COMMENTS

management of nonresponders (Feuerstein et al., 2017), and guidelines addressing proactive monitoring may be on the horizon. The psoriasis community will benefit from similar guidance, and parallel research across IMIDs may help to advance the field rapidly. We present a speculative framework (Figure 1) conceptually adapted from conditional recommendations in the field of IBD (Feuerstein et al., 2017). For all future guidelines focusing on anti-TNF therapy, cost will and should be a critical consideration. Given the money at stake, recommendations may be motivated by cost-effectiveness to an unprecedented degree.

CONFLICT OF INTERESTS
The authors state no conflict of interests.

REFERENCES


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KLHL24: Beyond Skin Fragility
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KLHL24 mutations have recently been associated with epidermolysis bullosa simplex. Initial studies focused on skin fragility. However, the picture of KLHL24 mutations causing extracutaneous human disease is emerging, with dilated cardiomyopathy as a strong association. In addition, neurological disease is suspected as well. Careful clinical follow-up and functional studies of (mutated) KLHL24 in these tissues are needed.


KLHL24, encoded by the gene KLHL24, is the “new kid on the block” in the hereditary skin fragility disorder epidermolysis bullosa simplex (EBS) (He et al., 2016; Lin et al., 2016). The basal variant of EBS with a level of blister formation through the basal keratinocytes is caused by mutations in genes encoding the basal cell keratins keratin (K) 5 or K14 in the majority of cases. However, approximately 25% of EBS cases were unsolved on the DNA level before next generation sequencing techniques emerged (Bolling et al., 2011). In 2016, Lin et al. (2016) and He et al. (2016), using whole-exome sequencing, discovered dominant acting point mutations in the start codon of KLHL24 caused basal cell skin fragility. Since then, several other patients with basal EBS caused by KLHL24 mutations, all affecting the same start codon, have been reported (Alkhalifa et al., 2018; Lee et al., 2017; Yanamandra et al., 2018). KLHL24, unlike K5 and K14, is not a structural protein. KLHL24 belongs to a family of proteins with a Kelch-like motif that is part of a ubiquitination-ligase complex and is involved in tight regulation of its substrate levels. Lin et al. (2016) suggested that KLHL24 was the substrate receptor of the cullin 3 (CUL3)–RBX1–KLHL24 ubiquitin-ligase complex, with K14 being the ubiquitination substrate based on in vitro experiments with recombinant proteins. They showed that the EBS-associated KLHL24 start codon mutation caused an N-terminal truncation of KLHL24,

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rendering the protein more stable because of abolished auto-ubiquitination, thereby causing increased breakdown and loss of K14 and K5 reduction as well (Lin et al., 2016). However, other groups (He et al., 2016; Lee et al., 2017) showed positive, although disorganized, staining of K14 in skin biopsy samples and increased amounts of K14 and K5 in keratinocyte cultures from patients with mutated KHL24, suggesting that the mutated proteins have a lower ability to promote keratin 14 degradation than the normal molecule, similar to the accumulation of undesired substrates seen in deficiencies of other members of the Kelch superfamily (Gupta and Beggs, 2014). We observed significantly reduced K14 expression the neonatal healthy-appearing skin of a KHL24-mutated patient, and staining of skin at the age of 14 years showed normal K14 (Yenamandra et al., 2018), suggesting that KHL24 regulation of K14 turnover may be more impaired during epidermal proliferation such as with body growth. The reported burn-like scars in patients with EBS due to KHL24 mutations are not seen in EBS caused by mutations in other EBS-associated genes (Alkhaliﬁa et al., 2018; He et al., 2016; Yenamandra et al., 2018). Additionally, Yenamandra et al. reported an abnormal basement membrane structure with thickening and thinning of the lamina densa with re-duplications and blind oﬀshoots typically encountered in poikiloderma, indicating other or additional skin pathology besides keratin filament fragility in basal keratinocytes in the case of perturbed KHL24. KHL24 is widely expressed in tissues other than skin, including brain, heart, skeletal muscle, kidney, liver, lung, and pancreas, with many tissues showing higher expression than skin (He et al., 2016; Lin et al., 2016). Recent reports strongly indicate extracutaneous sequelae of KHL24 mutations.

**Clinical Implications**

- **Dominant KHL24 mutations truncating the N-terminus of KHL24 are associated with a cardiocutaneous syndrome of epidermolysis bullosa simplex with dilated cardiomyopathy, warranting cardiac follow-up in every patient carrying a KHL24 mutation.**
- **Mutations in other regions of KHL24 besides the start codon are associated with human disease as well (cardiomyopathy and intellectual disability).**
- **Considering the high expression of KHL24 in neuronal and skeletal muscle tissue, and several reports of neurological problems like intellectual disability, developmental delay, memory problems, seizures, and muscular weakness, careful neurological evaluation is warranted in patients with mutated KHL24.**
- **The function of KHL24 and the effects of mutations in neuronal and cardiac tissue is unknown, and additional functional studies are needed.**

**KLHL24 mutations cause a cardiocutaneous syndrome with dilated cardiomyopathy**

In addition to an earlier case report (Yenamandra et al., 2018), Schwieger-Briel et al. (2018) report a cohort of 20 EBS patients with the known KHL24 missense mutations in which a strikingly high percentage of patients suffered from dilated cardiomyopathy (DCM): 85% had evidence of cardiac involvement with either elevated cardiac biomarkers or proven DCM (8/20 patients, 40%), leading to death at an early age in two patients. DCM may be a final common pathway of various cardiac pathologies; however, in all reported patients no signs of other causes were found. In addition, mutations in other DCM-associated genes have been carefully excluded. These data strongly indicate that dominant N-terminal truncating KHL24 mutations cause a potential severe and lethal DCM. In addition, a homozygous nonsense mutation in KHL24 was mentioned in two patients with severe hypertrophic cardiomyopathy (Hedberg-Oldfors et al., 2016), indicating that other KHL24 mutations than the EBS-causing ones may lead to cardiac pathology. However, no further functional data on this mutation were presented. Down-regulation of the KHL24 homologue in zebrafish resulted in defective heart development and early lethality; however, in mice this was not seen (Lin et al., 2016). This was not seen in mice, although mice were not subjected to cardiac stress, which could have elicited a phenotype (Lin et al., 2016). The pathomechanism of KHL24 mutations leading to DCM remains to be investigated. The suggestion that KHL24 is a regulator of controlled degradation of the intermediate filament keratin network in keratinocytes and vimentin in fibroblasts (He et al., 2016) makes it tempting to speculate that desmin is the substrate in cardiomyocytes. Schwieger-Briel et al. (2018) make an interesting suggestion that a defective interaction of mutated KHL24 with the COP9 signalosome, a critical regulator of cullin-RING family of ubiquitin ligase activity, could be a potential pathway, because defective COP9 signalosomes have been associated with DCM in mice.

Another interesting observation is the cell membrane localization and colocalization of KHL24 with desmoplakin in keratinocytes and cardiomyocytes (He et al., 2016; Yenamandra et al., 2018), because mutations in the desmosomal protein desmoplakin are associated with DCM, either nonsyndromic or syndromic, in Carvajal syndrome, as well.

Further cardiac phenotyping of the KHL24 mutation knock-in mouse model under stressed and unstressed conditions and additional mutational rescue would be of great value, as well as knock-in cardiomyocyte three-dimensional cultures and induced pluripotent stem cell-derived cardiac tissues from patients carrying KHL24 mutations. The substrate of KHL24 in cardiomyocytes could be identified using methods similar to those used by He et al. (2016) and Lin et al. (2016).

**KHL24 mutations and other extracutaneous involvement**

Reports of patients with EBS KHL24 mutation have mentioned neurological problems, like developmental delay, memory problems, learning difficulties, and seizures (Schwieger-Briel et al., 2018; Yenamandra et al., 2018). In addition, a homozygous KHL24 missense mutation in the Kelch domain has recently been associated with intellectual disability (Anazi et al., 2017). This is interesting considering the high
levels of expression of KLHL24 in neuronal tissue, especially the cortex and hippocampus, which are involved in memory and higher-order tasks (Laezza et al., 2007; Lin et al., 2016). KLHL24 was shown to bind to the C-terminal domain of the kainate receptor GluR6 in neuronal tissue and regulate its function by interacting with PICK1 (Laezza et al., 2007). These proteins are highly expressed and co-expressed in the hippocampus and cerebral cortex. Glutamate receptors mediate the majority of excitatory neurotransmission in the brain. The GluR6 receptor may have a role in synaptic plasticity, and it is suggested to be important for learning and memory. Additionally, fragmentation of vimentin, also a cytoskeleton protein in neuronal tissue, was observed in KLHL24-mutated fibroblasts (He et al., 2016).

Other symptoms reported in the patient with KLHL24-mutated EBS DCM reported by Yenamandra et al. (2018) are ptosis and muscular weakness. Myopathy was also mentioned in the case reported by Anazi et al. (2017). KLHL24 is clearly expressed in skeletal muscle as well (Lin et al., 2016; unpublished data). When KLHL24 plays a role in desmin turnover and degradation, as is suggested for keratins and vimentin, myopathy could result. Because many of the patients described are very young, additional phenotypic features may evolve. Altogether, attention should be paid to the possibility of neurological and muscular symptoms like intellectual disability, developmental delay, memory problems, and muscular weakness in patients with KLHL24 mutations, and when present a referral to a neurologist to objectify the symptoms is indicated. Further molecular characterization of the effects of KLHL24 mutations on neuronal and skeletal muscle tissue is needed as well.

In conclusion, the initial reports of KLHL24 mutations focused on skin fragility. However, the picture of KLHL24 mutations causing a syndromic phenotype with extracutaneous involvement is emerging. This is relevant for basic science and understanding of pathogenesis and for clinical follow-up and prognosis. Particularly, KLHL24-EB6 mutations show a strong association with DCM, which is directly relevant to the prognosis and management of patients carrying these mutations. Neurological problems like developmental delay, intellectual disability, memory problems, and myopathy should be sought as well. The molecular mechanisms by which KLHL24 mutations cause pathology in (extra)cutaneous tissues remain to be elucidated and deserve further attention.

**CONFLICT OF INTEREST**

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**REFERENCES**


