



King's Research Portal

DOI:

[10.1016/j.jid.2017.01.004](https://doi.org/10.1016/j.jid.2017.01.004)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Lee, J. Y. W., Liu, L., Hsu, C.-K., Aristodemou, S., Ozoemena, L., Ogboli, M., Moss, C., Martinez, A. E., Mellerio, J. E., & McGrath, J. A. (2017). Mutations in KLHL24 add to the molecular heterogeneity of epidermolysis bullosa simplex. *Journal of Investigative Dermatology*, 137(6), 1378-1380. Advance online publication. <https://doi.org/10.1016/j.jid.2017.01.004>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Accepted Manuscript

Mutations in *KLHL24* add to the molecular heterogeneity of epidermolysis bullosa simplex

John Y.W. Lee, Lu Liu, Chao-Kai Hsu, Sophia Aristodemou, Linda Ozoemena, Malobi Ogboli, Celia Moss, Anna E. Martinez, Jemima E. Mellerio, John A. McGrath

PII: S0022-202X(17)30032-5

DOI: [10.1016/j.jid.2017.01.004](https://doi.org/10.1016/j.jid.2017.01.004)

Reference: JID 697

To appear in: *The Journal of Investigative Dermatology*

Received Date: 2 December 2016

Revised Date: 3 January 2017

Accepted Date: 6 January 2017

Please cite this article as: Lee JYW, Liu L, Hsu C-K, Aristodemou S, Ozoemena L, Ogboli M, Moss C, Martinez AE, Mellerio JE, McGrath JA, Mutations in *KLHL24* add to the molecular heterogeneity of epidermolysis bullosa simplex, *The Journal of Investigative Dermatology* (2017), doi: 10.1016/j.jid.2017.01.004.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Mutations in *KLHL24* add to the molecular heterogeneity of epidermolysis bullosa simplex

John Y. W. Lee^{1,*}, Lu Liu^{2,*}, Chao-Kai Hsu^{1,3,4}, Sophia Aristodemou², Linda Ozoemena², Malobi Ogboli⁵, Celia Moss⁵, Anna E. Martinez⁶, Jemima E. Mellerio^{1,6}, John A. McGrath¹

¹St John's Institute of Dermatology, King's College London (Guy's Campus), London, United Kingdom; ²Viapath, St Thomas' Hospital, London, United Kingdom, ³Department of Dermatology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan; ⁴Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan; ⁵Department of Paediatric Dermatology, Birmingham Children's Hospital NHS Foundation Trust, Birmingham, United Kingdom; ⁶Department of Dermatology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom.

*These authors contributed equally to this work.

Corresponding author: John A. McGrath, Dermatology Research Labs, Floor 9 Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT, United Kingdom. Tel: 44-2071886409; Fax: 44-2071888050; E-mail: john.mcgrath@kcl.ac.uk

Short title: *KLHL24* mutations in epidermolysis bullosa simplex

Abbreviations used: EB, epidermolysis bullosa; EBS, epidermolysis bullosa simplex; *KLHL24*; Kelch-like family member 24.

Funding sources: The authors acknowledge financial support from the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London.

Conflicts of interest: The authors state no conflict of interest.

Word count: 1084 **Tables:** 1 **Figures:** 1

Keywords: Epidermolysis bullosa simplex; KLHL24; keratin 14; ubiquitination; genetic heterogeneity; mutation

TO THE EDITOR,

Over the last 25 years, enormous progress has been made in understanding the molecular basis of inherited skin fragility in the protean group of disorders known as epidermolysis bullosa (EB). Within the most recent classification of EB, mutations in 18 different genes were implicated in the four major categories of EB, simplex (EBS), junctional (JEB), dystrophic (DEB) and Kindler syndrome (KS), with the greatest genetic heterogeneity seen in EBS (Fine et al., 2014). Initially, mutations in *KRT5* (keratin 5) and *KRT14* (keratin 14) were reported in the most common autosomal dominant localized forms of EBS (for a review on keratin skin diseases, see Knobel et al., 2015), but the diverse phenotypes and genotypes of EBS may also involve mutations in *PLEC* (plectin), *ITGA6* ($\alpha 6$ integrin subunit), *ITGB4* ($\beta 4$ integrin subunit), *PKP1* (plakophilin-1), *DSP* (desmoplakin), *JUP* (plakoglobin), *TGM5* (transglutaminase 5), *EXPH5* (exophilin-5), and *DST* (dystonin, 230-kDa bullous pemphigoid antigen) (Fine et al., 2014). Despite the large number of genes and mutations described in

EBS, however, it is evident that many cases (perhaps up to 25%) do not harbor mutations in any of these known genes, and thus the molecular basis of EBS is not yet completely understood (Bolling et al., 2011).

Recently, a new candidate gene for dominant EBS has emerged. Lin et al. (2016) discovered heterozygous mutations in the methionine start codon of *KLHL24* (Kelch-like family member 24) in five unrelated individuals with EBS (China, Israel). Almost simultaneously, He et al. (2016) reported a further 14 cases from 10 families (Germany, Switzerland, Finland, Qatar, Italy). *KLHL24* is part of the family of >40 genes with a Kelch-like motif that forms part of a ubiquitin-ligase complex (Dhanoa et al., 2013). The mutations all resulted in the loss of the first 28 amino acids of the protein, leading to a truncated protein that is more stable than the wild-type protein. This mutant protein then promotes excessive ubiquitination and degradation of keratin 14 (Lin et al., 2016). These observations invoked a new mechanism germane to inherited skin blistering, namely dysregulation of autoubiquitination. He et al. (2016) also identified truncated *KLHL24* resulting from the start codon mutations and use of a downstream methionine initiation codon. These authors observed abnormal intermediate filaments in keratinocytes and fibroblasts, with evidence for irregular and fragmented keratin 14, and data to support an altered balance in the stability and degradation of this keratin (He et al., 2016).

Collectively, these two studies indicate that *KLHL24* is a new candidate gene for mutations in currently unresolved cases of EBS. Within our DNA archive at the UK National Diagnostic EB Laboratory, as of December 2016, there are 183 cases of EBS for which no pathogenic mutation has yet been identified (approximates to ~20% of all EBS referrals). Mutations in *KRT5* and *KRT14* have been excluded in these cases (in addition to selected other known candidates based on clinical or skin pathology). Following ethics' committee

approval and written informed consent, using PCR and Sanger sequencing in these samples (see Table S1 for primer details), we identified seven cases (six families) with four different heterozygous mutations in *KLHL24*, all affecting the first methionine codon, including a seemingly unreported mutation (c.1A>T) (Figures S1 and S2). We also reviewed the ultrastructural skin pathology and clinicopathologic features in the affected individuals (all skin biopsies were taken within the first month of life). Our data indicate that mutations in *KLHL24* contribute significantly to the molecular pathology of a subtype of EBS, and that *KLHL24* should be considered for inclusion as a “19th gene” in future classifications of EB.

The clinical characteristics of these seven individuals with *KLHL24* mutations are summarized in Table 1. Based on this cohort of cases, and also those published (Lin et al., 2016; He et al., 2016), the phenotype associated with pathogenic mutations in *KLHL24*, although subject to variability, tends to present with quite marked birth trauma – especially on the lower legs, with additional early blistering often involving the trunk and upper limbs. These lesions typically heal quickly with subtle atrophic scarring (not evident histologically) but blistering persists throughout childhood (Figure 1a), particularly in response to minor trauma. Nail defects (Figure 1b) and oral ulceration are common whereas transient milia also occur. Dyspigmentation is not a prominent feature. With increasing age, blistering severity tends to lessen. None of the individuals in our series manifested any hair abnormalities, including the only adult (case 7, Table 1), contrary to some other published cases where adults were found to have hair loss, particularly in the scalp (Lin et al., 2016; He et al., 2016).

Skin immunohistochemistry offered no specific clues to the diagnosis. Notably, in only one of the six cases biopsied was there any indication of a very slight reduction in the intensity of keratin 14 immunostaining (case 5, Table 1). In the majority of cases, however,

and in contrast to the observations made by Lin et al. (2016), the intensity of keratin 14 immunolabeling was not discernibly different from control skin (Figure S3), with just some unevenness in keratin 14 labeling within keratinocytes, as noted by He et al. (2016). However, in semithin sections, there was evident pallor in basal keratinocytes (Figure 1c) which were particularly prominent in perilesional skin, but only seen in occasional basal keratinocytes in nonlesional skin. Ultrastructurally, cleavage occurred within the lower poles of basal keratinocytes (Figure 1d), and perilesional basal keratinocytes showed a paucity of intermediate filaments (Figure 1e and f). In contrast, the microtubular network became much more visible in these cells. In addition, there were numerous autophagosomes and autolysosomes in basal cells, and mitochondria were very prominent. These findings are consistent with dysregulation of autoubiquitination. In suprabasal cells, some intermediate filaments were present although these were often sparse and condensed in appearance. In nonlesional skin, some basal keratinocytes also showed a lack of intermediate filaments – some filaments were seen inserting into hemidesmosomes and desmosomes but most of the cytoplasm was completely devoid of keratin filaments. Mitochondria were again notable in these keratinocytes although autolysosomes were much less prominent than in perilesional skin. Additional light and electron micrographs illustrating these observations are included in the Supplementary material (Figures S4-S10).

In summary, *KLHL24* is a new candidate gene implicated in autosomal dominant EBS with mutations affecting the start codon of this gene responsible for a sizable proportion of currently unsolved EBS cases. Our cohort study underscores the impact of *KLHL24* mutations in expanding the molecular basis of EBS, identifies one new mutation within the methionine start codon, and provides more detail on the ultrastructural pathology present in the skin of affected individuals.

Acknowledgments

The authors acknowledge financial support from the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London.

References

Bolling MC, Lemmink HH, Jansen GH, Jonkman MF. Mutations in KRT5 and KRT14 cause epidermolysis bullosa simplex in 75% of the patients. *Br J Dermatol* 2011;164:637-44.

Dhanoa BS, Cogliati T, Satish AG, Bruford EA, Friedman JS. Update on the Kelch-like (KLHL) gene family. *Hum Genomics* 2013;7:13.

Fine JD, Bruckner-Tuderman L, Eady RA, Bauer EA, Bauer JW, Has C, et al. Inherited epidermolysis bullosa: updated recommendations on diagnosis and classification. *J Am Acad Dermatol* 2014;70:1103-26.

He Y, Maier K, Leppert J, Hausser I, Schwieger-Briel A, Weibel L, et al. Monoallelic Mutations in the Translation Initiation Codon of KLHL24 Cause Skin Fragility. *Am J Hum Genet* 2016;99:1395-1404.

Knöbel M, O'Toole EA, Smith FJ. Keratins and skin disease. *Cell Tissue Res* 2015;360:583-9.

Lin Z, Li S, Feng C, Yang S, Wang H, Ma D, et al. Stabilizing mutations of KLHL24 ubiquitin ligase cause loss of keratin 14 and human skin fragility. *Nat Genet* 2016;48:1508-16.

Table

Table 1. Summary of clinical features of individuals with *KLHL24* mutations.

Case	Age (years)	Sex	<i>KLHL24</i> mutation	Areas of birth damage	Scarring at sites of birth damage	Milia	Nail involvement	Oral involvement	Hair involvement
1	9	M	c.3G>A	Legs	+	-	+	+	-
2	9	M	c.2T>C	Legs, wrists	+	+	+	-	-
3	7	F	c.1A>G	Legs, arms, chest, ear	+	+	+	+	-
4	10	M	c.1A>G	Legs, arms, chest	+	-	+	+	-
5	3	M	c.3G>A	Legs, genitalia, arms, ears	+	-	+	-	-
6	7	M	c.1A>T	Legs, wrists, chest	+	-	+	-	-
7*	39	F	c.1A>T	Legs, feet, abdomen, forehead	+	-	+	-	-

*This individual is the mother of Case 6.

Figure legend.**Figure 1. Clinicopathologic features of this newly characterized form of EB simplex.**

(a) Trauma-induced erosion on the knee and atrophic scarring on the shin (Case 4); (b) acral erosions and blistering as well as thickening of the great toenail (Case 4); (c) semithin section (Case 5) showing pallor within the basal layer and early blister formation (bar = 50 μm); (d) electron micrograph (Case 5) demonstrating blister formation through the lower pole of basal keratinocytes (bar = 2 μm); (e) electron micrograph revealing pallor and vesicles/vacuoles within the lower epidermis (bar = 5 μm); (f) enlargement of the boxed area shown in (e) discloses a paucity of keratin intermediate filaments, several autolysosomes and numerous mitochondria (bar = 0.5 μm).

