been less studied. An important reason for this discrepancy is the fact that nails undergo continuous growth under physiological conditions whereas the hair follicle undergoes cycling (anagen, catagen and telogen) and has thus been studied extensively as a general model of organ regeneration. Moreover, whereas hair follicles are abundant and more readily accessible, humans only have a total of twenty nails and are rarely biopsied.

The hair follicle and nail unit are related skin appendages, and they share various biological features and they tend to be affected together in certain genodermatoses (103). Achten proposed a model whereby the nail unit is likened to a longitudinal section of a hair follicle turned by 90 degrees (104). Hence, studies on nail organ stem cells have depended on the use of markers that have already been validated on the human hair follicle.

In 1968, Zaias and Alvarez showed using tritiated glycine injection into squirrel monkey and autoradiographic pulse-chase study that nail matrix is solely responsible for the formation of the nail plate (105).

A murine experiment used the label-retaining cells (LRC) method that relies on the specific characteristic of stem cells of being very slow cell cycling cells that retain labels in the nucleus for several weeks. The BrdU pulse-chase showed that LRCs reside in the basal layer of the nail matrix adjacent to the nail bed (6).

On the other hand, in a study using tamoxifen inducible lineage tracing of transgenic mice under the control of the keratin 14 promoter and labeling of keratin 14 positive basal epidermal cells with LacZ,

nail stem cells were found to reside in the proximal nail matrix (3).

An immunohistochemical study examining embryonic and fetal human nail samples using follicular stem cell markers (cytokeratin 15, cytokeratin 19 or *PHLDA1*) proposed that at least during nail embryogenesis the ventral aspect of the proximal nail fold represents the nail stem cell niche (7).

Supporting this is a recent study that detected using the LRC method stem cells within the basal layer of the nail proximal fold organized in a ring-like configuration around the nail root. They further showed that this stem cell population is bifunctional and can contribute to both the nail structure and peri-nail epidermis (5).

The subject of limb regeneration has fascinated the scientific community for over six decades where most of the work was performed on lower vertebrates (106, 107). Early on it was observed that ‘regeneration of lost body parts is an ability shared to a varying degree by all living things’ (108). However, this ability gradually decreased throughout evolution. In the early 1970s, scientific reports on regrowth following amputation of fingertips in children have emerged (4, 109). Two decades later, few studies showed that digit tip regeneration in mice is dependent on the presence of the nail organ (110–112); and that nail transplantation after proximal phalangeal amputation in rats induced bone growth (113).

Digit-tip regeneration depends on the coordinated regrowth of the nail organ, involving nail epithelial cells and the terminal phalanx. In amputations proximal to the nail, both the nail and the digit do not regenerate (3, 110, 112). However, it is not until very recently that the underlying biological mechanism by which the nail organ achieves digit regeneration has been elucidated.

Wnt signaling plays a key role in nail differentiation. In conditional *Wnt* knockout mice, the nail and bone failed to regenerate (3). WNT activation in the nail epithelium actually performs dual functions: It promotes nail regeneration and *RUNX2* (a key transcription factor associated with osteoblast differentiation) mesenchymal cell growth through its ability to induce nerve-dependent *FGF2* expression. Takeo et al. proposed the following model for regeneration.

Under homeostasis, WNT activation leads to nail stem cells giving rise to distal matrix cells. Concurrently, nail stem cells and distal matrix cells differentiate into the nail plate. The distal nail matrix expresses *WNTLESS* necessary for WNT signaling initiation. Following distal level amputation, nail epithelial cells cover the wound site. This in turn activates WNT signaling to differentiate into distal matrix cells and the nail plate. Moreover, this WNT activation induces blastema innervations, which is essential for *FGF2* expression in the regenerating nail epithelium. *FGF2* then leads to proliferation of *RUNX2* positive mesenchymal cells, eventually leading to digit regeneration.

Digit amputations within the nail stem cell region removes the distal matrix expressing *WNTLESS* required for initiation of Wnt signaling. Depletion of *WNTLESS* expressing nail epithelium leads to absence

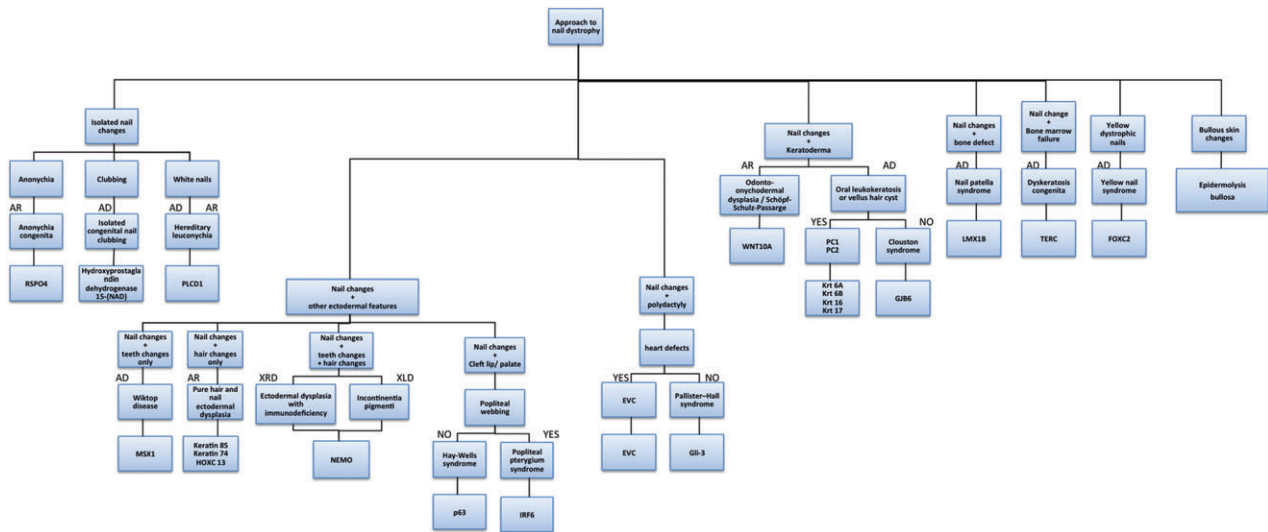


Fig. 2. Approach to nail dystrophy.

of Wnt activation in the nail epithelium and failure to regenerate the digit.

Conclusion

In this review, we have summarized the recent discoveries of mutations in syndromic and non-syndromic nail disorders. In Fig. 2, we provide an algorithm each dermatologist can refer to when approaching patients with hereditary nail dysplasia.

The last decade has witnessed considerable progress in nail research, including elucidating the molecular genetics of many rare genetic nail disorders, understanding the signaling pathways controlling differentiation, as well as the recent localization of nail stem cells. These recent advances in the field of nail research have revealed that the nail apparatus is a much more intricate system than once thought; indeed, it is a truly fascinating organ capable of orchestrating digit regeneration. New genes linked syndromic and nonsyndromic nail diseases are expected to be discovered to further clarify the biology of the nail unit. Moreover, more research is expected to further elucidate the homeostasis and function of nail stem cell; and hopefully such knowledge might be applied for the purpose of regenerative medicine and contribute to the development of novel therapeutic tools for amputees.

References

- de Berker DAR, Baran R. Science of the nail apparatus. In: Baran & Dawber's diseases of the nails and their management. Blackwell Publishing Ltd, Hoboken, New Jersey. 2012: 1–50.
- Paus R, Piker S, Sundberg JP. Biology of hair and nails. In: Dermatology, 2nd edn. St. Louis, MO: Elsevier, 2007.
- Takeo M, Chou WC, Sun Q et al. Wnt activation in nail epithelium couples nail growth to digit regeneration. *Nature* 2013; 499: 228–232.
- Douglas BS. Conservative management of guillotine amputation of the finger in children. *Aust Paediatr J* 1972; 8: 86–89.
- Leung Y, Kandyba E, Chen YB et al. Bifunctional ectodermal stem cells around the nail display dual fate homeostasis and adaptive wounding response toward nail regeneration. *Proc Natl Acad Sci USA* 2014; 111: 15114–15119.
- Nakamura M, Ishikawa O. The localization of label-retaining cells in mouse nails. *J Invest Dermatol* 2008; 128: 728–730.
- Sellheyer K, Nelson P. The ventral proximal nail fold: stem cell niche of the nail and equivalent to the follicular bulge – a study on developing human skin. *J Cutan Pathol* 2012; 39: 835–843.
- Sadler TW. *Limbs*. In: Langman's medical embryology, 13th edn. Wolters Kluwer Health, 2015.
- Cunningham TJ, Duester G. Mechanisms of retinoic acid signalling and its roles in organ and limb development. *Nat Rev Mol Cell Biol* 2015; 16: 110–123.
- Ohuchi H, Nakagawa T, Yamamoto A et al. The mesenchymal factor, FGF10, initiates and maintains the outgrowth of the chick limb bud through interaction with FGF8, an apical ectodermal factor. *Development* 1997; 124: 2235–2244.
- Vieux-Rochas M, Bouhali K, Mantero S et al. BMP-mediated functional cooperation between Dlx5/Dlx6 and Msx1/Msx2 during mammalian limb development. *PLoS One* 2013; 8: e51700.
- Ohuchi H, Nakagawa T, Itoh N et al. FGF10 can induce Fgf8 expression concomitantly with En1 and R-fng expression in chick limb ectoderm, independent of its dorsoventral specification. *Dev Growth Differ* 2000; 41: 665–673.
- Zeller R, Lopez-Rios J, Zuniga A. Vertebrate limb bud development: moving towards integrative analysis of organogenesis. *Nat Rev Genet* 2009; 10: 845–858.
- Cygan JA, Johnson RL, McMahon AP. Novel regulatory interactions revealed by studies of murine limb pattern in Wnt-7a and En-1 mutants. *Development* 1997; 124: 5021–5032.
- Zaias N. Embryology of the human nail. *Arch Dermatol* 1963; 87: 37–53.
- Cui CY, Klar J, Georgii-Hemming P et al. Frizzled6 deficiency disrupts the differentiation process of nail development. *J Invest Dermatol* 2013; 133: 1990–1997.
- Rice RH, Xia Y, Alvarado RJ, Phinney BS. Proteomic analysis of human nail plate. *J Proteome Res* 2010; 9: 6752–6758.
- Barthelemy NR, Bednarczyk A, Schaeffer-Reiss C et al. Proteomic tools for the investigation of human hair structural proteins and evidence of weakness sites on hair keratin coil segments. *Anal Biochem* 2012; 421: 43–55.
- Fleckman P, Jaeger K, Silva KA et al. Comparative anatomy of mouse and human nail units. *Anat Rec (Hoboken)* 2013; 296: 521–532.
- Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. *Genes Dev* 1998; 11: 3286–3305.
- Peifer M, Polakis P. Wnt signaling in oncogenesis and embryogenesis – a look outside the nucleus. *Science* 2000; 287: 1606–1609.
- Hao HX, Xie Y, Zhang Y et al. ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature* 2012; 485: 195–200.
- Niehrs C. The complex world of WNT receptor signalling. *Nat Rev Mol Cell Biol* 2012; 13: 767–779.

24. Ishii Y, Wajid M, Bazzi H et al. Mutations in R-spondin 4 (RSPO4) underlie inherited onychia. *J Invest Dermatol* 2008; 128: 867–870.
25. Blaydon DC, Ishii Y, O'Toole EA et al. The gene encoding R-spondin 4 (RSPO4), a secreted protein implicated in Wnt signaling, is mutated in inherited onychia. *Nat Genet* 2006; 38: 1245–1247.
26. Aoki M, Mieda M, Ikeda T et al. R-spondin3 is required for mouse placental development. *Dev Biol* 2006; 301: 218–226.
27. Bergmann C, Senderek J, Anhof D et al. Mutations in the gene encoding the Wnt-signaling component R-spondin 4 (RSPO4) cause autosomal recessive onychia. *Am J Hum Genet* 2006; 79: 1105–1109.
28. Khan TN, Klar J, Nawaz S et al. Novel missense mutation in the RSPO4 gene in congenital hyponychia and evidence for a polymorphic initiation codon (p.M11). *BMC Med Genet* 2012; 13: 120.
29. Kazanskaya O, Glinka A, del BarcoBarrantes I et al. R-Spondin2 is a secreted activator of Wnt/beta-catenin signaling and is required for Xenopus myogenesis. *Dev Cell* 2004; 7: 525–534.
30. Frojmark AS, Schuster J, Sobol M et al. Mutations in Frizzled 6 cause isolated autosomal-recessive nail dysplasia. *Am J Hum Genet* 2011; 88: 852–860.
31. Feenstra JM, Kanaya K, Pira CU et al. Detection of genes regulated by Lmx1b during limb dorsalization. *Dev Growth Differ* 2012; 54: 451–462.
32. Parr BA, McMahon AP. Dorsalizing signal Wnt-7a required for normal polarity of D-V and A-P axes of mouse limb. *Nature* 1995; 374: 350–353.
33. Chen H, Lun Y, Ovchinnikov D et al. Limb and kidney defects in Lmx1b mutant mice suggest an involvement of LMX1B in human nail patella syndrome. *Nat Genet* 1998; 19: 51–55.
34. Dai JX, Johnson RL, Ding YQ. Manifold functions of the Nail-Patella Syndrome gene Lmx1b in vertebrate development. *Dev Growth Differ* 2009; 51: 241–250.
35. Ghommid J, Petit F, Holder-Espinasse M et al. Nail-Patella Syndrome: clinical and molecular data in 55 families raising the hypothesis of a genetic heterogeneity. *Eur J Human Genet* 2015; 24: 44–50.
36. Marini M, Bocciardi R, Gimelli S et al. A spectrum of LMX1B mutations in Nail-Patella syndrome: new point mutations, deletion, and evidence of mosaicism in unaffected parents. *Genet Med* 2010; 12: 431–439.
37. Jiang S, Zhang J, Huang D et al. A microdeletion of chromosome 9q33.3 encompasses the entire LMX1B gene in a Chinese family with nail patella syndrome. *Int J Mol Sci* 2014; 15: 20158–20168.
38. Jumlongras D, Bei M, Stimson JM et al. A nonsense mutation in MSX1 causes Witkop syndrome. *Am J Hum Genet* 2001; 69: 67–74.
39. Reddy S, Andl T, Bagasra A et al. Characterization of Wnt gene expression in developing and postnatal hair follicles and identification of Wnt5a as a target of Sonic hedgehog in hair follicle morphogenesis. *Mech Dev* 2001; 107: 69–82.
40. Yamashiro T, Zheng L, Shitaku Y et al. Wnt10a regulates dentin sialophosphoprotein mRNA expression and possibly links odontoblast differentiation and tooth morphogenesis. *Differentiation* 2007; 75: 452–462.
41. Adaimy L, Chouery E, Megarbane H et al. Mutation in WNT10A is associated with an autosomal recessive ectodermal dysplasia: the odonto-onycho-dermal dysplasia. *Am J Hum Genet* 2007; 81: 821–828.
42. Pinheiro M, Pereira LC, Freire-Maia N. A previously undescribed condition: tricho-odonto-onycho-dermal syndrome. A review of the tricho-odonto-onychia subgroup of ectodermal dysplasias. *Br J Dermatol* 1982; 105: 371–382.
43. Fadhil M, Ghabra TA, Deeb M et al. Odontoonychodermal dysplasia: a previously apparently undescribed ectodermal dysplasia. *Am J Med Genet* 1983; 14: 335–346.
44. Mègarbané H, Haddad M, Delague V et al. Further delineation of the odonto-onycho-dermal dysplasia syndrome. *Am J Med Genet A* 2004; 129A: 193–197.
45. Bohring A, Stamm T, Spaich C et al. WNT10A mutations are a frequent cause of a broad spectrum of ectodermal dysplasias with sex-biased manifestation pattern in heterozygotes. *Am J Hum Genet* 2009; 85: 97–105.
46. Kurth I, Klopocki E, Stricker S et al. Duplications of noncoding elements 5' of SOX9 are associated with brachydactyly-anonychia. *Nat Genet* 2009; 41: 862–863.
47. Rangarajan A, Talora C, Okuyama R et al. Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. *EMBO J* 2001; 20: 3427–3436.
48. Lin MH, Kopan R. Long-range, nonautonomous effects of activated Notch1 on tissue homeostasis in the nail. *Dev Biol* 2003; 263: 343–359.
49. Cai J, Ma L. Msx2 and Foxn1 regulate nail homeostasis. *Genesis* 2011; 49: 449–459.
50. Shang L, Pruett ND, Awgulewitsch A. Hoxc12 expression pattern in developing and cycling murine hair follicles. *Mech Dev* 2002; 113: 207–210.
51. Pick L, Heffer A. Hox gene evolution: multiple mechanisms contributing to evolutionary novelties. *Ann N Y Acad Sci* 2012; 1256: 15–32.
52. Farooq M, Kurban M, Fujimoto A et al. A homozygous frameshift mutation in the HOXC13 gene underlies pure hair and nail ectodermal dysplasia in a Syrian family. *Hum Mutat* 2013; 34: 578–581.
53. Jave-Suarez LF, Winter H, Langbein L et al. HOXC13 is involved in the regulation of human hair keratin gene expression. *J Biol Chem* 2002; 277: 3718–3726.
54. Pruett ND, Tkatchenko TV, Jave-Suarez L et al. Krtap16, characterization of a new hair keratin-associated protein (KAP) gene complex on mouse chromosome 16 and evidence for regulation by Hoxc13. *J Biol Chem* 2004; 279: 51524–51533.
55. Tkatchenko AV, Visconti RP, Shang L et al. Overexpression of Hoxc13 in differentiating keratinocytes results in downregulation of a novel hair keratin gene cluster and alopecia. *Development* 2001; 128: 1547–1558.
56. Bazzi H, Demehri S, Potter CS, Barber AG et al. Desmoglein 4 is regulated by transcription factors implicated in hair shaft differentiation. *Differentiation* 2009; 78 (5): 292–300.
57. Pignata C, Fusco A, Amorosi S. Human clinical phenotype associated with FOXN1 mutations. *Adv Exp Med Biol* 2009; 665: 195–206.
58. Mangan S, Alon U. Structure and function of the feed-forward loop network motif. *Proc Natl Acad Sci USA* 2003; 100: 11980–11985.
59. Potter CS, Pruett ND, Kern MJ et al. The nude mutant gene Foxn1 is a HOXC13 regulatory target during hair follicle and nail differentiation. *J Invest Dermatol* 2011; 131: 828–837.
60. Gu LH, Coulombe PA. Keratin function in skin epithelia: a broadening palette with surprising shades. *Curr Opin Cell Biol* 2007; 19: 13–23.
61. Perrin C, Langbein L, Schweizer J. Expression of hair keratins in the adult nail unit: an immunohistochemical analysis of the onychogenesis in the proximal nail fold, matrix and nail bed. *Br J Dermatol* 2004; 151: 362–371.
62. Shimomura Y, Wajid M, Kurban M et al. Mutations in the keratin 85 (KRT85/hHb5) gene underlie pure hair and nail ectodermal dysplasia. *J Invest Dermatol* 2010; 130: 892–895.
63. Schweizer J, Langbein L, Rogers MA et al. Hair follicle-specific keratins and their diseases. *Exp Cell Res* 2007; 313: 2010–2020.
64. Rasool M, Nawaz S, Azhar A et al. Autosomal recessive pure hair and nail ectodermal dysplasia linked to chromosome 12p11.1-q14.3 without KRT85 gene mutation. *Eur J Dermatol* 2010; 20: 443–446.
65. Raykova D, Klar J, Azhar A et al. Autosomal recessive transmission of a rare KRT74 variant causes hair and nail ectodermal dysplasia: allelism with dominant woolly hair/hypotrichosis. *PLoS One* 2014; 9: e93607.
66. Langbein L, Rogers MA, Praetzel S et al. K6irs1, K6irs2, K6irs3, and K6irs4 represent the inner-root-sheath-specific type II epithelial keratins of the human hair follicle. *J Invest Dermatol* 2003; 120: 512–522.
67. Mazzalupo S, Wong P, Martin P et al. Role for keratins 6 and 17 during wound closure in embryonic mouse skin. *Dev Dyn* 2003; 226: 356–365.
68. Chamcheu JC, Siddiqui IA, Syed DN et al. Keratin gene mutations in disorders of human skin and its appendages. *Arch Biochem Biophys* 2011; 508: 123–137.
69. Gebhardt M, Fischer T, Claussen U et al. Monilethrix – improvement by hormonal influences? *Pediatr Dermatol* 1999; 16: 297–300.
70. Heydt GE. ON INFORMATION ON THE MONILETHRIX SYNDROME. *Arch Klin Exp Dermatol* 1963; 217: 15–29.
71. Bentley-Philips B. Monilethrix and pseudomonilethrix. In: Orfanos CE, ed. Hair and hair disorders. Stuttgart, Germany: G Fischer, 1979: 447–455.
72. Ramot Y, Zlotogorski A. Keratins: the hair shaft's backbone revealed. *Exp Dermatol* 2015; 24: 416–417.
73. Inada H, Izawa I, Nishizawa M et al. Keratin attenuates tumor necrosis factor-induced cytotoxicity through association with TRADD. *J Cell Biol* 2001; 155: 415–426.
74. Yoneda K, Furukawa T, Zheng YJ et al. An autocrine/paracrine loop linking keratin 14 aggregates to tumor necrosis factor alpha-mediated cytotoxicity in a keratinocyte model of epidermolysis bullosa simplex. *J Biol Chem* 2004; 279: 7296–7303.

75. Lugassy J, McGrath JA, Itin P et al. KRT14 haploinsufficiency results in increased susceptibility of keratinocytes to TNF-alpha-induced apoptosis and causes Naegeli-Franceschetti-Jadassohn syndrome. *J Invest Dermatol* 2008; 128: 1517–1524.
76. Itin PH, Lautenschlager S, Meyer R et al. Natural history of the Naegeli-Franceschetti-Jadassohn syndrome and further delineation of its clinical manifestations. *J Am Acad Dermatol* 1993; 28: 942–950.
77. Mills AA, Zheng B, Wang XJ et al. p63 is a p53 homologue required for limb and epidermal morphogenesis. *Nature* 1999; 398: 708–713.
78. Langlois S, Maher AC, Manias JL et al. Connexin levels regulate keratinocyte differentiation in the epidermis. *J Biol Chem* 2007; 282: 30171–30180.
79. Fujimoto A, Kurban M, Nakamura M et al. GJB6, of which mutations underlie Clouston syndrome, is a potential direct target gene of p63. *J Dermatol Sci* 2013; 69: 159–166.
80. Trzeciak WH, Koczorowski R. Molecular basis of hypohidrotic ectodermal dysplasia: an update. *J Appl Genet* 2016; 57: 51–61.
81. Durmowicz MC, Cui CY, Schlessinger D. The EDA gene is a target of, but does not regulate Wnt signaling. *Gene* 2002; 285: 203–211.
82. Laurikkala J, Pispala J, Jung HS et al. Regulation of hair follicle development by the TNF signal ectodysplasin and its receptor Edar. *Development* 2002; 129: 2541–2553.
83. Shimomura Y, Christiano AM. Biology and genetics of hair. *Annu Rev Genomics Hum Genet* 2010; 11: 109–132.
84. Bazzi H, Fantauzzo KA, Richardson GD et al. The Wnt inhibitor, Dickkopf 4, is induced by canonical Wnt signaling during ectodermal appendage morphogenesis. *Dev Biol* 2007; 305: 498–507.
85. Morlon A, Munnich A, Smahi A. TAB2, TRAF6 and TAK1 are involved in NF-kappaB activation induced by the TNF-receptor, Edar and its adaptor Edaradd. *Hum Mol Genet* 2005; 14: 3751–3757.
86. DiDonato JA, Hayakawa M, Rothwarf DM et al. A cytokine-responsive IkkappaB kinase that activates the transcription factor NF-kappaB. *Nature* 1997; 388: 548–554.
87. Deng L, Wang C, Spencer E et al. Activation of the IkkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* 2000; 103: 351–361.
88. Smahi A, Courtois G, Rabia SH et al. The NF-kappaB signalling pathway in human diseases: from incontinentia pigmenti to ectodermal dysplasias and immune-deficiency syndromes. *Hum Mol Genet* 2002; 11: 2371–2375.
89. Ensor CM, Tai HH. 15-Hydroxyprostaglandin dehydrogenase. *J Lipid Mediat Cell Signal* 1996; 12: 313–319.
90. Anggård E, Larsson C, Samuelsson B. The distribution of 15-hydroxy prostaglandin dehydrogenase and prostaglandin-delta 13-reductase in tissues of the swine. *Acta Physiol Scand* 1971; 81: 396–404.
91. Uppal S, Diggle CP, Carr IM et al. Mutations in 15-hydroxyprostaglandin dehydrogenase cause primary hypertrophic osteoarthropathy. *Nat Genet* 2008; 40: 789–793.
92. Tariq M, Azeem Z, Ali G et al. Mutation in the HPGD gene encoding NAD+ dependent 15-hydroxyprostaglandin dehydrogenase underlies isolated congenital nail clubbing (ICNC). *J Med Genet* 2009; 46: 14–20.
93. Farooq M, Kurban M, Abbas O et al. A novel mutation in the PLCD1 gene, which leads to an aberrant splicing event, underlies autosomal recessive leukonychia. *Br J Dermatol* 2012; 167: 946–949.
94. Kiuru M, Kurban M, Itoh M et al. Hereditary leukonychia, or porcelain nails, resulting from mutations in PLCD1. *Am J Hum Genet* 2011; 88: 839–844.
95. Nakamura Y, Ichinohe M, Hirata M et al. Phospholipase C-delta 1 is an essential molecule downstream of Foxn1, the gene responsible for the nude mutation, in normal hair development. *FASEB J* 2008; 22: 841–849.
96. Seo S, Fujita H, Nakano A et al. The forkhead transcription factors, Foxc1 and Foxc2, are required for arterial specification and lymphatic sprouting during vascular development. *Dev Biol* 2006; 294: 458–470.
97. Finegold DN, Kimak MA, Lawrence EC et al. Truncating mutations in FOXC2 cause multiple lymphedema syndromes. *Hum Mol Genet* 2001; 10: 1185–1189.
98. Rezaie T, Ghoroghchian R, Bell R et al. Primary non-syndromic lymphoedema (Meige disease) is not caused by mutations in FOXC2. *Eur J Hum Genet* 2008; 16: 300–304.
99. Itin PH, Eich G, Fistarol SK. V-shaped, longitudinal nail dystrophies in trichorhinophalangeal syndrome type I. *Dermatology* 2005; 211: 162–164.
100. Paus R, Cotsarelis G. The biology of hair follicles. *N Engl J Med* 1999; 341: 491–497.
101. Cotsarelis G. Epithelial stem cells: a folliculocentric view. *J Invest Dermatol* 2006; 126: 1459–1468.
102. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* 1990; 61: 1329–1337.
103. Sprecher E. Genetic hair and nail disorders. *Clin Dermatol* 2005; 23: 47–55.
104. Sellheyer K. Nail stem cells. *J Dtsch Dermatol Ges* 2013; 11: 235–239.
105. Zaias N, Alvarez J. The formation of the primate nail plate. An autoradiographic study in squirrel monkey. *J Invest Dermatol* 1968; 51: 120–136.
106. Mizell M. Limb regeneration: induction in the newborn opossum. *Science* 1968; 161: 283–286.
107. Singer M. Introduction of regeneration of forelimb of the frog by augmentation of the nerve supply. *Proc Soc Exp Biol Med* 1951; 76: 413–416.
108. Becker RO. The bioelectric factors in amphibian-limb regeneration. *J Bone Joint Surg Am* 1961; 43: 643–656.
109. Illingworth CM. Trapped fingers and amputated finger tips in children. *J Pediatr Surg* 1974; 9: 853–858.
110. Zhao W, Neufeld DA. Bone regrowth in young mice stimulated by nail organ. *J Exp Zool* 1995; 271: 155–159.
111. Vidal P, Dickson MG. Regeneration of the distal phalanx. A case report. *J Hand Surg Br* 1993; 18: 230–233.
112. Neufeld DA, Zhao W. Phalangeal regrowth in rodents: postamputational bone regrowth depends upon the level of amputation. *Prog Clin Biol Res* 1994; 383A: 243–252.
113. Mohammad KS, Day FA, Neufeld DA. Bone growth is induced by nail transplantation in amputated proximal phalanges. *Calcif Tissue Int* 1999; 65: 408–410.
114. Doffinger R, Smahi A, Bessia C et al. X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. *Nature Genet* 2001; 27: 277–285.
115. Courtois G, Smahi A, Reichenbach J et al. A hypermorphic IkkappaB mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency. *J Clin Invest* 2003; 112: 1108–1115.
116. Leslie EJ, O'Sullivan J, Cunningham ML et al. Expanding the genetic and phenotypic spectrum of popliteal pterygium disorders. *Am J Med Genet A* 2015; 167A: 545–552.
117. Clements SE, Techanukul T, Holden ST et al. Rapp-Hodgkin and Hay-Wells ectodermal dysplasia syndromes represent a variable spectrum of the same genetic disorder. *Br J Dermatol* 2010; 163: 624–629.
118. Kang S, Graham JM Jr, Olney AH, Biesecker LG. GLI3 frameshift mutations cause autosomal dominant Pallister-Hall syndrome. *Nat Genet* 1997; 15: 266–268.
119. Ruiz-Perez VL, Ide SE, Strom TM et al. Mutations in a new gene in Ellis-van Creveld syndrome and Weyers acrodermal dysostosis. *Nat Genet* 2000; 24: 283–286.
120. Vulliamy T, Marrone A, Szydlo R et al. Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. *Nat Genet* 2004; 36: 447–449.