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The keratin 16 null phenotype is modestly impacted by genetic strain background in mice

Abstract

The type I intermediate filament keratin 16 (K16) is constitutively expressed in ectoderm-derived appendages and is inducibly expressed in the epidermis upon barrier-compromising challenges. Dominantly acting missense alleles in *KRT16* are causative for pachyonychia congenita (PC), a genodermatosis involving debilitating palmoplantar keratoderma (PPK), nail dystrophy, oral lesions and, frequently, alterations in glands and hair. *C57Bl/6;Krt16*$^{-/-}$ mice develop oral lesions early after birth and PC-like PPK lesions as young adults. These PPK lesions have a marked dysregulation of skin barrier-related genes and innate immunity effectors (eg danger-associated molecular patterns) and are preceded by oxidative stress secondary to hypoactive Nrf2 signalling. These molecular features are present in PPK lesions of PC patients. Here, we report that all components of the *C57Bl/6;Krt16*$^{-/-}$ mouse phenotype occur as well in the *FVB* strain background, albeit less severely so, a significant observation in the light of variations in the clinical presentation of individuals harbouring disease-causing mutations in the *KRT16* gene.

1 | INTRODUCTION

Keratin 16 (K16), a type I intermediate filament gene and protein, is constitutively expressed in specific cell types in ectoderm-derived epithelial appendages (eg hair follicles, nail, glands, oral papillae etc.) and is inducibly expressed when stratified epithelia such as the epidermis are experiencing stress (eg UV exposure, wounding) or disease (eg psoriasis, carcinoma).[1] Genetic linkage analyses and DNA sequencing determined that dominantly acting missense alleles in *KRT16* are causative for pachyonychia congenita (PC),[2] a genodermatosis involving painful and debilitating palmoplantar keratoderma (PPK), nail dystrophy, oral lesions and, frequently, alterations in glands and hair as well.[3] Focal non-epidermolytic PPK lesions, which occur at pressure points in palms and soles, involve dramatic epidermal thickening and hyperkeratosis and are often significantly painful for patients. Of note, similar alleles in *KRT16* can elicit a presentation of focal non-epidermolytic PPK in the absence of other lesions characteristic of PC,[4] suggesting that the consequences associated with alterations in *KRT16* are subject to modifier gene(s) effects.

Mice null for *Krt16*, originally generated in the *C57Bl/6* background, develop oral lesions early after birth and PC-like PPK lesions as young adults.[5] Follow-up studies showed that the lesions in footpad skin involve a gross misregulation of skin barrier-related genes, including danger-associated molecular patterns (DAMPs; also known as alarmins) and effectors of innate immunity[6] and are preceded and caused by a state of oxidative stress secondary to misregulation of Keap1-Nrf2 signalling.[7] Several of the key features of PPK-like lesions in footpad skin of *C57Bl/6;Krt16*$^{-/-}$ mice are present in PPK lesions of individuals with PC.[6,7] Here, we describe the phenotype associated with the *Krt16* null allele in the *FVB* mouse strain background. This study was conducted for the primary purpose of defining the role of *Krt16* in HPV16-induced carcinogenesis in the *FVB* background (see Ref.[8]) but provided an opportunity to assess the impact of genetic strain background on expression of the *Krt16*$^{-/-}$ phenotype.

2 | RESULTS AND DISCUSSION

The *Krt16* null allele was transferred to the *FVB* strain background via six sequential backcrosses of *C57Bl/6;Krt16*$^{+/-}$ and wild-type *FVB* mice. Intercrosses between *FVB;Krt16*$^{+/-}$ were next conducted and the progeny analysed. A third of *FVB;Krt16*$^{-/-}$ offspring dies within a day after birth, while ~50% of these mice survive to adulthood (Figure 1A), which is slightly more than what had been observed in the *C57Bl/6* strain (by 17%;[5]). Macroscopic examination at postnatal day 5 (P5) reveals whitish and raised plaques (oral leukoplakia) in the posterior aspect of the dorsal tongue epithelium *FVB;Krt16*$^{-/-}$ mice, but not in *FVB;Krt16*$^{+/-}$ or *FVB;Krt16*$^{+/+}$ mice (Figure 1B). Histologically, examination of sagittal cross sections of this area of the dorsal tongue reveals a mildly thickened, disorganized and occasionally cytolytic epithelium (Figure 1C), with a normal complement of oral papilla (data not shown), at P5. Immunostaining of similar tissue sections reveals that expression of the differentiation-related K13 (Figure 1D) and its partner K4 (data not shown) is unaltered, whereas that of K17 is dramatically reduced in the dorsal tongue epithelium of *FVB;Krt16*$^{-/-}$ mice (Figure 1E). Altogether these observations, summarized in Figure S1, show that the survival at weaning and the character of oral lesions in *FVB;Krt16*$^{-/-}$ mice are similar to, but not as severe, as those previously reported for *C57Bl/6;Krt16*$^{-/-}$ mice.[5]

At the 2 months mark postbirth, surviving *FVB;Krt16*$^{-/-}$ mice still weigh less compared to *WT* littermates (Table S1). All surviving male and female *FVB;Krt16*$^{-/-}$ mice develop PPK-like lesions on their

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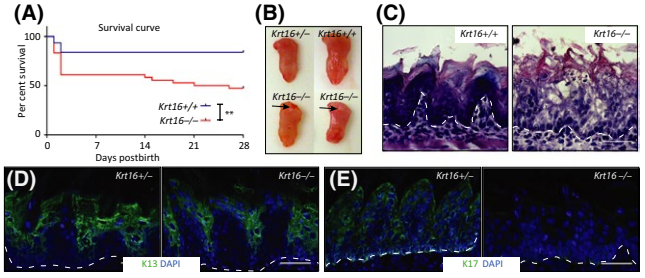


FIGURE 1 *FVB;Krt16*$^{-/-}$ mice have increased mortality compared to *WT* and develop oral lesions. (A) Survival curve of *FVB;Krt16* mice. n = 31-36 mice/genotype. ** denotes $P < .01$ determined by log-rank test. (B) Pictures of macroscopic tongue plaques that occur in *FVB;Krt16*$^{-/-}$ mice (both sexes) at P5. (C) H&E staining of P5 tongues to evaluate histology of female *FVB;Krt16*$^{-/-}$ tongue lesions. Scale bar, 50 μm (D and E) immunostaining for K13 and K17 in sections of fresh frozen tongue tissue from P5 male littermates

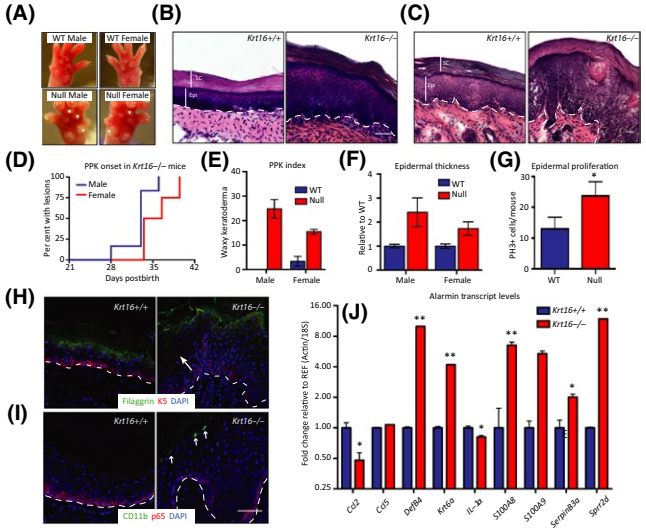


FIGURE 2 *FVB;Krt16*$^{-/-}$ mice develop PPK lesions. (A) Images of 6-week-old male (left) and female (right) *FVB;Krt16*$^{-/-}$ mice displaying macroscopic appearance of PPK lesions compared to controls. (B and C) H&E staining of front paw skin sections relating histological changes in 4- and 8-week-old *FVB;Krt16*$^{-/-}$ male mice. Dotted line corresponds to dermal/epidermal junction. Epi, epidermis; SC, stratum corneum. Scale bar, 50 μm. (D) Quantitation of PPK lesion onset in both sexes of *FVB;Krt16*$^{-/-}$ mice. Mice were scored as lesional when evidence of macroscopic lesions (waxy keratoderma) was present. n = 4-5 mice/sex. (E) Quantitation of PPK lesions severity based on presence of waxy keratoderma on front paws of 6-week-old *FVB;Krt16* mice. n = 2-4 mice/sex/genotype. (F) Sex-specific quantitation of epidermal thickness from 8-week-old *FVB;Krt16* front paws. n = 2-3 mice/genotype. (G) Quantitation of proliferation (measured by P-Histone H3 staining) in front paw skin from 8-week-old male *FVB;Krt16* mice. n = 3 mice/genotype. (H) Immunostaining for filaggrin (green) and keratin 5 (red) in front paw skin from 8-week-old male *FVB;Krt16* mice. (I) Immunostaining for CD11b (green) and p65 (red) in front paw skin from 8-week-old male *FVB;Krt16* mice. Scale bar, 50 μm. (J) Transcript levels for danger-associated molecular patterns (DAMPs) in front paw skin from 8-week-old *FVB;Krt16* null mice relative to *WT* controls. *denotes $P < .05$, and ** denotes $P < .01$ as determined by unpaired 2-tailed Student's *t* test on dCq values

front paws (Figure 2A), as we reported for *C57Bl/6;Krt16*$^{-/-}$ mice.[5] Male mice exhibit a slightly earlier onset of PPK relative to females (average 4.67 ± 0.37 vs 5.04 ± 0.41 weeks; see Figure 2D) and also exhibit a greater PPK index, a macroscopic and blinded measure of lesion severity, at 6 weeks of age (Figure 2,A,E). A similar sex bias for PPK onset also occurs in *C57Bl/6;Krt16*$^{-/-}$ mice.[9] Histologically, the paw skin of *FVB;Krt16*$^{-/-}$ mice exhibits thickened epidermis and modest hyperkeratosis starting at 4 weeks (Figure 2B) and peaking at 8 weeks (Figure 2C; sex-specific quantitation reported in Figure 2F). Lesional footpad skin shows decreased staining for filaggrin (Figure 2H), suggesting impaired epidermal differentiation, a ~2.5-fold increase in phosphorylated Histone H3-positive nuclei in the basal layer of *FVB;Krt16*$^{-/-}$ relative to *FVB;Krt16*$^{+/+}$ epidermis, indicating a mild state of hyperproliferation (Figure 2G), and cells positive for the innate immune cell marker CD11b, suggesting an inflammatory infiltrate (Figure 2I). These observations are qualitatively similar to those made when characterizing *C57Bl/6;Krt16*$^{-/-}$ mice [5] (see Figure S1).

Next, we assessed some of the key molecular readouts that had been shown to be misregulated in footpad skin of *C57Bl/6;Krt16*$^{-/-}$ mice. Emphasis was placed on mRNA levels for DAMPs (danger-associated molecular patterns; see[6]), key effectors of the glutathione-based antioxidant system and of the Keap1-Nrf2 system.[7] Transcript levels for *Krt6a*, the type II partner gene for *Krt16* and a sensitive indicator of cell and tissue stress, and of several DAMPS including *DefB4*, *S100A8* and *S100A9* were markedly elevated (Figure 2J). Additionally, the mRNA transcript levels for *Sprr2d* and *SerpinB3a*, which contribute to genesis of the cornified envelope, were also markedly elevated (Figure 2J). Some of these findings were corroborated at the protein level, for example, by Western blotting for S100A8 and the activated form of IL-1β (data not shown). These observations suggest that while qualitatively similar, the alterations occurring in the skin barrier are less severe in *FVB;Krt16*$^{-/-}$ relative to *C57Bl/6;Krt16*$^{-/-}$ mice.

One of the key justifications of this set of experiments was to assess the impact of *Krt16* loss for HPV16$^{tg/+}$-induced tumorigenesis in *FVB* mouse skin (see[8]). *FVB;Krt16*$^{+/-}$ mice were thus bred to the *FVB;HPV*$^{tg/+}$ mouse line as described.[8] Remarkably, 100% of the *Krt16*$^{-/-}$*;HPV*$^{tg/+}$ mice died within 2 weeks after birth (Table S2). While the cause of death remains undefined and calls for substantive follow-up studies, the outcome of this cross is indicative of a strong genetic interaction between the HPV16 transgene and the *Krt16* null allele.

In conclusion, our findings suggest that while genetic strain background does not impact the presentation of the *Krt16* null phenotype in mouse at a qualitative level, it does influence the intensity of virtually all aspects of the presentation, ranging from perinatal lethality to the severity of oral and PPK lesions. Differences in inflammatory responses between *C57Bl/6* and *FVB* strains may account for these observations.[10,11] These findings are thus consistent with the variable penetrance of the clinical presentation in individuals whose genome

harbour-mutated *KRT16* alleles,[2–4] and point to the *Krt16* null mouse model as a useful resource to define the determinants involved.

3 | MATERIALS AND METHODS

The methods used in this study are described under "Data S1."

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CONFLICT OF INTEREST

The authors have declared no conflicting interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

FIGURE S1 Comparison of *Krt16* null phenotypes between *C57Bl/6* and *FVB* strain backgrounds

FIGURE S2 PPK Index: Assessment of Paw Lesion Severity

DATA S1 Supplemental Materials

TABLE S1 Total body weight (±standard deviation) at 2 months' old

TABLE S2 Survival curve of *FVB;Krt16-/-* crossed with HPVtg/+ mice